The Development of the Pharyngeal Region in the Dog

Thesis for the Degree of Doctor of Philosophy

by

O.W. Sack, D.V.M., M.R.C.V.S.

May, 1962.

CONTENTS

Acknowledgements

The work for this thesis has been carried out in the Department of Anatomy, Royal (Dick) School of Veterinary Studies, University of Edinburgh, under the supervision of Professor T. Grahame, T.D., F.R.C.V.S., F.R.S.E.

The author wishes to express his sincere thanks to Professor Grahame for his unceasing great interest, good counsel and continuous support, and for having made available the facilities and equipment of his department. Through his assistance a number of valuable known-age specimens were obtained for study. Sincere thanks are also due to my veterinary colleagues of the Department of Anatomy for their generous help and encouragement.

The author is greatly indebted to Mr. R.C.W.S. Hood, F.I.B.P., F.I.S.T., who has devoted much care and time to the production of excellent photographs, and to Mr. E. Roberts, T.D., F.I.S.T., and Mr. A. Laing, A.I.S.T., for their valuable technical advice in the preparation of serial sections. He would also like to thank the many Canadian veterinary practitioners, and veterinary clinics both in Canada and in Scotland, for having collected the bulk of the embryos used in this study.

The writer wishes to acknowledge the financial assistance he received from the British Council for the first year of his study and from the Ontario Research Foundation for the second year. He is extremely grateful to these agencies for having been found worthy of such generous support.

The great care and thought given by Miss Margaret Nairn to the typing of the text is especially appreciated.

I. INTRODUCTION

The morphogenesis of the pharyngeal region is believed to be involved in the occurrence of so-called "Branchial Cysts", a condition affecting human and canine adolescents of comparable age, and which is characterised by the appearance of cystic lesions on the lateral aspects of the upper portion of the neck. These lesions are usually small in the human, while in the dog, due to the presence of a hair coat, they remain undiscovered until they have become large and pendulous. It is generally believed that branchial cysts arise from remnants of the branchial apparatus which are induced to proliferate by certain hormonal combinations present in the young individual. Investigators (Bhaskar and Bernier, 1959), who recently examined a large number of human lesions deny a purely branchiogenic origin however, and postulate that epithelial remnants of branchial or, what is more likely, parotid origin, after becoming trapped in developing lymph nodes, produce this condition, which according to their view should be renamed "Benign Lymphoepithelial Cysts".

Investigations into the etiology of branchial cysts in the dog, at present being carried out at the author's home university (Ontario Veterinary College, University of Toronto), became focused on the developmental processes of the pharyngeal region in that animal, because experience with the human condition suggested a developmental defect.

A search of the literature available to the author revealed that the development of the canine pharyngeal region was practically unknown, only some selected aspects such as the early development of the thyroid and parathyroids having been described by Godwin (1936, 1937 a) of the Kingsbury school. It was uncovered further that the developmental anatomy of the dog as a whole had scarcely been studied, which is surprising in view of this animal's importance to the veterinary surgeon and to the research worker.

The present study, then, was undertaken in an attempt to close the obvious gap in our knowledge of canine embryogenesis, and to provide those interested in the etiology of branchial cysts with what might be considered a working basis for further clinical and embryological examinations. It was found necessary to survey the entire field of pharyngeal development, from the appearance of a foregut to conditions found at full term, since confinement of the field, even though it could have been explored to much greater depth, would have proved useful neither to those interested in canine embryology nor to those studying branchial cysts. The former would find that the large gap in the knowledge of this region still persisted and the latter would not be able to select the particular anatomical region or developmental stage which may interest them for further scrutiny.

The lack of previous work on canine development and subsequent absence of known standards of developmental reference made it necessary to devote much space to the description and staging of the embryological material prior to sectioning. The bulk of the thesis however is devoted to the description of the developmental processes observed in the pharyngeal region, and minor points worthy of short discussion are dealt with here, because it was thought that due to the wide field covered in this study a separate discussion would appear rather disjointed. Major anatomical areas however have been extracted from this section and are presented at the end in the form of REGIONAL REVIEWS.

II. MATERIAL

This study is based on 88 prenatal canine specimens ranging from the blastocyst stage to full term. Of these, 62 specimens were obtained from veterinary practitioners and university clinics and the remaining 26, mostly of known copulation age, were collected by the writer himself (Table 1).

Table I List of embryos and fetuses used in this study, arranged according to size.

Serial No.	Log No.	Length	Plane of section	Copulation age in days	Remarks
1	-	1.2 mm. G.L.		18	blastocyst
2	-	1.3 mm. "		18	blastocyst
3	-	1.3 mm. "		18	blastocyst
4	D 6	1.5 mm. "	sagittal	18	blastocyst
5	-	1.5 mm. "		17	blastocyst
6	-	2.0 mm. "		17	blastocyst
7	-	2.0 mm. "		17	blastocyst
8	-	2.3 mm. "		17	blastocyst
9	D 12	2.5 mm. "	sagittal	21	
10	D 37	3.0 mm. "	whole mount	17	blastocyst
11	D 22	3.0 mm. "	sagittal	21	
12	D 5	3.5 mm. "	transverse	21	3 models
13	D 14	4.5 mm. "	sagittal		
14	D 15	4.5 mm. "	sagittal		2 models
15	D 23	4.5 mm. "	transverse		
16	D 76	5.0 mm.(!) "	horizontal		
17	D 8	5.0 mm. "	horizontal		
18	D 67	5.5 mm. "	sagittal	21	
19	D 75	6.0 mm.(!) "	transverse		1 model
20	D 68	6.0 mm. "	transverse	21	

Serial No.	Log No.	Length	Plane of section	Copulation age in days	Remarks
21	CC 6	6.0 mm. G.L.	sagittal	21	2 models
22	D 66	6.2 mm. "	horizontal	21	
23	D 17	6.3 mm. "	sagittal		
24	D 74	6.5 mm.(!) "	sagittal		
25	D 26	-	sagittal		2 models
26	D 27	-	transverse		
27	D 28	-	horizontal		
28	D 18	8.4 mm. "	sagittal		1 model
29	D 4	8.5 mm. "	horizontal		
30	D 1	8.5 mm. "	horizontal		1 model
31	D 31	8.6 mm. "	horizontal		
32	D 19	9.3 mm. "	sagittal		
33	D 19a	9.4 mm. "	sagittal		
34	D 25	-	sagittal		
35	D 24	9.5 mm.(!) "	transverse		
36	CC 4	9.6 mm. "	transverse		
37	D 32	9.8 mm. "	transverse		
38	CC 3	10.0 mm. "	sagittal		
39	D 21	10.3 mm. "	sagittal	23	
40	D 11	11.0 mm. "	sagittal	26	2 models
41	D 13	-	sagittal	22(?)	
42	D 36	-	horizontal	26	
43	D 41	14.5 mm. "	transverse		
44	CC 2	15.0 mm. "	horizontal		
45	D 35	15.0 mm. "	sagittal		
46	D 9	15.0 mm. "	horizontal		2 models
47	DD 1	15.0 mm. "	-		dissection
48	CC 1	15.5 mm. "	sagittal		
49	D 42	16.0 mm. "	horizontal		
50	D 40	21.5 mm. "	sagittal		
51	D 3	21.5 mm. "	horizontal		
52	D 39	22.0 mm. "	horizontal		
53	D 38	22.5 mm. "	transverse		
54	DD 2	23.0 mm. "	-		dissection

Serial No.	Log No.	Length		Plane of section	Copulation age in days	Remarks
55	D 44	25.0 mm.(!)	G.L.	transverse		
56	CC 7	28.0 mm.	"	sagittal		
57	DD 4	28.0 mm.	"	-		dissection
58	D 45	29.0 mm.	"	sagittal		
59	CC 8	29.0 mm.	"	horizontal		
60	D 43	29.0 mm.	"	horizontal		
61	DD 3	30 mm.	"	-		dissection
62	D 47	33 mm.	"	transverse		
63	D 46	41 mm.	C.R.L.	sagittal		
64	D 49	43 mm.	"	sagittal		
65	D 48	48 mm.	"	transverse		
66	D 50	50 mm.	"	transverse		
67	D 2	50 mm.	"	horizontal		
68	DS 3	50 mm.	"	sagittal		
69	DS 4	52 mm.	"	sagittal		
70	D 51	53 mm.	"	transverse		
71	D 53	66 mm.	"	transverse		
72	D 54	68 mm.	"	sagittal		
73	D 55	68 mm.	"	transverse		
74	D 58	70 mm.	"	sagittal		
75	D 59	-		transverse		
76	D 61	91 mm.	"	transverse		
77	D 60	92 mm.	"	sagittal		
78	D 63	93 mm.	"	horizontal		
79	D 65	118 mm.	"	sagittal		
80	D 71	118 mm.	"	-		dissection
81	D 64	120 mm.	"	transverse		
82	DD 8	178 mm.	"	-		dissection
83	D 73	180 mm.	"	sagittal		
84	DD 5	180 mm.	"	-		dissection
85	DD 7	180 mm.	"	-		dissection
86	D 72	183 mm.	"	transverse		
87	DD 9	184 mm.	"	-		dissection
88	DD 6	185 mm.	"	-		dissection

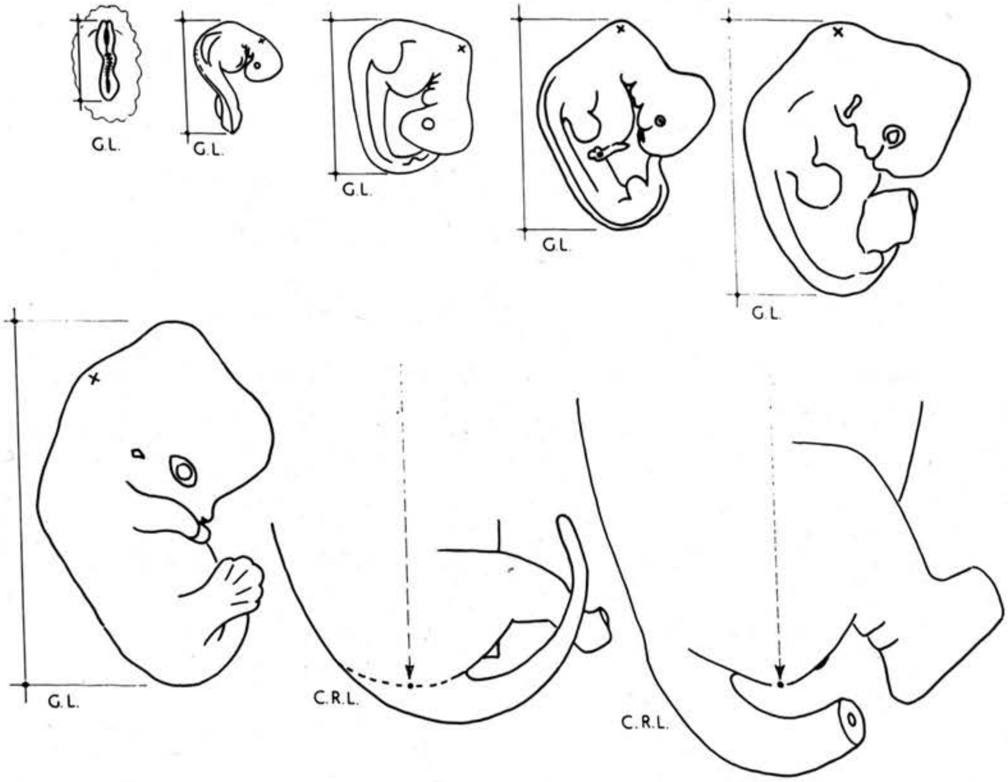
The prerequisite of a useful embryological investigation is an adequate description and staging of the examined material to permit meaningful comparisons with material of other investigations on the same species. This is especially important in species such as the dog whose intrauterine life has scarcely been explored, with the result that developmental standards of reference are not available. Unfortunately, size alone does not determine developmental stages sufficiently to be of comparative use, since different investigators employ different modes of collection, fixation and even measurements, each of which may have an influence on the crown-rump length of the specimen. Thread-measurements would appear to be unaffected by these variables, but experience shows that they are inaccurate, especially on small specimens, and that the procedure may cause damage.

It is clear, therefore, that more reliable criteria for the staging of embryos must be used. Fertilisation age, or what one might call the true age of an embryo, would of course be the ideal standard of reference for many species. Unfortunately, fertilisation does not manifest itself externally, hence this age can only be determined by calculation in relation to copulation time, and is therefore not too reliable. Copulation age, on the other hand, can easily be determined. However, experience during the collection of the specimens for the present study shows that it cannot be depended upon for the standardisation of developmental stages. A comparison between embryo D 5 (Fig. IV.7.) and embryo CC 6 (Fig. IV. 23.), both of the same copulation age, will be convincing on this point. The findings of three well known authorities on this subject show that this is not a unique occurrence. Harrop (1960) gives the length of an 18 - 21 day (copulation age?) embryo as being 13 mm.; Cole and Cupps (1959) give the length of a 23 day (copulation age?) embryo as being 5 mm.; and Evans (1956) gives the length of a 25 day (fertilisation age) embryo as being 13.6 mm. Somite count is an excellent indicator for young specimens and, such being the case, was used in this study for the first few developmental stages.

Cole and Cupps (1959) restate the well established fact that external features are the criteria of choice for the staging of embryos, anddeplore the lack of standards of reference based on these criteria for the domestic animals. The absence of such standards induces others (Zietzschmann and Kroelling, 1955) to maintain that greatest length (G.L.) measurements are the most practical indicators, at least for the first half of the gestation period. This view cannot be shared by the author, since in the present study, 6 mm. embryos (Fig. IV.17.) were found to be much less developed than 4.5 mm. embryos (Fig. IV.28.), although both were collected and fixed by the same technique. An exclamation mark (thus: 6.0 mm. (!)) warns the reader of specimens whose size, if considered alone, would be misleading. The classical work of Mall (1910) and Streeter (1942), which led to the establishment of embryological "horizons", is a very valuable outcome of this external-feature concept, but unfortunately available only for the human embryo. One must wonder that similar classifications have as yet not been devised for at least the more easily accessible species such as the pig, sheep or cat. Keibel's Normentafeln, which were compiled to overcome the same difficulties, include a number of mammalian species but regrettably not the dog. The study of canine embryos is made more difficult by the fact that this species is rarely examined by modern investigators. Therefore, such standards of developmental reference as have come into existence over years of intensive studies on the intrauterine life of the pig, for instance, are simply not available for the canine species; and the few existing publications cannot be relied upon, because of the reasons enumerated above.

It is necessary, therefore, to document each study of this embryologically rare species so well with regard to the examined material, that the reader can gain a clear picture of any developmental stage to which reference is made. This is even more important because of the great breed variation that exists in the canine species and reflects itself on fetal size, especially towards the end of the gestation period. One might even go so far as to say that canine embryological research can only be of meaning, if carried out on one pure breed. This might seem to be ideal, despite it being impractical and of great expense, but would it lead to embryological knowledge of the entire species or just to that of the breed selected? The canine species presents itself to us, granted, by our own intervention, as a heterogeneous group, and its embryology should be studied as such. The author admits that embryos from parents having approximately the same adult weight should be used for study, or such weight information should at least be stated (as has been done in the present study). Consideration of breed characteristics however, in studies of intrauterine development would seem to the writer as taking purist principles a little too far afield. The present study suggests that size variation in embryos due to breed cannot be detected before the 29 mm. stage.

With these aspects in mind, it became clear that as much information on each examined specimen as possible should be collected and presented. This, the writer believes, is the only way in which developmental standards of reference can be compiled over the years for a species as heterogeneous and as difficult to obtain as the canine. Because of the bulk of data collected for each specimen, this detailed information was placed in the section on OBSERVATIONS as a preface to each developmental stage. It consists of a photograph or sketch of nearly all the specimens used and a list of all the data that could be made available.



<u>Fig. II.1</u>. A series of canine embryos of different sizes to illustrate the manner in which measurements were taken in this investigation.

G.L. Greatest length; C.R.L. Crown-rump length; The cross (+) marks the neck hump.

The staging, therefore, of the material used here is based on the following: (a) external appearance, (b) size, (c) somite count, (d) copulation age, and (e) ancillary qualifying information such as breed, age and weight of the parents. This information, throughout the section on OBSERVATIONS, is augmented by continued reference to developmental landmarks such as the appearance of hair follicles, closure of eye lids and the appearance of ossification centres.

Despite the importance of external features, the use of crown-rump measurements is expedient for labelling of embryos or stages. Thus it is more natural to refer to a stage as "the 22 mm. stage" than as "that stage which is represented by embryo D 16 in Fig. 59" for example. Regrettably, one will have to continue in this fashion until equivalents to Streeter's horizons have been established for the canine embryo. The way in which the specimens used in this study have been measured is illustrated in the accompanying Fig. II.1. All measurements quoted in this work are of fixed material, unless otherwise stated. Crown-rump variations in specimens of the same developmental stage can occur for three reasons: (a) choice of fixative, Bouin's fluid, for instance, causes greater flexion of an embryo's long axis than formalin, (b) inexpert handling of specimens by collectors, resulting mainly in the uncurling of the embryo's curvature, and (c) a natural variation in sizes. The latter was found by Veau (1938) to be present in human embryos and by MacDonald (1928) in the mouse.

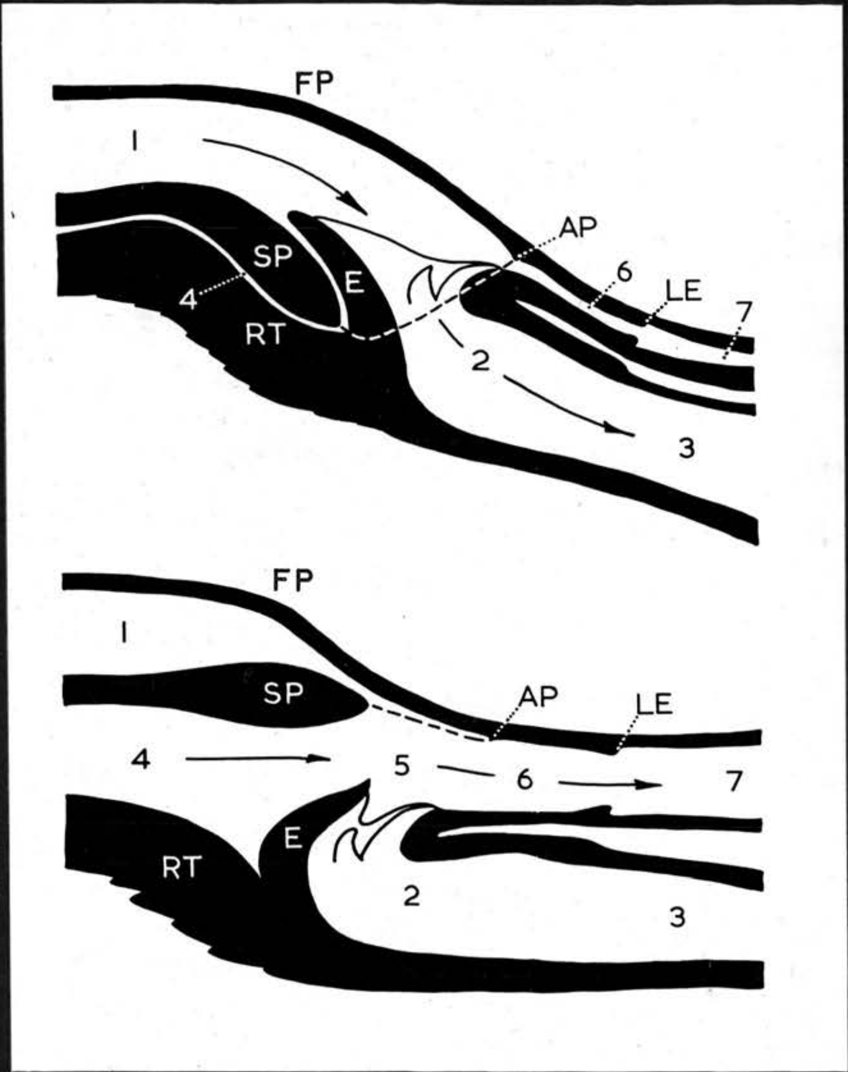


Fig. II.2. Schematic representation of the adult canine pharynx in nose-breathing and swallowing positions.
(Modified from Zietzschmann, 1939)

1 Nasopharynx; 2 Larynx; 3 Trachea; 4, 5, 6 Oropharynx; 4 Isthmus faucium; 5 Trachynx; 6 Vestibulum esophagi; 7 Esophagus.
AP Palatopharyngeal arch, the dotted line to the soft palate represents the pharyngeal isthmus or the Foramen intrapharyngicum;
E Epiglottis; FP Fornix pharyngis; LE Limen pharyngoesophagicum;
RT Root of tongue; SP Soft palate.

III. METHODS

Fixing

The embryos and fetuses not collected by the writer himself were fixed in a 10% formalin solution. Those that arrived inside their loculi and others that appeared to be improperly fixed were refixed in Bouin's fluid and then washed between five and ten times, depending on their size, in generous amounts of 50% alcohol, and finally transferred to 70% alcohol for storage. This mode of fixation is marked on the individual data sheets in the following manner: "10% Form./Bouin's Fluid". Specimens collected by the author himself were fixed either in 10% formalin solution or in Bouin's fluid according to standard techniques.

Measuring

Large specimens were measured with calipers. Smaller ones, especially those collected by the writer himself, were, while in a container filled with saline, placed on millimeter-ruled paper and measured visually (Size before fixation). After fixing they were remeasured in the same manner (Size after fixation).

Preparing Serial Sections

In general, standard techniques were employed, but a number of modifications were introduced to speed up the work. The specimens were taken through a series of alcohols, chloroform and soft paraffin wax (39 degrees C. melting point) into hard paraffin wax (56 degrees C. melting point), of which the blocks were made. The intention to reconstruct the pharynx in a number of embryos made accurate orientation and the inclusion of guide lines into the block necessary. Aligning specimens visually in relation to the sides of the paraffin block gave better results than attempting orientation on the chuck of the microtome at the time of sectioning. For this purpose an embedding mould consisting of two accurate Perspex (Plexiglass) angles and a base plate of the same material were made. The base plate was mounted with rubber cement on the flat side of a large tissue culture bottle (Fig. III.2.). A grid of 5 mm. squares was scratched on the inside surfaces of the angles and on the upper side of the base plate. These marks appear as lines on the sides and the bottom of the paraffin block and are very useful in orientating the block in relation to the microtome knife. To assure ample time for the embedding and simultaneous orientation of the embryos, the tissue culture bottle was connected to a hot and cold water supply plus an additional heating coil for the hot water in such manner that water of desired temperatures could be directed against the underside

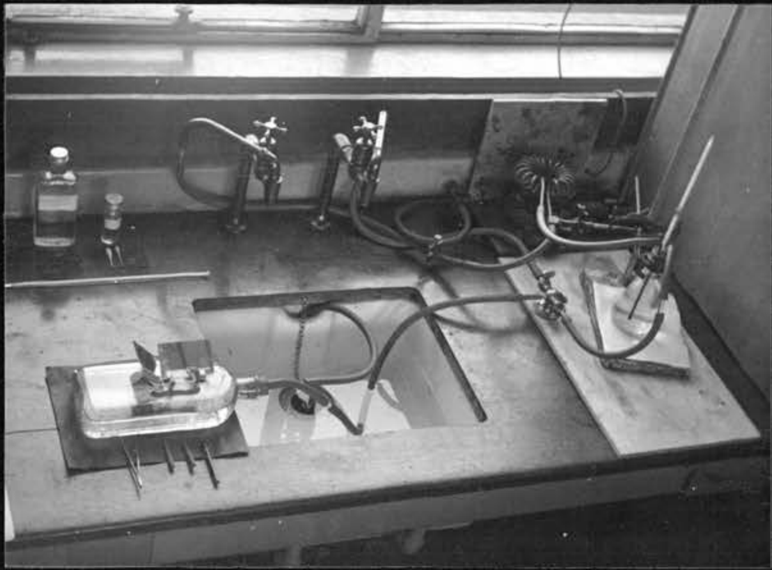


Fig. III.1. Simple embedding apparatus permitting controlled solidifying of the wax.

1 Hot water tap; 2 Cold water tap; 3 Heating coil; 4 Thermometer measuring temperature of hot water flowing through bottle; 5 Three-way junction; 6 Basin; 7 Bottle-mould assembly.

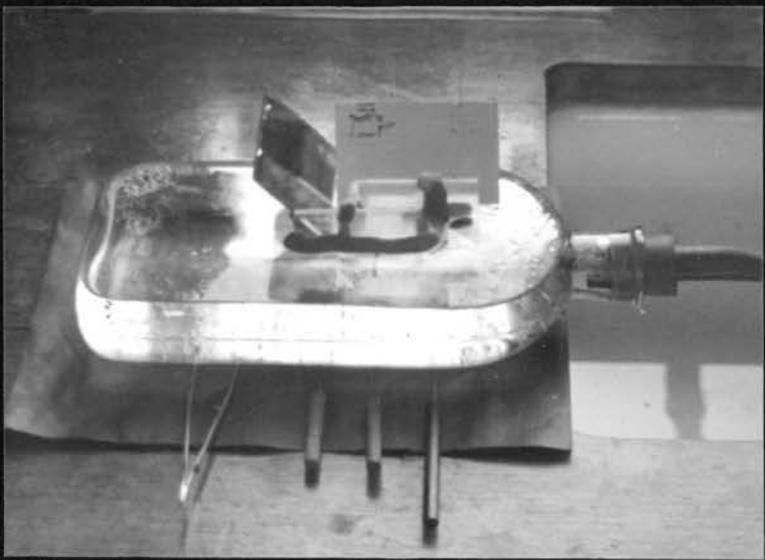
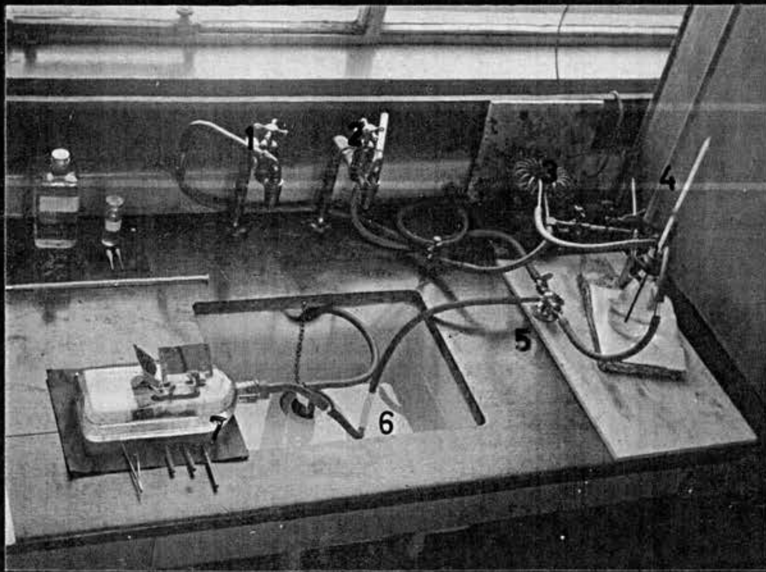


Fig. III.2. Close-up of bottle-mould assembly of Fig. III.1.

1 Large tissue culture bottle; 2 Hand mirrors; 3 Perspex mould; 4 Copper pipe directing water against undersurface of mould.



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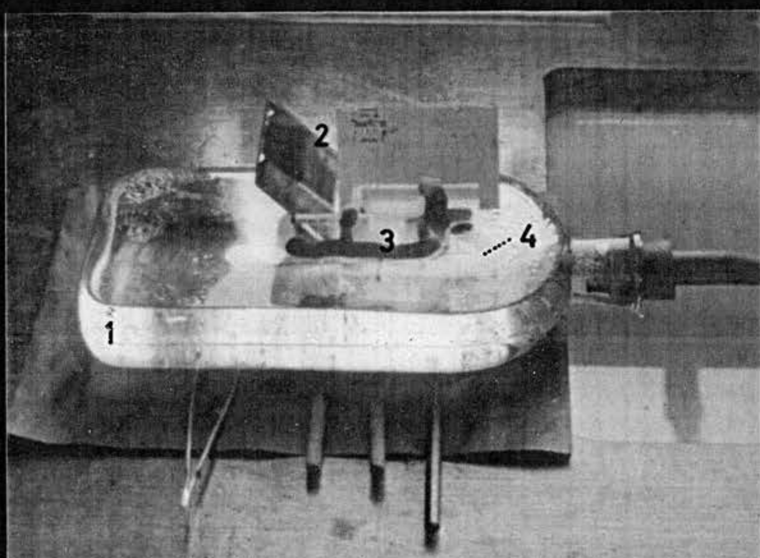


Fig. III.2. Close-up of bottle-mould assembly of Fig. III.1.

1 Large tissue culture bottle; 2 Hand mirrors; 3 Perspex mould; 4 Copper pipe directing water against undersurface of mould.

of the mould at any time and at a moment's notice (Fig.III. 1.).

At embedding time the bottle-mould assembly (7) was brought to a constant temperature by perfusing it with water of 59 degrees C. Then the mould was filled with stock wax at about 65 degrees C., which would drop to a temperature of around 58 degrees in a few minutes and be held at that level for as long as the hot water was kept flowing through the system. Next, the embryos (up to four were embedded in the same block) were placed in the mould and leisurely arranged with hot needles until they were correctly orientated in relation to the grid lines on the bottom of the mould. When they were all fairly well in place, the temperature of the water was lowered to 45 degrees C. by turning the gas tap under the heating coil (3) to a predetermined setting. This caused a fine opaque membrane to form on the bottom of the mould, thin enough for the grid lines to show through. The specimens, being fixed at the bottom by the membrane, could then easily be reorientated in relation to the bottom grid and also to the side grids, the latter being accomplished by thehelp of two hand mirrors mounted beside the mould at angles of 45 degrees (Fig. III.2./2). When all specimens were in the desired positions the two taps at the three-way junction (Fig. III.1./5) were turned which shut off the hot water supply and allowed cold water to flow against the bottom of the mould, immediately initiating the

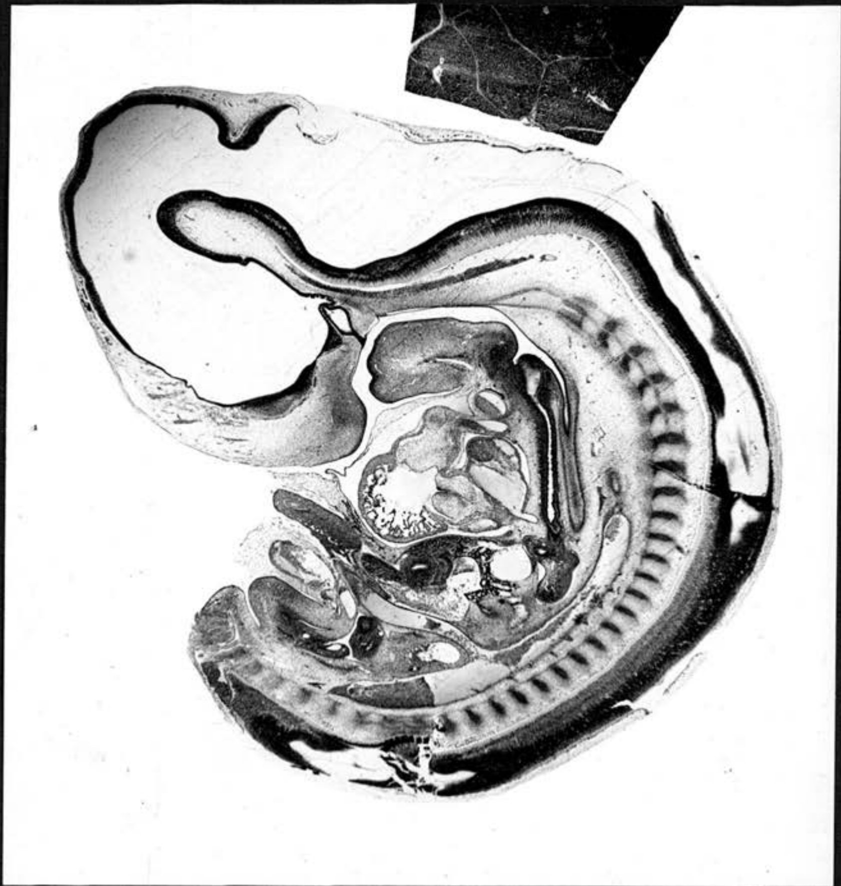


Fig. III.3. A piece of fetal liver used as guide line embedded alongside canine embryo D 11 (11.0 mm. G.L.).

Fig. III.4. Lacquer guide line alongside canine embryo D 36.

consolidation of the wax. A few minutes later the entire bottle-mould assembly was immersed in the adjacent basin (6), which had previously been filled with cold water to a predetermined height, allowing the water to rise upon submersion of the bottle only to the upper edge of the mould. In this way the contraction of the wax took place only on the upper surface, yielding a block with five straight sides.

Two methods to provide for guide lines in the sections were employed. One was the time-honoured straight piece of fetal liver embedded alongside the embryo (Fig. III.3.). The other was developed by Peter (1906) and employs a shoe lacquer which, when painted on the bottom surface of the block, appears as a fine line beside the section (Fig. III. 4.). The notches in these lines are the impressions of the grooves in the base plate of the mould.

The resulting block, then, has five even surfaces, adjacent ones standing at right angles to each other. On each of these sides is a grid of lines 5 mm. apart. The embryos inside the block are orientated according to these outside lines. Therefore, for orientation on the microtome chuck only the outside lines need be used, which automatically aligns the embryos in the desired planes. If several specimens are contained in a block, all of them were orientated during embedding in such manner that a single alignment on the microtome cuts them all, one after the other, in the predetermined planes.

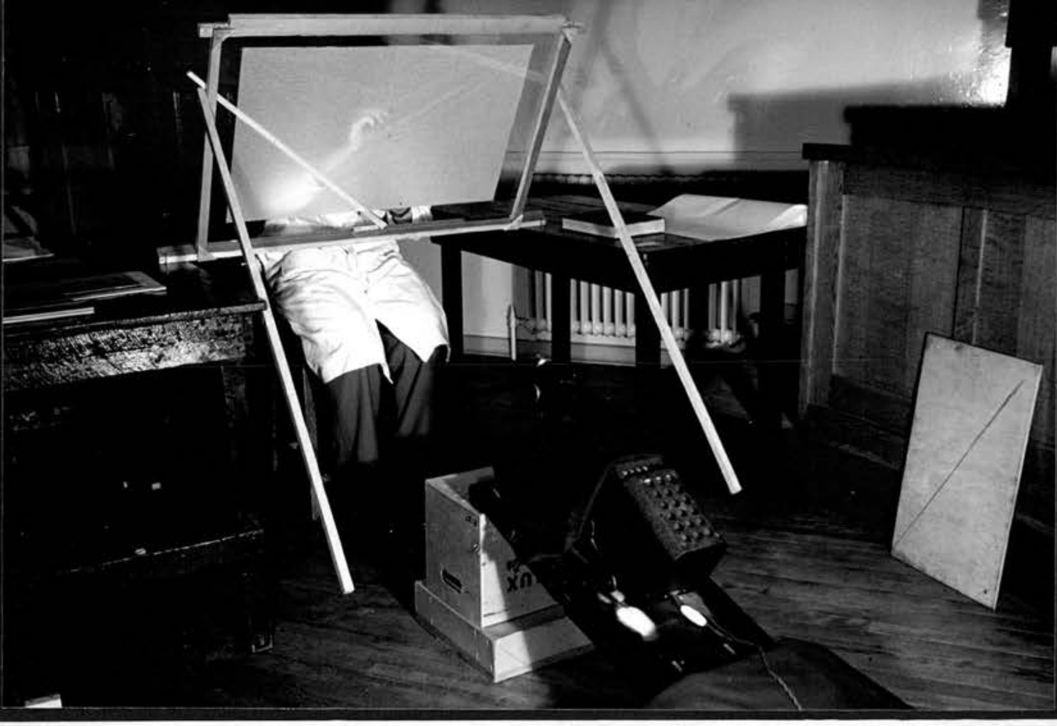


Fig. III.5. Simple drawing apparatus based on the principle of back-lighted projection.

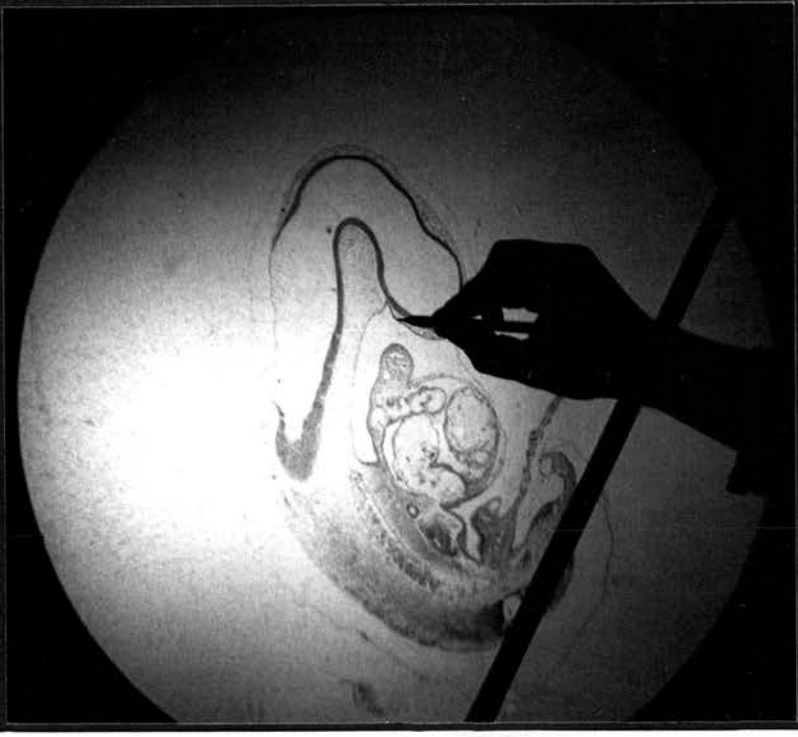


Fig. III.6. Image of embryo on drawing paper of drawing apparatus (Fig. III.5.). To the right is the silhouette of the hand, pencil and mahlstick without shadow.

Preparing Tracings for Drawings and Graphic Reconstructions

The outlines of many sections had to be traced for the illustration of this study. At first the camera lucida technique was thought to be suitable for this purpose, but the inadequacies of this method soon became apparent. Especially the crowded quarters inside the viewing box and the continuous struggle with the shadow of one's drawing hand and pencil became tiring with long sessions. Therefore, a simple drawing apparatus, based on the principle of back-lighted projection, was devised, and because it proved successful in simplifying the work, the method was found by the supervisor of this study to be suitable for publication and appeared subsequently in one of the technical journals (Sack, 1961). Fig. III.5. shows the operator sitting comfortably behind the tilted translucent drawing board of this apparatus, tracing the image of the desired object without being encumbered by the shadow of his hand and pencil (Fig. III.6.). The original tracings made on this apparatus can only be considered intermediary steps in the production of sharp black and white photographic reproductions. Moreover, they were made on thin tracing paper and drawn to a very large scale so as to obtain crisp replicas after photographic reduction. Under these circumstances they were not thought to be worthy of inclusion in the original copy of this thesis.

Making Wax Plate Models

The method originally devised by Born (1876 and 1883) and later modified by Strasser (1887) was employed at first for the making of reconstruction models. Here again, as was the case with the preparation of outline tracings, a number of shortcomings were encountered. Foremost among these were (a) the time-consuming intermediary paper tracings that have to be made, (b) the difficulty of incorporating these tracings into the wax plates at the time of rolling them out on the stone, and subsequent loss of drawings, (c) the difficulty of cutting wax plates that are enclosed by two sheets of paper, (d) the loss of accuracy by having to trace the same outlines twice, once on the drawing paper and a second time on the wax plate, and (e) the difficulty of uniting the plates when building up the model.

These difficulties were overcome and much time was saved by combining the principle of the Born-Strasser method with the back-lighted projection apparatus referred to in the preceding section. Pure wax plates were substituted for the tracing paper and the outlines of the projected images cut directly into the wax. These plates when built up to make the model can easily be welded together by plunging heated dissecting needles into the model and quickly withdrawing them. This method, the writer hopes, may by its simplicity help to revive wax plate modelling, which of late has fallen into disuse, largely because of its time-consuming attributes. It will, together with several additional innovations, be made the subject of a future publication.

IV. OBSERVATIONS

Introduction

The observations on the material are presented in 17 developmental stages of which the first, stage 0, deals with embryos in which a foregut, and therefore a pharyngeal region, has not yet appeared. The embryos were grouped into stages on the basis of their state of development, and by and large they had, within each group, approximately the same external dimensions. Deviations from the general rule, that an advance in development also infers an increase in size of an embryo, have occurred in this study, and the reasons for these are discussed in the section on MATERIAL. It is for the sake of these exceptions, and also for the purpose of simplifying the presentation of the findings, that an average C.R. length or, as the case may be, a range of C.R. lengths of round figures has been superimposed on the actual C.R. sizes measured in each stage. The discrepancies in the recording of the C.R. sizes were most often caused by the effacing of the embryo's curvature due to inexperienced handling by the collectors. The additions are summarised in the table on the following page.

In order to avoid undue repetition, each stage, especially from stage 10 onward, will only describe the changes that have occurred since the previous stage, and

TABLE II. Actual and superimposed lengths of specimens used in this study.

Stage	Nos. of specimens per stage	Actual range of lengths within each stage in mm.		Superimposed average length in mm.	
0	9	0 - 300 (microns)	G.L.	200 - 300 (microns)	G.L.
1	2	2.5 - 3.0	"	2 - 3	"
2	1	3.5 - 3.5	"	3 - 4	"
3	3	5.0 - 6.5	"	5 - 6(!)	"
4	8	4.5 - 6.2	"	4 - 6	"
5	4	6.3 - 9.5	"	6 - 8	"
6	3	- - -		7 - 9	"
7	6	8.4 - 10.3	"	8 - 10	"
8	6	8.6 - 11.0	"	9 - 12	"
9	7	14.5 - 16.0	"	15	"
10	5	21.5 - 22.5	"	22	"
11	7	28 - 29	"	29	"
12	2	33 - 41	"	40	"
13	7	43 - 55	C.R.L.	50	C.R.L.
14	5	67 - 71	"	70	"
15	3	93 - 94	"	90	"
16	3	114 - 120	"	120	"
17	7	178 - 185	"	Full term	
Total	88				

for this reason cannot be considered an independent descriptive unit, although the attempt has been made to present each stage as a readable entity.

Each developmental stage is preceded by a detailed presentation of all the available data, including, with very few exceptions, photographs, relating to the embryos of a particular stage. The subsequent observations are made upon three aspects:

1) The Shape of the Pharynx

2) The Structure of the Pharyngeal Wall

3) The Relationships of the Pharynx

In the first section the configuration of the pharyngeal tube is described. This includes the area between the primitive mouth and the esophagus for embryos up to the 8 - 10 mm. group. In embryos beyond this length, the mouth moves anteriorly with the development of the oral cavity, thus the description confines itself to the area homologous with the adult pharynx. Rathke's pocket and the remnant of its stalk, the pharyngeal hypophysis, act as excellent landmarks for the anterior limit of the pharynx throughout the range of the specimens examined, with the exception, perhaps, of those of the last two or three stages. The initial portion of the esophagus has always been included in the description, and the anterior components of the larynx, as they affect the shaping of the pharyngeal floor, are portrayed.

The structure of the pharyngeal epithelium and the changes it undergoes are described in the second section. To this is added, in older specimens, a description of the development of the pharyngeal and palatine muscles and the glandular and tonsillar elements. It should be stated at this point that the author, in describing the evolution of the different tissues that make up the pharyngeal wall, did not intend to trespass into the realm of histogenesis but continued to move, generally, on morphological ground. The sections from which the observations are made were cut, with one exception, at a thickness of 10 microns and were all stained with Hematoxylin and Eosin, conditions not very suitable for the study of histogenesis. Frequently, however, for the sake of rounding off a morphological description, the appearance of cells and their formations are described, and it is hoped the author will be pardoned for not having attained all that the circumstances have permitted.

The third section gives an account of the changes, both in position and substance, that go on in neighbouring structures. This gives a better appreciation of the relative movements the pharynx and attendant structures perform, and also contributes in fixing more accurately any given developmental stage. The latter is very important when one considers that such standards of developmental reference are virtually non-existing for the dog embryo. Each developmental stage is concluded by a short review, which will enable the reader to follow the

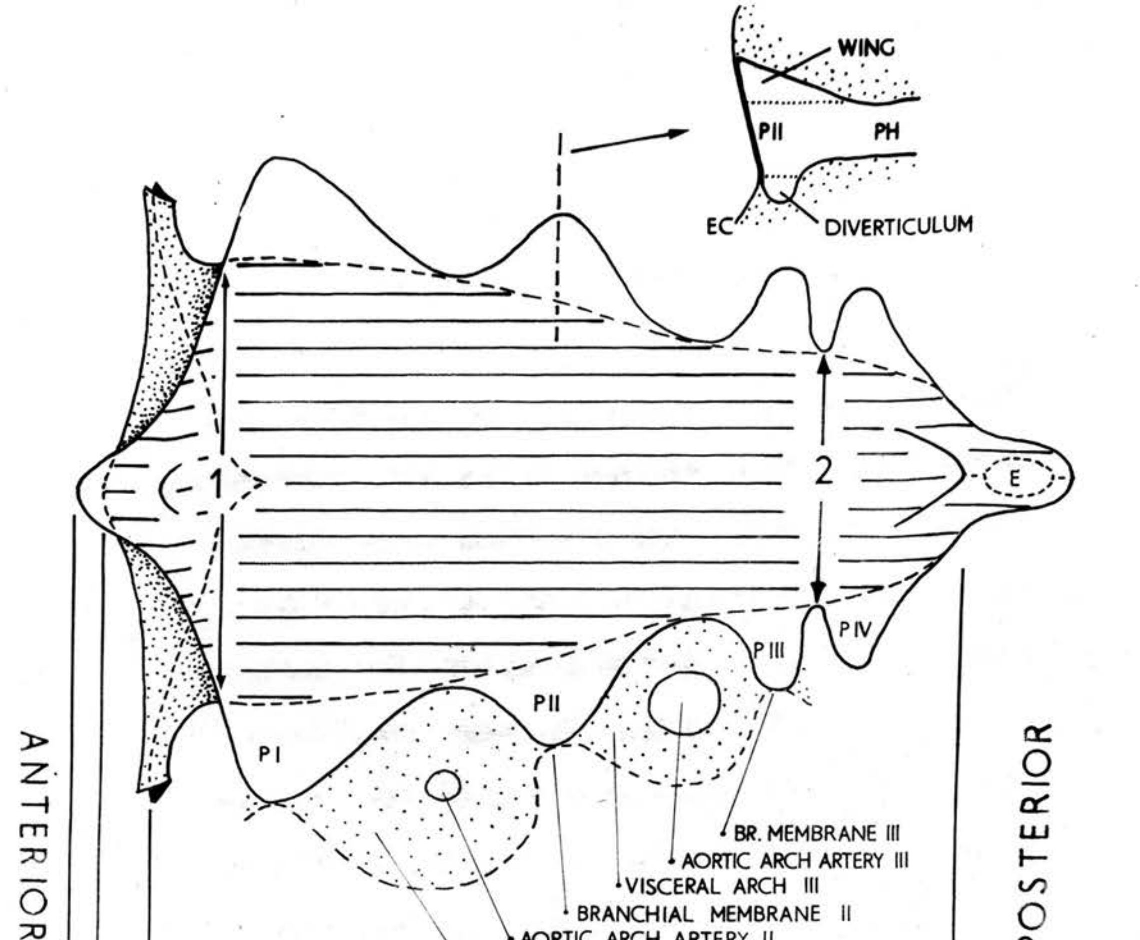


Fig. IV.1. Schematic representation of typical embryonic pharynx. At the upper right corner: transverse section through pouch II; in the centre: dorsal view of pharynx; at the bottom: midsagittal section.

1 Width of pharynx in front of pouch I; 2 Width of pharynx between pouches III and IV; 3 Longitudinal measurement of pharynx, arching from the posterior border of Rathke's pocket to the entrance into the esophagus; 4 Distance between the apex of the pharyngeal recess and the entrance into the esophagus.

E Esophagus; EC Ectoderm; M Mouth; PH Pharynx; PH R Pharyngeal recess; PI - PIV Pharyngeal pouches I - IV; R Rathke's pocket; S Seessel's pocket; T Trachea.

continuity of the work to the best advantage.

The flat upper surface of the primitive pharynx and, in later stages, the base of the chondrocranium have been selected as horizontal reference for all directional information. This, together with other information on position, terminology and measurements, is illustrated in Fig. IV.1. The longitudinal measurement of the pharynx (Fig. IV.1./3) was selected as arching from Rathke's pocket to the entrance into the esophagus, because both these points will remain determinable until the end of intrauterine development. It should, however, be mentioned that the posterior point of reference, now quite correctly called the entrance into the esophagus, becomes in later embryonic life the entrance into the Vestibulum esophagi which is that portion of the pharynx overlying the cricoid cartilage.

STAGE 0

(0 - 300 microns for the embryonic disc)

The observations for this stage are based on 9 blastocysts belonging to two litters as follows:

Blastocyst	-	1.2 mm. G.L.	litter 1
Blastocyst	-	1.3 mm. G.L.	litter 1
Blastocyst	-	1.3 mm. G.L.	litter 1
Blastocyst	D 6	1.5 mm. G.L.	litter 1
Blastocyst	-	1.5 mm. G.L.	litter 0
Blastocyst	-	2.0 mm. G.L.	litter 0
Blastocyst	-	2.0 mm. G.L.	litter 0
Blastocyst	-	2.3 mm. G.L.	litter 0
Blastocyst	D 37	3.0 mm. G.L.	litter 0

The measurements for litter 0 were taken when the blastocysts were floating in saline before fixation, those of litter 1 were made after fixation in Bouin's fluid. Although the specimens listed above have not advanced far enough for a foregut to have appeared, they are included in this study, because material describing canine embryos of any developmental stage is extremely scarce.

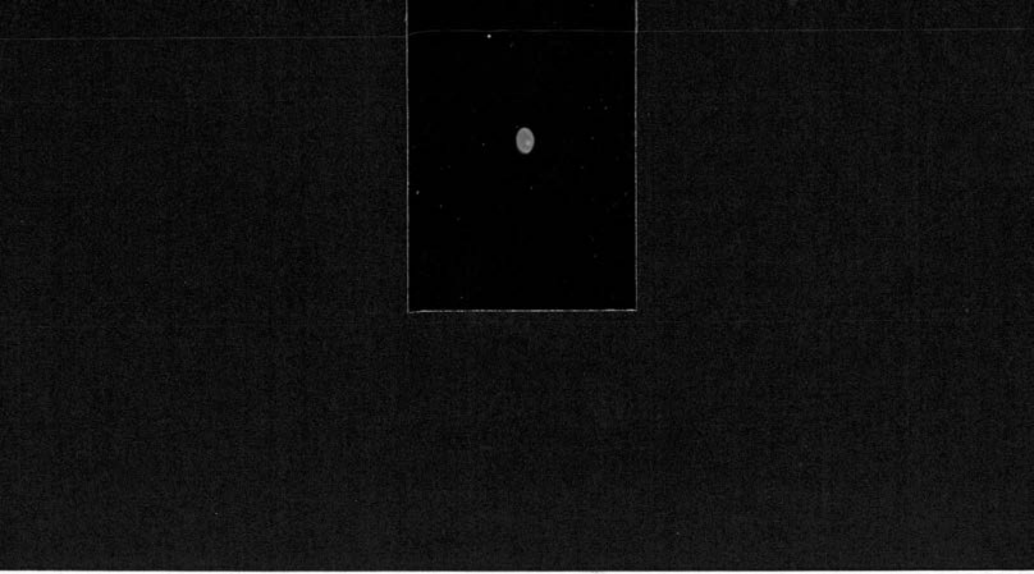


Fig. IV.2. Canine embryo (blastocyst) D 6 (1.5 mm. G.L.)

The white spot is the embryonic disc.

Number of embryo:	D 6	Date of processing:	June 25, 1960
Size before fixation:	-	Block:	Paraffin
Size after fixation:	1.5 mm. (blastocyst) 0.3 mm. (embryo)	Stain:	H. & E.
Age:	18 days	Plane of section:	Sagittal
Position on uterus:	-	Thickness of section:	10 microns
Size of loculus:	No loculi on uterus	Series made:	D 6 (1 - 11)
Fixed in:	Bouin's Fluid		
Stored in:	70% Alcohol		

From litter:		1
Number of embryos in litter:		3
DAM	Breed:	-
	Age:	-
	Weight:	-
SIRE	Breed:	-
	Age:	-
	Weight:	-
Number of services:		2
Date of 1st service:		May 14, 1960
Date of 2nd service:		May 16, 1960
Date of collection:		June 1, 1960

At time of collection Dam was alive ( ), dead (x).

Embryos collected 2 hour(s) after death.

Litter received from: Edinburgh Clinic

Remarks: After comparison with Embryo D 37 of litter O it was assumed that conception occurred on the 1st service, 18 days prior to collection.

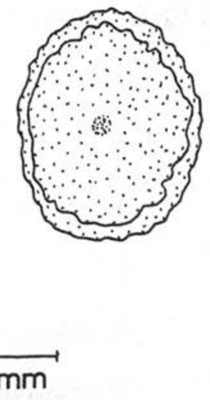


Fig. IV.3. Sketch of canine embryo (blastocyst) D 37 (3.0 mm. G.L.)

The outer layer is the Zona pellucida and the inner vesicle the trophoblast, lined on its inside by endoderm which is not shown here. The embryonic disc is in the centre.

Number of embryo:	D 37	Date of processing:	March 23, 1961
Size before fixation:	3.0 mm. (blastocyst) 0.2 mm. (embryo)	Block:	-
Size after fixation:	3.0 mm. (blastocyst)	Stain:	Haematoxylin
Age:	17 days	Plane of section:	Wholemount
Position on uterus:	-	Thickness of section:	-
Size of loculus:	No loculi on uterus	Series made:	-
Fixed in:	10% Formalin		
Stored in:	10% Formalin		

From litter:		O
Number of embryos in litter:		4
DAM	Breed:	X German Shepherd *
	Age:	10 months
	Weight:	40 lb.
SIRE	Breed:	X Greyhound
	Age:	2 years
	Weight:	40 lb.
Number of services:		1
Date of 1st service:		February 22, 1961
Date of 2nd service:		-
Date of collection:		March 11, 1961

At time of collection Dam was alive ( ), dead (X).

Embryos collected 1 hour(s) after death.

Litter received from: Edinburgh Clinic

Remarks:

* Cross-bred dog, resembling German Shepherd.

By the 17th day after copulation the developing canine ova have grown into small oval blastocysts ranging in length from 1.5 to 3.0 mm. (fresh measurements for litter O). The specimens of this litter were found to be floating freely in the uterine horns, from which they were recovered in two ways. They were flushed from the one horn with warm saline injected into the ovarian end, and by careful dissection from the other. Having observed special caution not to disturb the uterus prior to dissection, it was possible to locate the blastocysts at the points to which they had migrated during the previous 17 days. The uterine mucosa at these sites was distinguishably redder than above and below, and a transverse histological section showed a functional congestion, especially in the vessels associated with the straight portions of the uterine glands. There was an increase in the diameter of the glands and a lowering of the uterine epithelium. In the light of this evidence it should be safe to conclude that the blastocysts have reached their final destination in the uterine horns by the 17th day and are preparing to embed. Litter O consisted of five blastocysts, two of which were in the right horn and three in the left. They were all of different sizes ranging from 1.5 x 1.0 mm. to 3.0 x 2.3 mm. (fresh measurements).

The 17 day canine blastocyst is a triple-layered, closed, oval vesicle. Its outer layer is the acellular

Zona pellucida, originally surrounding the ovum and now thin and greatly expanded; in hematoxylin-eosin sections it appears as a light-red staining, irregular band. The middle layer is the trophoblast, separated from the Zona pellucida by an irregular narrow space. It is the perpetuation of the blastular wall and, as its name suggests, concerns itself in later embryonic progress with the formation of the membranes through which food stuffs for the embryo are absorbed from the uterus. The cells of this layer are flat, containing small darkly staining nuclei in which the karyoplasm is heavily and evenly distributed. They form a well organised distinct sheet. In the three larger embryos of this litter the trophoblasts bear a minute area of cell density half way between the poles. This is the embryonic disc from which, in later stages, the body of the embryo will develop. (In the largest embryo of litter O the disc has a diameter of about 200 microns (Fig. IV.3.)). The innermost layer of the blastocystic vesicle is endoderm that has originated from the under-surface of the embryonic disc and expanded to line the entire inner surface of the trophoblast. This lining adheres to the trophoblast quite closely with the exception of the area below the embryonic disc, where a moderate space, containing a few free cells and debris, exists between the two structures. The cells of the endodermal layer distinguish themselves from the cells in the trophoblast by being much more loosely arranged, especially on the periphery where contact with the trophoblast is intimate. They contain larger nuclei than those of the trophoblastic cells and show only scant amounts of intranuclear material, except for one or two large irregular nucleoli.

Litter 1 was recovered 18 days after copulation, and what has been said with regard to description and size of the 17 day old embryos can be applied in general here. The embryonic disc of blastocyst D 6, which was sectioned serially in a sagittal plane, had a diameter of some 300 microns. This blastocyst itself measured 1.5 mm. in length after fixation in Bouin's fluid for one hour.

STAGE 1

(2 - 3 mm. G.L.)

The first stage in the development of the pharynx is represented by two embryos, both of the same litter:

Embryo D 12	2.5 mm. G.L.	litter 3
Embryo D 22	3.0 mm. G.L.	

As will be seen on the following pages they are six-somite embryos just beginning to constrict away from the blastocyst.

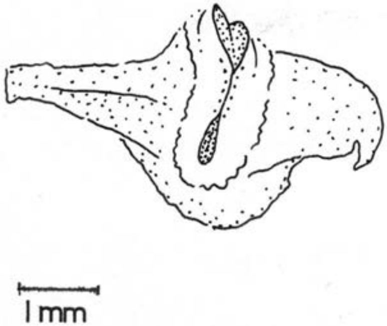


Fig. IV.4. Sketch of canine embryo D 12 (2.5 mm. G.L.).

The head process is at the top of the picture, the blastocyst has collapsed.

Number of embryo:	D 12	Date of processing:	August 10, 1960
Size before fixation:	-	Block:	Paraffin
Size after fixation:	2.5 mm. G.L.	Stain:	H. & E.
Age:	21 days	Plane of section:	Sagittal
Position on uterus:	-	Thickness of section:	10 microns
Size of loculus:	13 x 10 mm.	Series made:	D 12 (1 - 12)
Fixed in:	Bouin's Fluid		
Stored in:	70% Alcohol		

From litter:		3
Number of embryos in litter:		3
DAM	Breed:	-
	Age:	-
	Weight:	-
SIRE	Breed:	-
	Age:	-
	Weight:	-
Number of services:		1
Date of 1st service:		June 1, 1960
Date of 2nd service:		-
Date of collection:		June 22, 1960

At time of collection Dam was alive ( ), dead (X).

Embryos collected 1 hour(s) after death.

Litter received from: Edinburgh Clinic

Remarks: Embryo lying on blastocyst, counted 6 somites.

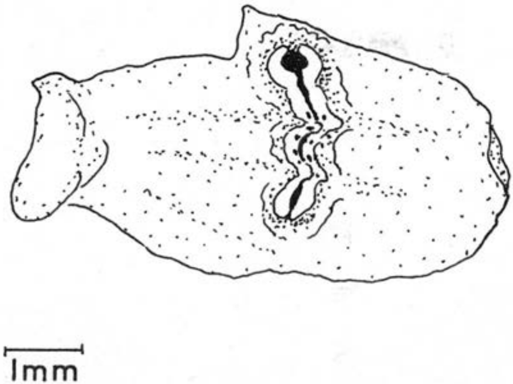


Fig. IV.5. Sketch of canine embryo D 22 (3.0 mm. G.L.).

The head process is at the top of the picture, the blastocyst has collapsed.

Number of embryo:	D 22	Date of processing:	January 19, 1961
Size before fixation:	-	Block:	Paraffin
Size after fixation:	3.0 mm. G.L.	Stain:	H. & E.
Age:	21 days	Plane of section:	Sagittal
Position on uterus:	-	Thickness of section:	10 microns
Size of loculus:	13 x 10 mm.	Series made:	D 22 (1 - 8)
Fixed in:	Bouin's Fluid		
Stored in:	70% Alcohol		

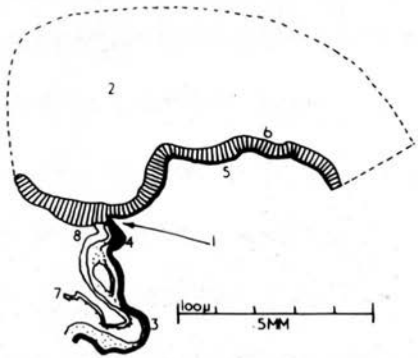
From litter:		3
Number of embryos in litter:		3
DAM	Breed:	-
	Age:	-
	Weight:	-
SIRE	Breed:	-
	Age:	-
	Weight:	-
Number of services:		1
Date of 1st service:		June 1, 1960
Date of 2nd service:		-
Date of collection:		June 22, 1960

At time of collection Dam was alive ( ), dead (X).

Embryos collected 1 hour(s) after death.

Litter received from: Edinburgh Clinic

Remarks: Embryo lying on blastocyst, counted 6 somites.



<u>Fig. IV.6.</u> Median section of head region of embryo D 12 (2.5 mm. G.L.), (semischematic).

1 Arrow pointing into developing foregut; 2 Outline of forebrain; 3 Endoderm; 4 Prochordal plate; 5 Endoderm forming roof of midgut; 6 Floor of neural groove; 7 Ectoderm; 8 Stomodeum anterior to oral membrane.

The Shape of the Pharynx

During the blastocyst stage, endoderm covered the flat undersurface of the embryonic disc before it turned ventrally in the formation of the yolk sac. As the embryo lifts away from the blastocyst, and the head process especially pushes forward to form the head fold, the endodermal undersurface is carried along in the forward movement and forms a shallow pocket in this area. This pocket is the first indication of the foregut (Fig. IV.6./1). Prior to this, the endodermally enclosed cavity below the disc-like embryo was called the primitive gut; now it is termed the midgut, because it is situated between the foregut pocket and the hindgut, an evagination similar to the one just described but later in appearance.

The conditions encountered in embryos D 12 and D 22 are illustrated in Fig. IV.6., a mid-sagittal section of the head region of embryo D 12. The heavy solid line (3) represents endoderm and covers the ventral surface of the embryo, here being closely attached to the floor of the neural groove (5). The arrow points into the foregut pocket (1). Just in front of this is an ectodermal recess, the future stomodeum (8). Between it and the foregut, only a layer of ectoderm and endoderm intervene as the oral membrane. Immediately below the oral membrane the endoderm thickens markedly for about 80 microns (4) before it returns to its normal thickness. This is thought to be the prochordal plate. It is difficult to do so with certainty, because only three specimens are available for the description of the first two stages. Both Aasar (1931) and Johnston (1940), the former for the rabbit and the latter for the human embryo, imply that a relationship exists between the prochordal plate and the oral membrane, although in Aasar's illustrations the plate remains always anterior to the membrane and does not actually take part in its formation. In the dog this thickening seems to overlie the oral membrane in all of the few specimens examined.

The Structure of the Pharyngeal Wall

The roof of the primitive pharynx consists of a single layer of cuboidal cells, the boundaries of which are very indistinct, a cell membrane seen only on the free border of the epithelium. Rather large vacuoles are present in the cytoplasm of some cells. In areas where the epithelium appears stretched, the cells become spindle-shaped. The nuclei are large and vesicular, showing eccentrically placed nucleoli and small amounts of karyoplasm. The epithelium of the floor is much thicker than that of the roof, being composed of three irregular layers of polyhedral cells with large nuclei; cell outlines again are indistinct. In embryo D 12 several darkly staining solid bodies of nucleolus size were observed.

The Relationships of the Pharynx

Dorsally, the primitive pharynx is related to the floor of the neural groove, ventrally to the ectodermal component of the oral plate.

In the two specimens of the 2 - 3 mm. stage, the primitive foregut is only a shallow transverse groove under the forebrain and serves the observer to mark the position where, in the following stage, the more definite foregut will be found.

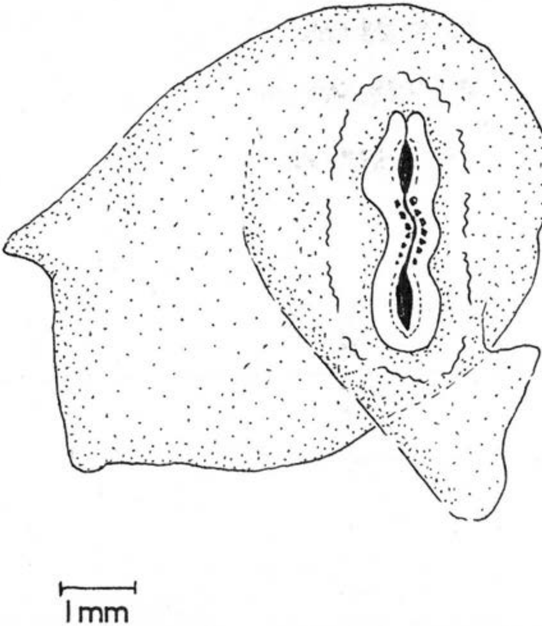
STAGE 2

(3 - 4 mm. G.L.)

The second stage in the development of the pharynx is represented by an embryo from the same litter as those annotated D 12 and D 22 in stage 1.

Embryo D 5 3.5 mm. G.L. litter 3

This specimen is better developed than its litter mates, so much so that the foregut is now a quite definitive entity (Fig. IV.8./1).



[redacted]

<u>Fig. IV.7</u>. Sketch of embryo D 5 (3.5 mm. G.L.).

The blastocyst has collapsed.

Number of embryo:	D 5	Date of processing:	June 25, 1960
Size before fixation:	-	Block:	Paraffin
Size after fixation:	3.5 mm. G.L.	Stain:	H. & E.
Age:	21 days	Plane of section:	Transverse
Position on uterus:	-	Thickness of section:	10 microns
Size of loculus:	13 x 10 mm.	Series made:	D 5 (1 - 11)
Fixed in:	Bouin's Fluid		
Stored in:	70% Alcohol		

From litter: 3

Number of embryos in litter: 3

DAM Breed: -

Age: -

Weight: -

SIRE Breed: -

Age: -

Weight: -

Number of services: 1

Date of 1st service: June 1, 1960

Date of 2nd service: -

Date of collection: June 22, 1960

At time of collection Dam was alive ( ), dead (X).

Embryos collected 1 hour(s) after death.

Litter received from: Edinburgh Clinic

Remarks: Embryo lying on blastocyst, counted 7 somites.

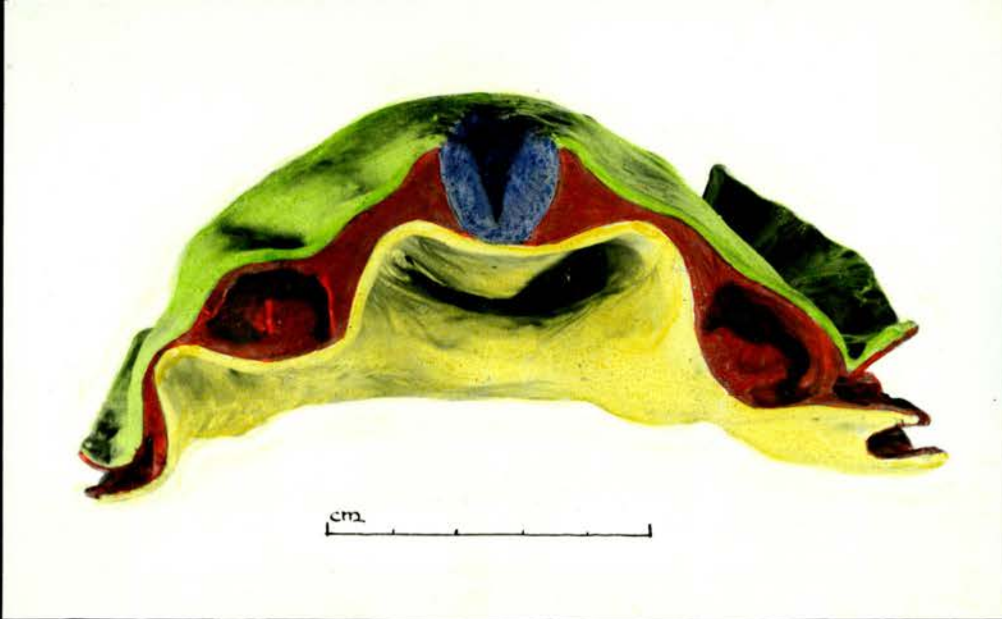


Fig. IV.8. Wax model of head segment of embryo D 5 (3.5 mm. G.L.), posterior view, (model X 100).

1 Foregut; 2 Swelling produced by expanding forebrain; 3 Neural groove; 4 Ectoderm; 5 Endoderm.

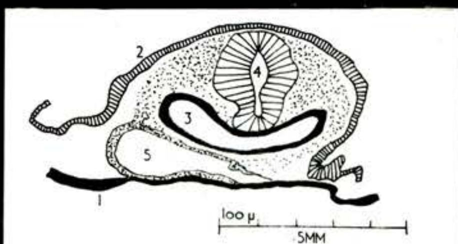


Fig. IV.9. Transverse section of head region of embryo D 5 (3.5 mm. G.L.), (semischematic).

1 Endoderm; 2 Ectoderm; 3 Foregut; 4 Neural tube; 5 Pericardial celom.

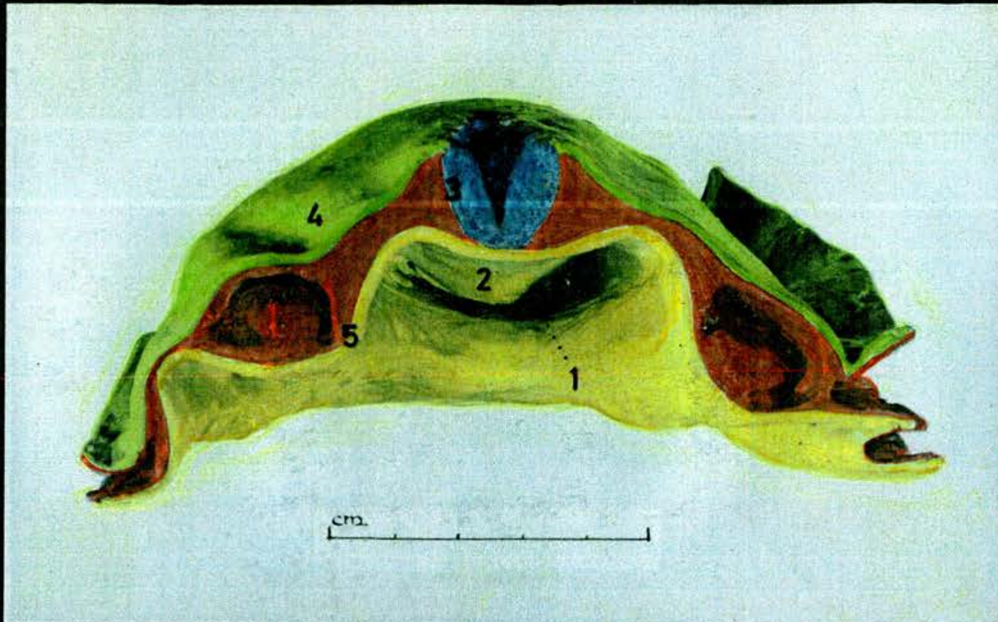


Fig. IV.8. Wax model of head segment of embryo D 5 (3.5 mm. G.L.), posterior view, (model X 100).

1 Foregut; 2 Swelling produced by expanding forebrain; 3 Neural groove; 4 Ectoderm; 5 Endoderm.

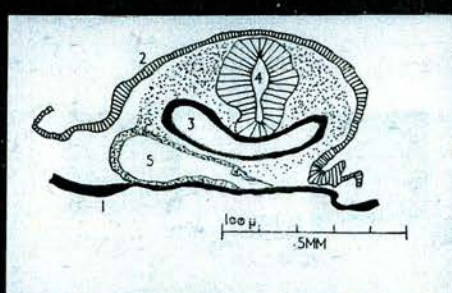


Fig. IV.9. Transverse section of head region of embryo D 5 (3.5 mm. G.L.), (semischematic).

1 Endoderm; 2 Ectoderm; 3 Foregut; 4 Neural tube; 5 Pericardial celom.

The Shape of the Pharynx

In this specimen the foregut can be described as a dorsoventrally flattened pouch projecting anteriorly under the forebrain (Fig. IV.14.). Communication with the midgut is achieved via the anterior intestinal portal. Its depth, measured from the anterior extremity to the level of the anterior intestinal portal, was found to be approximately 200 microns, while the greatest lateral extent was in the region of 500 microns at the intestinal portal. The foregut as a whole is concave dorsally and, in transverse section, has the appearance of a double-walled bowl in which the neural tube rests (Fig. IV.9.).

The floor of the foregut is semicircular in outline, showing a general concavity dorsally which conforms with that of the roof. At the intestinal portal, the central portion of the floor, about 200 microns wide transversely, folds abruptly under and runs forward to cover the heart region from below, thereafter continuing ventrally as the yolk sac (Fig. IV.8.). The anterior area of the floor is characterised by a local thickening (prochordal plate) which results in the formation of a rounded elevation of roughly 100 microns diameter. This projects visibly into the lumen from below (Figs. IV.10./3; IV.11./5; IV.12./5; IV.15./4); together with the ectoderm underlying the anterior section of the floor it forms the oral membrane (Fig. IV.11./6). The roof of the foregut is also semicircular and generally convex when viewed from below.

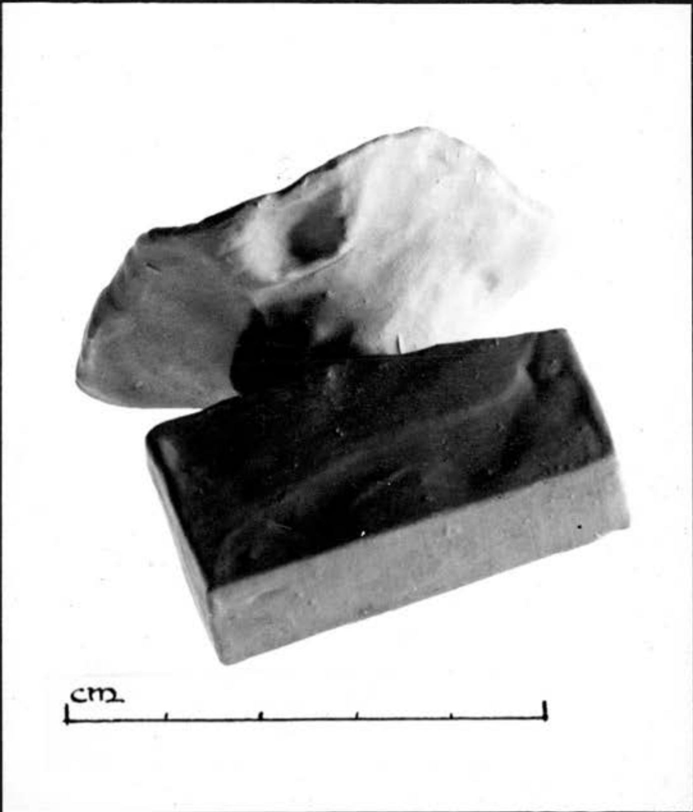


Fig. IV.10. Wax model cast of foregut lumen, embryo D 5 (3.5 mm. G.L.), ventral view, (model X 100).

1 Lateral margin; 2 Caudally directed depression in anterior margin; 3 Depression caused by prochordal plate; 4 Base of model.

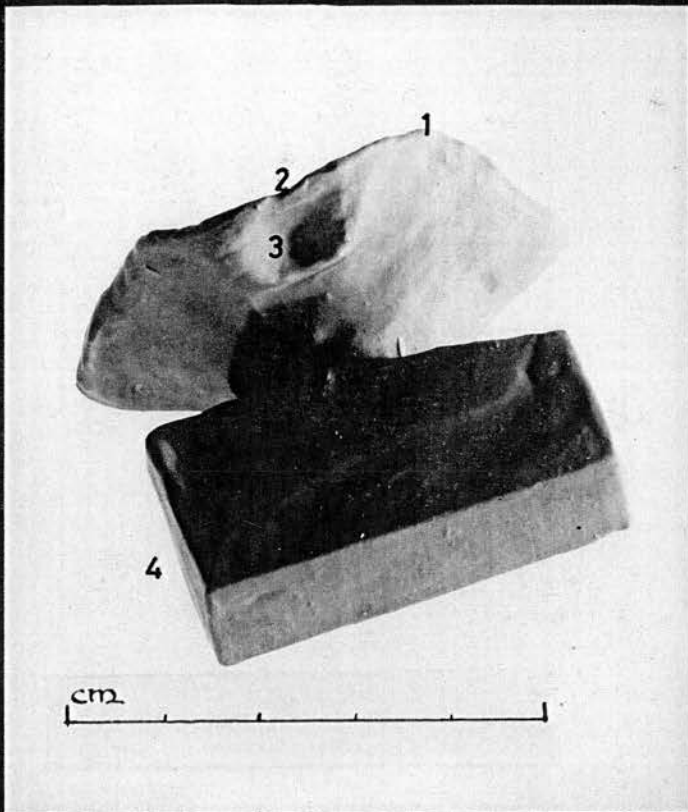
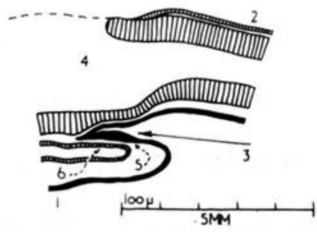


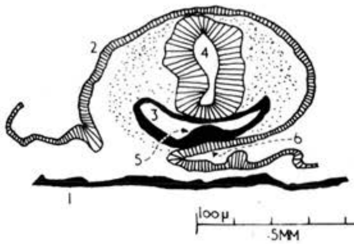
Fig. IV.10. Wax model cast of foregut lumen, embryo D 5 (3.5 mm. G.L.), ventral view, (model X 100).

1 Lateral margin; 2 Caudally directed depression in anterior margin; 3 Depression caused by prochordal plate; 4 Base of model.



<u>Fig. IV.11</u>. Median section of head region of embryo D 5 (3.5 mm. G.L.), (semischematic).

1 Endoderm; 2 Ectoderm; 3 Arrow pointing into foregut; 4 Neural tube; 5 Prochordal plate; 6 Oral membrane.



<u>Fig. IV.12</u>. Transverse section of head region of embryo D 5 (3.5 mm. G.L.). This section is about 100 microns anterior to that in Fig. IV.9. (semischematic).

1 Endoderm; 2 Ectoderm; 3 Foregut; 4 Neural tube; 5 Prochordal plate; 6 Oral membrane.

This convexity, as it happens, is more marked anteriorly where pressure of the rapidly expanding forebrain pushes the endoderm ventrally, producing a rounded elevation of approximately 300 microns diameter (Fig. IV.8./2). Its summit is nearly opposite the smaller protuberance that arises from below. Thus between the two the lumen is compressed to a narrow slit (Fig. IV.15.). Caudally, the roof of the foregut is continued without demarcation as the roof of the midgut. The rounded margins of the foregut start at the lateral extremities of the anterior intestinal portal and curve anteromedially to meet in the midline in a shallow, caudally directed concavity (Fig. IV.10./2). As they encircle the anterior portion of the foregut they become progressively sharper and, being quite smooth, do not as yet show any evidence of pharyngeal pouch formation (1).

<u>The Structure of the Pharyngeal Wall</u>

The roof consists of a single layer of cuboidal cells with round vesicular nuclei. The basement membrane is indistinct and seems to be interrupted in many places. The cell boundaries form a continuous line however, on the unattached border. The epithelium of the floor is similar to that of the roof of the foregut with the exception of the prochordal plate, where it is composed of three to four irregular layers of cells. Here the

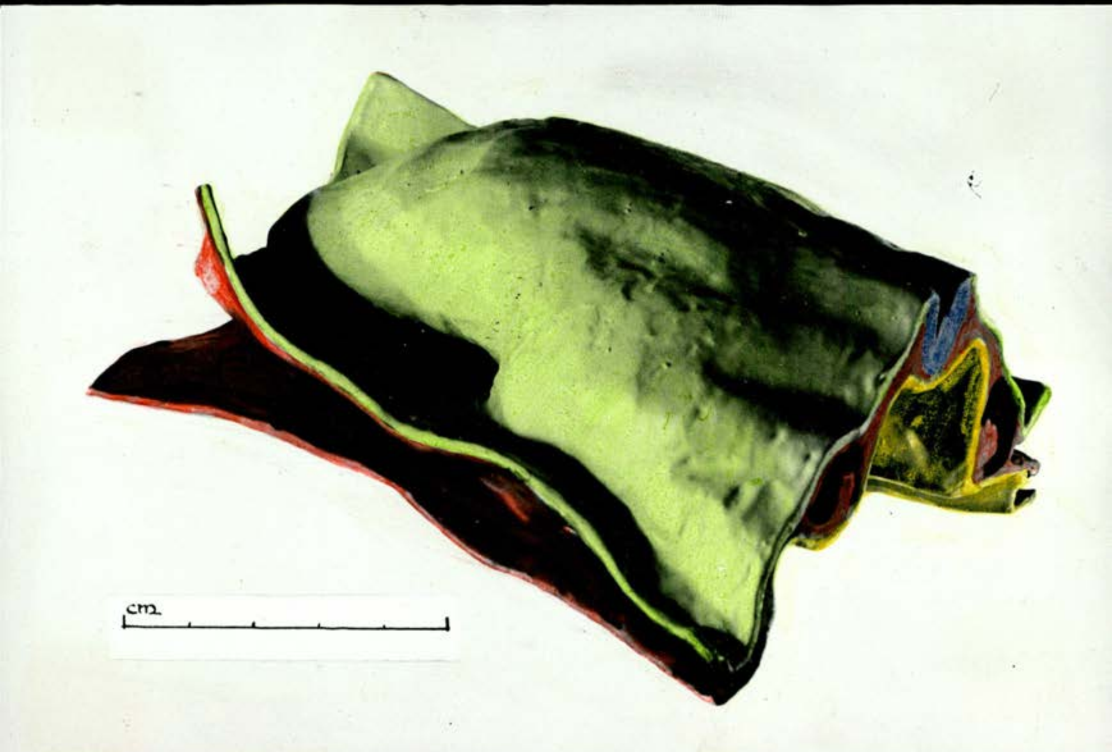


Fig. IV.13. Wax model of the head region of embryo D 5 (3.5 mm. G.L.), dorsolateral view, (model X 100).

1 Splanchnopleure of yolk sac; 2 Amnion; 3 Subcephalic pocket; 4 Neural groove; 5 Midgut.

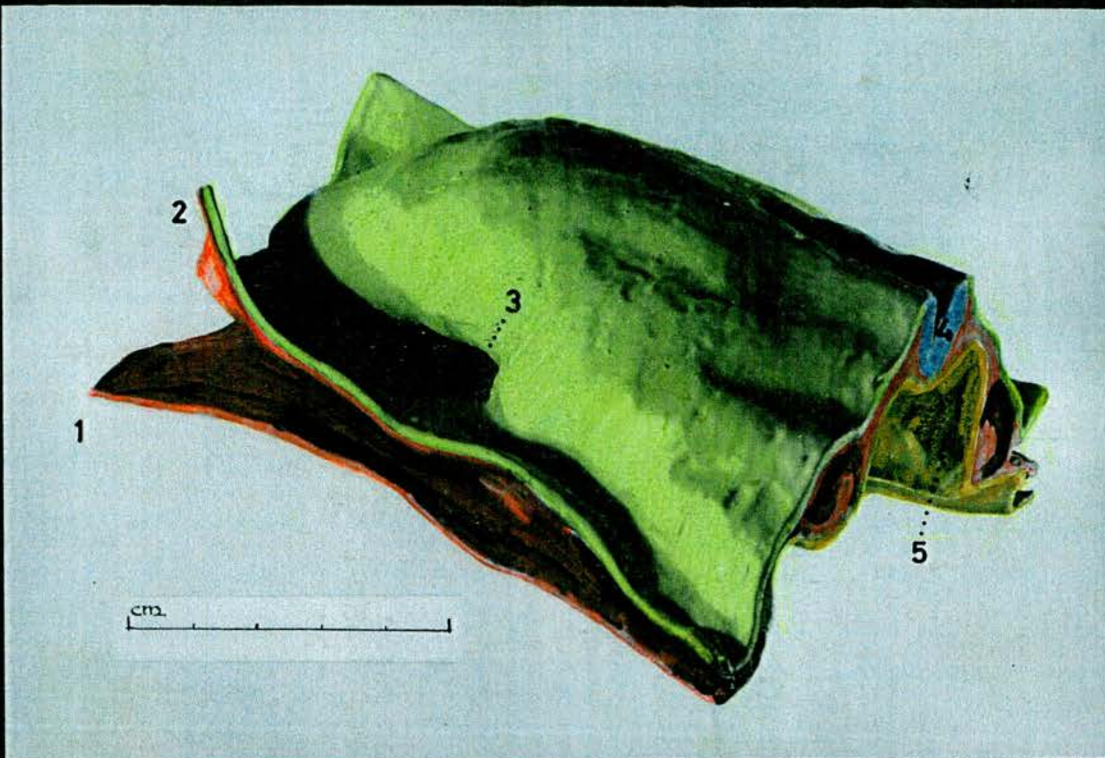


Fig. IV.13. Wax model of the head region of embryo D 5 (3.5 mm. G.L.), dorsolateral view, (model X 100).

1 Splanchnopleure of yolk sac; 2 Amnion; 3 Subcephalic pocket; 4 Neural groove; 5 Midgut.

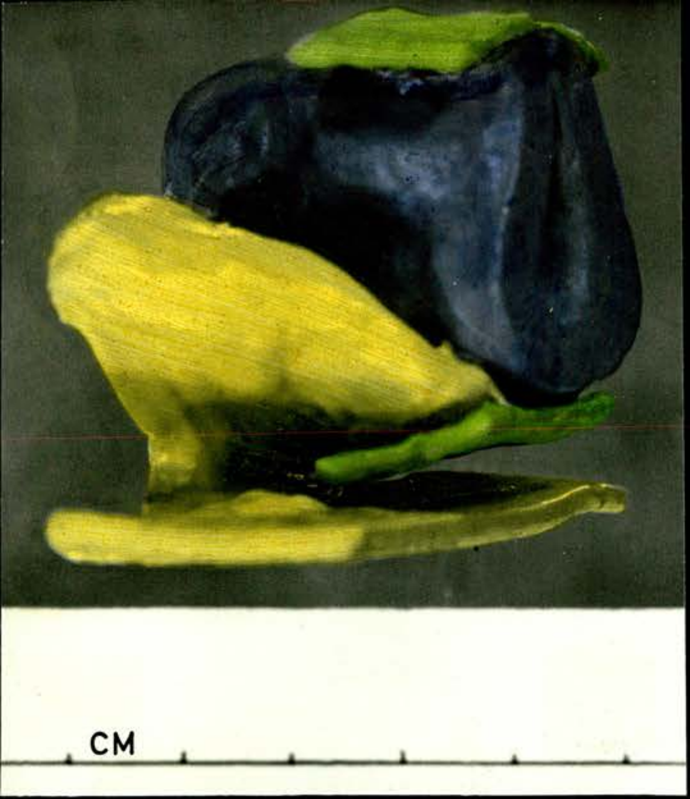


Fig. IV.14. Wax model of foregut and related structures of embryo D 5 (3.5 mm. G.L.), (model X 100).

1 Foregut; 2 Oral membrane; 3 Forebrain; 4 Neural canal; 5 Endoderm of yolk sac.

Fig. IV.15. Transverse section of foregut, embryo D 5 (3.5 mm. G.L.), (X 520).

1 Neural canal; 2 Lumen of foregut; 3 Notochordal cells; 4, 5, 6 Oral membrane; 4 Prochordal plate; 5 Ectoderm; 6 Basement membrane separating endoderm from ectoderm.

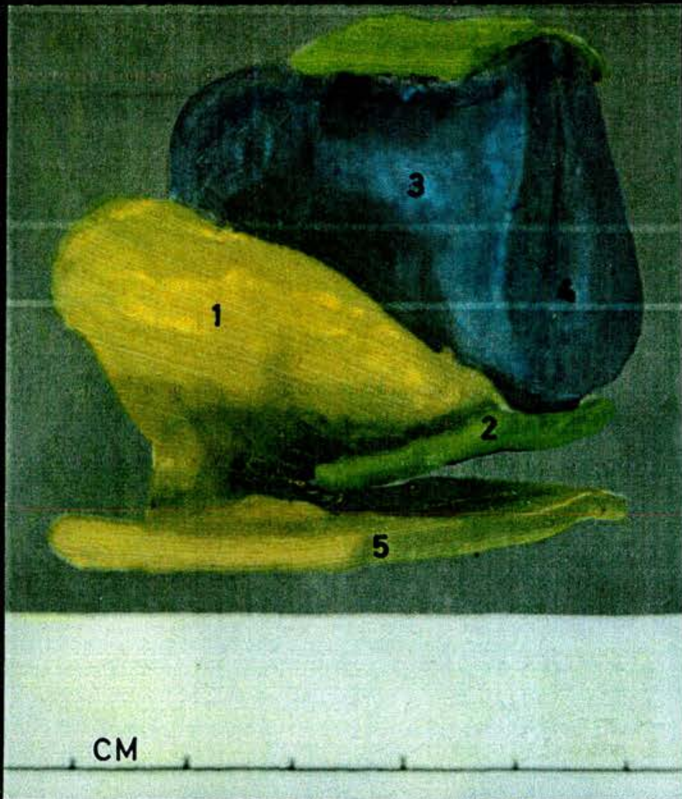


Fig. IV.14. Wax model of foregut and related structures of embryo D 5 (3.5 mm. G.L.), (model X 100).

1 Foregut; 2 Oral membrane; 3 Forebrain; 4 Neural canal; 5 Endoderm of yolk sac.

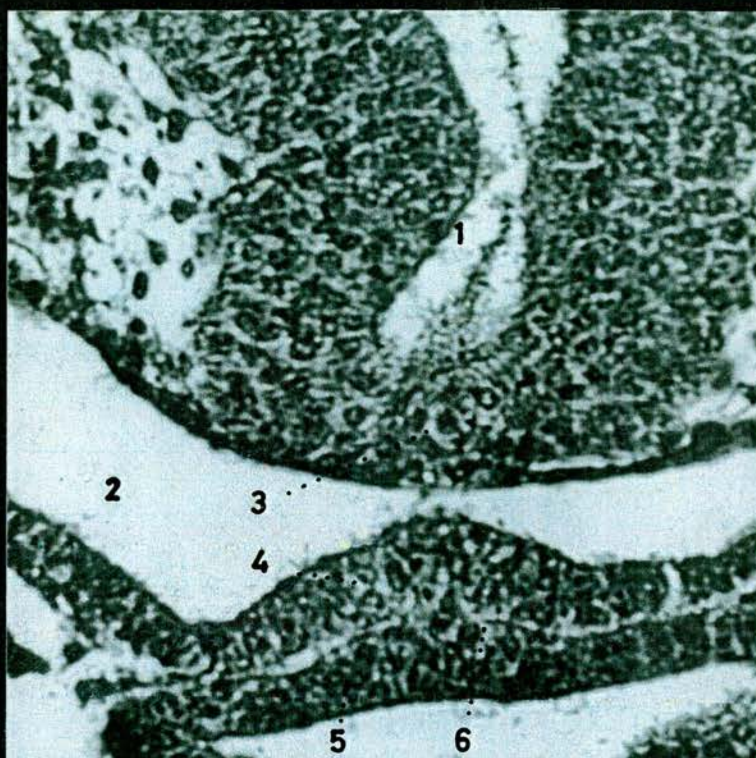


Fig. IV.15. Transverse section of foregut, embryo D 5 (3.5 mm. G.L.), (X 520).

1 Neural canal; 2 Lumen of foregut; 3 Notochordal cells; 4, 5, 6 Oral membrane; 4 Prochordal plate; 5 Ectoderm; 6 Basement membrane separating endoderm from ectoderm.

basement membrane is well formed and, combining with that of the underlying ectoderm, produces a conspicuous line which separates the two germ layers of the oral plate (Fig. IV.15./6).

The Relationships of the Pharynx

Dorsally, the principal relation is the closed neural tube of the future forebrain. Flanking this, the roof is in contact with loose mesenchyme, while between roof and neural tube flattened groups of cells of the yet unorganised notochord can be observed at varying levels (Fig. IV.15./3). On the ventral side the pharynx can be related to the ectoderm of the oral membrane in front (Fig. IV.14./2) while caudally it is in contact with pericardium.

Although well established as a structural unit, the foregut is merely a flat pouch under the forebrain with little resemblance to the configuration so characteristic of the pouch-bearing embryonic pharynx.

STAGE 3

(5 - 6 mm. (!) G.L.)

The observations for this developmental stage were carried out on three embryos all of the same litter:

Embryo D 74	6.5 mm. (!) G.L.	litter 3 c
Embryo D 75	6.0 mm. (!) G.L.	
Embryo D 76	5.0 mm. (!) G.L.	

The dorsal aspects of these embryos were applied to the uterine mucosa. The head processes, however, had freed themselves from the uterine lining and had begun to bend ventrally into the lumen of the loculus. 18 - 20 somites were counted.

Fig. IV.16. Canine embryo D 74 (6.5 mm. (!) G.L.), embryo is still attached to uterine mucosa, ventral view.

1 Forebrain; 2 First visceral arches; 3 Heart; 4 Anterior intestinal portal.

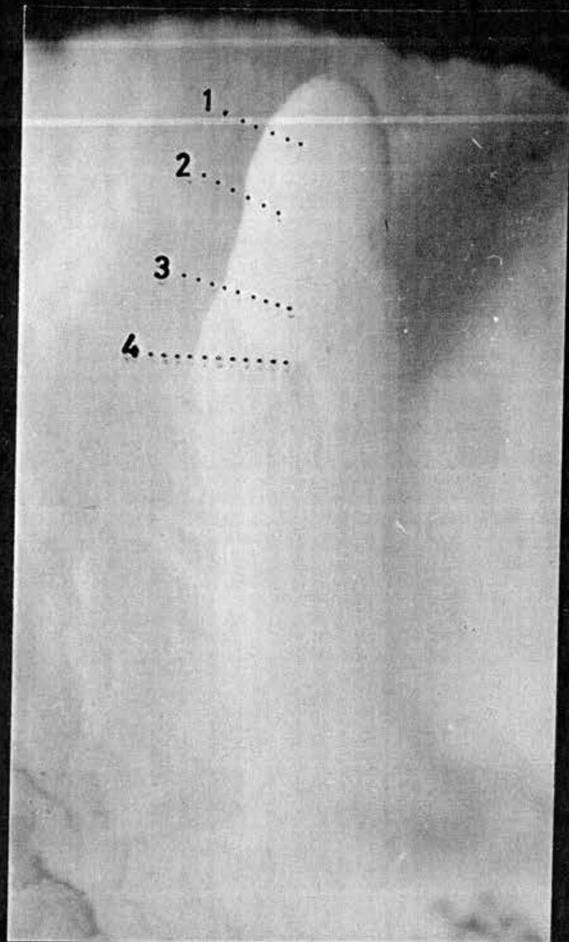


Fig. IV.16. Canine embryo D 74 (6.5 mm. (!) G.L.), embryo is still attached to uterine mucosa, ventral view.

1 Forebrain; 2 First visceral arches; 3 Heart; 4 Anterior intestinal portal.

Number of embryo:	D 74	Date of processing:	February 7, 1962
Size before fixation:	8.0 mm. (!) G.L.	Block:	Paraffin
Size after fixation:	6.5 mm. (!) G.L.	Stain:	H. & E.
Age:	-	Plane of section:	Sagittal
Position on uterus:	-	Thickness of section:	10 microns
Size of loculus:	13 x 15 mm.	Series made:	D 74 (1 - 4)
Fixed in:	Bouin's Fluid		
Stored in:	70% Alcohol		

From litter:		3 c
Number of embryos in litter:		3
DAM	Breed:	X Irish Terrier
	Age:	2 years
	Weight:	35 lb.
SIRE	Breed:	-
	Age:	-
	Weight:	-
Number of services:		-
Date of 1st service:		-
Date of 2nd service:		-

Date of collection: January 25, 1962

At time of collection Dam was alive (X), dead ( ).

Embryos collected hour(s) after death.

Litter received from: Edinburgh Surgery

Remarks:

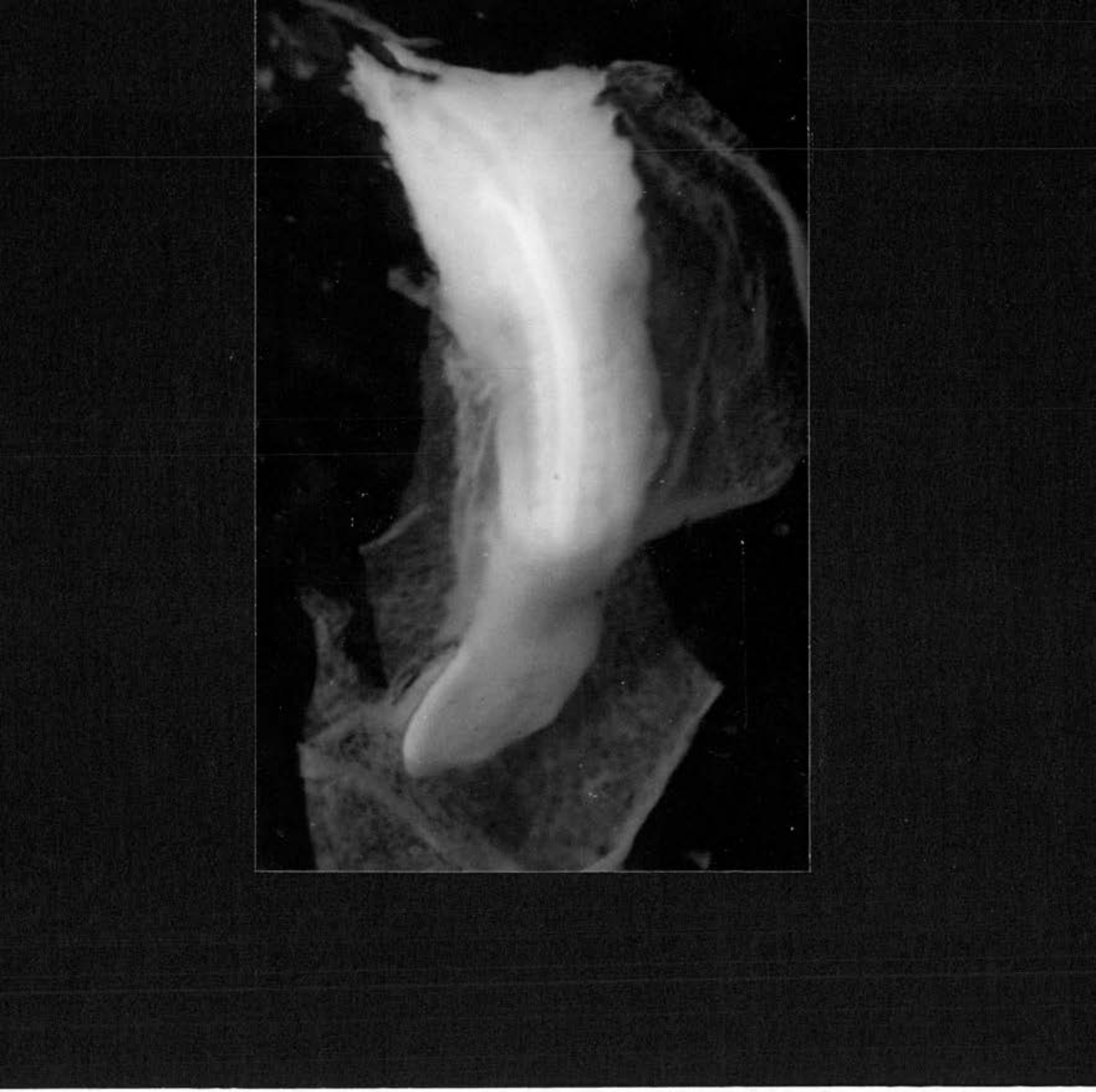


Fig. IV.17. Canine embryo D 75 (6.0 mm. (!) G.L.), some uterine mucosa still adheres to the head region, dorsal view.

Number of embryo:	D 75	Date of processing:	February 7, 1962
Size before fixation:	7.0 mm. (!) G.L.	Block:	Paraffin
Size after fixation:	6.0 mm. (!) G.L.	Stain:	H. & E.
Age:	-	Plane of section:	Transverse
Position on uterus:	-	Thickness of section:	10 microns
Size of loculus:	14 x 18 mm.	Series made:	D 75 (1 - 10)
Fixed in:	Bouin's Fluid		
Stored in:	70% Alcohol		

From litter:		3 c
Number of embryos in litter:		3
DAM	Breed:	X Irish Terrier
	Age:	2 years
	Weight:	35 lb.
SIRE	Breed:	-
	Age:	-
	Weight:	-
Number of services:		-
Date of 1st service:		-
Date of 2nd service:		-

Date of collection: January 25, 1962

At time of collection Dam was alive (X), dead ( ).

Embryos collected hour(s) after death.

Litter received from: Edinburgh Surgery

Remarks:

Fig. IV.18. Canine embryo D 76 (5.0 mm. (!) G.L.), the head-heart segment is bent to the embryo's left, ventral view.

Number of embryo:	D 76	Date of processing:	February 7, 1962
Size before fixation:	7.0 mm. (!) G.L.	Block:	Paraffin
Size after fixation:	5.0 mm. (!) G.L.	Stain:	H. & E.
Age:	-	Plane of section:	Horizontal
Position on uterus:	-	Thickness of section:	10 microns
Size of loculus:	14 x 18 mm.	Series made:	D 76 (1 - 6)
Fixed in:	Bouin's Fluid		
Stored in:	70% Alcohol		

From litter:		3 c
Number of embryos in litter:		3
DAM	Breed:	X Irish Terrier
	Age:	2 years
	Weight:	35 lb.
SIRE	Breed:	-
	Age:	-
	Weight:	-
Number of services:		-
Date of 1st service:		-
Date of 2nd service:		-

Date of collection: January 25, 1962

At time of collection Dam was alive (X), dead ( ).

Embryos collected hour(s) after death.

Litter received from: Edinburgh Surgery

Remarks:

Fig. IV.19. Transverse section at the level of the first pharyngeal pouches, embryo D 75 (6.0 mm. (!) G.L.), (X 160).

1 Branchial cleft I; 2 Branchial membrane I; 3 Right dorsal aorta; 4 Right cardinal vein; 5 Wall of otic vesicle; 6 Neural tube; 7 Left otic vesicle; 8 Dorsal extremity of second visceral arch; 9 Pharyngeal pouch I; 10 Notochord; 11 First aortic arch; 12 First visceral arch.

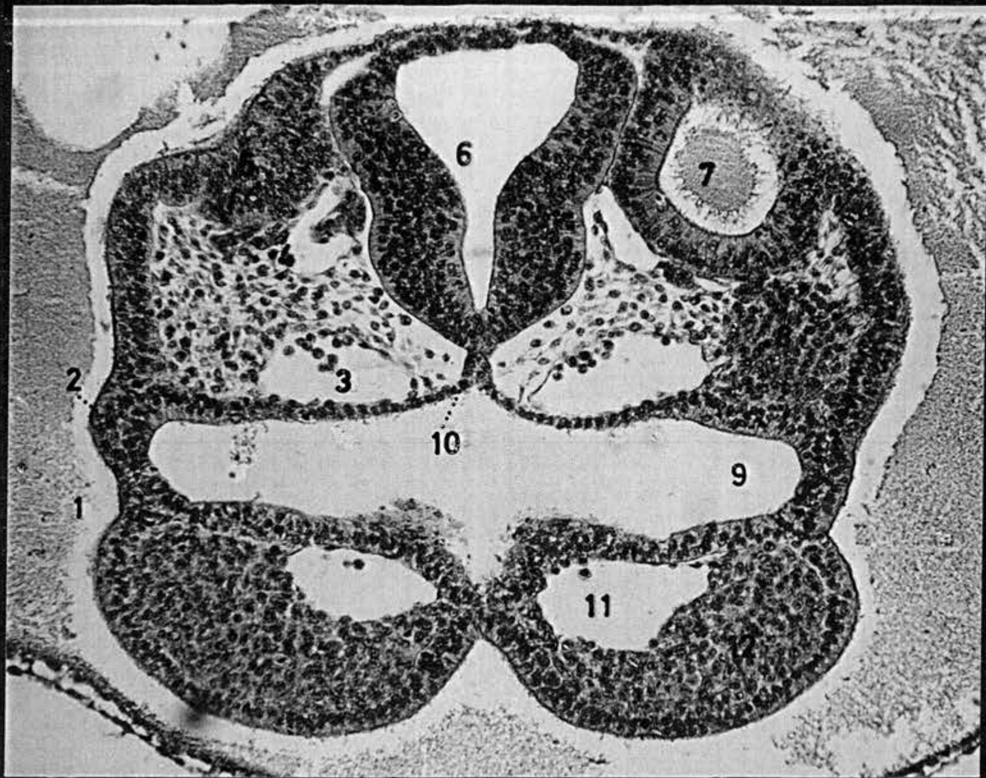


Fig. IV.19. Transverse section at the level of the first pharyngeal pouches, embryo D 75 (6.0 mm. (!) G.L.), (X 160).

1 Branchial cleft I; 2 Branchial membrane I; 3 Right dorsal aorta; 4 Right cardinal vein; 5 Wall of otic vesicle; 6 Neural tube; 7 Left otic vesicle; 8 Dorsal extremity of second visceral arch; 9 Pharyngeal pouch I; 10 Notochord; 11 First aortic arch; 12 First visceral arch.

The Shape of the Pharynx

The foregut is a dorsoventrally flattened, long tube situated between the neural tube and the primitive heart (Fig. IV.22.). It has a length of 1.2 mm., and at its widest measures 0.55 mm. This represents a marked increase in length, while the width has remained almost constant. Such growth tendencies are borne out by the accompanying pictures of the examined specimens taken before they were sectioned, in which the head portion of the embryos seems to have shot forwards without an attendant increase in width (Fig. IV.16.). The tube opens posteriorly at the anterior intestinal portal, through which its interior communicates with the midgut (4) (Fig. IV.22./12). The roof of the primitive pharynx can be divided into two symmetrical halves by a median ridge extending along its entire length (Figs. IV.19.; IV.21.). The notochord overlies the ridge, and, being attached to it as well as to the undersurface of the neural tube, may have been instrumental in its formation (Fig. IV.19./10). At the anterior extremity the roof is pointed and from there backwards increases rapidly in width to form the first pair of pharyngeal pouches at its widest point, behind which it decreases gradually in width towards the anterior intestinal portal (Fig. IV.20.). The floor conforms with the general outline of the roof, but lacks the longitudinal median ridge. Instead, it presents an elevated triangular area found mid-way between the first

<u>Fig. IV.20</u>. Wax model cast of pharyngeal lumen, embryo D 75 (6.0 mm. (!) G.L.), (model X 100), ventrolateral view.

1 Right pharyngeal pouch I; 2 Preoral gut; 3 Area of oral membrane; 4 Impression made by first visceral arch; 5 Left pharyngeal pouch I; 6 Indentation in lateral pharyngeal edge made by second visceral arch; 7 Depression made by aortic bulb; 8 Longitudinal impression made by second visceral arch; 9 Pharyngeal pouch II; 10 Impression made by immature third visceral arch.

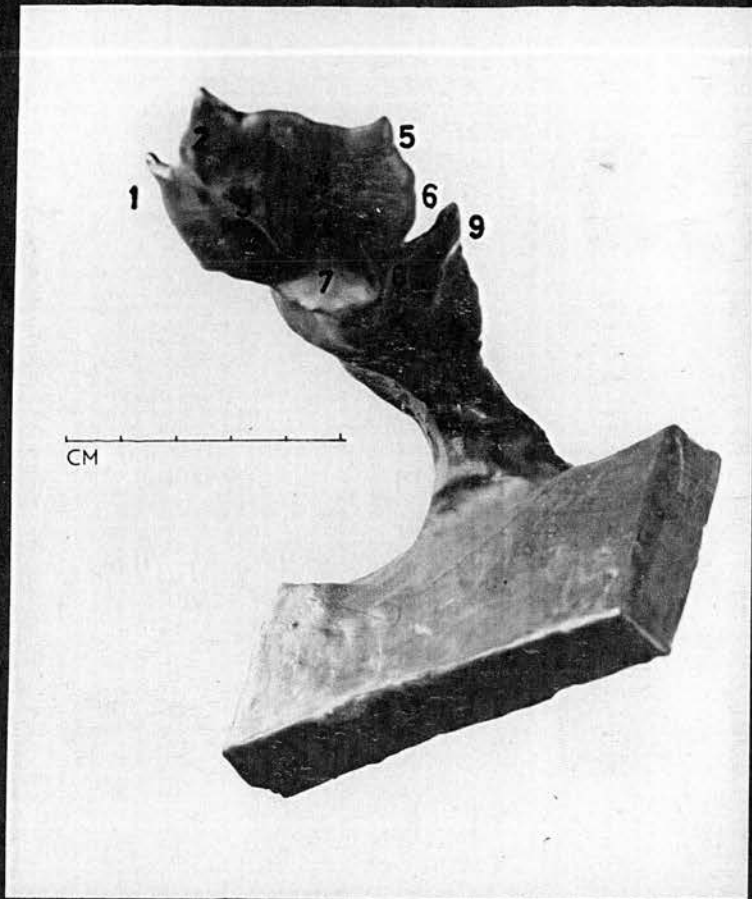


Fig. IV.20. Wax model cast of pharyngeal lumen, embryo D 75 (6.0 mm. (!) G.L.), (model X 100), ventrolateral view.

1 Right pharyngeal pouch I; 2 Preoral gut; 3 Area of oral membrane; 4 Impression made by first visceral arch; 5 Left pharyngeal pouch I; 6 Indentation in lateral pharyngeal edge made by second visceral arch; 7 Depression made by aortic bulb; 8 Longitudinal impression made by second visceral arch; 9 Pharyngeal pouch II; 10 Impression made by immature third visceral arch.

pouches (3). Here it forms the oral membrane with an equally triangular section of overlying ectoderm (Fig. IV.22./5). The portion of foregut anterior to this is commonly termed Preoral Gut (2) (Fig. IV.20./2). Behind the oral membrane follows a deep rounded depression which harbours the Bulbus arteriosus (7). The plate-like anlage of the thyroid can be located at the posterior pole of this depression (Fig. IV.22./10). From here backwards the floor presents no special features and decreases gradually in width, being slightly convex from side to side when viewed from below (Fig. IV.20.).

The lateral edges of the pharynx are sharp and indented at about their middle (6). Thus, two lateral projections are produced, the one in front of the indentation being Pouch I (5) and the one behind, Pouch II (9). The indentation is caused by the expanding second visceral arch of which a further impression can be noted for a short distance along the floor of the tube (8). The first visceral arch occupies an area on the floor just lateral to the triangle of the oral membrane (4). Further backwards, a second but much shallower indentation is visible on the lateral edge, which again is followed by an elongated impression on the floor, caused this time by the as yet immature third visceral arch (10). From here backwards, the lateral edges are smooth, giving no indication of further pouch formation. Thus, the embryonic pharynx at this stage of development has produced the first two pouches of the eventual complement of four. The long axis of each pouch is almost parallel to that of the tube itself, a position that will soon change, since in the following stage the pouches stand at right angles to the pharyngeal tube.

The Structure of the Pharyngeal Wall

The pharyngeal roof is composed of a single layer of cuboidal cells containing round vesicular nuclei. Towards the lateral margins of the tube, the epithelium thickens slightly, because the cells here are taller or have formed two layers (Fig. IV.19.). The epithelium of the pharyngeal floor is generally thicker than that of the roof. The cells are of the columnar type, again with round vesicular nuclei. The epithelial lining along the lateral edges of the primitive pharynx has lost the simple organisation of both roof and floor and consists of two to three rows of irregular cells. Also, in that section of the pharyngeal floor which contributes to the formation of the oral membrane, the epithelium is thicker and composed of several layers of irregular cells. The basement membrane, separating the endodermal from the ectodermal components of the oral membrane, is only visible in fragments and is perhaps already beginning to disappear, soon to be followed by the entire membrane itself. The circumscribed epithelial thickening of the prochordal plate observed in the foregut floor in the preceding stage has been retained in this group of embryos and is found again in the oral membrane (Fig. IV.22./5).

An area, roughly 130 microns in diameter, of especially tall columnar cells is situated immediately behind the elevation in the floor caused by the bulging aortic bulb. Its cells are very slender and crowded and are arranged in such manner that if their long axes were to be continued beyond the free cell border they would meet at a focal point somewhere within the pharyngeal lumen. The nuclei are round and oval, most of them occupying a basal position. The plate-like area of differentiated cells rests on a well defined basement membrane and is slightly concave when viewed from above. It represents the anlage of the thyroid gland (10) (Fig. IV.21./10).

The Relationships of the Pharynx

The anterior portion of the neural tube has continued to enlarge and begun to bend ventrally, so as to be situated in front of, and its anterior tip even somewhat below, the preoral portion of the foregut (Fig. IV.22./1). For this reason the pharynx forms much more extensive relationships with the neural tube than was the case in the preceding stage. The immediate dorsal relation is the notochord, which overlies the mid-sagittal ridge of the

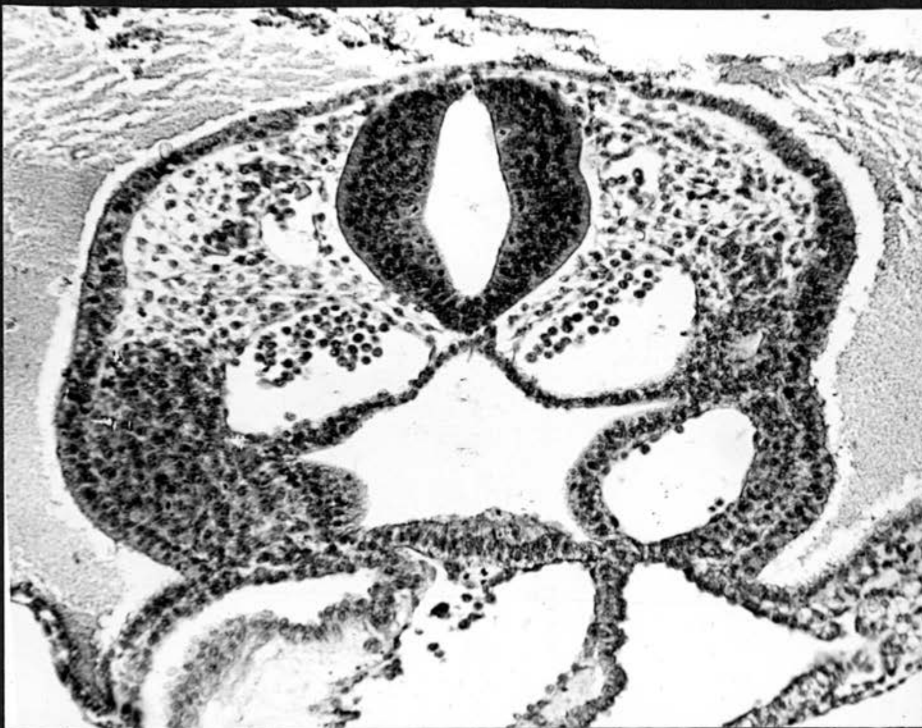


Fig. IV.21. Transverse section at the level of the second visceral arches, embryo D 75 (6.0 mm. (!) G.L.), (X 160).

1 Right second visceral arch; 2 Right dorsal aorta; 3 Right cardinal vein; 4 Neural tube; 5 Left dorsal aorta; 6 Dorsal extremity of left second pharyngeal pouch; 7 Branchial cleft II; 8 Notochord; 9 Pharyngeal lumen; 10 Thyroid plate; 11 Aortic arch II; 12 Bulbus arteriosus.

Fig. IV.21. Transverse section at the level of the second visceral arches, embryo D 75 (6.0 mm. (!) G.L.), (X 160).

1 Right second visceral arch; 2 Right dorsal aorta; 3 Right cardinal vein; 4 Neural tube; 5 Left dorsal aorta; 6 Dorsal extremity of left second pharyngeal pouch; 7 Branchial cleft II; 8 Notochord; 9 Pharyngeal lumen; 10 Thyroid plate; 11 Aortic arch II; 12 Bulbus arteriosus.

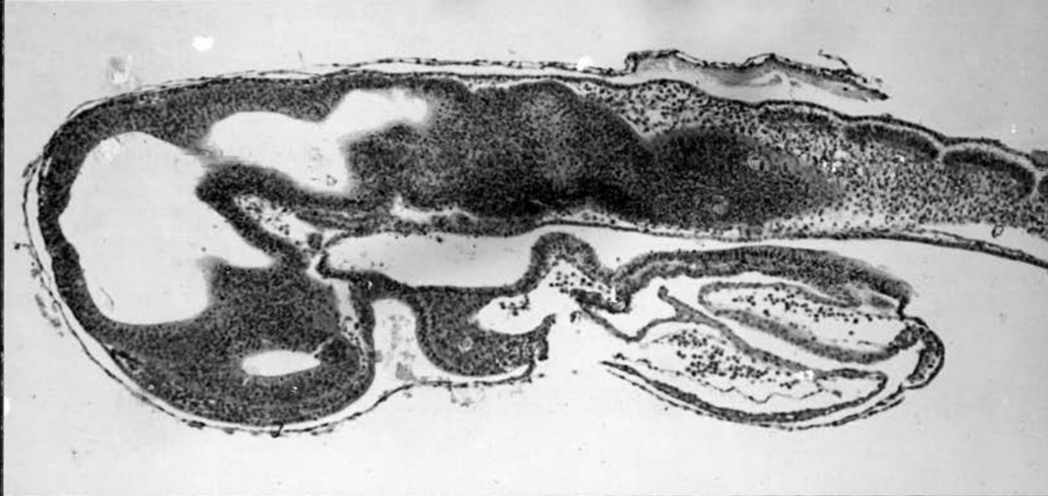


Fig. IV.22. Median section of head process of embryo D 74 (6.5 mm. (!) G.L.), (X 70).

1 Forebrain; 2 Preoral gut; 3 Notochord; 4 Stomodeum; 5 Oral membrane, note thickened anterior portion (prochordal plate); 6 First visceral arch; 7 Pharynx; 8 Hindbrain; 9 Bulbus arteriosus; 10 Thyroid anlage; 11 Heart; 12 Anterior intestinal portal.

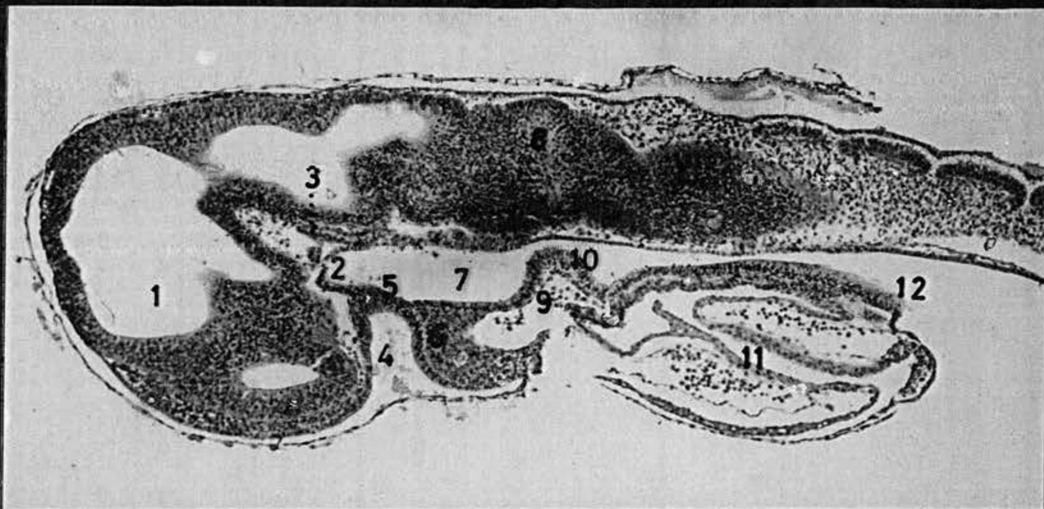


Fig. IV.22. Median section of head process of embryo D 74 (6.5 mm. (!) G.L.), (X 70).

1 Forebrain; 2 Preoral gut; 3 Notochord; 4 Stomodeum; 5 Oral membrane, note thickened anterior portion (prochordal plate); 6 First visceral arch; 7 Pharynx; 8 Hindbrain; 9 Bulbus arteriosus; 10 Thyroid anlage; 11 Heart; 12 Anterior intestinal portal.

pharyngeal roof (3). To either side of the notochord the pharynx is in contact with the dorsal aortae which are embedded in loose mesenchyme (Fig. IV.21./2, 5). These vessels are connected with the aortic bulb by the first two pairs of aortic arches, of which the first arch arteries are slightly larger in diameter. Above and overshadowing the notochord in mass is the tubular hind-brain (4), and overlying the preoral portion of the foregut, the midbrain. The forebrain and the first aortic arches, the latter lying embedded in the first visceral arches, form the anterior relationships. Ventrally, the relations from before backwards are as follows. Under the preoral gut is the most anterior tip of the formerly straight neural tube (Fig. IV.22.). The anterior neuropore is in the process of closing. Posterior to the tip is the stomodeum (4), bounded in front by the fore-brain (1) and behind and to the sides by the mandibular arches (6). The oral membrane forms the roof of this depression (5). Rathke's pocket has not yet appeared. Underlying the floor of the foregut behind the oral membrane are the ventral aortae contained in the ventral portion of the mandibular arches. This is followed first by the aortic bulb (9) and then by the second aortic arches which lie in the centres of the second visceral arches. Subsequent to these is the primitive pericardium which is flanked on either side by the short stumps of the third aortic arch arteries and the as yet very narrow third visceral arches. From this level to the intestinal portal the pericardial celom forms the principal ventral relationship (11). Laterally, the pharynx is bounded by the visceral arches I and II, harbouring the respective aortic arch arteries. Between the arches, pouch tissue is in contact with overlying ectoderm, forming branchial membranes. The postbranchial portion of the gut is coextensive with pericardial celom.

When compared to the short crescent-shaped foregut of the previous stage, the present pharynx has changed markedly both in shape and size. Most notable is the sharp increase in length. The lateral edges of the flat foregut have been indented by the first and second visceral arches and to a minor degree by the third. The areas left standing between the expanding mesenchymal arches represent the pharyngeal pouches, two of them being recognisable on either side. The plate-like thyroid anlage has appeared in the floor immediately behind the raised bulge caused by the aortic bulb. The prechordal cells, previously observed above the pharynx, have organised to form the notochord which is still attached to the pharyngeal roof. Due to the development of the forebrain, its concurrent ventral turn and lateral expansion, the neural tube forms a more extensive relationship with the pharynx as a whole.

STAGE 4

(4 - 6 mm. G.L.)

The observations for the fourth stage in the development of the pharynx were made on eight embryos taken from two litters as follows:

Embryo CC 6	6.0 mm. G.L.	litter 3 a
Embryo D 66	6.2 mm. G.L.	litter 3 a
Embryo D 67	5.5 mm. G.L.	litter 3 a
Embryo D 68	6.0 mm. G.L.	litter 3 a
Embryo D 8	5.0 mm. G.L.	litter 4
Embryo D 14	4.5 mm. G.L.	litter 4
Embryo D 15	4.5 mm. G.L.	litter 4
Embryo D 23	4.5 mm. G.L.	litter 4

The members of litter 4, although smaller, are slightly more developed than those of litter 3 a.

Fig. IV.23. Canine embryo CC 6 (6.0 mm. G.L.).

Number of embryo:	**CC 6**	Date of processing:	**June 5, 1961**
Size before fixation:	**-**	Block:	**Paraffin**
Size after fixation:	**6.0 mm. G.L.**	Stain:	**H. & E.**
Age:	**21 days**	Plane of section:	**Sagittal**
Position on uterus:	**-**	Thickness of section:	**10 microns**
Size of loculus:	**22 x 16 mm.**	Series made:	**CC 6 (1 - 5)**
Fixed in:	**10% Formalin**		
Stored in:	**10% Formalin**		

From litter:		**3 a**
Number of embryos in litter:		**9**
DAM	Breed:	**X Bull Terrier**
	Age:	**16 months**
	Weight:	**50 lb.**
SIRE	Breed:	**X Collie**
	Age:	**2 years**
	Weight:	**50 lb.**
Number of services:		**1**
Date of 1st service:		**May 8, 1961**
Date of 2nd service:		**-**

Date of collection: **May 29, 1961**

At time of collection Dam was alive ( ), dead (**X**).

Embryos collected **2** hour(s) after death.

Litter received from: **Edinburgh Clinic**

Remarks:

Fig. IV.24. Canine embryo D 66 (6.2 mm. C.L.).

Number of embryo:	**D 66**	Date of processing:	**June 5, 1961**
Size before fixation:	**-**	Block:	**Paraffin**
Size after fixation:	**6.2 mm. G.L.**	Stain:	**H. & E.**
Age:	**21 days**	Plane of section:	**Frontal**
Position on uterus:	**-**	Thickness of section:	**10 microns**
Size of loculus:	**22 x 17 mm.**	Series made:	**D 66 (1 - 11)**
Fixed in:	**10% Formalin**		
Stored in:	**10% Formalin**		

From litter:		**3 a**
Number of embryos in litter:		**9**
DAM	Breed:	**X Bull Terrier**
	Age:	**16 months**
	Weight:	**50 lb.**
SIRE	Breed:	**X Collie**
	Age:	**2 years**
	Weight:	**50 lb.**
Number of services:		**1**
Date of 1st service:		**May 8, 1961**
Date of 2nd service:		**-**

Date of collection: **May 29, 1961**

At time of collection Dam was alive ( ), dead (**X**).

Embryos collected **2** hour(s) after death.

Litter received from: **Edinburgh Clinic**

Remarks:

<u>Fig. IV.25</u>. Canine embryo D 67 (5.5 mm. G.L.).

Number of embryo:	D 67	Date of processing:	June 5, 1961
Size before fixation:	-	Block:	Paraffin
Size after fixation:	5.5 mm. G.L.	Stain:	H. & E.
Age:	21 days	Plane of section:	Sagittal
Position on uterus:	-	Thickness of section:	10 microns
Size of loculus:	22 x 16 mm.	Series made:	D 67 (1 - 7)
Fixed in:	10% Formalin		
Stored in:	10% Formalin		

From litter:		3 a
Number of embryos in litter:		9
DAM	Breed:	X Bull Terrier
	Age:	16 months
	Weight:	50 lb.
SIRE	Breed:	X Collie
	Age:	2 years
	Weight:	50 lb.
Number of services:		1
Date of 1st service:		May 8, 1961
Date of 2nd service:		-

Date of collection: May 29, 1961

At time of collection Dam was alive ( ), dead (X).

Embryos collected 2 hour(s) after death.

Litter received from: Edinburgh Clinic

Remarks:

Fig. IV.26. Canine embryo D 68 (6 mm. G.L.).

Number of embryo:	D 68	Date of processing:	June 5, 1961
Size before fixation:	-	Block:	Paraffin
Size after fixation:	6.0 mm. G.L.	Stain:	H. & E.
Age:	21 days	Plane of section:	Transverse
Position on uterus:	-	Thickness of section:	10 microns
Size of loculus:	23 x 16 mm.	Series made:	D 68 (1 - 9)
Fixed in:	10% Formalin		
Stored in:	10% Formalin		

From litter:		3 a
Number of embryos in litter:		9
DAM	Breed:	X Bull Terrier
	Age:	16 months
	Weight:	50 lb.
SIRE	Breed:	X Collie
	Age:	2 years
	Weight:	50 lb.
Number of services:		1
Date of 1st service:		May 8, 1961
Date of 2nd service:		-
Date of collection:		May 29, 1961

At time of collection Dam was alive ( ), dead (X).

Embryos collected 2 hour(s) after death.

Litter received from: Edinburgh Clinic

Remarks:

Fig. IV.27. Canine embryo D 8 (5 mm. G.L.).

Number of embryo:	**D 8**	Date of processing:	**August 1, 1960**
Size before fixation:	**-**	Block:	**Paraffin**
Size after fixation:	**5.0 mm. G.L.**	Stain:	**H. & E.**
Age:	**-**	Plane of section:	**Frontal**
Position on uterus:	**-**	Thickness of section:	**5 microns**
Size of loculus:	**28 x 15 mm. (fixed)**	Series made:	**D 8 (1 - 18)**
Fixed in:	**10% Form./Bouin's Fluid**		
Stored in:	**70% Alcohol**		

From litter:		**4**
Number of embryos in litter:		**5**
DAM	Breed:	**X Terrier**
	Age:	**-**
	Weight:	**13 lb.**
SIRE	Breed:	**Small Cocker Spaniel**
	Age:	**-**
	Weight:	**20 lb.**
Number of services:		**-**
Date of 1st service:		**-**
Date of 2nd service:		**-**

Date of collection: **February 5, 1960**

At time of collection Dam was alive (**X**), dead ( ).

Embryos collected hour(s) after death.

Litter received from: **Dr. McKay, Whitby, Ontario.**

Remarks:

Number of embryo:	**D 14**	Date of processing:	**August 10, 1960**
Size before fixation:	**-**	Block:	**Paraffin**
Size after fixation:	**4.5 mm. G.L.**	Stain:	**H. & E.**
Age:	**-**	Plane of section:	**Sagittal**
Position on uterus:	**-**	Thickness of section:	**10 microns**
Size of loculus:	**27 x 15 mm. (fixed)**	Series made:	**D 14 (1 - 12)**
Fixed in:	**10% Form./Bouin's Fluid**		
Stored in:	**70% Alcohol**		

From litter:		**4**
Number of embryos in litter:		**5**
DAM	Breed:	**X Terrier**
	Age:	**-**
	Weight:	**13 lb.**
SIRE	Breed:	**Small Cocker Spaniel**
	Age:	**-**
	Weight:	**20 lb.**
Number of services:		**-**
Date of 1st service:		**-**
Date of 2nd service:		**-**

Date of collection: **February 5, 1960**

At time of collection Dam was alive (**X**), dead ( ).

Embryos collected hour(s) after death.

Litter received from: **Dr. McKay, Whitby, Ontario.**

Remarks: **For a picture see those of litter mates D 8 and D 15 of this stage (Figs.IV.27; IV.28.)**

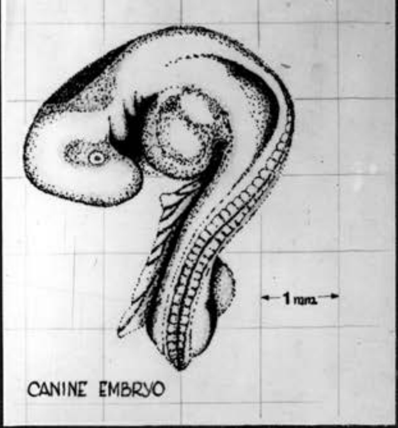


Fig. IV.28. Canine embryo D 15 (4.5 mm. G.L.).

Number of embryo:	D 15	Date of processing:	September 8, 1960
Size before fixation:	-	Block:	Paraffin
Size after fixation:	4.5 mm. G.L.	Stain:	H. & E.
Age:	-	Plane of section:	Sagittal
Position on uterus:	-	Thickness of section:	10 microns
Size of loculus:	26 x 14 mm. (fixed)	Series made:	D 15 (1 - 8)
Fixed in:	10% Form./Bouin's Fluid		
Stored in:	70% Alcohol		

From litter:		4
Number of embryos in litter:		5
DAM	Breed:	X Terrier
	Age:	-
	Weight:	13 lb.
SIRE	Breed:	Small Cocker Spaniel
	Age:	-
	Weight:	20 lb.
Number of services:		-
Date of 1st service:		-
Date of 2nd service:		-

Date of collection: February 5, 1960

At time of collection Dam was alive (X), dead ( ).

Embryos collected hour(s) after death.

Litter received from: Dr. McKay, Whitby, Ontario.

Remarks:

Number of embryo:	**D 23**	Date of processing:	**January 23, 1961**
Size before fixation:	**-**	Block:	**Paraffin**
Size after fixation:	**4.5 mm. G.L.**	Stain:	**H. & E.**
Age:	**-**	Plane of section:	**Transverse**
Position on uterus:	**-**	Thickness of section:	**10 microns**
Size of loculus:	**27 x 14 mm. (fixed)**	Series made:	**D 23 (1 - 11)**
Fixed in:	**10% Form./Bouin's Fluid**		
Stored in:	**70% Alcohol**		

From litter:		**4**
Number of embryos in litter:		**5**
DAM	Breed:	**X Terrier**
	Age:	**-**
	Weight:	**13 lb.**
SIRE	Breed:	**Small Cocker Spaniel**
	Age:	**-**
	Weight:	**20 lb.**
Number of services:		**-**
Date of 1st service:		**-**
Date of 2nd service:		**-**

Date of collection: **February 5, 1960**

At time of collection Dam was alive (X), dead ( ).

Embryos collected hour(s) after death.

Litter received from: **Dr. McKay, Whitby, Ontario.**

Remarks: **For a picture see those of litter mates D 8 and D 15 (Figs.IV.27; IV.28.)**

The Shape of the Pharynx

Considerable growth has taken place in the pharyngeal area, and although the foregut has retained its dorsoventrally flattened shape, it varies greatly from the configuration of the previous stage. The oral membrane has disintegrated and communication between the foregut and the amniotic cavity is established through the primitive mouth. When viewed from above, the pharynx displays a triangular shape of which the wide, ventrally directed mouth forms the base, and the opening into the esophagus, which also points downward, forms the apex (Fig. IV.29.). The longitudinal axis, arching from Rathke's pocket to the esophagus (Fig. IV.1./3), is 1.2 mm. long. Transversely, the measurements are 700 microns in front of pouch I and 500 microns just anterior to pouch IV (1, 2). The pharyngeal tube at this stage can be likened to a funnel of which the wide part and the stem have been flattened in planes lying at right angles to each other, and which thereafter has been bent once where the stem joins the wide part and a second time just behind the mouth in such manner that the two openings point in the same direction and the long axes of these openings, at the same time, are at right angles to each other (Fig. IV.29./3, 13). The anterior bend, the one above the mouth, is caused by the cephalic flexure and the posterior one, above the entrance into the esophagus, by the cervical flexure of the embryo. Consequently, they

Fig. IV.29. Wax model of the left half of the pharyngeal tube, embryo D 15 (4.5 mm. G.L.), ventral view, (model X 100).

P1 - P4 Pharyngeal pouches I - IV; 1 Rathke's pocket; 2 Pharyngeal roof; 3 Mouth; 4 Floor forming median indentation; 5 Thyroid plate; 6, 7, 8 Ridges connecting ventral wings of pharyngeal pouches I - III with pharyngeal floor; 9 Pharyngeal floor; 10 Laryngotracheal groove; 11, 12 Right and left lung buds; 13 Esophagus.

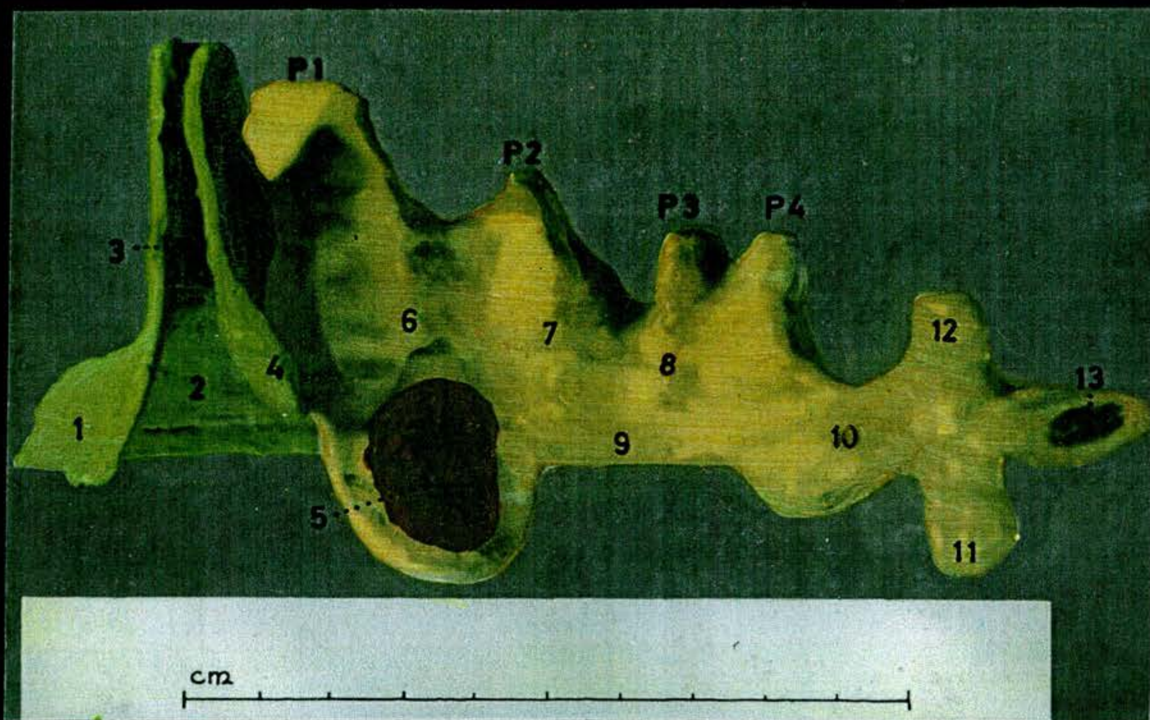


Fig. IV.29. Wax model of the left half of the pharyngeal tube, embryo D 15 (4.5 mm. G.L.), ventral view, (model X 100).

P1 - P4 Pharyngeal pouches I - IV; 1 Rathke's pocket; 2 Pharyngeal roof; 3 Mouth; 4 Floor forming median indentation; 5 Thyroid plate; 6, 7, 8 Ridges connecting ventral wings of pharyngeal pouches I - III with pharyngeal floor; 9 Pharyngeal floor; 10 Laryngotracheal groove; 11, 12 Right and left lung buds; 13 Esophagus.

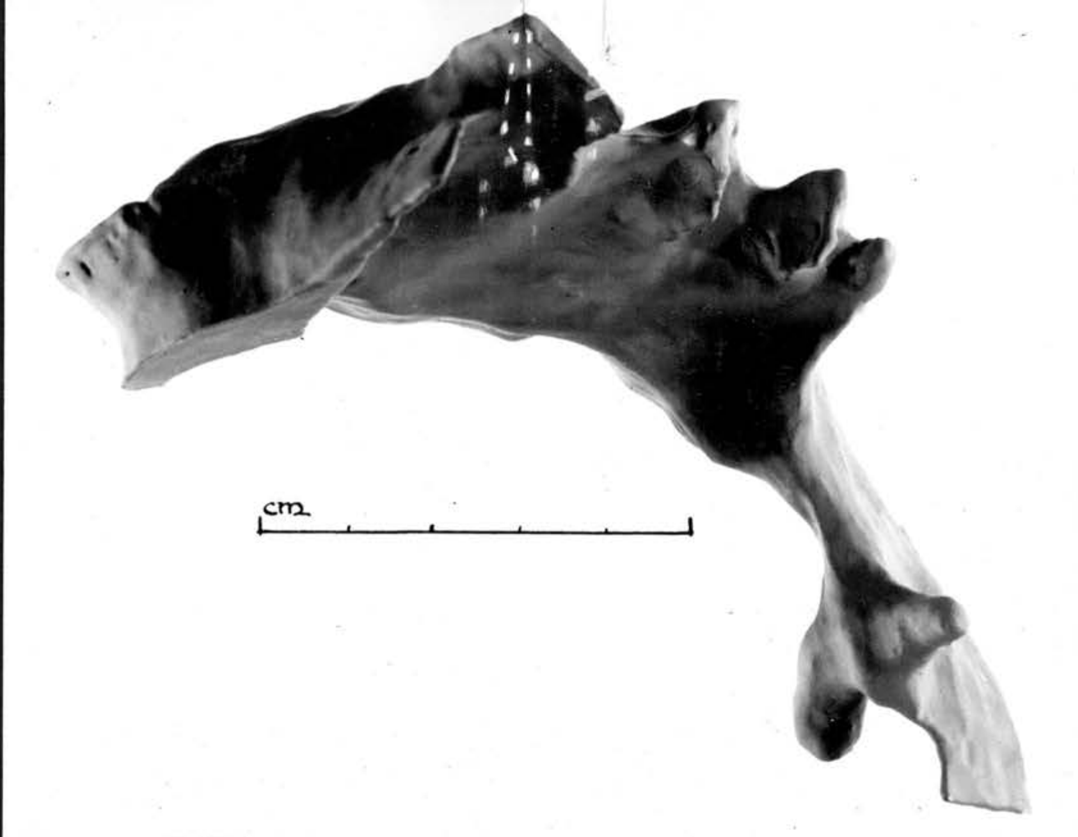


Fig. IV.30. Wax model cast of the left half of the pharyngeal tube, embryo D 15 (4.5 mm. G.L.), ventrolateral view, (model X 100).

P1 - P4 Pharyngeal pouches I - IV; 1 Rathke's pocket; 2 Lateral edge of primitiv oral cavity; 3 Laryngotracheal groove; 4, 5 Right and left lung buds; 6 Esophagu

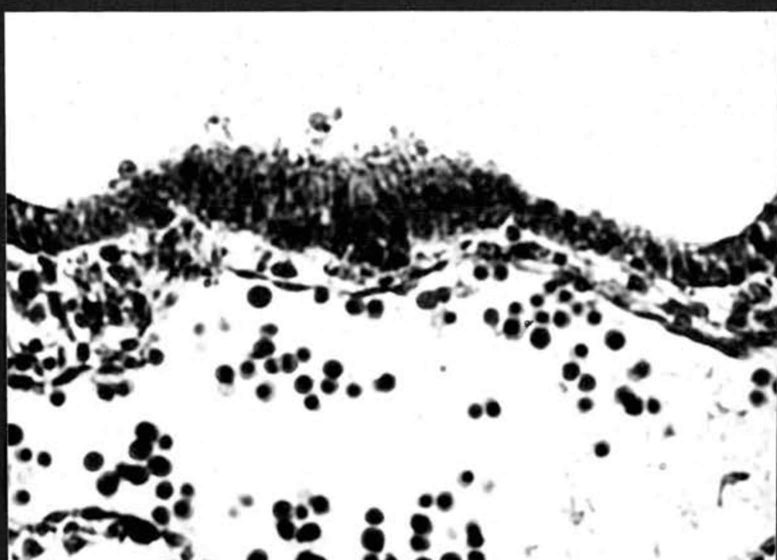


Fig. IV.31. Transverse section of thyroid plate, embryo D 23 (4.5 mm. G.L.), (X 520)

1 Rough dorsal surface with cell debris; 2 Beginning of cell cord formation; 3 Aortic sac; 4 Pericardium; 5 Epithelium of pharyngeal floor; 6 Lumen of pharynx.

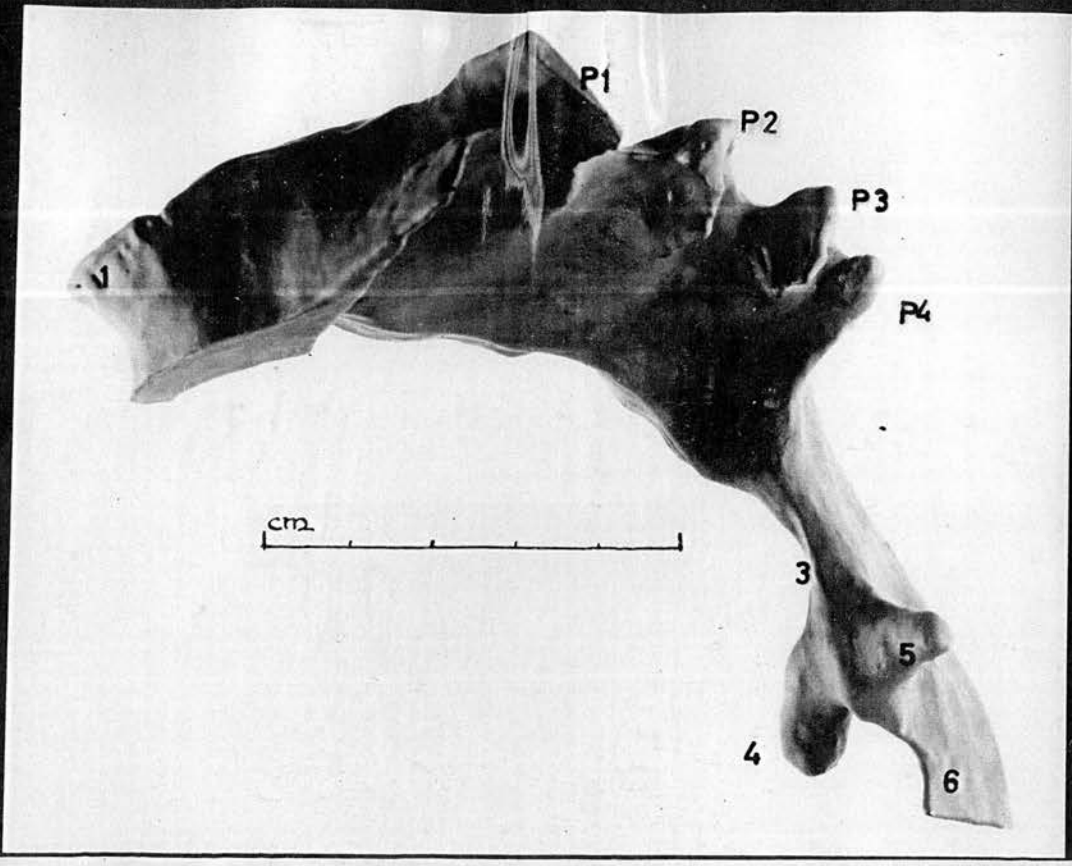


Fig. IV.30. Wax model cast of the left half of the pharyngeal tube, embryo D 15 (4.5 mm. G.L.), ventrolateral view, (model X 100).

P1 - P4 Pharyngeal pouches I - IV; 1 Rathke's pocket; 2 Lateral edge of primiti oral cavity; 3 Laryngotracheal groove; 4, 5 Right and left lung buds; 6 Esophag

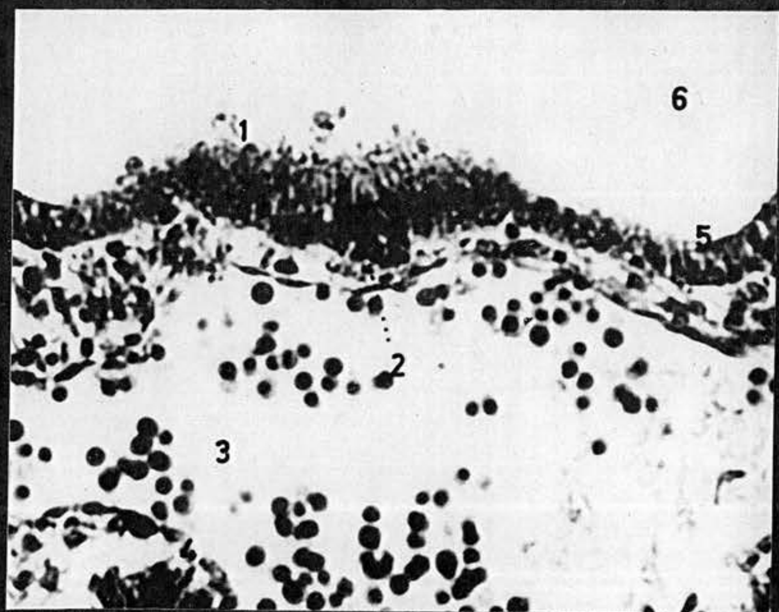


Fig. IV.31. Transverse section of thyroid plate, embryo D 23 (4.5 mm. G.L.), (X 520

1 Rough dorsal surface with cell debris; 2 Beginning of cell cord formation; 3 Aort sac; 4 Pericardium; 5 Epithelium of pharyngeal floor; 6 Lumen of pharynx.

are called the cephalic and the cervical flexures of the pharynx (Fig. IV.34./3, 10).

The floor of the pharynx is triangular in outline and slightly convex both from front to back and from side to side. Anteriorly, the endodermal wall curves sharply downwards, and, while covering the anterior aspects of the mandibular arches, forms the caudal border of the mouth opening (Fig. IV.37./8). At this point the endoderm of the foregut is continued by ectoderm, because it was here that the oral membrane had its caudal attachment on the expanding mandibular arches. The ventral portions of the latter run somewhat posteriorly before they converge in the midline, which causes the floor of the pharynx (Area mesobranchialis) to show a caudally directed indentation at its anterior margin (Fig. IV.29./4). Posteriorly, the floor narrows and also bends ventrally to continue as the lining of the esophagus. Laterally, the continuity of the floor is interrupted by three transversely directed grooves which originate from the ventral wings of pouch I, II and III and run towards the median plane (6, 7, 8). To conform with the somewhat radial convergence of the visceral arches in the ventral midline, the first of these furrows is directed slightly caudally, the second exactly transversely, and the third slightly anteriorly. None of them reaches the midline at this stage. The second groove is deepest, whereas the first is the shallowest, indicating the relative ventral development of the pouches.

At the point where the furrows from the first and second pharyngeal pouches would, if continued, meet in the median plane, the plate-like anlage of the thyroid gland can be detected (5). This is a local circumscribed thickening in the endoderm, oval in outline, with its long axis in the transverse plane. In embryo D 15 this oval thyroid plate measures 220 by 160 microns. In embryo D 23 the thyroid plate is raised slightly above the level of the floor and consists of an aggregation of heavily crowded columnar cells (Fig. IV.31.). The dorsal surface of the plate is somewhat concave and presents a rough appearance (1), which is caused by cell debris and some entire cells that have apparently been squeezed upwards into the chamber of the pharynx. The lower surface of the plate bulges toward the aortic sac and in some places is coextensive with it. The beginning of cell cord formation is visible at one point (2). In embryo D 14 the thickening of the plate has progressed, and downward growth is already apparent. Short finger-like cell cords emanate from the entire surface of the anlage (Fig. IV.37/10). In none of the embryos of this group have any cell cords lost contact with the pharyngeal floor.

The pharyngeal roof is smooth, also triangular in outline, and convex from front to back and from side to side. The longitudinal convexity is most marked anteriorly and posteriorly where the mucosa turns sharply downwards to take part in the formation of the mouth and

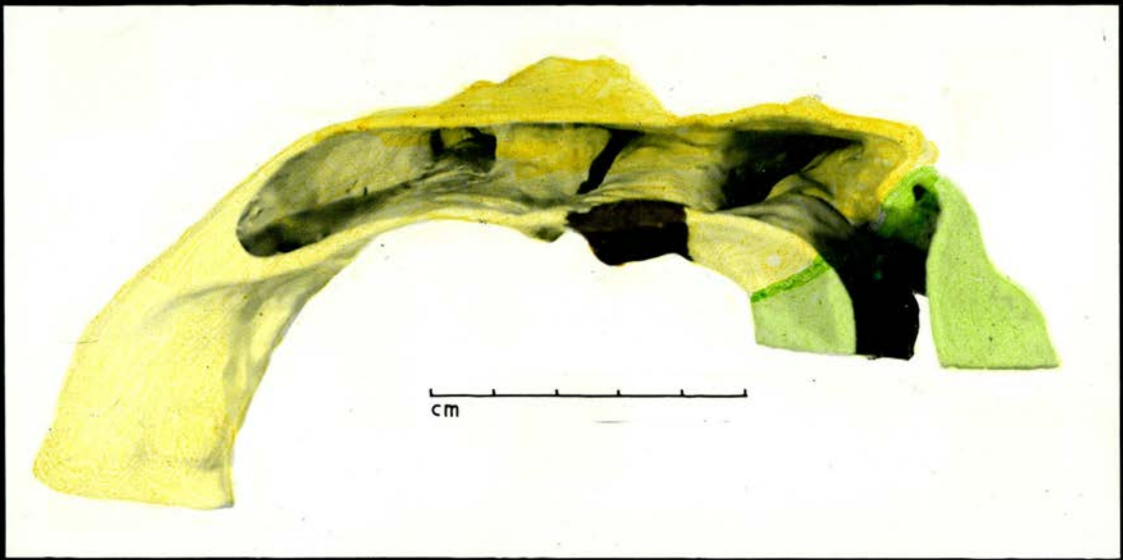


Fig. IV.32. Wax model of left half of pharyngeal tube, embryo CC 6 (6.0 mm. G.L.), medial view, (model X 100).

P1 - P4 Pharyngeal pouches I - IV; 1 Esophagus; 2 Right lung bud; 3 Floor; 4 Thyroid plate; 5 Roof; 6 Seessel's pocket; 7 Remnant of oral membrane; 8 Rathke's pocket; 9 Mouth.

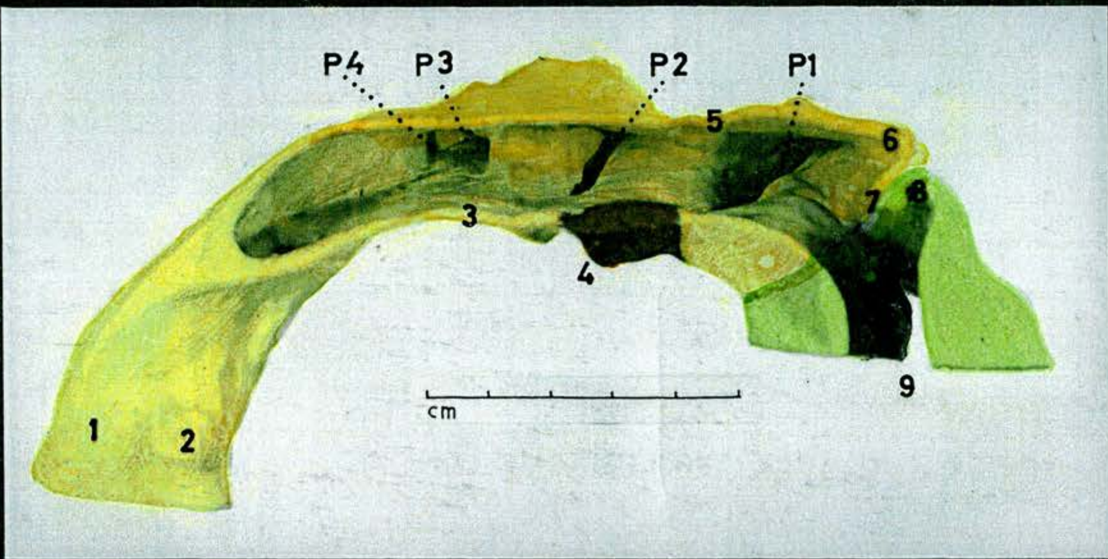


Fig. IV.32. Wax model of left half of pharyngeal tube, embryo CC 6 (6.0 mm. G.L.), medial view, (model X 100).

P1 - P4 Pharyngeal pouches I - IV; 1 Esophagus; 2 Right lung bud; 3 Floor; 4 Thyroid plate; 5 Roof; 6 Seessel's pocket; 7 Remnant of oral membrane; 8 Rathke's pocket; 9 Mouth.

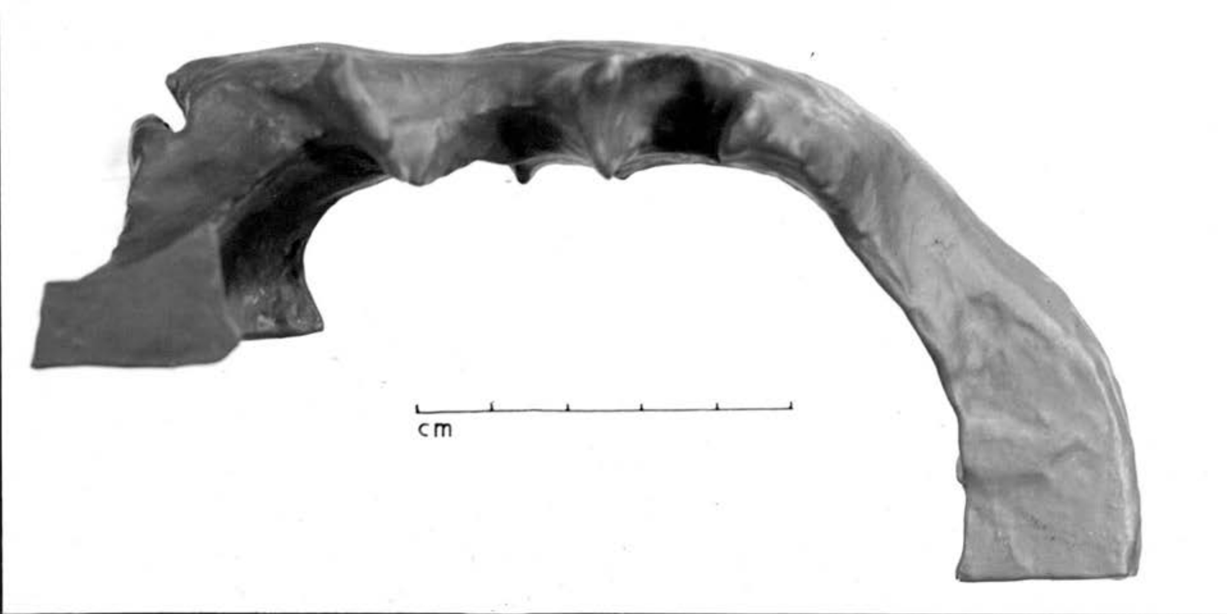


Fig. IV.33. Wax model cast of the pharyngeal lumen, embryo CC 6 (6.0 mm. G.L.), lateral view, (model X 100).

P1 - P4 Pharyngeal pouches I - IV; 1 Rathke's pocket; 2 Seessel's pocket; 3 Foramen cecum; 4 Esophagus.

the esophagus respectively. Anterior to the still visible line of attachment of the oral membrane (Fig. IV.32/7), Rathke's pocket presents a crescent-shaped recess, the long axis of which runs transversely and the ridge it forms on the outside of the epithelium is directed anteriorly (Fig. IV.30./1). The shallow, transversely running depression immediately behind the oral membrane attachment is Seessel's pocket (Fig. IV.36./11). At the posterior end the narrowing roof bulges caudally, producing a medially located recess above the entrance into the esophagus (Fig. IV.34./9). In embryo D 15 the distance between the apex of this pharyngeal recess and the entrance into the esophagus (Fig. IV.1./4) measures 550 microns. In the embryos from the less advanced litter the pharyngeal recess has not yet formed (Fig. IV.33.).

The lateral walls of the pharynx, observed to be rounded and smooth in the 3 - 4 mm. stage, have undergone the most striking modification. In the preceding 5 - 6 mm. (!) stage the first two pharyngeal pouches had developed. Now, two more have budded from the pharynx and pushed laterally to gain contact with the ectoderm between the bars of the visceral arches. In the 4 - 6 mm. stage the full complement of these outpocketings is reached, although the last one, pouch IV, is still poorly developed, especially in the members of litter 3 a (Fig. IV.33./P4). They are designated as pouch I to

Fig. IV.34. Wax model of the left half of the pharyngeal tube, embryo D 15 (4.5 mm. G.L.), lateral view, (model X 100).

P1 - P4 Pharyngeal pouches I - IV; 1 Rathke's pocket; 2 Mouth; 3 Cephalic flexure; 4, 6, 7 Branchial membranes; 5 Thyroid plate showing beginning of cell cord formation; 8 True diverticulum of pouch III; 9 Pharyngeal recess; 10 Cervical flexure; 11 Laryngotracheal groove; 12, 13 Left and right lung buds; 14 Esophagus.

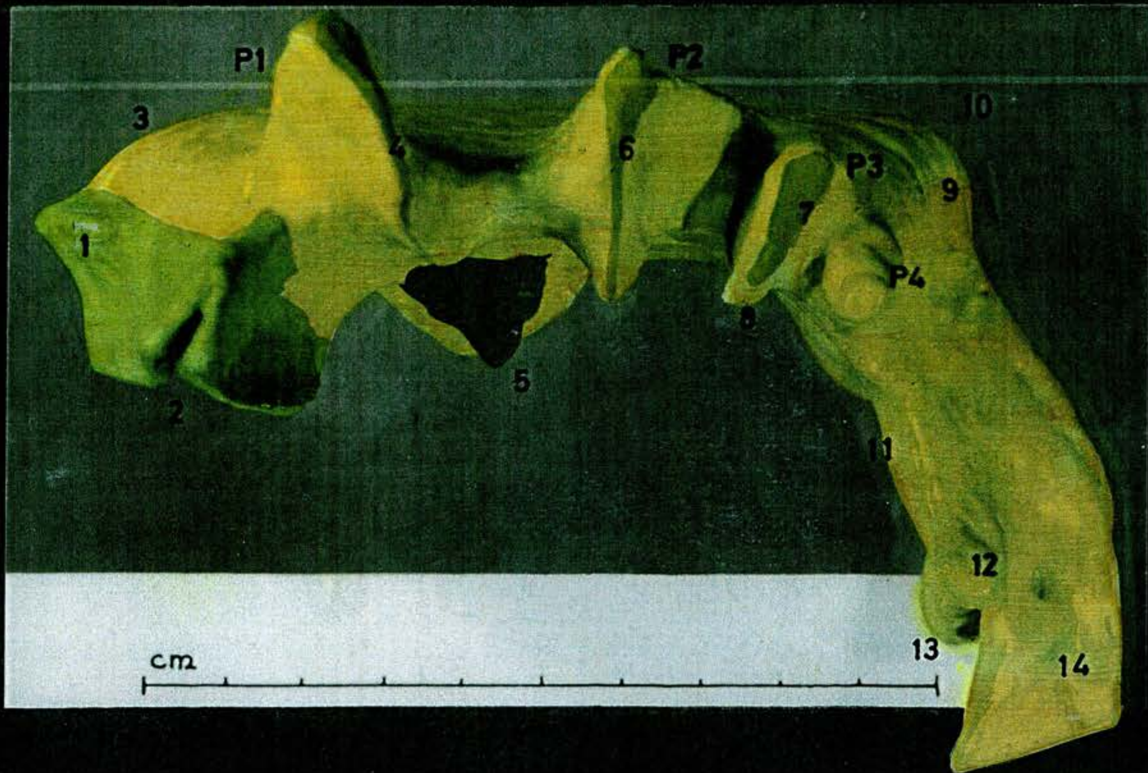


Fig. IV.34. Wax model of the left half of the pharyngeal tube, embryo D 15 (4.5 mm. G.L.), lateral view, (model X 100).

P1 - P4 Pharyngeal pouches I - IV; 1 Rathke's pocket; 2 Mouth; 3 Cephalic flexure; 4, 6, 7 Branchial membranes; 5 Thyroid plate showing beginning of cell cord formation; 8 True diverticulum of pouch III; 9 Pharyngeal recess; 10 Cervical flexure; 11 Laryngotracheal groove; 12, 13 Left and right lung buds; 14 Esophagus.

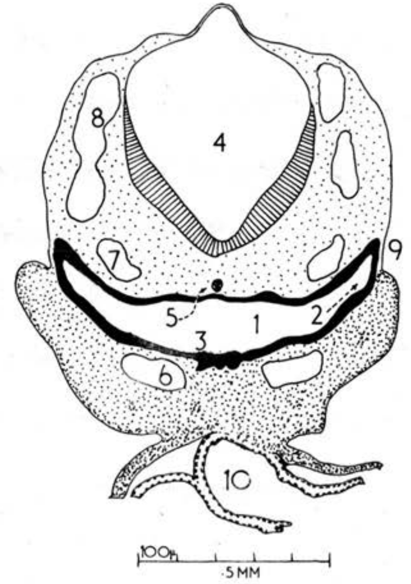


Fig. IV.35. Transverse section of head at the level of pouch I, embryo D 23 (4.5 mm. G.L.), (semischematic).

1 Pharynx; 2 Lumen of pouch I; 3 Thyroid plate; 4 Hindbrain; 5 Notochord; 6 Left aortic arch I; 7 Left dorsal aorta; 8 Left anterior cardinal vein; 9 Right branchial membrane I; 10 Heart.

pouch IV, I being the most anterior. The visceral clefts, visible on the lateral side of the head (Fig. IV.25.), are ectodermal furrows whose depths meet the endoderm of the pharyngeal pouches and form at each point of contact a branchial membrane (Figs. IV.34./4, 6, 7; IV.35./9). A pouch and a cleft lying on either side of a branchial membrane have the same number. Similarly, the visceral arches and the aortic arch arteries contained by them are specified by numerals from I - IV; and it is the established rule that the arch always precedes the pouch of like number. The surface area of the branchial membrane is small when compared to that of each pouch. None of them had ruptured in this group of embryos. Since the pouches push outward between the visceral arches, which at this point run practically parallel, they are by necessity flattened between them. Most of the pouches grow dorsal and ventral wings, projecting above and below the main body of the pharynx. Only the third pouch of embryo D 15 has a true ventral diverticulum at this stage of development (Fig. IV.34./8)(see also Fig. IV.1.). Axes laid through dorsal and ventral wings of each pouch on a given side have the following directions: pouch I - ventrally, medially and slightly posteriorly; pouch II - ventrally and slightly medially; pouch III - ventrally and slightly anteriorly. Pouch IV is devoid of wings at this stage and, being the least advanced, represents only a short finger-like outgrowth pointing laterally and somewhat ventrally (Fig. IV.34./P4). It communicates with the pharyngeal cavity in common with pouch III (Fig. IV.29.). The dorsal and ventral wings of the pouches that form them differ in relative size: in pouch I the dorsal wing is larger than the ventral, in pouch II and III the reverse is true. The relative size of each pouch decreases from front to back; pouch IV is too short to make contact with ectoderm. The walls of the fourth pouch were observed to be considerably thicker than those of the other pouches; in embryo D 14 this was only true of the caudal wall. In none of the pouches in this group could any evidence of cell differentiation be detected.

Lung buds are developing from the caudal end of the laryngotracheal groove (Fig. IV.30./3) on the antero-lateral face of the esophagus. In embryo D 15 they have attained a length of about 100 microns, the right being slightly larger than the left (Fig. IV.29./11, 12). In embryo CC 6 only the bud of the right lung produces a bulge on the laterally flattened esophageal portion of the foregut; on the opposite side there is merely a thickening in the epithelium. In the other members of litter 3 a,only thickened areas in the epithelium mark the beginning of lung bud formation. From the examination of these two litters it appears that, unlike the human and the pig where, according to Arey (1948) and Patten (1948, 1958), a central unpaired lung bud grows from the posterior

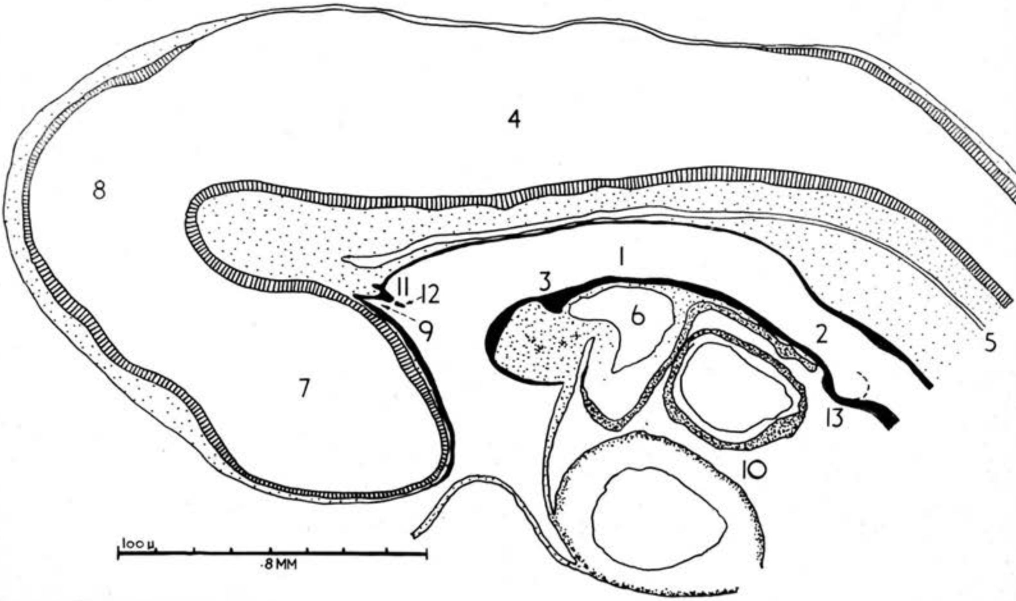


Fig. IV.36. Median section of head region, embryo D 14 (4.5 mm. G.L.), (semischematic).

1 Pharynx; 2 Esophagus; 3 Thyroid plate; 4 Hindbrain; 5 Notochord; 6 Aortic sac; 7 Forebrain; 8 Midbrain; 9 Rathke's pocket; 10 Heart; 11 Seessel's pocket; 12 Remnant of oral membrane; 13 Position of lung bud.

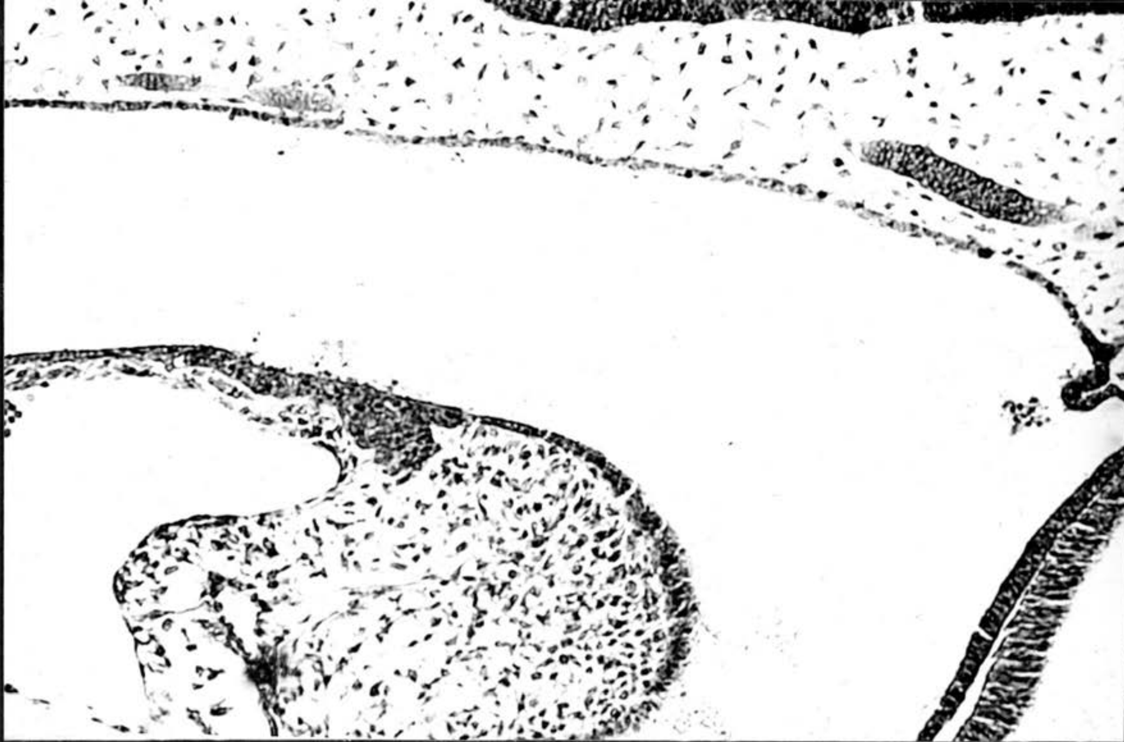
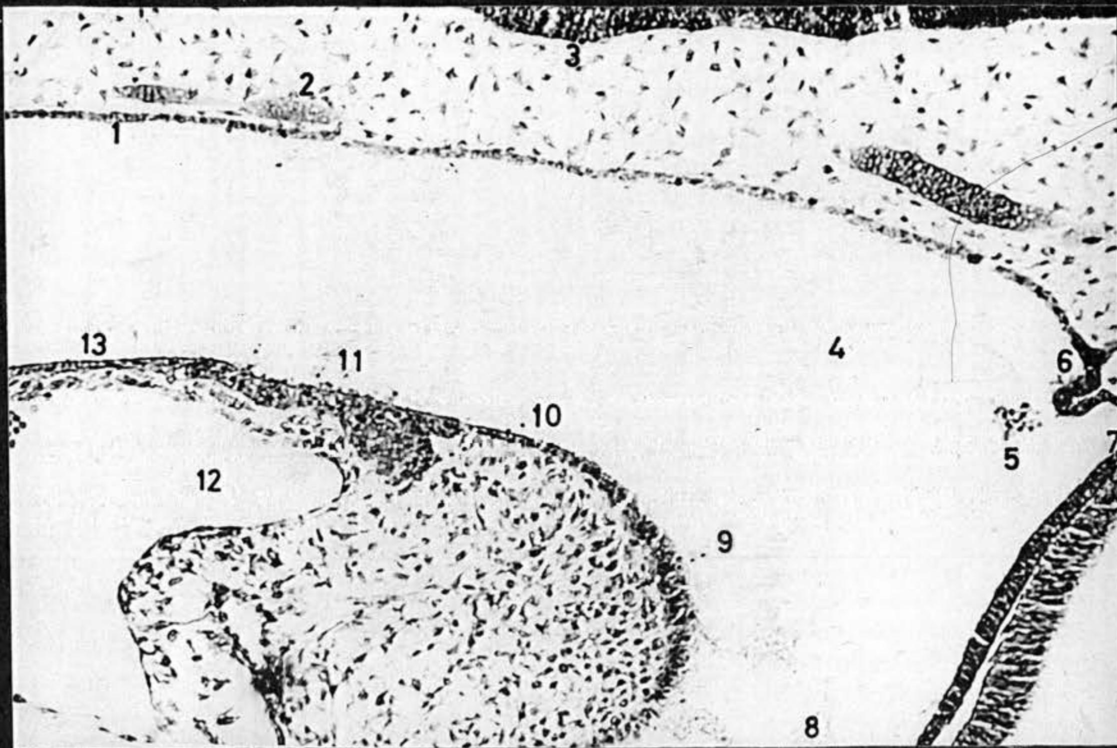


Fig. IV.37. Median section of anterior pharynx, embryo D 14 (4.5 mm. G.L.), (X 270).

1 Roof of the pharynx; 2 Notochord; 3 Floor of hindbrain; 4 Lumen of pharynx; 5 Fragment of oral membrane; 6 Seessel's pocket; 7 Rathke's pocket; 8 Mouth; 9 Ventral end of mandibular arch; 10 Beginning of cell cord formation; 11 Thyroid plate; 12 Aortic sac; 13 Floor of pharynx.



<u>Fig. IV.37</u>. Median section of anterior pharynx, embryo D 14 (4.5 mm. G.L.), (X 270).

1 Roof of the pharynx; 2 Notochord; 3 Floor of hindbrain; 4 Lumen of pharynx; 5 Fragment of oral membrane; 6 Seessel's pocket; 7 Rathke's pocket; 8 Mouth; 9 Ventral end of mandibular arch; 10 Beginning of cell cord formation; 11 Thyroid plate; 12 Aortic sac; 13 Floor of pharynx.

end of the laryngotracheal groove, in the dog this evagination is already paired before it is split off from the ventral surface of the foregut. In fact, the right lung bud may bulge from the lateral aspect of the flattened esophagus before there is any evidence of the left (Fig. IV.32./2).

The Structure of the Pharyngeal Wall

The roof of the pharynx is formed by a single layer of low epithelial cells on a basement membrane resting on loose mesenchymal tissue. The cell membranes on the free border of the epithelium appear thickened and form a continuous line. The nuclei are vesicular and oval and, filling almost the entire cells, lie rather crowdedly with their long axes parallel to the basement membrane. The epithelium of the floor is thicker, being composed of taller, almost columnar, cells in the posterior regions, and what appears to be two irregular layers of cells anteriorly where it covers the first visceral arches. In general, the cells are more crowded in the pharyngeal floor. The basement membrane is distinct and so is the free border of the cells. The mesenchyme underlying the floor is denser than that upon which the epithelium of the roof rests (Fig. IV.37.). The epithelium gradually thickens and becomes denser towards the lateral borders and into the pharyngeal pouches. The cells are stratified low columnar and the long axes of the nuclei stand at right angles to the basement membrane. The ectodermal and endodermal epithelial layers that oppose each other to constitute a branchial membrane lose their identities and seem to fuse in the greater portion of the sections. The cells have abandoned their original orientation and occupy the space between the two free borders which are on either side of the membrane, in very irregular fashion. Only occasionally can fragments of the former basement membranes be seen separating the two layers of cells.

The Relationships of the Pharynx

A wide cushion of loose mesenchyme has developed between the roof of the pharynx and the tubular hindbrain (Fig. IV.36.). The notochord is embedded in this cushion in the median plane and runs nearly parallel to the roof only a short distance from it (5). In embryo D 14 the notochord is in contact with the epithelium of the roof at three points, all lying at the level of the thyroid anlage; in embryo D 23 there are six such communications of which four are at the thyroid level and two are caudal to it; and in embryo D 15 there are four contacts at the thyroid level and only one more caudally. As a rule the pharyngeal epithelium is slightly thickened where contact with the notochord is made. According to Huber (1912) similar contacts occur in human embryos and are thought to be instrumental in the formation of the pharyngeal bursa. Whilst a pharyngeal bursa is not present in the dog, these contacts will be followed in this study until such time when their fate or possible function is established. Laterally, the roof is related to the two dorsal aortae which receive the first, second and third aortic arches at this stage (Fig. IV.35./7). On the lateral and ventral sides, the pharynx is bounded by the four visceral arches of which the first three contain fully developed aortic arch arteries. Below the centre of the floor is the aortic sac and behind it the pericardium is in contact with the pharynx (Fig. IV.36.).

In the 4 - 6 mm. stage the foregut has transformed into a typical embryonic pharynx, bearing a full complement of four pouches on either side. With the disappearance of the oral membrane and the formation of the mouth, the pharyngeal tube is open in front and in communication with the amniotic cavity. The dorsoventrally flattened pharynx is bent on itself in conformity with the flexures of the overlying neural tube at this time. The first pharyngeal derivative, the thyroid gland, has appeared in the centre of the pharyngeal floor, while caudal to it the laryngotracheal groove foreshadows the development of the postbranchial respiratory elements, two lung buds having already formed at its caudal extremity.

The following 6 - 8 mm. stage concerns itself mainly with the transformations of the pharyngeal pouches.

S T A G E 5

(6 - 8 mm. G.L.)

This stage is represented by four embryos all of the same litter as follows:

Embryo D 17	6.3 mm. G.L.	litter 5
Embryo D 1	8.5 mm. G.L.	
Embryo D 24	9.5 mm. G.L.	
Embryo D 25	-	

Embryo D 25 is not an entire embryo, consisting only of the head-heart segment; therefore, no measurements can be given. The unusually wide range of measurements for the members of this litter is due, unfortunately, to rough handling before they were received by the author. Had the normal body flexure of embryo D 24, for example, not accidentally been uncurled, its length would certainly lie within the 6 - 8 mm. range assigned to this group.

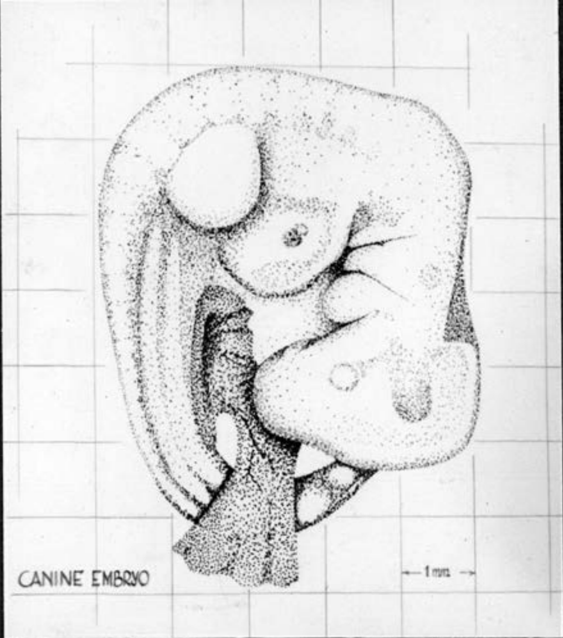


Fig. IV.38. Canine embryo D 17 (6.3 mm. G.L.).

Number of embryo:	D 17	Date of processing:	October 6, 1960
Size before fixation:	-	Block:	Paraffin
Size after fixation:	6.3 mm. G.L.	Stain:	H. & E.
Age:	-	Plane of section:	Sagittal
Position on uterus:	-	Thickness of section:	10 microns
Size of loculus:	-	Series made:	D 17 (1 - 11)
Fixed in:	10% Formalin		
Stored in:	10% Formalin		

From litter:		5
Number of embryos in litter:		4
DAM	Breed:	-
	Age:	-
	Weight:	-
SIRE	Breed:	-
	Age:	-
	Weight:	-
Number of services:		2
Date of 1st service:		-
Date of 2nd service:		-
Date of collection:		-

At time of collection Dam was alive ( ), dead (x).

Embryos collected 2 hour(s) after death.

Litter received from: Edinburgh Clinic

Remarks:

Fig. IV.39. Canine embryo D 1 (8.5 mm. G.L.).

Number of embryo:	D 1	Date of processing:	June 13, 1960
Size before fixation:	-	Block:	Paraffin
Size after fixation:	8.5 mm. G.L.	Stain:	H. & E.
Age:	-	Plane of section:	Horizontal
Position on uterus:	-	Thickness of section:	10 microns
Size of loculus:	-	Series made:	D 1 (1 - 14)
Fixed in:	10% Formalin		
Stored in:	10% Formalin		

From litter:		5
Number of embryos in litter:		4
DAM	Breed:	-
	Age:	-
	Weight:	-
SIRE	Breed:	-
	Age:	-
	Weight:	-
Number of services:		2
Date of 1st service:		-
Date of 2nd service:		-
Date of collection:		-

At time of collection Dam was alive ( ), dead (x).

Embryos collected 2 hour(s) after death.

Litter received from: Edinburgh Clinic

Remarks:

Fig. IV.40. Canine embryo D 24 (9.5 mm. G.L.) mutilated during collection.

Number of embryo:	D 24	Date of processing:	February 2, 1961
Size before fixation:	-	Block:	Paraffin
Size after fixation:	9.5 mm. G.L.	Stain:	H. & E.
Age:	-	Plane of section:	Transverse
Position on uterus:	-	Thickness of section:	10 microns
Size of loculus:	-	Series made:	D 24 (1 - 24)
Fixed in:	10% Formalin		
Stored in:	10% Formalin		

From litter:		5
Number of embryos in litter:		4
DAM	Breed:	-
	Age:	-
	Weight:	-
SIRE	Breed:	-
	Age:	-
	Weight:	-
Number of services:		2
Date of 1st service:		-
Date of 2nd service:		-
Date of collection:		-

At time of collection Dam was alive ( ), dead (X).

Embryos collected 2 hour(s) after death.

Litter received from: Edinburgh Clinic

Remarks:

Fig. IV.41. Head-heart segment of canine embryo D 25.

Number of embryo:	**D 25**	Date of processing:	**February 2, 1961**
Size before fixation:	-	Block:	**Paraffin**
Size after fixation:	-	Stain:	**H. & E.**
Age:	-	Plane of section:	**Sagittal**
Position on uterus:	-	Thickness of section:	**10 microns**
Size of loculus:	-	Series made:	**D 25 (1 - 6)**
Fixed in:	**10% Formalin**		
Stored in:	**10% Formalin**		

From litter: 5

Number of embryos in litter: 4

<u>DAM</u> Breed: -

Age: -

Weight: -

<u>SIRE</u> Breed: -

Age: -

Weight: -

Number of services: 2

Date of 1st service: -

Date of 2nd service: -

Date of collection: -

At time of collection Dam was alive ( ), dead (x).

Embryos collected 2 hour(s) after death.

Litter received from: **Edinburgh Clinic**

Remarks:

The Shape of the Pharynx

Not much change has taken place in the configuration of the pharynx. It is still a dorsoventrally flattened, funnel-shaped cavity with a wide, ventrally directed mouth and a tapering posterior portion which continues as the downward pointing trachea and esophagus (Fig. IV.42.). The long axis, arching from Rathke's pocket to the entrance into the esophagus, is approximately 1.8 mm. long, whereas the transverse measurements have increased to 0.9 mm. at pouch I and 0.4 mm. at pouch IV (Fig. IV.1./1, 2, 3).

The floor of the pharynx when viewed from below has the appearance of a triangle of which the base is anterior and represented by the mouth, and the apex posterior and formed by the entrance into the trachea. In contrast to the previous stage, the floor now extends beyond the level of the cervical flexure of the pharynx, and can therefore be divided into a horizontal and a vertical portion, the former running from the cephalic to the cervical flexure and the latter proceeding ventrocaudally from the cervical flexure to where the now well established trachea commences (Figs. IV.43./6, 12; IV.1.). On the vertical portion of the floor, the median longitudinal ridge, which is the external manifestation of the laryngotracheal groove, has grown much longer. It starts gradually opposite the cervical angle of the pharynx and extends caudoventrally for about 500 microns until it widens to be continued as

Fig. IV.42. Wax model of the left half of the pharynx, embryo D 1 (8.5 mm. G.L.), ventral view, (model X 100).

P1 - P4 Pharyngeal pouches I - IV; 1 Rathke's pocket; 2 Mouth; 3 Median indentation of pharyngeal floor; 4 Tuberculum impar; 5 Thyroid; 6 Copula; 7 Laryngotracheal groove; 8 Trachea; 9 Esophagus; 10, 11 Ridges connecting the diverticula of pouches I and II with pharyngeal floor; 12 Visceral cleft II; 13 Anlage of parathyroid III; 14 Appendicular portion of pouch IV (ultimobranchial body); 15 Horizontal portion of floor; 16 Vertical portion of floor.

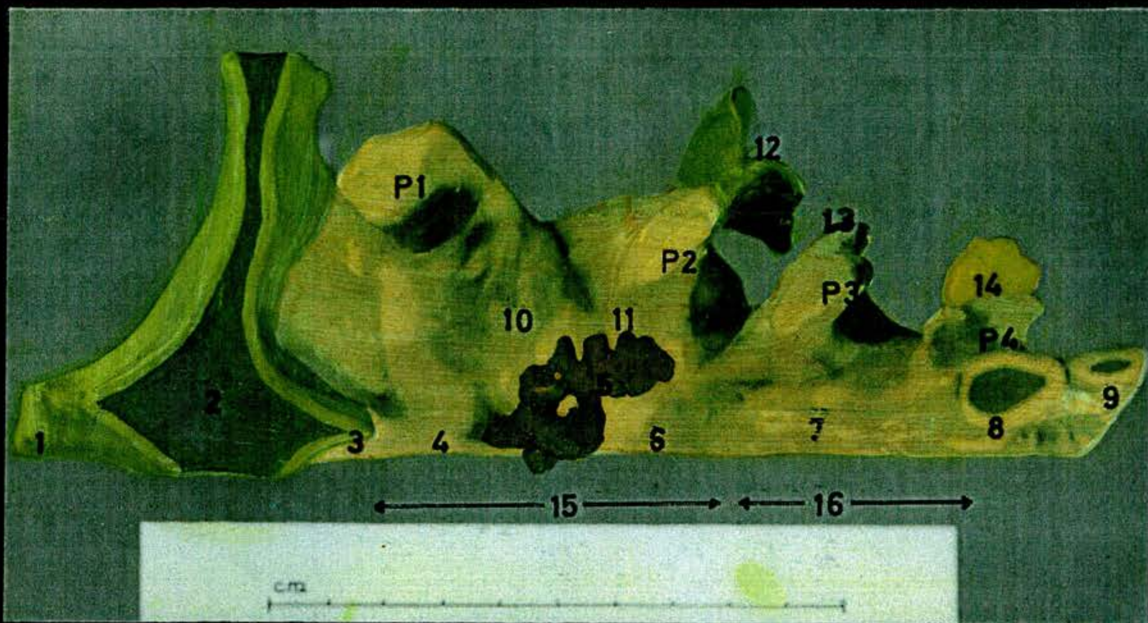


Fig. IV.42. Wax model of the left half of the pharynx, embryo D 1 (8.5 mm. G.L.), ventral view, (model X 100).

P1 - P4 Pharyngeal pouches I - IV; 1 Rathke's pocket; 2 Mouth; 3 Median indentation of pharyngeal floor; 4 Tuberculum impar; 5 Thyroid; 6 Copula; 7 Laryngotracheal groove; 8 Trachea; 9 Esophagus; 10, 11 Ridges connecting the diverticula of pouches I and II with pharyngeal floor; 12 Visceral cleft II; 13 Anlage of parathyroid III; 14 Appendicular portion of pouch IV (ultimobranchial body); 15 Horizontal portion of floor; 16 Vertical portion of floor.

Fig. IV.43. Wax model of the left half of the pharynx, embryo D 1 (8.5 mm. G.L.), lateral view, (model X 100).

P1 - P4 Pharyngeal pouches I - IV; 1 Rathke's pocket; 2 Seessel's pocket; 3 Lateral edge of primitive oral cavity; 4 Thyroid; 5 Cephalic flexure; 6 Horizontal portion of pharynx; 7 Cervical flexure; 8 Pharyngeal recess; 9 Position of rupture in pharyngeal membrane II; 10 Anlage of parathyroid III; 11 Visceral cleft II; 12 Vertical portion of pharynx; 13 Appendicular portion of pouch IV (ultimobranchial body); 14 Trachea; 15 Esophagus.

Fig. IV.43. Wax model of the left half of the pharynx, embryo D 1 (8.5 mm. G.L.), lateral view, (model X 100).

P1 - P4 Pharyngeal pouches I - IV; 1 Rathke's pocket; 2 Seessel's pocket; 3 Lateral edge of primitive oral cavity; 4 Thyroid; 5 Cephalic flexure; 6 Horizontal portion of pharynx; 7 Cervical flexure; 8 Pharyngeal recess; 9 Position of rupture in pharyngeal membrane II; 10 Anlage of parathyroid III; 11 Visceral cleft II; 12 Vertical portion of pharynx; 13 Appendicular portion of pouch IV (ultimobranchial body); 14 Trachea; 15 Esophagus.

the anterior aspect of the trachea (Fig. IV.42./7). It marks the future position of the larynx. The trachea is well formed in this stage and extends into the body of the embryo to end by separating into two primary lung buds. These have not subdivided and bear distal club-shaped thickenings of which the right one is larger. On either side of the laryngotracheal groove, longitudinal ridges are present which are similar to each other. They originate also opposite the cervical flexure but take a more lateral course towards the ventral diverticula of the third pouches. Their length was found to be about 300 microns. Identical ridges, but on a smaller scale, appear farther caudad opposite the caudal end of the laryngotracheal groove. They terminate in the poorly developed ventral diverticula of the fourth pouches.

The much enlarged thyroid occupies the centre of the horizontal portion of the floor (Fig. IV.42./5). Much of its bulk is rapidly growing away from the pharynx in a ventrocaudal direction (Fig. IV.43./4). The original thyroid plate has become smaller and constitutes now a narrow oval (50 microns wide and from 80 - 140 microns long), lying with its long axis in the median plane. From it arise four finger-like cell cords (embryo D 25 and D 24). One of the cords is very well developed and the other three are small in embryo D 25 (Fig. IV.44.), whereas in embryo D 24 three long and one short cell cord have formed. Embryo D 1 is somewhat farther advanced in

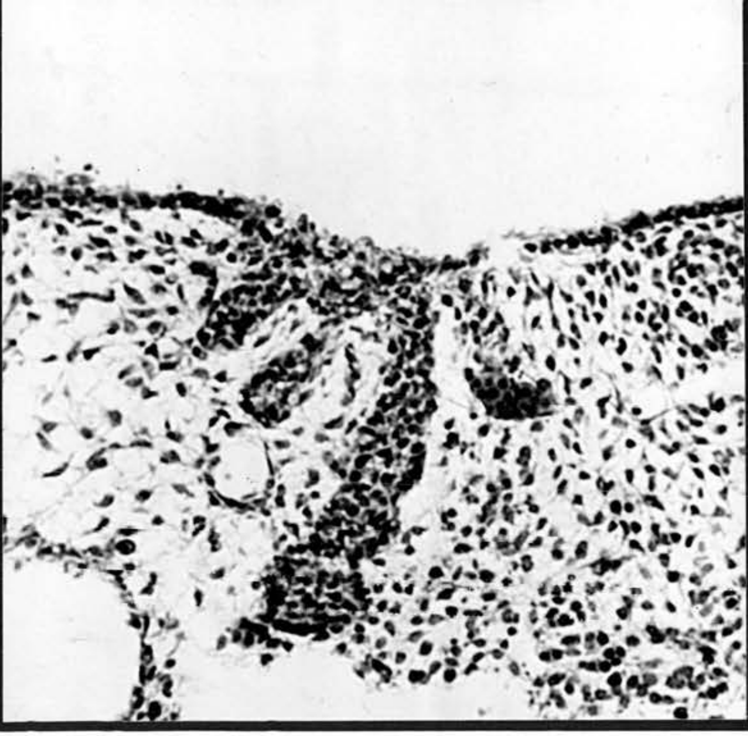


Fig. IV.44. Paramedian section of thyroid, embryo D 25, (X 380).

1 Lumen of pharynx; 2 Thyroid plate; 3 Cell cords emanating from plate; 4 Left aortic arch II; 5 Aortic sac.

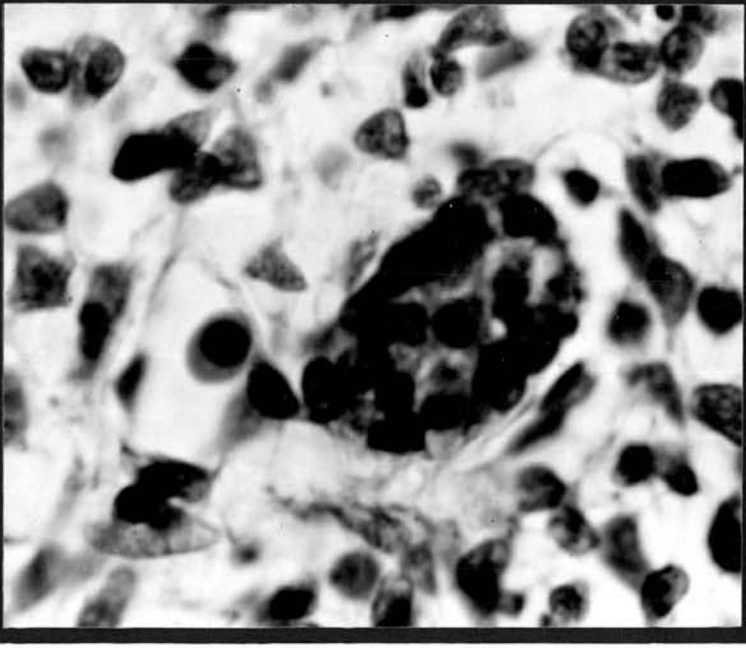


Fig. IV.45. Transverse section of thyroglossal stalk, embryo D 1 (8.5 mm. G.L.), (X 1500).

1 Thyroglossal stalk; 2 Capillary.

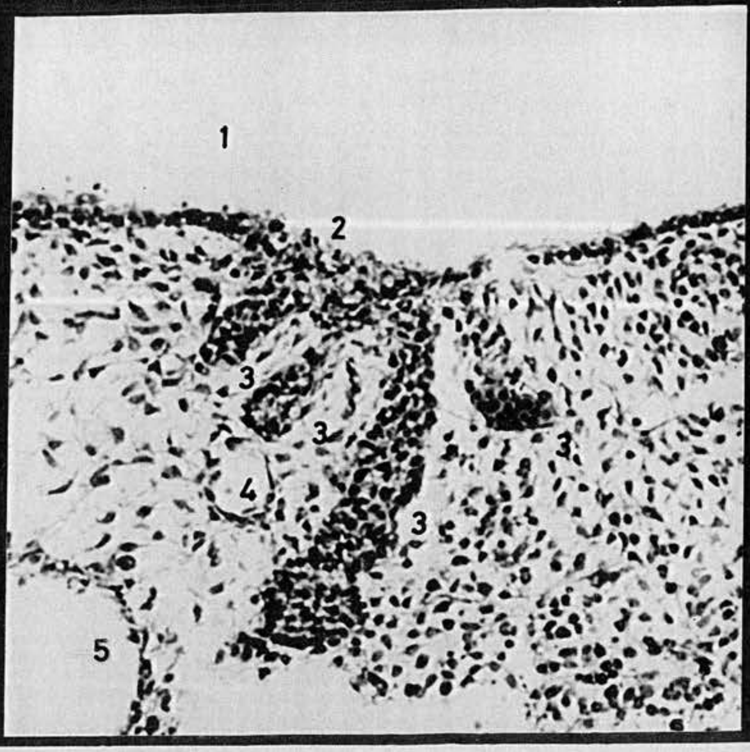


Fig. IV.44. Paramedian section of thyroid, embryo D 25, (X 380).

1 Lumen of pharynx; 2 Thyroid plate; 3 Cell cords emanating from plate; 4 Left aortic arch II; 5 Aortic sac.

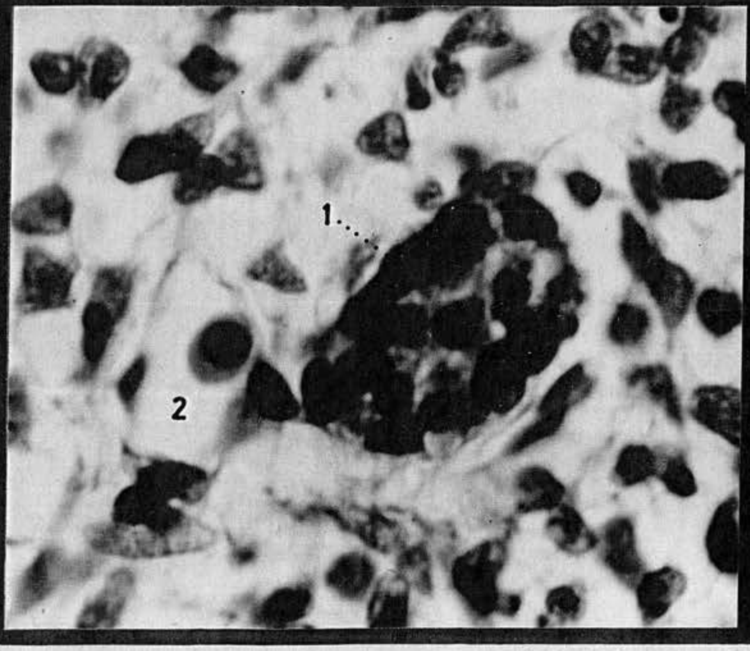


Fig. IV.45. Transverse section of thyroglossal stalk, embryo D 1 (8.5 mm. G.L.), (X 1500).

1 Thyroglossal stalk; 2 Capillary.

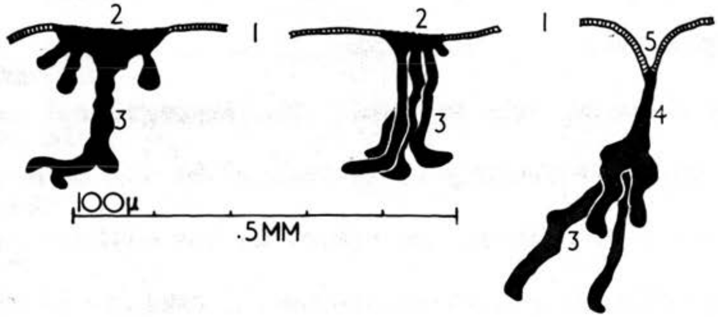


Fig. IV.46. Median sections of thyroids of embryos D 25, D 24 (9.5 mm. G.L.) and D 1 (8.5 mm. G.L.) (from left to right), (for thyroid of embryo D 17 see Fig. IV.49.).

1 Floor of pharynx; 2 Thyroid plate; 3 Cell cords; 4 Thyroglossal stalk; 5 Transitory foramen cecum linguae and lingual duct.

this region, with the result that most of the glandular tissue has moved away from the pharynx, leaving only a short stalk, the thyroglossal duct of the human, in connection with the floor (Fig. IV.46.). There is no thyroid plate in this embryo. The thyroglossal stalk unites with the pharyngeal epithelium in the same manner as the stem of a funnel is joined to the conical part. The infundibular recess on the dorsal surface of the floor and opposite the stalk could be regarded as a transitory Foramen cecum linguae and lingual duct (5). The thyroglossal stalk is narrowest proximately, having a diameter there of 20 microns, and widens gradually to a diameter of 28 microns. In embryo D 1 its length is about 70 microns. The stalk, on cross section, is slightly oval and consists of a number of faintly staining cuboidal cells arranged radially around a potential lumen which at some levels is occupied by a few irregular cells. No patent lumen could be detected (Fig. IV.45.). In the cell cords of the thyroid the cells appear similarly arranged, they are, however, much more closely packed.

From the pharyngeal attachment of the thyroid two rather blunt ridges proceed laterally and slightly cranially to end in the shallow diverticula of the first pharyngeal pouches (Fig. IV.42./10). A short distance caudal to this, another pair of short ridges connect the greatly enlarged ventral diverticula of the second pouches with the floor of the pharynx (11). The direction of the latter ridges is transversely and somewhat ventrally. The medial extremities of the latter pair do not reach the midline. In front of the thyroid attachment there is a small rounded depression in the epithelium which appears as the Tuberculum impar on the inside of the pharyngeal cavity (4) (Fig. IV.49./9). Immediately behind the thyroid attachment is a similar, but larger, depression which bulges into the pharyngeal cavity and is known as the Copula (Figs. IV.42./6; IV.49./10). These two structures contribute to the tissues of the adult tongue and will therefore receive only cursory mention in this study. Anteriorly, the floor bends sharply downward to form the caudal boundary of the mouth, being again markedly indented in the median plane (Fig. IV.42./3).

The roof of the pharynx, conforming to the triangular shape of the floor, can also be divided into a horizontal and a vertical portion. At the line of division between the two areas is the now more pronounced pharyngeal recess, a caudally directed bulge in the epithelium (Fig. IV.43./8). This bulge causes the cervical flexure to appear much more marked in the roof than in the floor. In embryo D 1 the distance between the vertex of the pharyngeal recess and the entrance into the esophagus is 700 microns (Fig. IV.1./4). Corresponding to the cephalic flexure of the embryo, the horizontal portion of the roof turns downwards anteriorly to contribute to the formation of the mouth. In the midline of this area, Seessel's pocket can still be identified as a small rounded protuberance pointing craniodorsally (Fig. IV.43./2). Anterior to it is Rathke's pocket. It has become a quadrilateral evagination of the epithelium in the midline (1). Between Seessel's and Rathke's pockets the epithelium changes from endoderm to ectoderm without visible demarcation as was the case in the previous stage. In its horizontal part, the roof is nearly straight from front to back and only slightly convex from side to side. Proceeding ventrally from the cervical flexure, the vertical portion of the roof is at first concave and later convex when viewed from behind, after which it continues as the caudal aspect of the esophagus. Laterally, the roof is convex and relatively narrow, especially between the second and third pouches.

The pouches on the lateral border of the pharynx have all increased in size and dominate the appearance of this region much more than in the preceding stage. All pouches, with the exception of the first, have enlarged and elongated in a ventral direction, giving the impression of hanging from the edge of the pharynx (Fig. IV.43.).

Pouch I has also taken part in the general rapid enlargement, but in a dorsal direction, so that its dorsal wing projects now well above the roof of the pharynx. Its ventral wing, however, has retained the same size as was observed in the previous stage, a small diverticulum having formed on it (Pl); in embryo D 24 the ventral wings have completely disappeared (Fig. IV.50.). Pouch I has preserved its flattened shape, and the direction of its plane is similar to what was seen before. The area of contact with the ectoderm is approximately 450 microns long and very narrow, extending the entire length of the sharp lateral border of the pouch, except for a short distance below.

Pouch II has become a very flat appendage with a well developed ventral diverticulum, but still protruding above the roof a fair amount. The direction of its plane is vertical and about midway between transverse and sagittal. Similar to pouch I, the area of contact with the ectoderm of the second visceral cleft is long and narrow. It extends for about 450 microns along the lateral border of the pouch, leaving a diverticulum ventrally, but reaching almost to the apex of the dorsal wing (Fig. IV.43./P2). In all of the embryos of this stage the second pharyngeal membrane is ruptured at its lower extremity, creating a slit-like opening, 60 microns in length in embryo D 1, through which the pharynx and the amniotic cavity communicate (9) (Fig. IV.47./5, 6).

Pouch III is very similar to pouch II both in outline and direction, with the exception that it is only half its size. It is attached to the lateral border of the pharynx immediately behind the level of the cervical flexure. The dorsal diverticulum, although present, is

Fig. IV.47. Horizontal section of pharynx, embryo D 1 (8.5 mm. G.L.), (X 180). All structures labelled are on the left side.

1 Mandibular arch (I); 2 Visceral cleft I; 3 Hyoid arch (II); 4 Second aortic arch artery; 5 Pharyngeal pouch II; 6 Visceral cleft II showing rupture of pharyngeal membrane II; 7 Visceral cleft III; 8 Aortic arch artery III contained in third visceral arch; 9 Lumen of pharynx; 10 Foramen cecum.

Fig. IV.47. Horizontal section of pharynx, embryo D 1 (8.5 mm. G.L.), (X 180). All structures labelled are on the left side.

1 Mandibular arch (I); 2 Visceral cleft I; 3 Hyoid arch (II); 4 Second aortic arch artery; 5 Pharyngeal pouch II; 6 Visceral cleft II showing rupture of pharyngeal membrane II; 7 Visceral cleft III; 8 Aortic arch artery III contained in third visceral arch; 9 Lumen of pharynx; 10 Foramen cecum.

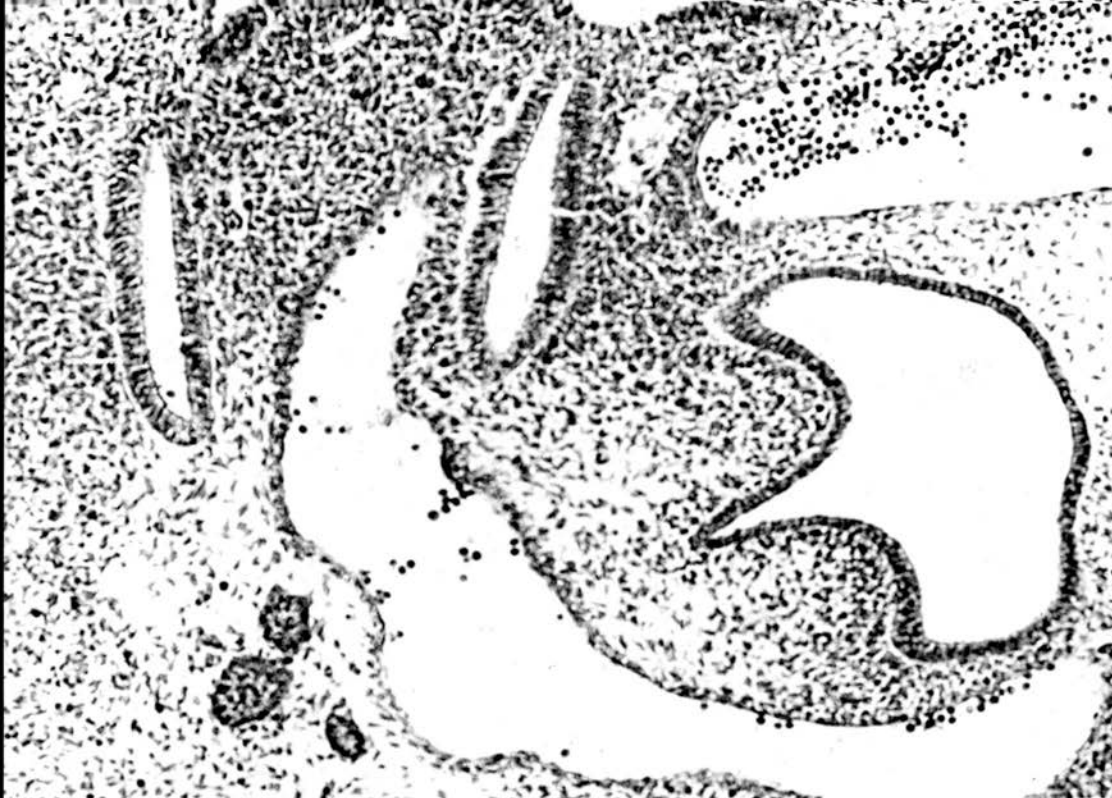


Fig. IV.48. Horizontal section of pharynx, embryo D 1 (8.5 mm. G.L.), (X 180). All structures labelled are on the right side.

1 Ventral diverticulum of pouch II; 2 Visceral cleft II; 3 Visceral cleft III; 4 Anlage of parathyroid III; 5 Ventral wing of pouch III; 6 Aortic sac; 7 Thyroid cords in cross section; 8 Laryngotracheal groove; 9 Vertical portion of pharynx in cross section.

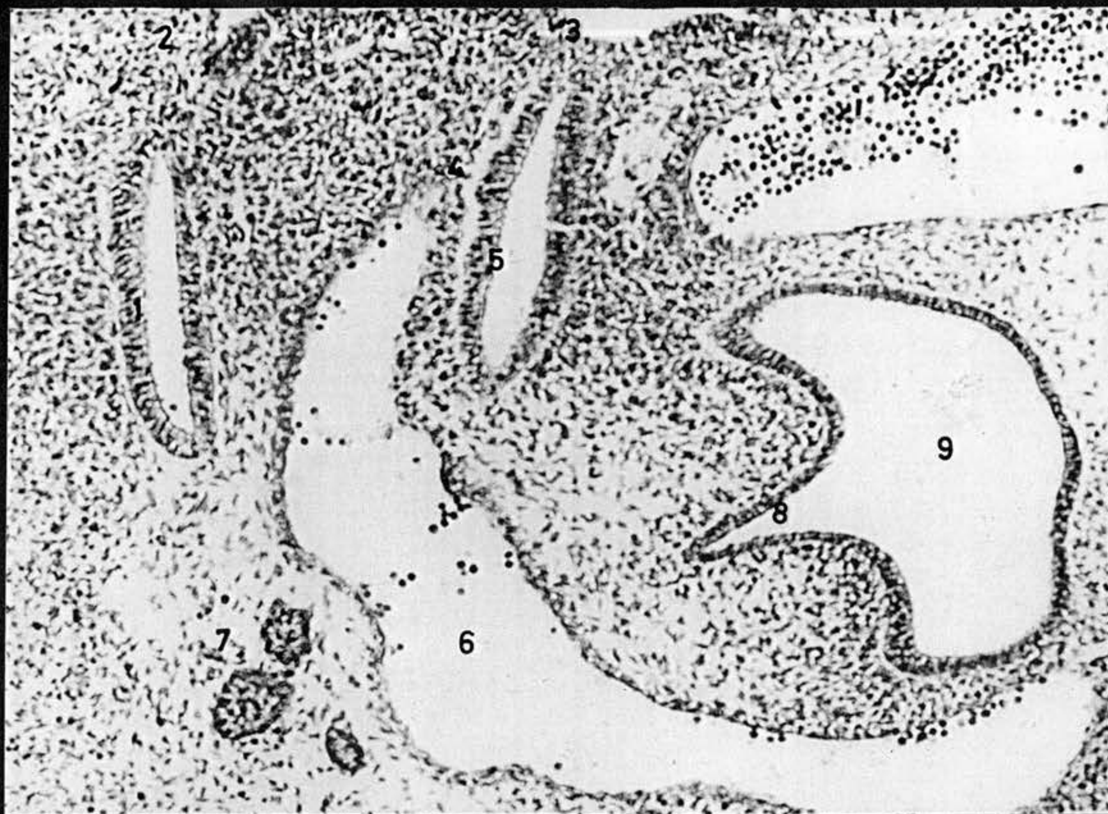


Fig. IV.48. Horizontal section of pharynx, embryo D 1 (8.5 mm. G.L.), (X 180). All structures labelled are on the right side.

1 Ventral diverticulum of pouch II; 2 Visceral cleft II; 3 Visceral cleft III; 4 Anlage of parathyroid III; 5 Ventral wing of pouch III; 6 Aortic sac; 7 Thyroid cords in cross section; 8 Laryngotracheal groove; 9 Vertical portion of pharynx in cross section.

insignificant, while the ventral diverticulum is well developed and makes up about one half of the bulk of the entire pouch. The lateral border of the pouch contacts the ectoderm at two places, first for about 100 microns in the area of junction between the upper and middle thirds of the lateral border, and secondly for a distance of about 30 microns towards the ventral end of the border (Fig. IV.43./P3). Immediately anterior to the longer ectodermal contact is a thickened area on the anterior face of the pouch. This is the anlage of parathyroid III (10) (Fig. IV.48./4). No other cell growth or differentiation could be detected on this pouch.

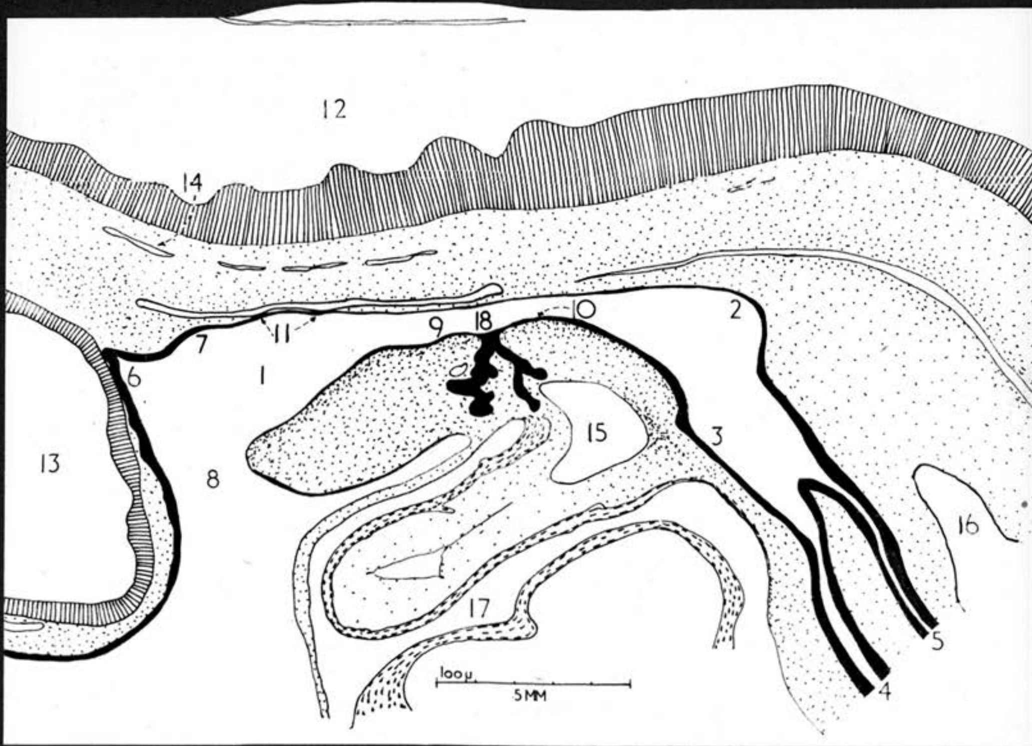
Pouch IV is the smallest of the pharyngeal pouches. It is roughly rectangular in outline and flat, and is attached to the pharynx by one of its smaller sides (Fig. IV.43./P4). The direction of its long axis is ventral and slightly lateral and that of its transverse axis is posterior and slightly lateral. Pouch IV is dissimilar to the other three pouches in that it seems to consist of two parts. The more proximal portion is the pouch proper, carrying both small dorsal and ventral wings, of which the former makes brief contact with the ectoderm. Attached to this portion by a slight constriction is a larger appendicular part. This extends ventrally into the embryo, running almost parallel to esophagus and trachea. Its ventral extremity is in close proximity to the epicardium. The appendicular part is the ultimobranchial body (13).

The Structure of the Pharyngeal Wall

The horizontal portion of the roof is composed of a single layer of low cuboidal cells, resting on a cushion of loose, undifferentiated mesenchyme. The round to oval nuclei almost fill the entire cells and due to an increase in the amount of karyoplasm do not appear as vesicular as in the preceding stages. Below the pharyngeal recess the roof of the vertical portion of the pharynx thickens and resembles the make-up of the floor in this region. The epithelium of the floor is generally thicker and denser than that of the roof and overlies relatively dense mesenchyme. In the anterior portion where the epithelium covers the mandibular arches it consists of three to four irregular layers of flattened cells, rather similar to stratified squamous epithelium. In the caudal portions of the floor and lining the pharyngeal pouches, the epithelium consists of a single layer of tall cells, containing oval nuclei whose long axes stand at right angles to the well formed basement membrane. The latter disintegrates however at the pharyngeal membranes and both endoderm and ectoderm oppose each other without demarcation.

The Relationships of the Pharynx

The mesenchymal cushion between the horizontal portion of the pharynx and the hindbrain has increased in



<u>Fig. IV.49</u>. Median section of pharyngeal region, embryo D 17 (6.3 mm. G.L.), (semischematic).

1 Lumen of pharynx; 2 Pharyngeal recess; 3 Laryngotracheal groove; 4 Trachea; 5 Esophagus; 6 Rathke's pocket; 7 Seessel's pocket; 8 Mouth; 9 Tuberculum impar; 10 Copula; 11 Contacts between notochord and pharyngeal roof; 12 Hindbrain; 13 Forebrain; 14 Basilar artery; 15 Aortic sac; 16 Dorsal aorta; 17 Heart, 18 Thyroid.

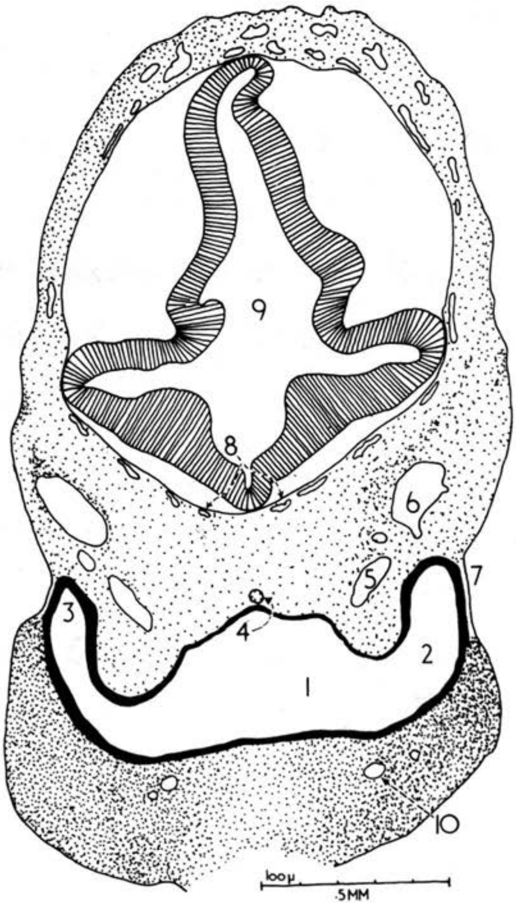


Fig. IV.50. Transverse section of the head at the level of pouch I, embryo D 24 (9.5 mm. G.L.), (semischematic).

1 Pharynx; 2 Pouch I; 3 Dorsal wing of pouch I; 4 Notochord; 5 Dorsal aorta; 6 Anterior cardinal vein; 7 Pharyngeal membrane I; 8 Paired basilar arteries; 9 Hindbrain; 10 Remnant of aortic arch artery I.

thickness and grown somewhat denser. The notochord is embedded in this cushion and runs parallel and in close relation to the roof(Fig. IV.49.). It unites twice with the pharyngeal epithelium cranial to the thyroid level in embryo D 17 and D 1, and only once in embryo D 24. In embryo D 25, in addition to one cranial contact there is also one union behind the thyroid. Further dorsal and in close proximity to the myelencephalon are the still paired basilar arteries which, when followed anteriorly, join the dorsal aortae under the metencephalon(Fig.IV.50/8). More laterally, the roof of the pharynx is related to the dorsal aortae(5) which receive the second, third, fourth and pulmonary arches along their pharyngeal course. The anterior cardinal veins(6) are situated just dorsally and laterally to these arteries. A mass of very loose mesenchyme overlies the vertical portion of the pharyngeal roof and appears wedged between it and the hindbrain dorsally, and the dorsal aortae and nodose ganglia on either side (Fig. IV.50.).

The mesenchymal bars of the visceral arches, of which the first and second are the most prominent at this stage, enclose the pharynx laterally and ventrally. The mandibular nerve in the first arch is fairly well developed but reaches ventrally only to the level of the pharyngeal floor. It is accompanied by a meshwork of fine blood vessels, which, when followed in serial sections, still forms a patent connection between the dorsal aorta and the ventral portion of the second aortic arch. The second visceral arch harbours a fully developed, though small, second arch artery and anterior to it the short facial nerve, the latter barely reaching the level of the pharyngeal roof. Branching of this nerve could not be observed in this group of embryos. The third visceral arch is much smaller as it passes the lateral pharyngeal border and contains the larger third arch artery. The glossopharyngeal nerve issuing from the petrosal ganglion is still very short and projects into the third visceral arch only to the level of the pharyngeal roof. The petrosal ganglion lies close to the dorsolateral edge of the pharynx between the second and the third pouches. Apart from the glossopharyngeal nerve, a few short nerve fibres leave its ventral end and turn towards the second pouch. They form the beginning of the tympanic nerve. The lateral relations of the vertical portion of the pharynx are the small fourth visceral arches with the large fourth arch arteries and the tip of the anterior laryngeal nerve coming from the nodose ganglion, and ventral to pouch IV an anteromedial thickening of the distal portion of the nodose ganglion which represents the posterior laryngeal (recurrent) nerve developing just below the pulmonary arch. The vagus nerve continues ventrally from the nodose ganglion and lies lateral and parallel to the esophagus and trachea.

The pharynx is bounded ventrally by the converging visceral arches in which their respective aortic arch arteries travel until they unite in the aortic sac. Caudal to the sac, the laterally flattened anlage of the larynx forms the ventral relation of the pharynx.

In the 6 - 8 mm. group the growth emphasis is laid upon appendicular development, while the body of the pharynx maintains its characteristic shape. Foremost among the changes is the down-growth of the pouches, giving the impression of their hanging from the lateral borders of the pharyngeal tube. The first pouch does not take part in this ventral movement; on the contrary, it is drawn upwards above the roof of the pharynx. The second branchial membrane was found to have ruptured in all specimens of this group, simulating, for a short period, conditions found in the gill-breathing lower vertebrates. The pharyngeal derivatives parathyroid III and the ultimo-branchial body have appeared, while the thyroid anlage is already moving away from the pharyngeal floor. The post-branchial respiratory elements have advanced also with the establishment of the trachea, the latter still sharing a common lumen with the esophagus at the level of the laryngotracheal groove.

STAGE 6

(7 - 9 mm. G.L.)

The observations for this stage were made on the following three embryos:

Embryo D 26	-	litter 5 a
Embryo D 27	-	
Embryo D 28	-	

Unfortunately, these specimens were mutilated when obtained, their trunks had been severed above the leg buds and the crown of embryo D 28 had also been detached; but in view of the scarcity of material it was decided to include them in the study, especially since the pharyngeal regions seemed to be remarkably well preserved. Thus, no measurements are recorded for these particular specimens. They would, in the author's opinion, have fallen into the 7 - 9 mm. range of this stage, had they not been injured.

Fig. IV.51. Canine embryo D 26, severed above hind leg buds during collection, (X 10).

Number of embryo:	D 26	Date of processing:	February 3, 1961
Size before fixation:	-	Block:	Paraffin
Size after fixation:	-	Stain:	H. & E.
Age:	-	Plane of section:	Sagittal
Position on uterus:	-	Thickness of section:	10 microns
Size of loculus:	-	Series made:	D 26 (1 - 13)
Fixed in:	10% Form./Bouin's Fluid		
Stored in:	70% Alcohol		

From litter:		5 a
Number of embryos in litter:		3
DAM	Breed:	X Terrier
	Age:	-
	Weight:	20 lb.
SIRE	Breed:	-
	Age:	-
	Weight:	-
Number of services:		-
Date of 1st service:		-
Date of 2nd service:		-

Date of collection: June 28, 1960

At time of collection Dam was alive (X), dead ( ).

Embryos collected hour(s) after death.

Litter received from: Ontario Veterinary College

Remarks:

Fig. IV.52. Canine embryo D 27, severed above hind leg buds, as in previous figure, (X 10).

Number of embryo:	D 27	Date of processing:	February 3, 1961
Size before fixation:	-	Block:	Paraffin
Size after fixation:	-	Stain:	H. & E.
Age:	-	Plane of section:	Transverse
Position on uterus:	-	Thickness of section:	10 microns
Size of loculus:	-	Series made:	D 27 (1 - 10)
Fixed in:	10% Form./Bouin's Fluid		
Stored in:	70% Alcohol		

From litter:		5 a
Number of embryos in litter:		3
DAM	Breed:	X Terrier
	Age:	-
	Weight:	20 lb.
SIRE	Breed:	-
	Age:	-
	Weight:	-
Number of services:		-
Date of 1st service:		-
Date of 2nd service:		-

Date of collection: June 28, 1960

At time of collection Dam was alive (x), dead ( ).

Embryos collected hour(s) after death.

Litter received from: Ontario Veterinary College

Remarks:

Fig. IV.53. Canine embryo D 28, severed during collection both anterior to hind leg buds and in the crown region, (X 10).

Number of embryo:	D 28	Date of processing:	February 3, 1961
Size before fixation:	-	Block:	Paraffin
Size after fixation:	-	Stain:	H. & E.
Age:	-	Plane of section:	Horizontal
Position on uterus:	-	Thickness of section:	10 microns
Size of loculus:	-	Series made:	D 28 (1 - 13)
Fixed in:	10% Form./Bouin's Fluid		
Stored in:	70% Alcohol		

From litter:		5 a
Number of embryos in litter:		3
DAM	Breed:	X Terrier
	Age:	-
	Weight:	20 lb.
SIRE	Breed:	-
	Age:	-
	Weight:	-
Number of services:		-
Date of 1st service:		-
Date of 2nd service:		-

Date of collection: June 28, 1960

At time of collection Dam was alive (X), dead ( ).

Embryos collected hour(s) after death.

Litter received from: Ontario Veterinary College

Remarks:

The Shape of the Pharynx

The pharynx has retained its flattened, triangular shape but has now become considerably wider in its horizontal portion (Fig. IV.54.). At the same time, the long axis of the pharynx, from Rathke's pocket to the commencement of the esophagus, has in fact decreased from 1.8 mm. in stage 4, to 1.6 mm. The reason for this can be found in an upward movement of the esophageal opening, caused by a disintegrative growth process that separates the trachea from the esophagus in an anterior and dorsal direction. These two structures had previously been observed to share a common lumen for some distance (Fig. IV.43./14, 15), but by this process of separation the original tube has been split; and this is responsible for the increase in length of esophagus and trachea against the general direction of growth in this area (Fig. IV.55./19, 20). The width of the pharynx, as measured at the base of pouch I, is 1.3 mm., nearly twice that of the previous stage. Between the third and fourth pouches it measures 0.5 mm.

Viewed from above, the horizontal portion of the roof is flat from before backwards and only slightly convex from side to side. Anteriorly, it makes the well recognised abrupt downward turn, to continue as the anterior boundary of the mouth. At this point Seessel's pocket is still distinguishable in the midline (Fig. IV.56./2). Immediately below it arises Rathke's

Fig. IV.54. Wax model of the left half of the pharynx with some of the major structures surrounding it, embryo D 26, (model X 100), ventral view.

P1, P2, P3, P4 Pharyngeal pouches I, II, III, IV; 1 Rathke's pocket; 2 Mouth; 3 Semilunar ganglion; 4 Stump of ophthalmic nerve; 5 Stump of maxillary nerve; 6 Mandibular nerve; 7 Ridge on ventral surface of pharyngeal floor, marking boundary between first and second visceral arches; 8 Chorda tympani nerve; 9 Facial nerve; 10 Second visceral cleft; 11 Rupture of pharyngeal membrane II; 12 Remnant of second arch artery, source of external carotid; 13, 14, 15 Aortic arch arteries III, IV and VI; 16 Cervical sinus; 17 Nodose ganglion; 18 Spinal accessory nerve; 19 Primordium of posterior laryngeal nerve (recurrent); 20 Aortic sac; 21 Thyroid; 22 Trachea; 23 Esophagus; 24 Vagus; 25 Left dorsal aorta.

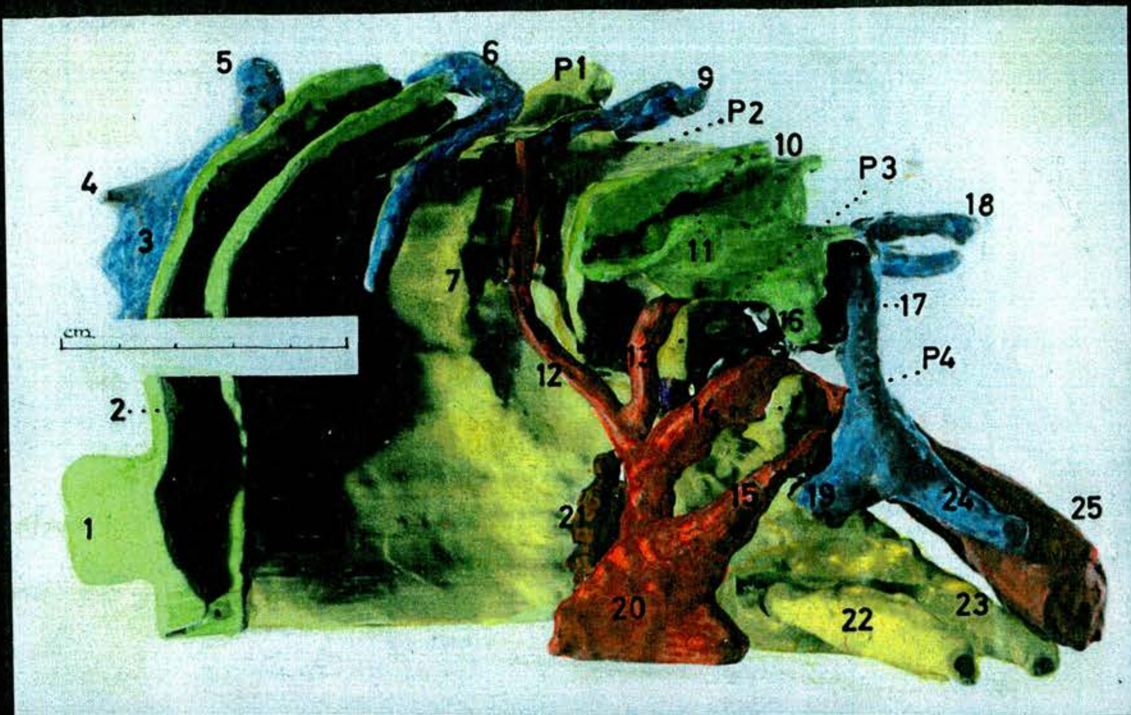


Fig. IV.54. Wax model of the left half of the pharynx with some of the major structures surrounding it, embryo D 26, (model X 100), ventral view.

P1, P2, P3, P4 Pharyngeal pouches I, II, III, IV; 1 Rathke's pocket; 2 Mouth; 3 Semilunar ganglion; 4 Stump of ophthalmic nerve; 5 Stump of maxillary nerve; 6 Mandibular nerve; 7 Ridge on ventral surface of pharyngeal floor, marking boundary between first and second visceral arches; 8 Chorda tympani nerve; 9 Facial nerve; 10 Second visceral cleft; 11 Rupture of pharyngeal membrane II; 12 Remnant of second arch artery, source of external carotid; 13, 14, 15 Aortic arch arteries III, IV and VI; 16 Cervical sinus; 17 Nodose ganglion; 18 Spinal accessory nerve; 19 Primordium of posterior laryngeal nerve (recurrent); 20 Aortic sac; 21 Thyroid; 22 Trachea; 23 Esophagus; 24 Vagus; 25 Left dorsal aorta.

Fig. IV.55. Wax model of the left half of the pharynx with some of the major structures surrounding it, embryo D 26, (model X 100), lateral view.

P1, P2 Pharyngeal pouches I, II; 1 Rathke's pocket; 2 Mouth; 3 Left dorsal aorta; 4 Semilunar ganglion; 5 Stump of ophthalmic nerve; 6 Stump of maxillary nerve; 7 Mandibular nerve; 8 Ganglia for facial and acoustic nerves; 9 Otic vesicle; 10 Jugular ganglion; 11 Facial nerve; 12 Chorda tympani nerve; 13 Remnant of aortic arch artery II; 14 Thyroid; 15 Aortic sac; 16 Cervical sinus; 17 Nodose ganglion; 18 Spinal accessory nerve; 19 Esophagus; 20 Trachea; 21 Vagus.

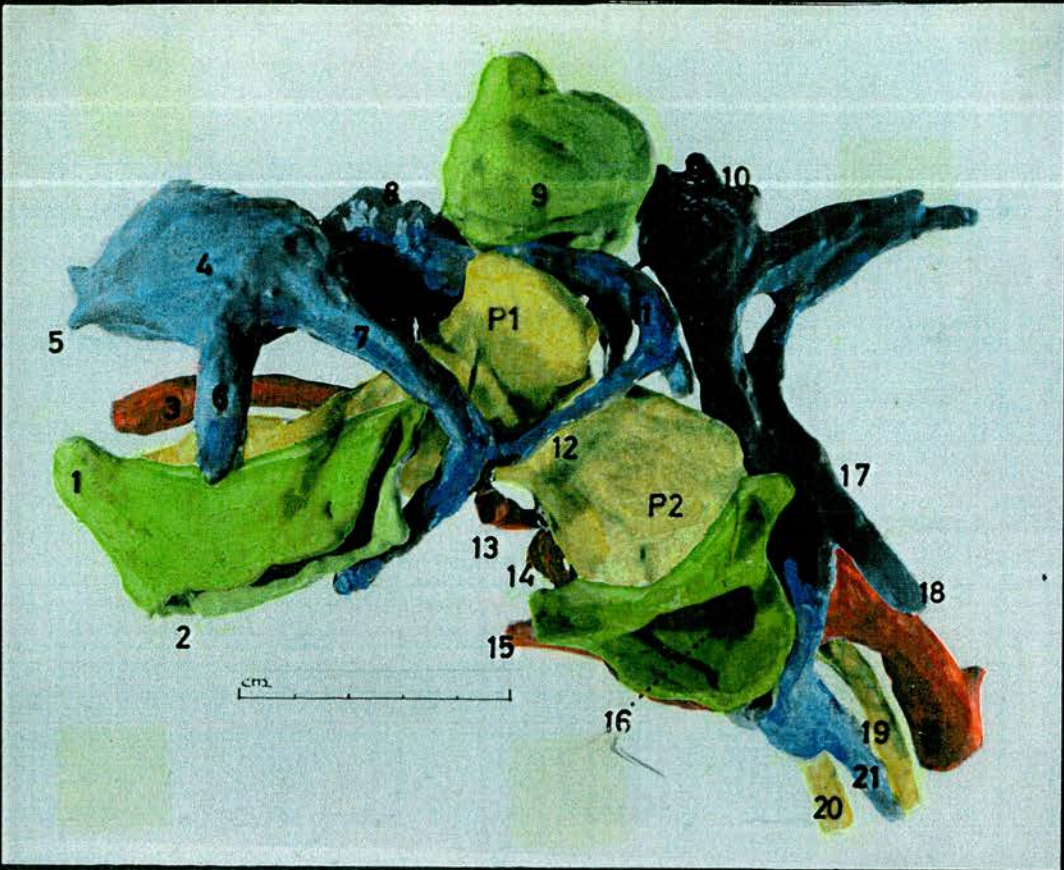


Fig. IV.55. Wax model of the left half of the pharynx with some of the major structures surrounding it, embryo D 26, (model X 100), lateral view.

P1, P2 Pharyngeal pouches I, II; 1 Rathke's pocket; 2 Mouth; 3 Left dorsal aorta; 4 Semilunar ganglion; 5 Stump of ophthalmic nerve; 6 Stump of maxillary nerve; 7 Mandibular nerve; 8 Ganglia for facial and acoustic nerves; 9 Otic vesicle; 10 Jugular ganglion; 11 Facial nerve; 12 Chorda tympani nerve; 13 Remnant of aortic arch artery II; 14 Thyroid; 15 Aortic sac; 16 Cervical sinus; 17 Nodose ganglion; 18 Spinal accessory nerve; 19 Esophagus; 20 Trachea; 21 Vagus.

pocket (3) (Figs. IV.54./1; IV.55./1). It has developed into a dorsoventrally flattened epithelial outgrowth, pointing anterodorsally towards the posterior wall of the diencephalon, with which it is in contact below the freshly formed shallow infundibulum (Fig. IV.56./11). Between Seessel's and Rathke's pockets the epithelium changes from endoderm to ectoderm. The pharyngeal recess at the cervical flexure is very prominent and rises about 200 microns above the general level of the pharynx in embryo D 26. It is directed caudally, its dorsal surface being the direct continuation of the flat horizontal portion of the roof (5). The gradual disappearance of the dorsal diverticula of either third pouch leaves the vertical portion of the roof flat throughout, though immediately below the pharyngeal recess a slight anteriorly directed concavity can be noted.

The floor at this stage represents a fairly even surface and is slightly concave both from before backwards and transversely when viewed from below. Two pairs of transverse ridges break the continuity of its horizontal portion however. The anterior pair extend medially and somewhat backwards from the now almost indistinguishable ventral diverticula of the first pouches and fade out after a length of about 500 microns (Figs. IV.54./7; IV.57./1). The remaining pair of ridges, springing from the ventral diverticula of the second pouches, lie in a transverse plane to blend into the floor after roughly

300 microns (2). The thyroid has lost all connection with the floor and, as can be observed from embryo D 26, leaves no trace of its previous origin and attachment on the pharyngeal epithelium. In the remaining two embryos a small infundibular evagination (Foramen cecum) persists for a short while at the point of earlier contact. Furthermore, in embryo D 27 the thyroglossal stalk remains attached to the ventral end of the evagination, which indicates that the separation must have occurred at that point where the stalk became united with the body of the gland. The cell cords of the thyroid parenchyma have combined to form an irregular cluster of plates and cords, which, as a body, envelope the anterior face of the aortic sac to a width of about 500 microns (Figs. IV.54./21; IV.55./14; IV.56./13; IV.58./5). Tuberculum impar in front and the copula behind the transitory Foramen cecum are less prominent in this stage. Opposite the cervical flexure of the pharynx, the ridges springing from the ventral diverticula of either third pouch are directed medially and slightly anteriorly. They are quite rudimentary and fade out at some distance from the midline. In the midline of the vertical portion of the floor, the laryngotracheal groove, which was previously recognised from the outside as a long sharp ridge, has widened and, in doing so, has become shorter and more blunt in the process of transition to form the larynx. At the same time, the trachea has become well separated in this stage

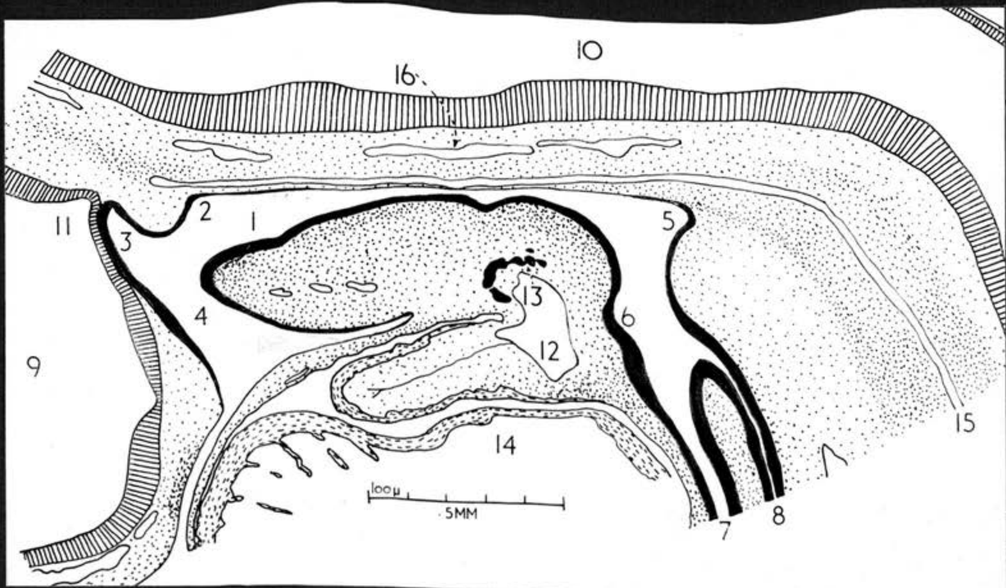


Fig. IV.56. Median section of pharyngeal region, embryo D 26, (semischematic).

1 Lumen of pharynx; 2 Seessel's pocket; 3 Rathke's pocket; 4 Mouth; 5 Pharyngeal recess; 6 Laryngotracheal groove; 7 Trachea; 8 Esophagus; 9 Forebrain; 10 Hindbrain; 11 Infundibulum; 12 Aortic sac; 13 Thyroid; 14 Heart; 15 Notochord; 16 Basilar artery.

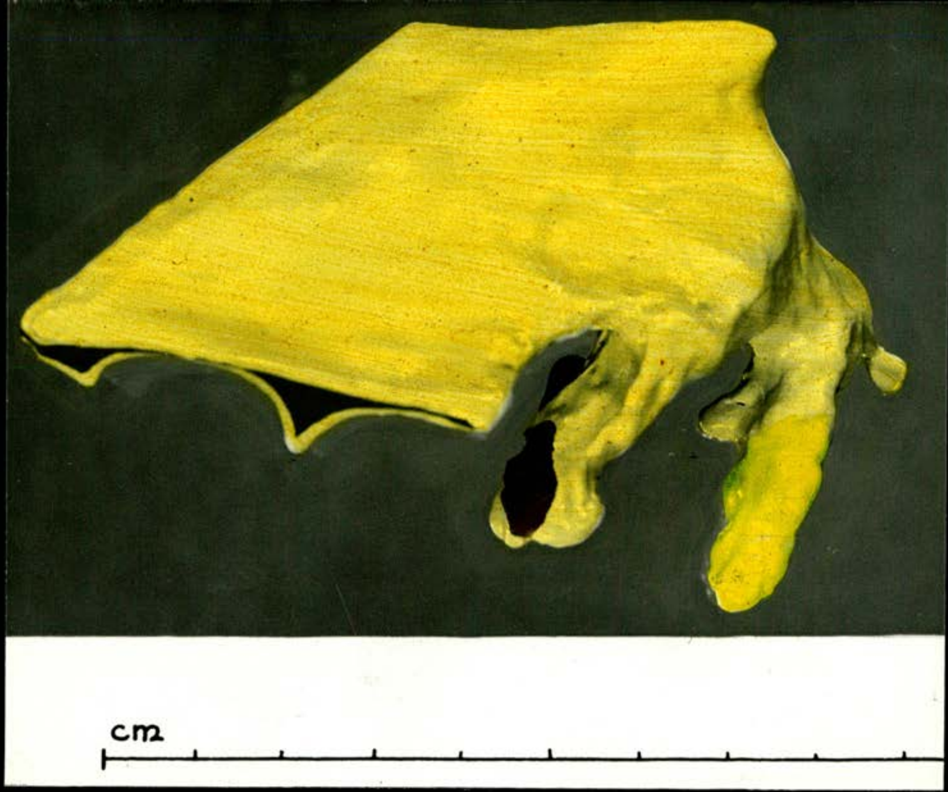
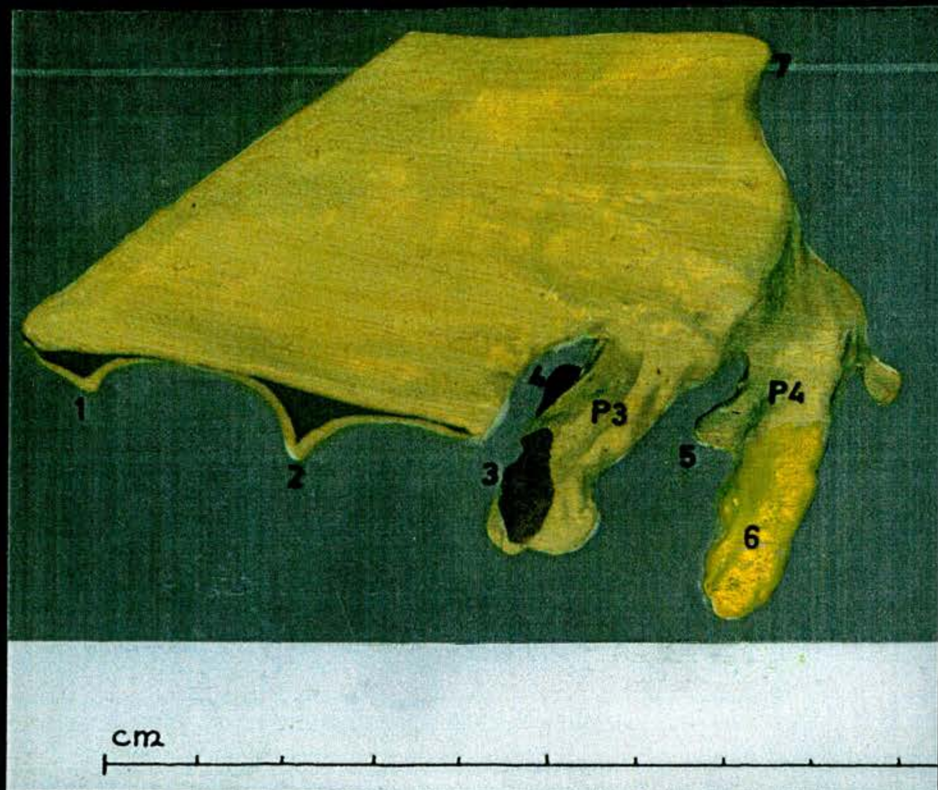


Fig. IV.57. Wax model of the left third and fourth pharyngeal pouches, embryo D 26, dorsolateral view.

P3, P4 Pharyngeal pouches III, IV; 1 Ridge on ventral surface of pharyngeal floor marking boundary between mandibular and hyoid arches; 2 Similar ridge marking boundary between hyoid and third visceral arches; 3 Parathyroid III, 4 Anlage of thymus; 5 Ventral diverticulum of pouch IV; 6 Ultimobranchial body; 7 Part of pharyngeal recess.



<u>Fig. IV.57</u>. Wax model of the left third and fourth pharyngeal pouches, embryo D 26, dorsolateral view.

P3, P4 Pharyngeal pouches III, IV; 1 Ridge on ventral surface of pharyngeal floor marking boundary between mandibular and hyoid arches; 2 Similar ridge marking boundary between hyoid and third visceral arches; 3 Parathyroid III, 4 Anlage of thymus; 5 Ventral diverticulum of pouch IV; 6 Ultimobranchial body; 7 Part of pharyngeal recess.

from the esophagus, the distance between the commencement of the esophagus and the apex of the pharyngeal recess, as observed from embryo D 26, now measuring 450 microns.

The pharyngeal pouches remain the dominant features of the lateral aspects of the pharynx. None has lost contact with the pharyngeal epithelium, and the lumen of each communicates freely with the interior. Nonetheless, it is evident that constrictions are making their appearance at the attachments of the third and fourth pouches (Fig. IV.57.).

The ventral diverticulum of pouch I is rapidly disappearing and can only be detected by a small ventral protuberance in the floor. Concomitantly, the large flattened dorsal diverticulum, which, by virtue of its upward inclination, has been observed previously to project well beyond the roof, has now suffered a ventral deflection, thus gaining a rather more horizontal plane. In the light of this change it becomes increasingly difficult to consider pouch I as a true pharyngeal appendage, and it would appear more appropriate to think of it as a lateral extension or continuation of the pharynx. The pharyngeal membrane I has shortened, measuring, as it extends along the ventral two-thirds of the sharp lateral border, 250 microns in embryo D 26.

With the exception of having become more flattened between the second and third visceral arches, pouch II has not markedly changed in appearance. The area of

Fig. IV.58. Paramedian section of posterior pharynx, embryo D 26, (X 190).

1 Floor of hindbrain; 2 Basilar artery; 3 Notochord; 4 Primitive jaw; 5 Thyroid; 6 Aortic sac; 7 Pharyngeal lumen; 8 Pharyngeal recess; 9 Heart.

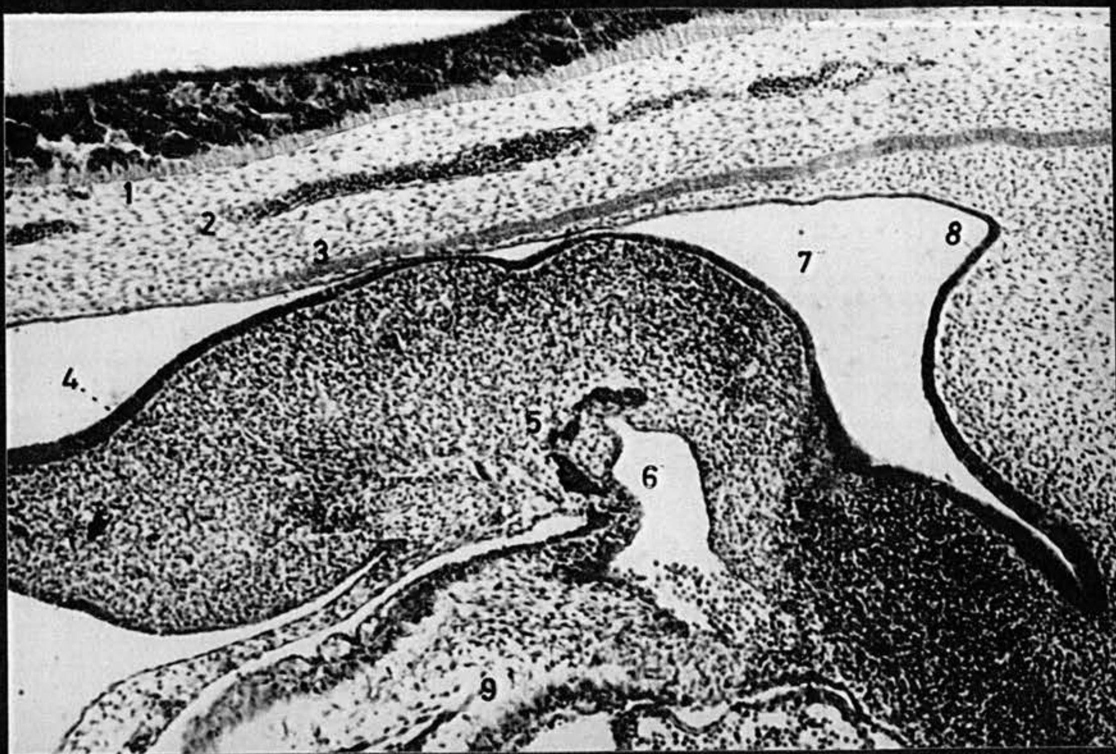


Fig. IV.58. Paramedian section of posterior pharynx, embryo D 26, (X 190).

1 Floor of hindbrain; 2 Basilar artery; 3 Notochord; 4 Primitive jaw; 5 Thyroid; 6 Aortic sac; 7 Pharyngeal lumen; 8 Pharyngeal recess; 9 Heart.

Fig. IV.59. Sagittal section of pharynx at the level of the third pharyngeal pouch, embryo D 26, (X 190).

1 Ventral tip of otic vesicle; 2 Dorsal aorta; 3 Nodose ganglion; 4 Cervical sinus; 5 Pouch III; 6 Parathyroid III; 7 Third aortic arch artery; 8 Third visceral arch; 9 Hyoid arch; 10 Lumen of pharynx; 11 Mandibular arch; 12 Heart.

Fig. IV.59. Sagittal section of pharynx at the level of the third pharyngeal pouch, embryo D 26, (X 190).

1 Ventral tip of otic vesicle; 2 Dorsal aorta; 3 Nodose ganglion; 4 Cervical sinus; 5 Pouch III; 6 Parathyroid III; 7 Third aortic arch artery; 8 Third visceral arch; 9 Hyoid arch; 10 Lumen of pharynx; 11 Mandibular arch; 12 Heart.

contact with ectoderm is 450 microns long, narrow and extending the entire length of its sharpened lateral border. In all embryos of this group, the narrow pharyngeal membrane was again found to be ruptured for a short distance (Fig. IV.54./11).

The dorsal diverticulum of pouch III can no longer be identified at this stage. What was regarded to be the ventral diverticulum in the previous stage has grown downwards, producing a neck-like constriction, the branchio-pharyngeal duct III, by which it finds attachment to the body of the pharynx. This pendulous pouch (complex III) bears a medially directed, short, finger-like process on its medial border, representing the rudimentary thymus (Figs. IV.54./P3; IV.57./4). The primordium of para-thyroid III has enlarged on the anterior face of this pouch, and now presents a raised area of roughly 100 microns diameter (3) (Fig. IV.59./6). Its cells differ little, however, from those of the abutting pouch III tissue, other than that their staining reaction tends to be slightly more critical. Their overall appearance is that of columnar epithelium with oval to long nuclei arranged at 90 degrees to the lumen of the pouch. A narrow area of contact, roughly 70 microns long, persists with the ectoderm of the anterior wall of the cervical sinus along the lateral border of the pouch.

Pouch IV (complex IV) also shows signs of undergoing similar constrictive growth away from the pharynx and has become pendulous. Its connection with the body of the pharynx is maintained by a narrow, neck-like portion, later to be known as the branchiopharyngeal duct IV. The small dorsal diverticulum has been carried down with the pouch and can still be identified as a minute tubercle, borne by the lateral border of the pouch. The ventral diverticulum is also present in the shape of a small evagination from the anterior face of the complex (Fig. IV.57./5). Thus it would appear that the entire fourth pouch, in effect, migrates from the pharynx. In the case of pouch III however, it is only the former ventral diverticulum that pulls away, leaving behind the dorsal diverticulum on the body of the pharynx. The distance between the fourth pouch and the thyroid gland was observed to be 350 microns.

The Structure of the Pharyngeal Wall

The horizontal portion of the roof consists of a single layer of low cuboidal cells, resting on a cushion of loose mesenchyme. The cell nuclei are oval in outline and lie with their long axes parallel to the plane of the well developed basement membrane. The epithelium of the vertical portion of the roof is similar to that of the caudal areas of the floor described below. The anterior region of the pharyngeal floor is composed of four to five irregular layers of flat to cuboidal cells. Their nuclei are round and fill the entire cells, especially in the deeper strata. Progressing caudally, the nature of the epithelium changes to stratified columnar. As the epithelium approaches the pharyngeal pouches, it changes to low stratified columnar similar to that of the pouch walls. Along the narrow pharyngeal membranes cellular organisation disappears in both ectodermal and endodermal components, the two irregular cellular layers now lying in apposition without the interposition of a basement membrane. The mesenchyme underlying the floor is much denser than that above the roof (Fig. IV.58.). Some cell differentiation can be observed, especially laterally, but despite the fact that numerous small vessels have made their appearance in the deeper layers of the mesenchyme, there is as yet no indication of their having penetrated as far as the subepithelial tissue. Concurrently, the mesenchyme surrounding the epithelial tubes of both esophagus and trachea is beginning to become denser, in the case of the esophagus the cells having arranged themselves circularly around the tube.

The Relationships of the Pharynx

The mesenchymal cushion intervening between hindbrain and horizontal portion of the pharynx is denser close to the pharyngeal epithelium and more loosely arranged in the vicinity of the brain (Fig. IV.58.). This condensed layer continues anteriorly beyond the limits of the pharynx and sweeps up on either side of Rathke's pocket into the space between diencephalon in front and metencephalon behind. Similarly, this dense layer of mesenchyme extends caudally beyond the posterior limits of the pharynx until, having pursued a horizontal course for some short length, it curves downwards to become the ventral layer of the developing cartilaginous vertebrae (Fig. IV.56.). The cells have arranged themselves in rows in the portions of this mesenchymal sheet, that overlie and proceed from Seessel's pocket anteriorly, and from the pharyngeal recess posteriorly. It seems that these cell cords permit the transmission of tensions, both from in front and behind, to the anterior and posterior extremities of the pharyngeal roof. The head chorda is embedded in this thickened mesenchymal layer and can be observed running parallel to the horizontal portion of the pharyngeal roof (15). In embryo D 26 there is one contact between the two structures at the thyroid level; in embryo D 27 no union occurs anywhere along the roof; and in embryo D 28 three contacts are present, one in front of, one at, and one behind the thyroid. The paired basilar arteries notable in the loose mesenchymal layer are now moving towards each other and at some levels have united to form a single trunk (16) (Fig. IV.60./5). The dorsal aortae are the dorsal relations of the pharynx along the length of its lateral borders (6) (Fig. IV.55./3). These arteries receive the third, fourth and the pulmonary arch

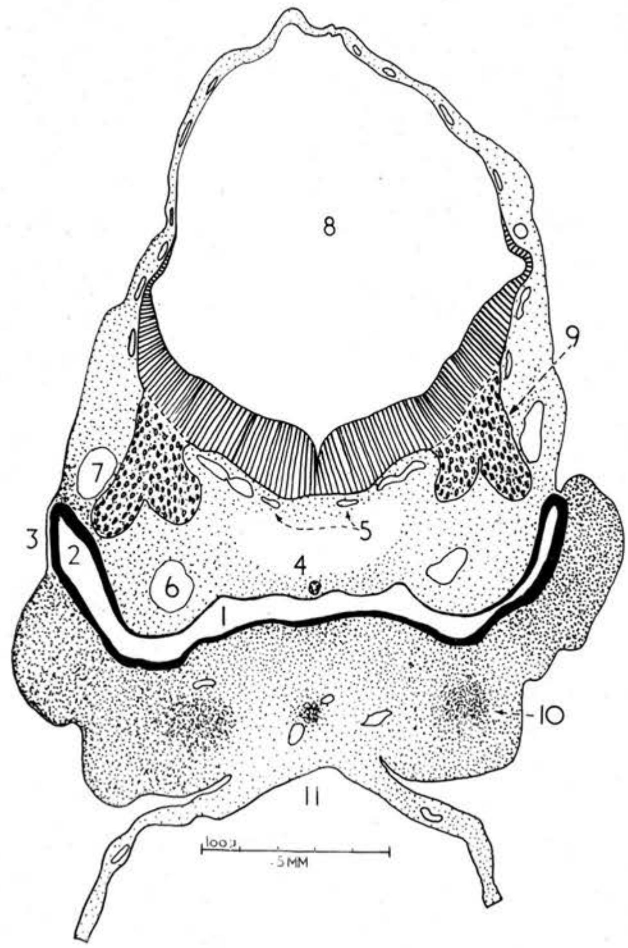


Fig. IV.60. Transverse section of the head at the level of pouch I, embryo D 27, (semischematic).

1 Pharynx; 2 Pouch I; 3 Branchial membrane I; 4 Notochord; 5 Basilar arteries; 6 Dorsal aorta; 7 Anterior cardinal vein; 8 Hindbrain; 9 Combined ganglia of seventh and eighth cranial nerves; 10 Hypoglossal pre-muscle mass.

Fig. IV.61. Transverse section of the head at the level of pouch II, embryo D 27, (X 95).

1 Otocyst; 2 Tympanic nerve; 3 Dorsal aorta; 4 Pouch II; 5 Branchial cleft II; 6 Thyroid; 7 Heart.

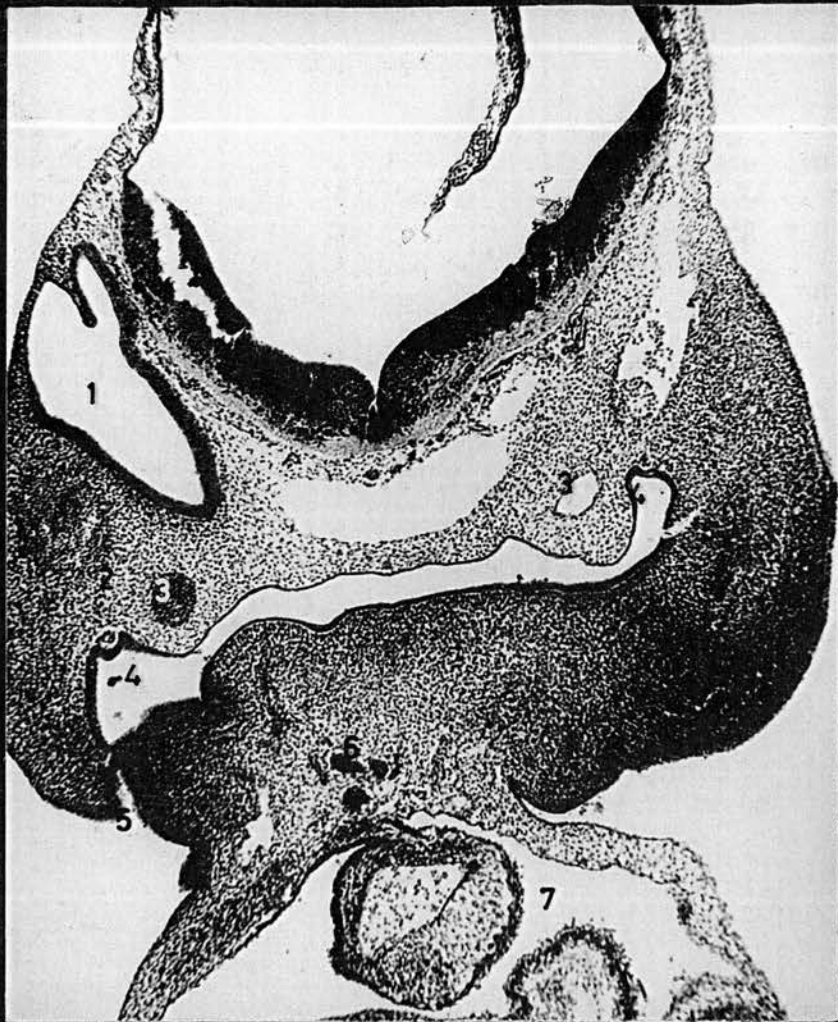


Fig. IV.61. Transverse section of the head at the level of pouch II, embryo D 27, (X 95).

1 Otocyst; 2 Tympanic nerve; 3 Dorsal aorta; 4 Pouch II; 5 Branchial cleft II; 6 Thyroid; 7 Heart.

arteries, of which the fourth is the largest and the third the weakest in the group of embryos under review (Fig. IV.54./13, 14, 15). That segment of dorsal aorta on either side between the third and fourth arch arteries has thinned drastically foreshadowing its disintegration to allow for the formation of the internal carotid artery. Dorsolaterally to the dorsal aortae are the anterior cardinal veins of the head (Fig. IV.60./7). At the level of the second pouch the ventral tip of the otic vesicle (Fig. IV.59/1) lies just above the lateral border of the pharynx, while the petrosal ganglion can be noted some short distance caudalwards between the second and third pouch. The tympanic nerve has now elongated and reaches beyond the level of pouch II. As it crosses the dorsal aspect of the pouch, rather peculiar circumstances cause it to sink into the pouch from above, pushing pouch epithelium ahead of it (Fig. IV.61./2, 4). This is a surprising fact when one considers that the tympanic nerve at this stage is very short and freely suspended in loose mesenchyme, which circumstance provides it with no weight of its own, by means of which it could make an impression upon the pouch wall. Furthermore, the growth tendencies with regard to pouch II are downwards and away from the nerve, rather than in the opposite direction with the object of engulfing it. Be this as it may, the indentation encountered quite regularly in the 7 - 9 mm. embryos may serve to explain an unusual cell cord formation associated with pouch II, which will be seen with equal regularity in the following stage.

The lateral relations of the pharynx at this stage are the visceral arches and their attendant arteries and nerves. In the first arch the mandibular nerve is large and as yet unbranched. After having passed the anterior cardinal vein on its lateral aspect, the nerve curves around the lateral border of the pharynx to terminate between it and the midline (Fig. IV.54./6). It lies in the centre of the visceral arch throughout its course. Accompanying it, can be found a meshwork of small blood vessels which however no longer make connection with the dorsal aorta. In the second visceral arch the facial nerve, passing the anterior cardinal vein on the medial side, bifurcates at the level of the pharyngeal cavity; the longer branch (Chorda tympani nerve) appears thin and, being rather closely applied to the underside of the pharynx, runs anteromedially to end below pouch I in the proximity of the mandibular nerve (8) (Fig. IV.55./12). The remaining branch is much thicker and pursues a ventral course for a short distance within the centre of the arch, accompanied by the remnants of the second aortic arch artery. The glossopharyngeal nerve contained in the third arch has also developed two branches, of which the anterior, the future glossal branch, passes forwards closely related to the pharyngeal floor; the posterior branch, on the other hand, is short and continues ventrally in the centre of the visceral arch. The artery of the third aortic arch has decreased in diameter. The anterior laryngeal nerve in the fourth visceral arch is still short and runs a common course with the fourth aortic arch artery, which by now is the largest of the remaining aortic arches. The cervical sinus has invaded the space of the fourth visceral arch and comes to lie immediately lateral to the anterior laryngeal nerve and accompanying arch artery (16) (Fig. IV.54./16). Its connection with the ectoderm of the hyoid arch and that of the heart bulge persists, but it is already evident that the deep portion of the sinus is soon destined to be isolated by the overgrowing hyoid arch. Inferior to the fourth pharyngeal pouch the posterior laryngeal nerve (recurrent) is as yet represented merely by a dense mass of spindle-shaped cells arranged medial to the pulmonary arch artery but connected below the artery to the ventral portion of the nodose ganglion (19).

The rapidly expanding ventral portion of the mandibular arch represents the chief inferior pharyngeal relationship. The aortic sac has moved somewhat ventrally from its previous position and occupies now a place just anterior to the future larynx (20). The remnant of the second arch artery courses forwards and outwards from it to terminate below pouch I as the external carotid artery (12). Ventral to either lateral pharyngeal border two pre-muscle masses have made their appearance. They originate in the centre of the primitive jaw and curve outwards, downwards, and backwards on to the wall of the heart bulge to make connection with the hypoglossal nerve immediately lateral to the fourth pharyngeal pouches (Fig. IV.60./10).

The close succession of developmental stages covering the gestation life of 5 to 10 mm. embryos is designed to portray in detail the changes that occur during the modification of the pharyngeal pouches. The body of the pharynx remains unchanged as a whole, although its horizontal portion has begun to widen, due doubtless to the rapid outgrowth and expansion of the first two visceral arches. Concomitant with such accelerated growth, the third and fourth arches lose pace and thus give the impression of a slow submergence within the depths of the cervical sinus, itself a by-product of these morphological changes. The thymic primordium has appeared on the third pouch, while that of the thyroid has lost all contact with the pharyngeal epithelium and is now an independent entity within the subpharyngeal mesenchyme. Constrictions have also become evident between the pharyngeal chamber and the third and fourth pouches, foreshadowing their eventual separation from the body of the pharynx. Finally, the so-called hypoglossal pre-muscular masses, now present within the primitive jaw, represent the first definitive muscular elements of the pharyngeal region as a whole.

STAGE 7

(8 - 10 mm. G.L.)

This stage is represented by six embryos taken from three litters as follows:

Embryo D 18	8.4 mm. G.L.	litter 6
Embryo D 4	8.5 mm. G.L.	litter 6
Embryo D 19	9.3 mm. G.L.	litter 7 a
Embryo D 19a	9.4 mm. G.L.	litter 7 a
Embryo D 32	9.8 mm. G.L.	litter 7 a
Embryo D 21	10.3 mm. G.L.	litter 7 b

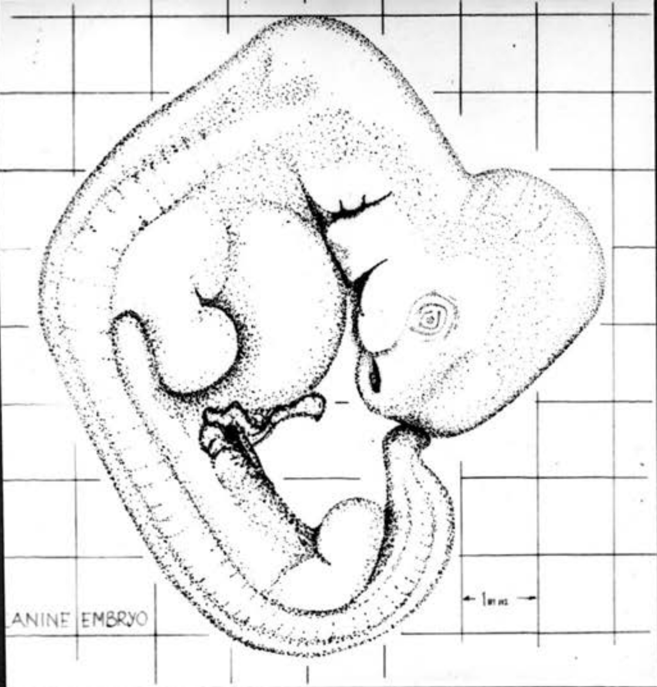


Fig. IV.62. Canine embryo D 18 (8.4 mm. G.L.).

Number of embryo:	**D 18**	Date of processing:	**October 3, 1960**
Size before fixation:	**-**	Block:	**Paraffin**
Size after fixation:	**8.4 mm. G.L.**	Stain:	**H. & E.**
Age:	**-**	Plane of section:	**Sagittal**
Position on uterus:	**-**	Thickness of section:	**10 microns**
Size of loculus:	**-**	Series made:	**D 18 (1 - 17)**
Fixed in:	**10% Form./Bouin's Fluid**		
Stored in:	**70% Alcohol**		

From litter:		**6**
Number of embryos in litter:		**2**
DAM	Breed:	**-**
	Age:	**-**
	Weight:	**-**
SIRE	Breed:	**-**
	Age:	**-**
	Weight:	**-**
Number of services:		**-**
Date of 1st service:		**-**
Date of 2nd service:		**-**

Date of collection: **April 15, 1960**

At time of collection Dam was alive (**X**), dead ( ).

Embryos collected hour(s) after death.

Litter received from: **Dr. Laforet, Windsor, Ontario.**

Remarks:

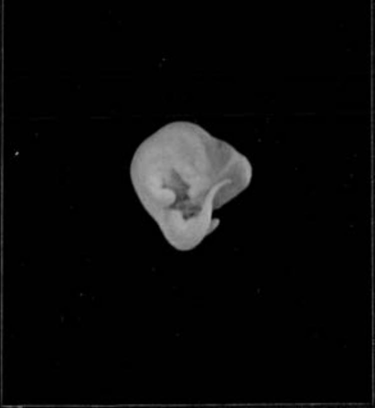


Fig. IV.63. Canine embryo D 4 (8.5 mm. G.L.).

Number of embryo:	D 4	Date of processing:	July 12, 1960
Size before fixation:	-	Block:	Paraffin
Size after fixation:	8.5 mm. G.L.	Stain:	H. & E.
Age:	-	Plane of section:	Horizontal
Position on uterus:	-	Thickness of section:	10 microns
Size of loculus:	-	Series made:	D 4 (1 - 22)
Fixed in:	10% Form./Bouin's Fluid		
Stored in:	70% Alcohol		

From litter:		6
Number of embryos in litter:		2
DAM	Breed:	-
	Age:	-
	Weight:	-
SIRE	Breed:	-
	Age:	-
	Weight:	-
Number of services:		-
Date of 1st service:		-
Date of 2nd service:		-

Date of collection: April 15, 1960

At time of collection Dam was alive (X), dead ( ).

Embryos collected hour(s) after death.

Litter received from: Dr. Laforet, Windsor, Ontario.

Remarks:

Fig. IV.64. Canine embryo D 19 (9.3 mm. G.L.).

Number of embryo:	D 19	Date of processing:	January 26, 1961
Size before fixation:	-	Block:	Paraffin
Size after fixation:	9.3 mm. G.L.	Stain:	H. & E.
Age:	-	Plane of section:	Sagittal
Position on uterus:	-	Thickness of section:	10 microns
Size of loculus:	35 x 20 mm. (fixed)	Series made:	D 19 (1 - 17)
Fixed in:	10% Formalin		
Stored in:	10% Formalin		

From litter:		7 a
Number of embryos in litter:		6
DAM	Breed:	-
	Age:	-
	Weight:	-
SIRE	Breed:	-
	Age:	-
	Weight:	-
Number of services:		-
Date of 1st service:		-
Date of 2nd service:		-
Date of collection:		-

At time of collection Dam was alive ( ), dead ( ).

Embryos collected hour(s) after death.

Litter received from: Department of Anatomy, Ontario Veterinary College.

Remarks:

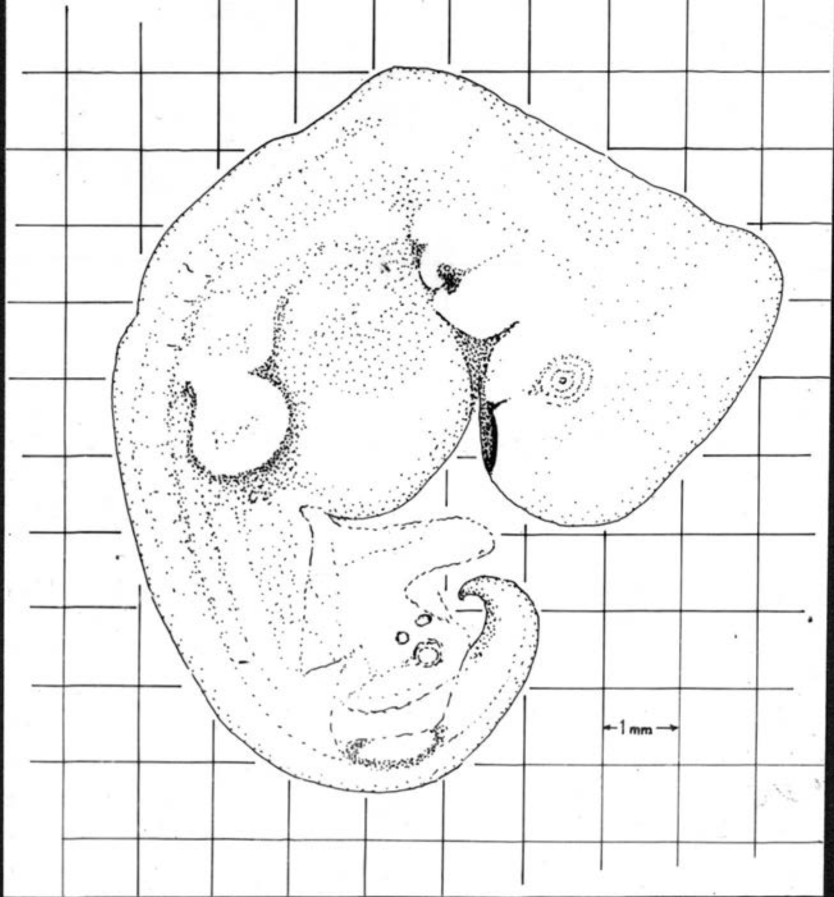


Fig. IV.65. Canine embryo D 19a (9.4 mm. G.L.).

Number of embryo:	D 19a	Date of processing:	January 16, 1961
Size before fixation:	- -	Block:	Paraffin
Size after fixation:	9.4 mm. G.L.	Stain:	H. & E.
Agė:	-	Plane of section:	Sagittal
Position on uterus:	-	Thickness of section:	10 microns
Size of loculus:	35 x 20 mm. (fixed)	Series made:	D 19a (1 - 8)
Fixed in:	10% Formalin		
Stored in:	10% Formalin		

From litter: 7 a

Number of embryos in litter: 6

DAM	Breed:	-
	Age:	-
	Weight:	-
SIRE	Breed:	-
	Age:	-
	Weight:	-

Number of services: -

Date of 1st service: -

Date of 2nd service: -

Date of collection:

At time of collection Dam was alive ( ), dead ( ).

Embryos collected hour(s) after death.

Litter received from: Department of Anatomy, Ontario Veterinary College.

Remarks:

Fig. IV.66. Canine embryo D 32 (9.8 mm. G.L.).

Number of embryo:	D 32	Date of processing:	March 24, 1961
Size before fixation:	-	Block:	Paraffin
Size after fixation:	9.8 mm. G.L.	Stain:	H. & E.
Age:	-	Plane of section:	Transverse
Position on uterus:	-	Thickness of section:	10 microns
Size of loculus:	35 x 20 mm. (fixed)	Series made:	D 32 (1 - 23)
Fixed in:	10% Formalin		
Stored in:	10% Formalin		

From litter:		7 a
Number of embryos in litter:		6
DAM	Breed:	-
	Age:	-
	Weight:	-
SIRE	Breed:	-
	Age:	-
	Weight:	-
Number of services:		-
Date of 1st service:		-
Date of 2nd service:		-
Date of collection:		-

At time of collection Dam was alive ( ), dead ( ).

Embryos collected hour(s) after death.

Litter received from: Department of Anatomy, Ontario Veterinary College.

Remarks:

Fig. IV.67. Canine embryo D 21 (10.3 mm. G.L.).

The Shape of the Pharynx

The pharynx does not differ much in general outline from that seen in the previous stage. It is a dorso-ventrally flattened, triangularly shaped, epithelial tube, having an anterior (cephalic) flexure, from whence it continues ventrally to open as the wide oral cavity, and a posterior (cervical) flexure, posterior to which it narrows to become the esophagus (Figs. IV.68.; IV.69.). The longitudinal measurement from the entrance to Rathke's pocket to the beginning of the esophagus has increased again to 1.8 mm., while the transverse measurements at the base of pouch I and between the third and fourth pouches are 1.8 mm. and 0.9 mm. respectively. At the cervical flexure the prominent pharyngeal recess bulges caudally, lending emphasis to the bend there (Fig. IV.68./3). Similarly, at the anterior aspect Seessel's pocket accentuates the cephalic flexure (2) (Fig. IV.75./2), immediately below which Rathke's pocket springs from the subjacent ectoderm. It has grown two lateral wings extending from the central body, that was previously observed; and the more or less quadrilateral tube conjoining it to the pharynx is about 100 microns wide in this group of embryos (3) (Fig. IV.69./1). The pharyngeal roof is smooth, and, with the exception of the aforementioned pharyngeal recess, presents no features worthy of note. The uniformity of the underside of the floor however, is interrupted by two transverse ridges which run

Number of embryo:	**D 21**	Date of processing:	**February 21, 1961**
Size before fixation:	**-**	Block:	**Paraffin**
Size after fixation:	**10.3 mm. G.L.**	Stain:	**H. & E.**
Age:	**23 days**	Plane of section:	**Sagittal**
Position on uterus:	**-**	Thickness of section:	**10 microns**
Size of loculus:	**42 x 25 mm. (fresh)**	Series made:	**D 21 (1 - 21)**
Fixed in:	**10% Formalin**		
Stored in:	**10% Formalin**		

From litter:		**7 b**
Number of embryos in litter:		**5**
DAM	Breed:	**X Terrier**
	Age:	**1.5 years**
	Weight:	**14 lb.**
SIRE	Breed:	**X Terrier**
	Age:	**-**
	Weight:	**15 lb.**
Number of services:		**1**
Date of 1st service:		**December 14, 1960**
Date of 2nd service:		**-**

Date of collection: **January 6, 1961**

At time of collection Dam was alive ( ), dead (**X**).

Embryos collected **2** hour(s) after death.

Litter received from: **Edinburgh Clinic**

Remarks:

Fig. IV.68. Wax model of the left half of the pharynx, embryo D 18 (8.4 mm. G.L.), (model X 100), lateral view.

P1, P2, P4 Pharyngeal pouches I, II, IV; 1 Rathke's pocket; 2 Seessel's pocket; 3 Pharyngeal recess; 4 Remnant of branchial membrane II; 5 Parathyroid III; 6 Thymus; 7 Cervical sinus; 8 Parathyroid IV; 9 Anlage of larynx; 10 Trachea; 11 Esophagus.

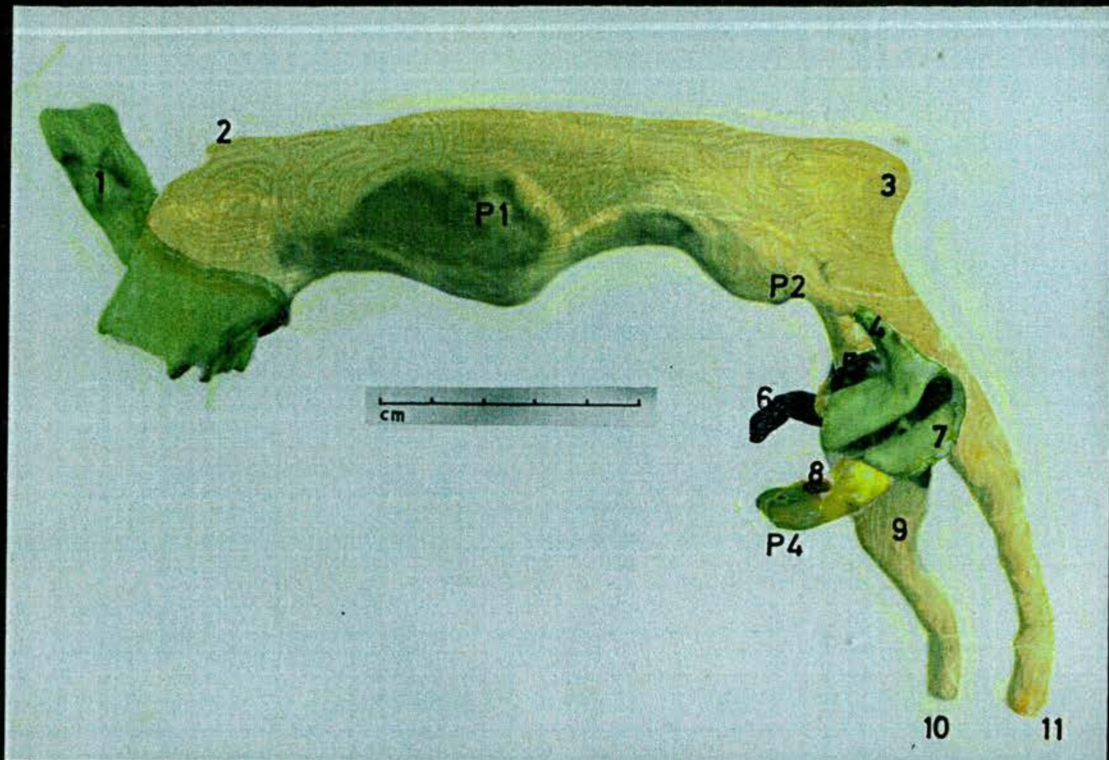


Fig. IV.68. Wax model of the left half of the pharynx, embryo D 18 (8.4 mm. G.L.), (model X 100), lateral view.

P1, P2, P4 Pharyngeal pouches I, II, IV; 1 Rathke's pocket; 2 Seessel's pocket; 3 Pharyngeal recess; 4 Remnant of branchial membrane II; 5 Parathyroid III; 6 Thymus; 7 Cervical sinus; 8 Parathyroid IV; 9 Anlage of larynx; 10 Trachea; 11 Esophagus.

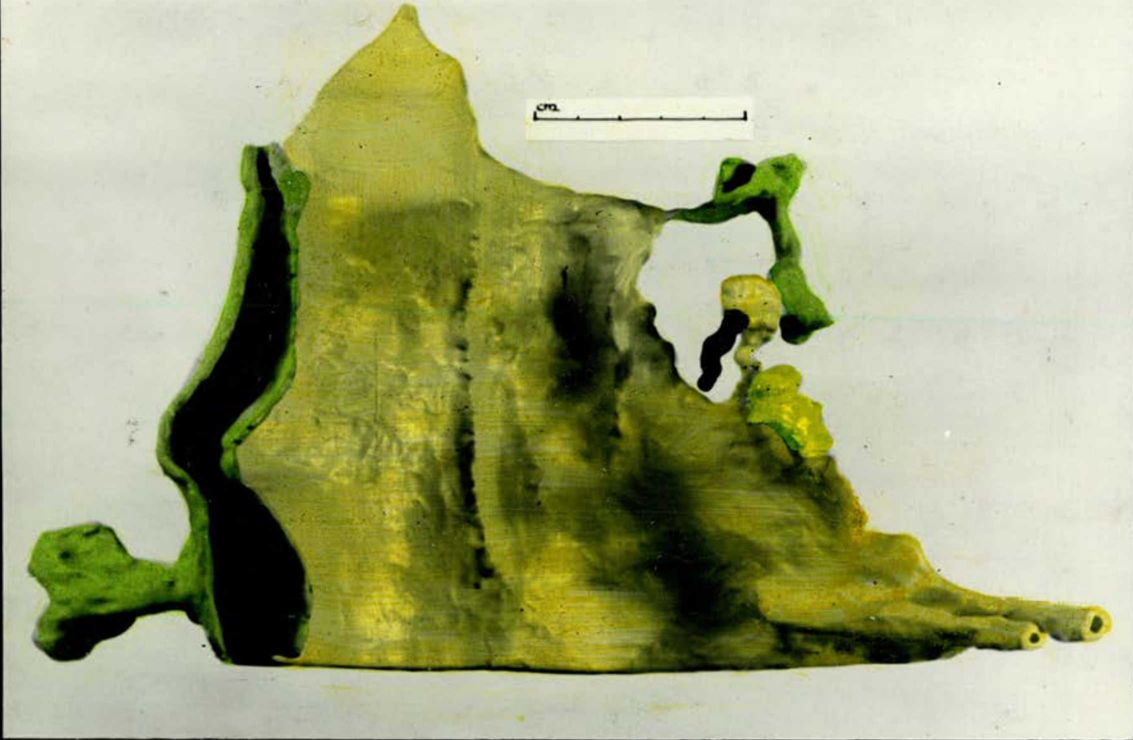
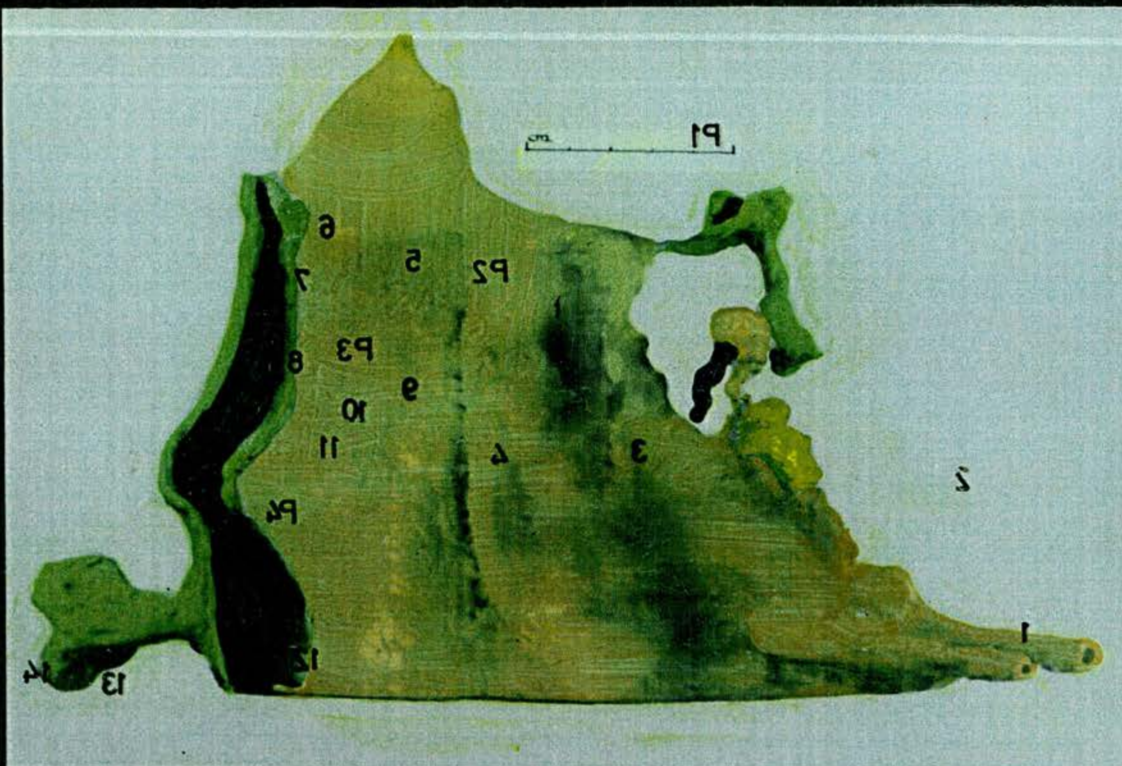


Fig. IV.69. Wax model of the left half of the pharynx, embryo D 18 (8.4 mm. G.L.), (model X 100), ventral view.

P1 - P4 Pharyngeal pouches I - IV; 1 Rathke's pocket; 2 Oral cavity and mouth; 3 Ridge on ventral surface of floor, marking boundary between first and second visceral arches; 4 Similar ridge between second and third visceral arches; 5 Remnant of branchial membrane II; 6 Cervical sinus; 7 Constricted portion of cervical sinus (cervico-branchial duct); 8 Deep portion of cervical sinus (cervical vesicle); 9 Thymus; 10 Pharyngobranchial duct III; 11 Ultimobranchial body; 12 Anlage of larynx; 13 Trachea; 14 Esophagus.



<u>Fig. IV.69</u>. Wax model of the left half of the pharynx, embryo D 18 (8.4 mm. G.L.), (model X 100), ventral view.

P1 - P4 Pharyngeal pouches I - IV; 1 Rathke's pocket; 2 Oral cavity and mouth; 3 Ridge on ventral surface of floor, marking boundary between first and second visceral arches; 4 Similar ridge between second and third visceral arches; 5 Remnant of branchial membrane II; 6 Cervical sinus; 7 Constricted portion of cervical sinus (cervico-branchial duct); 8 Deep portion of cervical sinus (cervical vesicle); 9 Thymus; 10 Pharyngobranchial duct III; 11 Ultimobranchial body; 12 Anlage of larynx; 13 Trachea; 14 Esophagus.

medially from the ventral diverticula of the first and second pouches (3, 4). There is no longer any evidence of thyroidal attachment. The future larynx arises in the midline of the vertical portion of the floor, being recognisable as a laterally flattened, oval structure, which, pointing ventrally, is continued by the trachea (12) (Fig. IV.68./9, 10).

With the possible exception of the first, the pharyngeal pouches can now be said to have lost much of their prominence as a result of their retarded growth, which fails to keep pace with the rapidly enlarging pharyngeal tube; indeed such is their appearance that they may not have grown at all, and, as in the case of the second pouch, may actually have suffered a loss in size. Pouch I is now a flattened, lateral extension of the pharynx (Fig. IV.69./P1). Its plane does not have as pronounced an upward inclination however, as was seen in previous embryos. Its apex, at the same time, is pointed and makes but scant contact with the overlying ectoderm (Fig. IV.74./1, 4). Pouch II can no longer be recognised as an appendage by virtue of its appearing to have been resorbed by pharyngeal tissue, until, in this group of embryos, it is evidenced only by a thickening within the lateral margin of the pharynx (Figs. IV.68./P2; IV.69./P2). Its previous position can however be determined by the transverse ridge persistent on the floor of the pharynx, originating from its former ventral

Fig. IV.70. Transverse section of posterior pharynx, a short distance anterior to that of Fig. IV.72., embryo D 32 (9.8 mm. G.L.), (X 95).

1 Cervical sinus; 2 Pouch II containing cell cord; 3 Petrosal ganglion; 4 Dorsal aorta; 5 Third aortic arch artery; 6 Pouch III (thymus); 7 Pharyngeal recess; 8 Notochord; 9 Pouch III and parathyroid III; 10 Fourth aortic arch artery; 11 Pouch IV and parathyroid IV; 12 Sixth aortic arch artery; 13 Pericardium.

Fig. IV.70. Transverse section of posterior pharynx, a short distance anterior to that of Fig. IV.72., embryo D 32 (9.8 mm. G.L.), (X 95).

1 Cervical sinus; 2 Pouch II containing cell cord; 3 Petrosal ganglion; 4 Dorsal aorta; 5 Third aortic arch artery; 6 Pouch III (thymus); 7 Pharyngeal recess; 8 Notochord; 9 Pouch III and parathyroid III; 10 Fourth aortic arch artery; 11 Pouch IV and parathyroid IV; 12 Sixth aortic arch artery; 13 Pericardium.

Fig. IV.71. Sagittal section of lateral pharyngeal region, embryo D 19 (9.3 mm. G.L.), (X 95).

1 Semilunar ganglion; 2 Anterior cardinal vein; 3 Pouch I; 4 Combined ganglia of seventh and eighth cranial nerves; 5 Otic vesicle; 6 Ninth cranial nerve; 7 Jugular ganglion; 8 Petrosal ganglion; 9 Tympanic nerve; 10 Pouch II containing cell cord; 11 Hyoid arch; 12 Mandibular arch.

Fig. IV.71. Sagittal section of lateral pharyngeal region, embryo D 19 (9.3 mm. G.L.), (X 95).

1 Semilunar ganglion; 2 Anterior cardinal vein; 3 Pouch I; 4 Combined ganglia of seventh and eighth cranial nerves; 5 Otic vesicle; 6 Ninth cranial nerve; 7 Jugular ganglion; 8 Petrosal ganglion; 9 Tympanic nerve; 10 Pouch II containing cell cord; 11 Hyoid arch; 12 Mandibular arch.

diverticulum, and, in addition, by a strand of cells which quite regularly connects the apex of this now rudimentary pouch with the overlying ectoderm (5). This cord of cells would appear to be the remnant of the second branchial membrane, joining the ectoderm immediately anterior to the cleft between neck and heart bulge (6), from the depth of which the cervical vesicle is in the process of being pinched off (7, 8). Four of the six embryos of this group demonstrated cell cords of 40 microns diameter within the greatly reduced lumina of the second pouches (Figs. IV.70./2; IV.71./10). In one instance the cord was embedded in the epithelium of the pouch wall, protruding into the mesenchyme from the periphery of the pouch. As a rule such cords are short and almost without exception attached to the pouch wall. They are most probably a by-product of the involution of the pouch, and the indentations the tympanic nerve was observed to have made in the wall of the second pouch in the preceding stage may perhaps explain their formation (Fig. IV.61./2, 4). It should be noted, however, that these features are transitory, no traces being evident in the following stage. This is unfortunate, for it was the author's hope that the cell cords by virtue of their attachment to the identical area of each second pouch would lend themselves to being regarded as labelled areas, which could be traced through succeeding stages. Such would have been of especial value in putting forward a valid explanation of that perennial problem ...... the precise fate of the second mammalian pouch. In the 15, 22 and 29 mm. stages of this work similar cell cords will frequently be seen in the vicinity of the developing tonsillar swellings (Fig. IV.106./7). But the fact that the intervening 9 - 12 mm. range of embryos revealed no trace of the fasciculi in question, unfortunately puts an end to the thesis that the cords both in the present stage and in those of the 15 - 29 mm. range are identical, despite their similar general disposition. Ramsay (1935) observed similar cell cords in the 11 mm. cat embryo and explained them as being infoldings of the compressed second pouches. He attached, however, no further significance to them.

Pouch III is connected to the lateral margin of the pharynx by the pharyngobranchial duct III (Fig. IV.69./10) and has rearranged itself into a cuboid hollow mass (P3). Posteriorly, this mass abuts on the cervical vesicle (8), a contact that must have been effected only of late, because in the previous stage a cushion of mesenchyme, about 80 microns thick, intervened between the two structures (Fig. IV.54./P3, 16). The body of pouch III has lost all apparent connection with the ectoderm, with the exception of embryo D 32, in which a strand-like remnant of the third branchial membrane still persists (Fig. IV.70./6). This latter cell cord connection however, would appear to be an exception when compared with the regularity with which the same feature persists with regard to the second pharyngeal pouch. On the dorsal face of pouch III the primordium of parathyroid III has now enlarged, at the same time becoming slightly elevated from the pouch (Fig. IV.72./13). The thymus is a rapidly lengthening cell tube, which, measuring roughly 200 microns in length, is growing away from the antero-medial aspect of the pouch (Figs. IV.68./6; IV.69./9). The cervical vesicle still maintains its contact with the ectoderm by means of a duct (7), which, directed laterally, opens into the depth of the cleft between the neck and heart bulge of the embryo. The caudal end of the cell strand emerging from the second pouch, incidentally, can be noted immediately anterior to the opening of this duct (5).

Pouch IV has not undergone much change. It is connected to the lateral border of the pharynx by a constricted portion and presents now a rather club-shaped appearance (Fig. IV.68./P4). Dorsal and ventral diverticula have disappeared and most of its tissue can be regarded as belonging to the ultimobranchial body (Fig. IV.69./11). Parathyroid IV is now also conspicuous as a group of darkly staining cells situated on the anterodorsal aspect of the pouch (Fig. IV.73./9) or, if further advanced, as a raised cluster of cells, as can be noted in embryo D 32 (Fig. IV.72./8). The distance between pouch IV and the thyroid is still 350 microns (embryo D 19).

The Structure of the Pharyngeal Wall

As observed in previous stages, the epithelium of the pharyngeal roof consists of a single layer of low cuboidal cells, whose oval nuclei have their long axes lying parallel to the fairly well developed basement membrane. Such mesenchyme as overlies the roof is as yet undifferentiated, although it is growing denser. The cells of this region have extended oval nuclei arranged with long axes parallel to the pharyngeal roof. Cytoplasmic connections leaving either nuclear pole give a stretched appearance to the region as a whole from before backwards. Such would appear to be the result of tension applied both from in front and behind of this mesenchymal cushion immediately overlying the pharyngeal roof, and can best be appreciated when seen in median section (Fig. IV.75.). This arrangement is naturally not evident on transverse section, but a mesenchymal density about the head chorda leads one to believe that this phenomenon of mesenchymal tension is in fact concentrated about the median plane (Fig. IV.74.).

The epithelium of the pharyngeal floor is thicker than that of the roof and consists of two to four irregular layers of round-nucleated cells. The subjacent mesenchyme is considerably denser than its counterpart on the pharyngeal roof, notably so in the region of the cervical flexure, where this cellular condensation can be taken to foreshadow the internal organisation of (a) in the

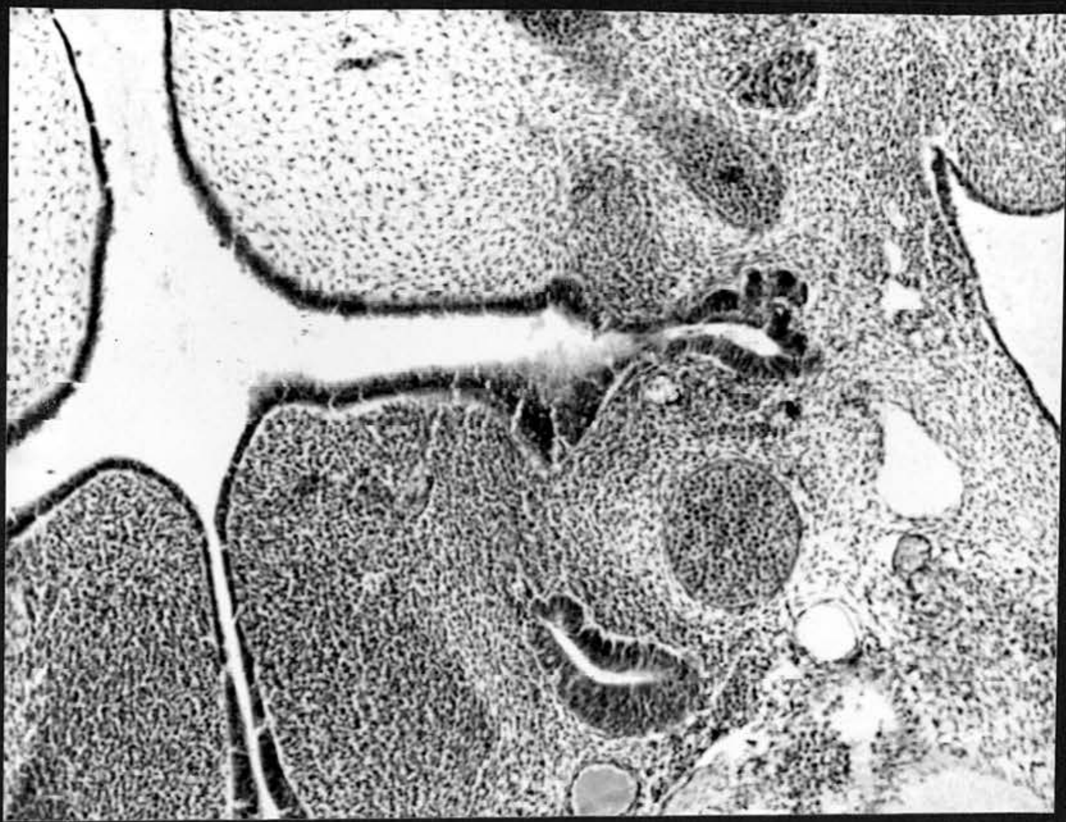


Fig. IV.72. Transverse section of posterior pharynx, embryo D 32 (9.8 mm. G.L.), (X 190).

P3, P4 Pharyngeal pouches III, IV; 1 Pharyngeal recess; 2 Lumen of pharynx; 3 Arytenoid swelling; 4 Entrance into larynx and trachea; 5 Sixth aortic arch artery; 6 Heart; 7 Ultimobranchial body; 8 Parathyroid IV; 9 Fourth aortic arch artery; 10 Hypoglossal nerve and associated pre-muscle mass; 11 Anterior laryngeal nerve; 12 Pharyngobranchial duct III; 13 Parathyroid III; 14 Pre-muscle mass of pharyngeal musculature; 15 Third aortic arch artery; 16 Ventral pole of petrosal ganglion.

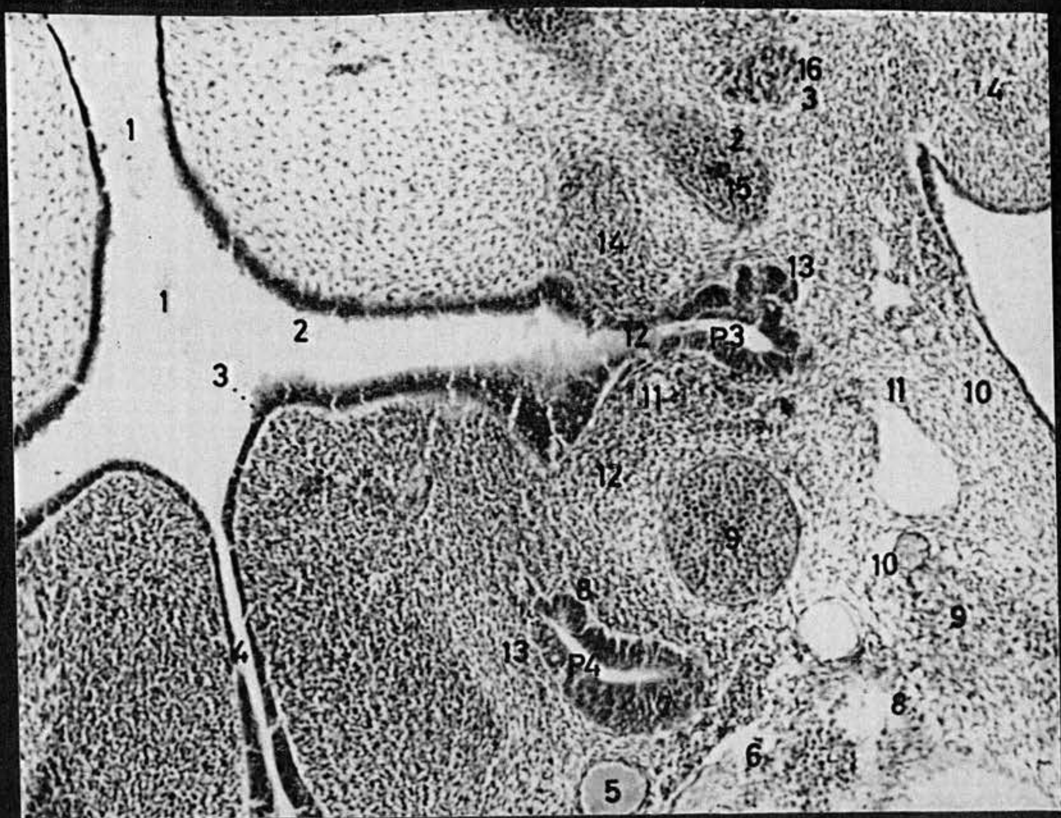


Fig. IV.72. Transverse section of posterior pharynx, embryo D 32 (9.8 mm. G.L.), (X 190).

P3, P4 Pharyngeal pouches III, IV; 1 Pharyngeal recess; 2 Lumen of pharynx; 3 Arytenoid swelling; 4 Entrance into larynx and trachea; 5 Sixth aortic arch artery; 6 Heart; 7 Ultimobranchial body; 8 Parathyroid IV; 9 Fourth aortic arch artery; 10 Hypoglossal nerve and associated pre-muscle mass; 11 Anterior laryngeal nerve; 12 Pharyngobranchial duct III; 13 Parathyroid III; 14 Pre-muscle mass of pharyngeal musculature; 15 Third aortic arch artery; 16 Ventral pole of petrosal ganglion.

Fig. IV.73. Sagittal section of posterior pharynx at the level of pouch IV, embryo D 19 (9.3 mm. G.L.), (X 190).

1 Primitive jaw; 2 Glossal branch of glossopharyngeal nerve; 3 Lumen of pharynx; 4 Third aortic arch artery; 5 Nodose ganglion; 6 Fourth aortic arch artery; 7 Sixth aortic arch artery (pulmonary arch); 8 Ultimobranchial body; 9 Parathyroid IV; 10 Proximal end of pharyngobranchial duct III; 11 Anterior laryngeal nerve; 12 Hypoglossal nerve and associated pre-muscle mass; 13 Heart.

Fig. IV.73. Sagittal section of posterior pharynx at the level of pouch IV, embryo D 19 (9.3 mm. G.L.), (X 190).

1 Primitive jaw; 2 Glossal branch of glossopharyngeal nerve; 3 Lumen of pharynx; 4 Third aortic arch artery; 5 Nodose ganglion; 6 Fourth aortic arch artery; 7 Sixth aortic arch artery (pulmonary arch); 8 Ultimobranchial body; 9 Parathyroid IV; 10 Proximal end of pharyngobranchial duct III; 11 Anterior laryngeal nerve; 12 Hypoglossal nerve and associated pre-muscle mass; 13 Heart.

midline, the upward growing epiglottic swelling, and (b) somewhat lateral and behind, the two arytenoid swellings (Fig. IV.72./3). At this point the mesenchyme is profuse with, for the most part, rounded cells, which, in sharp contrast to the mesenchymal picture observed above the pharynx, create the impression of a desire to proliferate further, at the same time, however, being prevented from doing so by the bulk of the heart below, and the general downward and inward flexion of the developing head in front. At this stage of development, there are no obvious signs of cell differentiation immediately subjacent to the basement membrane, but capillaries are seen to be invading the subepithelial layers from below. Towards either lateral margin of the pharynx, the epithelial linings of both roof and floor assume gradually a tall cuboidal appearance, which increases finally to an irregular stratified columnar type which is also seen predominantly in the walls of the pharyngeal pouches.

Parathyroid III, for the first time growing more independent of its parent pouch, does as yet differentiate little from the cytological make-up of the remainder of the complex, and thus it rests largely on position, shape, and absence of lumen for its distinguishing features (Fig. IV.72./13). It should be recorded however, that early parathyroid tissue in some specimens has a less dense cellular arrangement and smaller-appearing nuclei, while alternative preparations evidenced identical tissue definitely denser and more deeply stained (Fig. IV.73./9). Much of this also holds true of the cellular structure of the primordial thymus, which has only recently appeared on the third pouch. Simultaneous with the above changes, the mesenchymal envelope of the trachea is also growing denser, especially at its proximal end where the different laryngeal elements will soon make their appearance. The organisation of mesenchyme around the primordium of the esophagus however is peculiar in that the epithelial tube is at first surrounded by a relatively loose cellular layer; this is quickly followed by a ring of concentrated mesenchymal tissue, in which the proliferation of the cytoplasmic components suggests pre-muscle formation (Fig. IV.75./7).

The Relationships of the Pharynx

It is hoped that during the processes involving the rapidly altering morphology of the pharyngeal pouches which have been recorded in detail, it is obvious that there has been little appreciable modification in morphology and position of the remainder of the pharyngeal cavity and its neighbouring structures. Consequently, the description of the pharyngeal relationships as observed in the preceding stage proves adequate as yet, which is why only major subsequent changes will be recorded in the text following.

The basilar arteries have united, forming a single

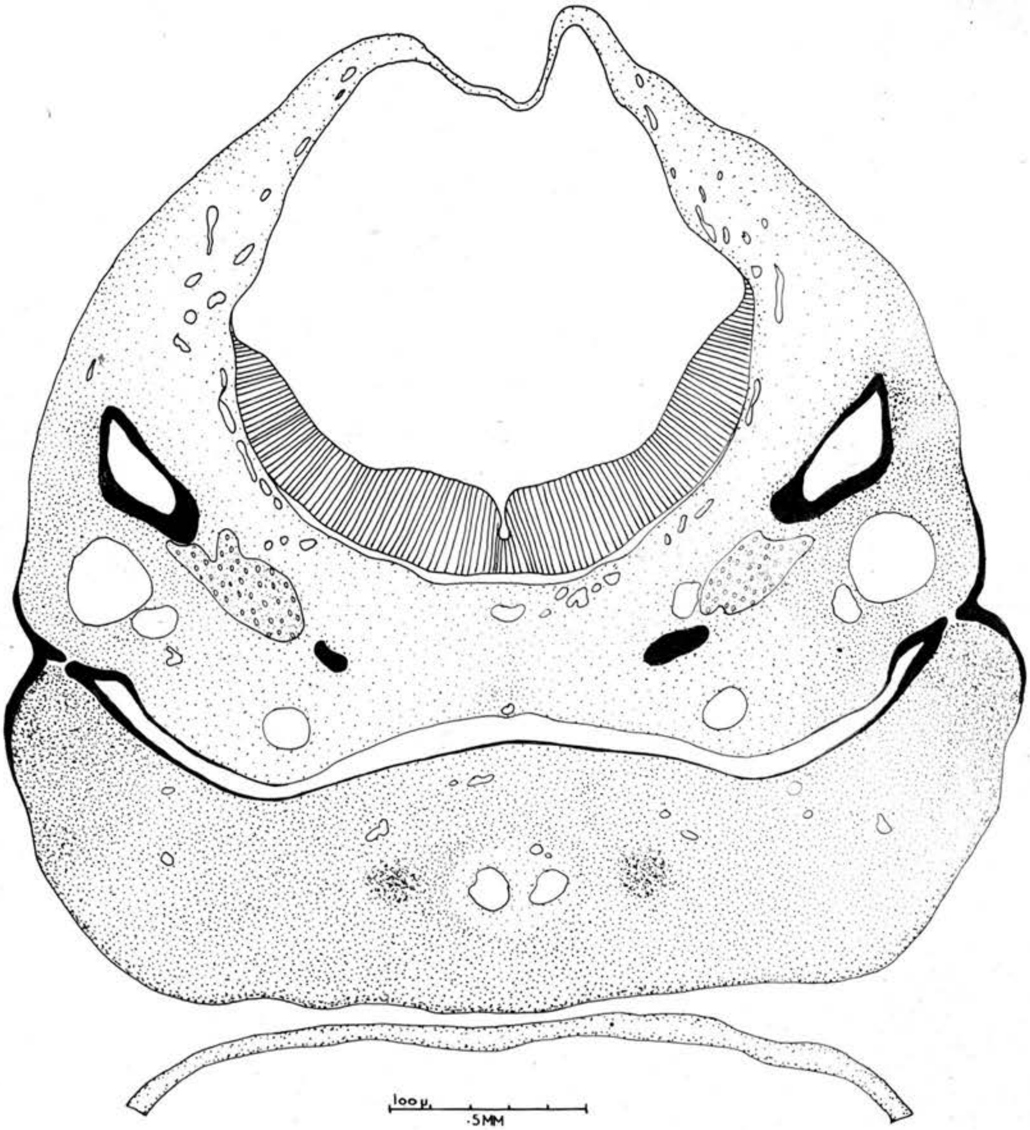


Fig. IV.74. Transverse section of head at the level of pouch I, embryo D 32 (9.8 mm. G.L.), (semischematic).

1 Branchial membrane I; 2 Anterior cardinal vein; 3 Facial nerve; 4 Pouch I; 5 Dorsal aorta; 6 Pharynx; 7 Notochord; 8 Basilar artery; 9 Hindbrain; 10 Ventral tip of otic vesicle; 11 Combined ganglion for seventh and eighth cranial nerves; 12 Otic vesicle; 13 Chorda tympani nerve; 14 Hypoglossal pre-muscle mass; 15 Heart.

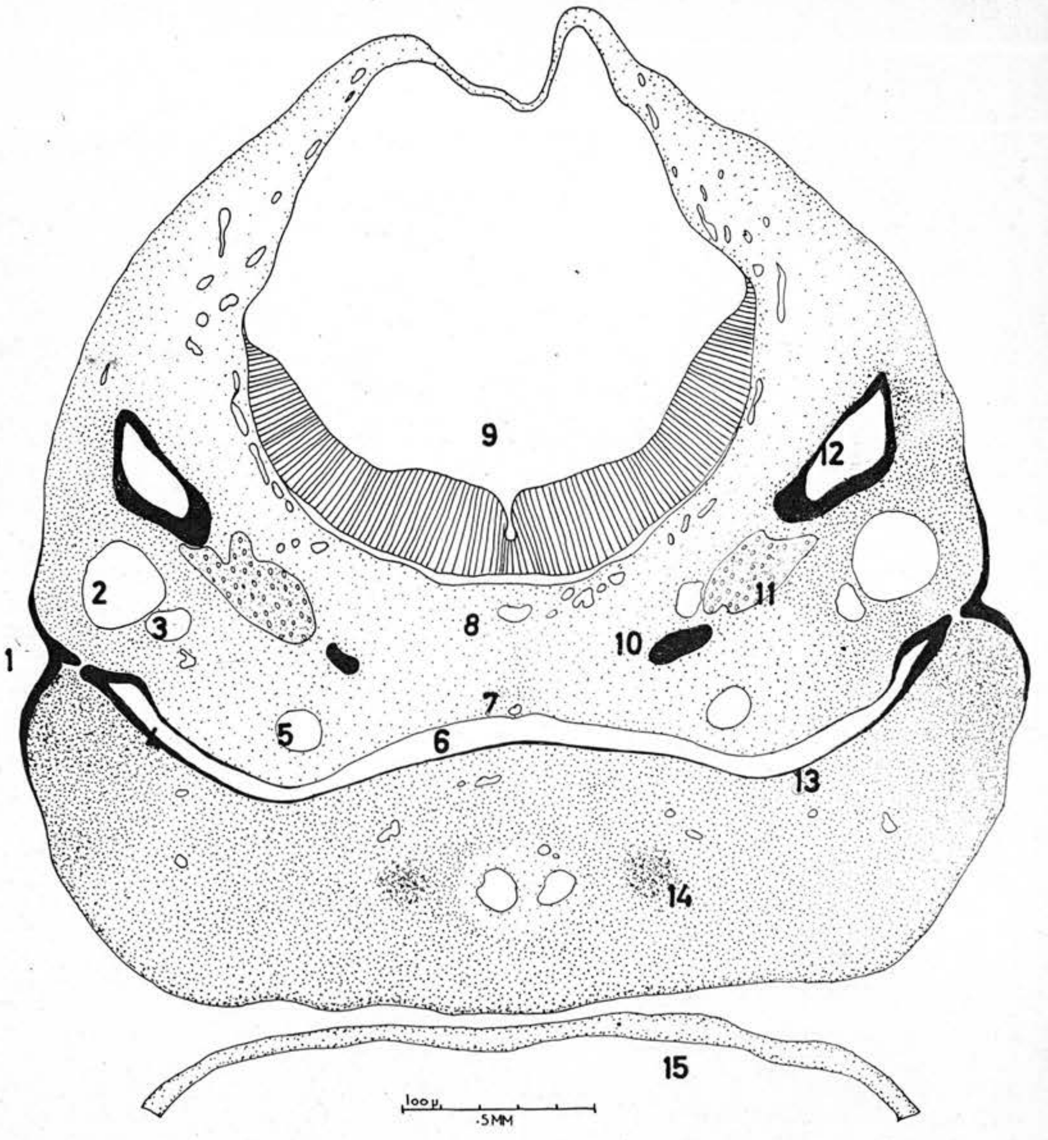


Fig. IV.74. Transverse section of head at the level of pouch I, embryo D 32 (9.8 mm. G.L.), (semischematic).

1 Branchial membrane I; 2 Anterior cardinal vein; 3 Facial nerve; 4 Pouch I; 5 Dorsal aorta; 6 Pharynx; 7 Notochord; 8 Basilar artery; 9 Hindbrain; 10 Ventral tip of otic vesicle; 11 Combined ganglion for seventh and eighth cranial nerves; 12 Otic vesicle; 13 Chorda tympani nerve; 14 Hypoglossal pre-muscle mass; 15 Heart.

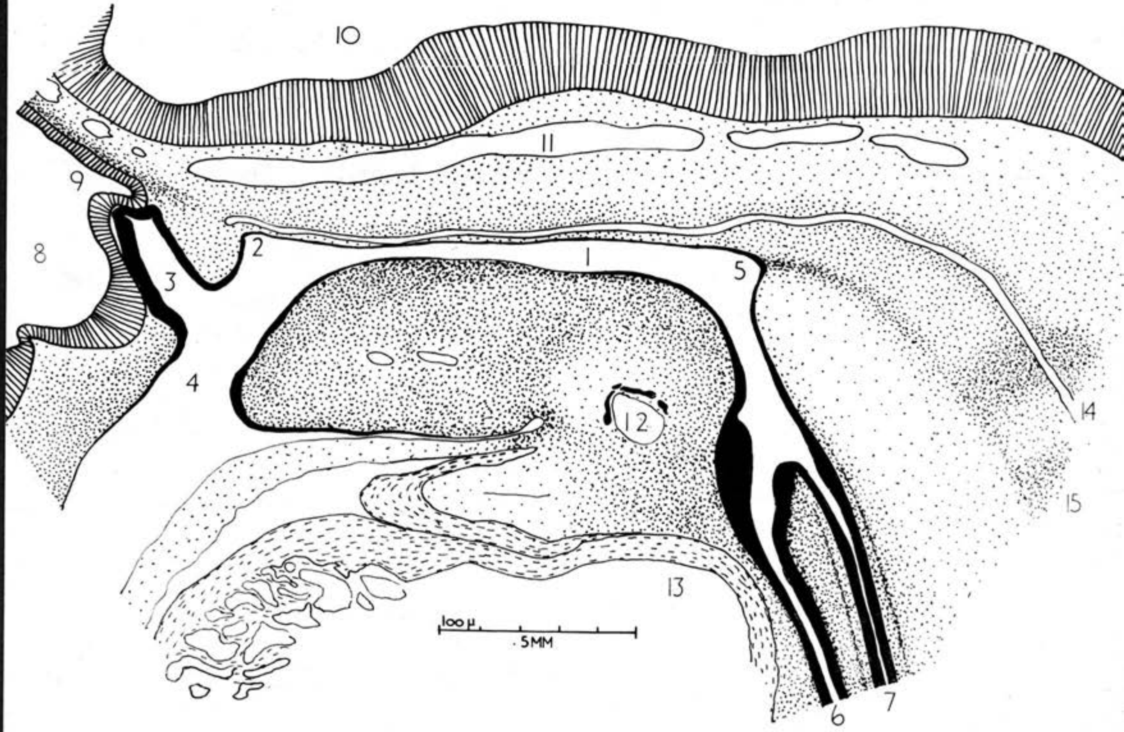


Fig. IV.75. Median section of pharyngeal region, embryo D 18 (8.4 mm. G.L.), (semischematic).

1 Pharynx; 2 Seessel's pocket; 3 Rathke's pocket; 4 Oral cavity and mouth; 5 Pharyngeal recess; 6 Trachea; 7 Esophagus; 8 Forebrain; 9 Infundibulum; 10 Hindbrain; 11 Basilar artery; 12 Aortic sac with thyroid; 13 Heart; 14 Notochord; 15 Developing cervical vertebrae.

trunk above the horizontal portion of the pharynx (Fig. IV.75./11). Only in two embryos of this group (D 18 and D 4) does the overlying head chorda make any contact with the pharyngeal roof; in both instances these contacts are in front of the thyroid level. The tympanic nerve, having left the petrosal ganglion to pass forwards and slightly outwards superior to the body of the pharynx, has crossed the root of pouch II and reaches now almost to the root of pouch I. From the depth of the cervical sinus the cervical vesicle has been pinched off and, now completely isolated between the third and fourth pharyngeal pouches, forms one of the lateral relations in this area. On the medial side of the facial nerve, after it has given off the Chorda tympani nerve (post-trematic to cleft I), a sheet-like concentration of mesenchyme has appeared. This is also evident with regard to the mandibular and glossopharyngeal nerves, but to a lesser degree. Further attention will be payed to these concentrations in the succeeding 9 - 12 mm. stage, when it becomes clear that such are the anlagen of the muscles destined to be innervated by the particular trunk with which they are associated. In the subpharyngeal region the aortic sac has descended further towards the future larynx, carrying with it the cluster of thyroid plates (12). In the group of embryos under review, the primordium of the latter gland envelopes the anterior face of the aortic sac. All its cell cords and plates lie in the same plane, none obviously having been left behind during the ventral migration with the aortic sac.

Again in this stage the pharyngeal pouches have undergone the greatest modifications, while the body of the pharynx, apart from general enlargement, has retained its characteristic shape. The pouches, however, taken as a whole, have ceased to dominate the appearance of the pharynx, principally by virtue of their failure to keep pace with the rapid growth rate of the parent organ at that time. Pouch I is a dorsoventrally flattened extension of the pharyngeal body, drawn out to a point with which it only just touches the ectoderm of the first branchial cleft. For its part, the second pouch is in the process of being resorbed into the body of the pharynx. It does maintain connection with the cervical sinus however, via a thin epithelial strand. The third and fourth pouches on the other hand have begun to move away from the lateral edge of the pharyngeal tube, retaining connection with the pharynx through their respective branchiopharyngeal ducts. The derivatives of the third pouch are, at the same time, more highly differentiated, an especially striking feature being the anlage of the thymus, which is now a blind epithelial tube all but equal in length to the entire pouch itself. Simultaneous with these changes along the pharyngeal edge, the depth of the cervical sinus, the future cervical vesicle, is being constricted off. Its cervicobranchial duct still maintains the connection between it and the ectoderm however.

It will be remembered that the hypoglossal pre-muscle masses were the first muscular elements to be observed in the preceding stage. To these this stage now adds pre-muscle densities on the medial aspects of the mandibular, facial and glossopharyngeal nerves, which are the nerves associated with the first three visceral arches. A tubular mesenchymal density surrounding the esophagus hints at the laying-down of muscular tissue there as well.

In the following stage which covers embryos of sizes up to 12 mm. G.L., these densities will be observed to have taken on a more definitive character; and similar ones will be added of which the primordia of the pharyngeal musculature - since they become part of the pharyngeal wall - are of particular interest.

STAGE 8

(9 - 12 mm. G.L.)

This stage is represented by six embryos from three litters as follows:

Embryo	D 31	8.6 mm. G.L.	litter 7 d
Embryo	CC 3	10.0 mm. G.L.	litter 7 d
Embryo	CC 4	9.6 mm. G.L.	litter 7 d
Embryo	D 13	-	litter 7
Embryo	D 11	11.0 mm. G.L.	litter 6 a
Embryo	D 36	-	litter 6 a

Due to their arriving in a mutilated condition, no measurements can be given for embryos D 13 and D 36.

Fig. IV.76. Canine embryo D 31 (8.6 mm. G.L.).

Number of embryo:	D 31	Date of processing:	March 23, 1961
Size before fixation:	-	Block:	Paraffin
Size after fixation:	8.6 mm. G.L.	Stain:	H. & E.
Age:	-	Plane of section:	Transverse
Position on uterus:	-	Thickness of section:	10 microns
Size of loculus:	-	Series made:	D 31 (1 - 22)
Fixed in:	10% Form./Bouin's Fluid		
Stored in:	70% Alcohol		

From litter:		7 d
Number of embryos in litter:		8
DAM	Breed:	Boxer
	Age:	-
	Weight:	45 lb.
SIRE	Breed:	Mixed Breed
	Age:	-
	Weight:	-
Number of services:		1
Date of 1st service:		-
Date of 2nd service:		-

Date of collection: August 25, 1960

At time of collection Dam was alive (x), dead ( ).

Embryos collected hour(s) after death.

Litter received from: Dr. D. O'Connor, Stouffville, Ontario.

Remarks:

Fig. IV.77. Canine embryo CC 3 (10.0 mm. G.L.).

Number of embryo:	CC 3	Date of processing:	February 12, 1961
Size before fixation:	-	Block:	Paraffin
Size after fixation:	10.0 mm. G.L.	Stain:	H. & E.
Age:	-	Plane of section:	Sagittal
Position on uterus:	-	Thickness of section:	10 microns
Size of loculus:	-	Series made:	CC 3 (1 - 18)
Fixed in:	10% Form./Bouin's Fluid		
Stored in:	70% Alcohol		

From litter:		7 d
Number of embryos in litter:		8
DAM	Breed:	Boxer
	Age:	-
	Weight:	45 lb.
SIRE	Breed:	Mixed Breed
	Age:	-
	Weight:	-
Number of services:		1
Date of 1st service:		-
Date of 2nd service:		-

Date of collection: August 25, 1960

At time of collection Dam was alive (X), dead ( ).

Embryos collected hour(s) after death.

Litter received from: Dr. D. O'Connor, Stouffville, Ontario.

Remarks:

Fig. IV.78. Canine embryo CC 4 (9.6 mm. G.L.).

Number of embryo:	CC 4	Date of processing:	February 12, 1961
Size before fixation:	-	Block:	Paraffin
Size after fixation:	9.6 mm. G.L.	Stain:	H. & E.
Age:	-	Plane of section:	Transverse
Position on uterus:	-	Thickness of section:	10 microns
Size of loculus:	-	Series made:	CC 4 (1 - 28)
Fixed in:	10% Form./Bouin's Fluid		
Stored in:	70% Alcohol		

From litter:		7 d
Number of embryos in litter:		8
DAM	Breed:	Boxer
	Age:	-
	Weight:	45 lb.
SIRE	Breed:	Mixed Breed
	Age:	-
	Weight:	-
Number of services:		1
Date of 1st service:		-
Date of 2nd service:		-

Date of collection: August 25, 1960

At time of collection Dam was alive (X), dead ( ).

Embryos collected hour(s) after death.

Litter received from: Dr. D. O'Connor, Stouffville, Ontario.

Remarks:

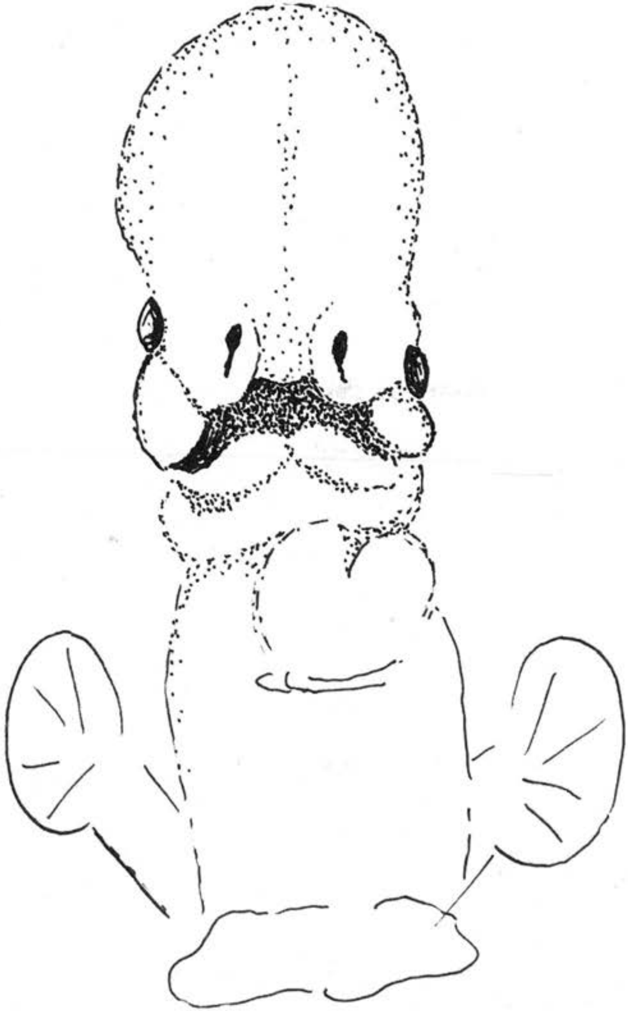


Fig. IV.79. Sketch of head-heart segment of canine embryo D 13.

Number of embryo:	D 13	Date of processing:	August 10, 1960
Size before fixation:	-	Block:	Paraffin
Size after fixation:	-	Stain:	H. & E.
Age:	22 days (?)	Plane of section:	Sagittal
Position on uterus:	-	Thickness of section:	10 microns
Size of loculus:	-	Series made:	D 13 (1 - 16)
Fixed in:	Bouin's Fluid		
Stored in:	70% Alcohol		

From litter:		7
Number of embryos in litter:		1
DAM	Breed:	-
	Age:	-
	Weight:	-
SIRE	Breed:	-
	Age:	-
	Weight:	-
Number of services:		2
Date of 1st service:		April 22, 1960
Date of 2nd service:		April 24, 1960
Date of collection:		May 14, 1960

At time of collection Dam was alive ( ), dead (X).

Embryos collected 2 hour(s) after death.

Litter received from: Edinburgh Clinic

Remarks:

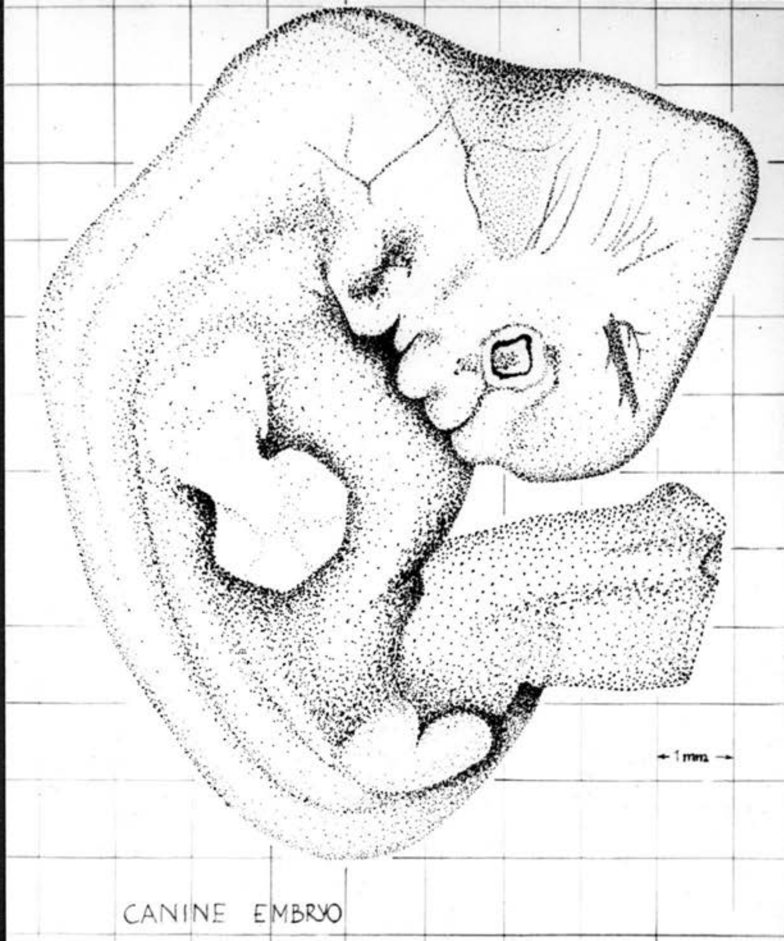


Fig. IV.80. Canine embryo D 11 (11.0 mm. G.L.).

Number of embryo:	**D 11**	Date of processing:	**September 24, 1960**
Size before fixation:	**-**	Block:	**Paraffin**
Size after fixation:	**11.0 mm. G.L.**	Stain:	**H. & E.**
Age:	**26 days**	Plane of section:	**Sagittal**
Position on uterus:	**-**	Thickness of section:	**10 microns**
Size of loculus:	**38 x 26 mm.**	Series made:	**D 11 (1 - 22)**
Fixed in:	**Bouin's Fluid**		
Stored in:	**70% Alcohol**		

From litter:		**6 a**
Number of embryos in litter:		**5 (2 were partially disintegrated)**
DAM	Breed:	**Collie**
	Age:	**2 years**
	Weight:	**20 lb.**
SIRE	Breed:	**-**
	Age:	**-**
	Weight:	**20 - 25 lb.**
Number of services:		**1**
Date of 1st service:		**June 16, 1960**
Date of 2nd service:		**-**

Date of collection: **July 12, 1960**

At time of collection Dam was alive ( ), dead (**X**).

Embryos collected **1** hour(s) after death.

Litter received from: **Edinburgh Clinic**

Remarks:

Number of embryo:	D 36	Date of processing:	March 23, 1961
Size before fixation:	-	Block:	Paraffin
Size after fixation:	-	Stain:	H. & E.
Age:	26 days	Plane of section:	Transverse
Position on uterus:	-	Thickness of section:	10 microns
Size of loculus:	38 x 26 mm.	Series made:	D 36 (1 - 26)
Fixed in:	Bouin's Fluid		
Stored in:	70% Alcohol		

From litter:		6 a
Number of embryos in litter:		5 (2 were partially disintegrated)
DAM	Breed:	Collie
	Age:	2 years
	Weight:	20 lb.
SIRE	Breed:	-
	Age:	-
	Weight:	20 - 25 lb.
Number of services:		1
Date of 1st service:		June 16, 1960
Date of 2nd service:		-

Date of collection: July 12, 1960

At time of collection Dam was alive ( ), dead (X).

Embryos collected 1 hour(s) after death.

Litter received from: Edinburgh Clinic

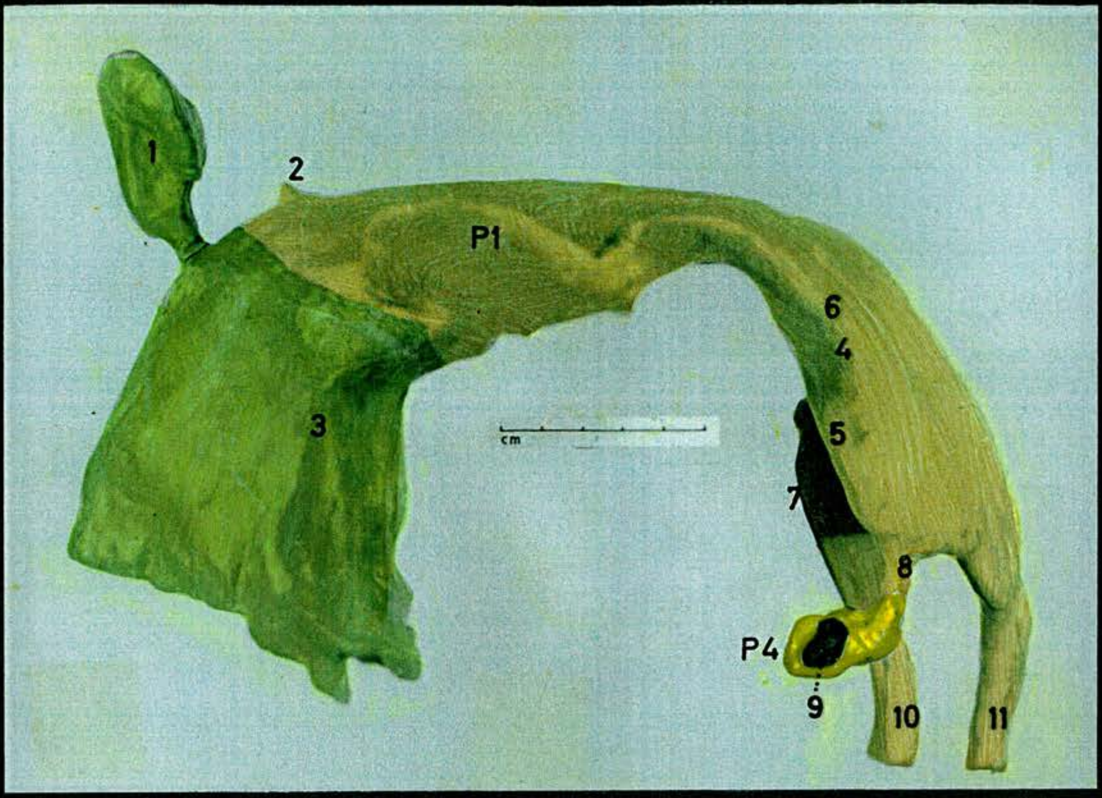
Remarks: For a picture see that of litter mate D 11 (Fig. IV.80.)

The Shape of the Pharynx

The flattened pharyngeal tube, although having continued to enlarge, has not undergone significant changes when compared to that of the previous stage. In embryo D 11, the largest of the present group, the long axis from Rathke's pocket to the esophageal entrance measures 2.3 mm. The transverse measurements are 2 mm. just anterior to pouch I, and 1 mm. at the level of pouch IV, when measured along the dorsal convexity of the roof. Throughout the range of this group, the pharyngeal recess at the cervical flexure of the pharynx is decreasing in size, it actually having disappeared in specimen D 11 (Fig. IV.81.). Concurrent with this regression in growth, the lateral edges of the vertical portion of the pharynx are bending ventrally, thereby causing the hitherto flat roof of this portion to take on a convex appearance, which caps, as it were, the upward-pushing anterior components of the developing larynx (Fig. IV.81.). It is perhaps not by chance that these two processes coincide, because from a purely mechanical point of view one seems to aid the other. At the cephalic flexure a small vestigial protuberance in the midline is all that remains of Seessel's pocket in the larger embryos of this stage (2) (Fig. IV.90./5). The hollow stalk connecting Rathke's pocket (2) with the pharyngeal mucosa has contracted even further and at its narrowest diameter measures 50 microns. In this group of embryos, the tongue (8) makes its first

Fig. IV.81. Wax model of the left half of the pharyngeal tube, embryo D 11 (11.0 mm. G.L.), (model X 100), lateral view.

P1, P4 Pharyngeal pouches I, IV; 1 Rathke's pocket; 2 Seessel's pocket; 3 Lateral edge of oral cavity; 4 Lateral edge of pharynx; 5 Secondary lateral edge of pharynx; 6 Previous position of pouch II; 7 Larynx; 8 Pharyngobranchial duct IV; 9 Parathyroid IV; 10 Trachea; 11 Esophagus.



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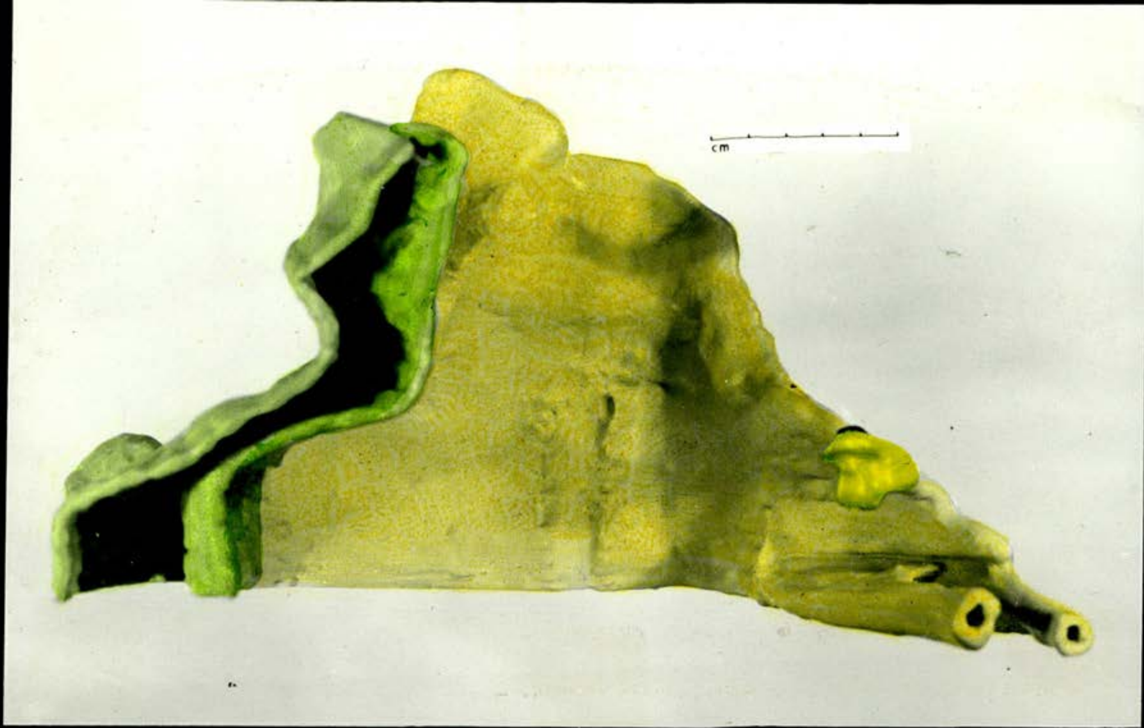


Fig. IV.82. Wax model of the left half of the pharyngeal tube, embryo D 11 (11.0 mm. G.L.), (model X 100), ventral view.

P1, P4 Pharyngeal pouches I, IV; 1 Rathke's pocket; 2 Position of lateral palatine processes anterior to mouth; 3 Mouth; 4 Ridge on the ventral surface of the pharyngeal floor, marking the boundary between the first and second visceral arches; 5 Similar ridge marking the boundary between the second and third visceral arches; 6 Previous position of pouch II; 7 Ultimobranchial body; 8 Parathyroid IV; 9 Trachea; 10 Esophagus.

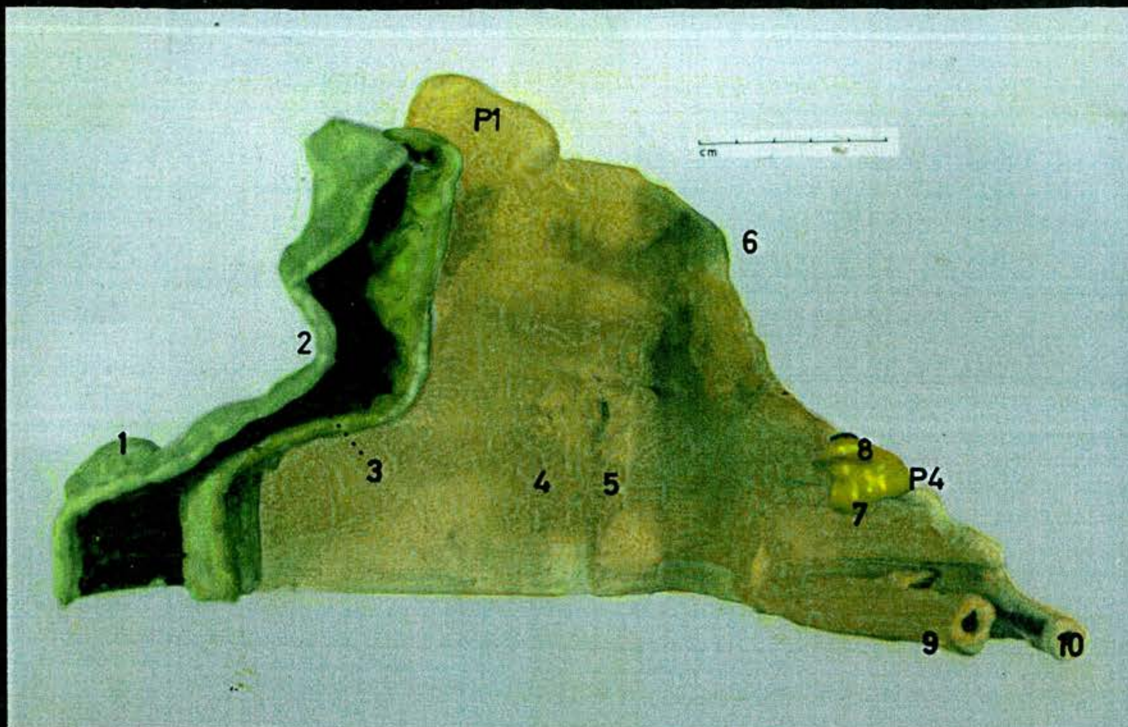


Fig. IV.82. Wax model of the left half of the pharyngeal tube, embryo D 11 (11.0 mm. G.L.), (model X 100), ventral view.

P1, P4 Pharyngeal pouches I, IV; 1 Rathke's pocket; 2 Position of lateral palatine processes anterior to mouth; 3 Mouth; 4 Ridge on the ventral surface of the pharyngeal floor, marking the boundary between the first and second visceral arches; 5 Similar ridge marking the boundary between the second and third visceral arches; 6 Previous position of pouch II; 7 Ultimobranchial body; 8 Parathyroid IV; 9 Trachea; 10 Esophagus.

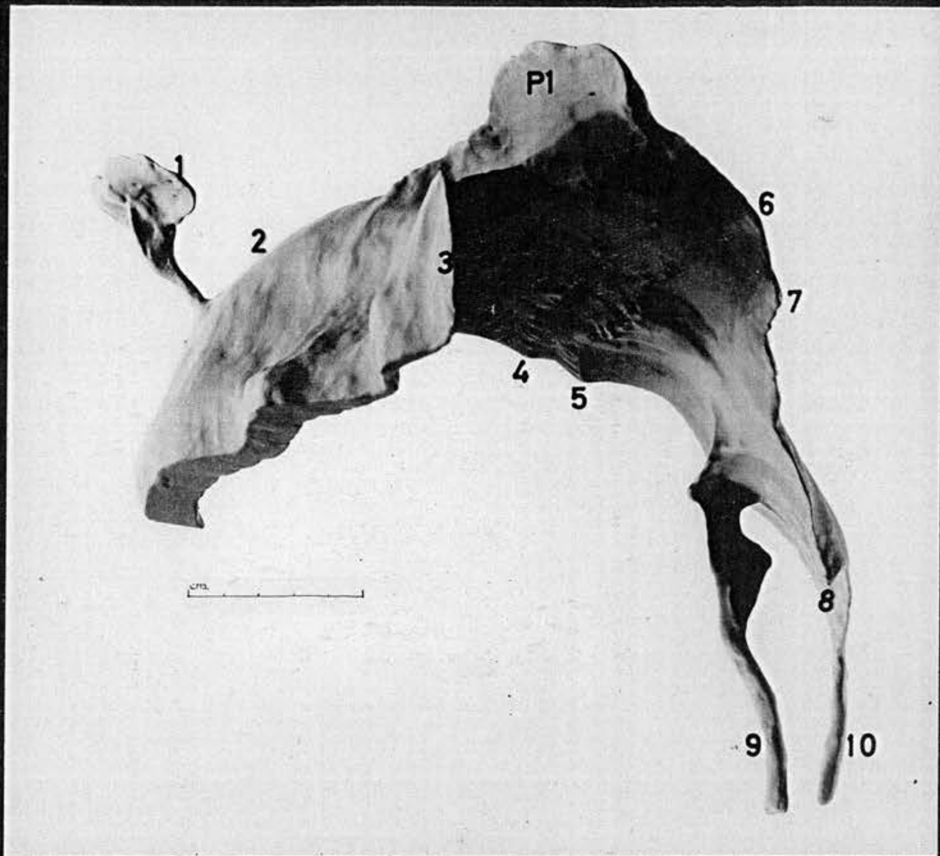
distinct appearance on the floor of the pharynx. In the area underlying the cephalic flexure, the body of the primitive tongue initiates a relatively rapid dorsal growth, which, as it meets the non-yielding hypophysial region, pushes the lower jaw ventrally. At the same time, the anterior portion of the tongue lifts from the hitherto smooth floor, thus creating a semicircular groove (6) which circumscribes the periphery of the lingual anlage. The groove commences immediately inferior to either first pouches, then courses ventrally and anteromedially to converge with its fellow in the midline roughly 350 microns below the stalk of Rathke's pocket (embryo D 11). It continues to undercut the primitive tongue, and thus forms the anterior part of the floor of the mouth cavity. The two transverse grooves, noted in previous stages to have originated from the ventral diverticula of pouches I and II, persist yet in the centre of the horizontal portion of the floor (Figs. IV.82./4, 5; IV.83./4, 5).

Referring back to the ventral aspects of those models made for the preceding two stages (Figs. IV.54.; IV.69.), the mouth can be noted as a more or less straight transverse opening. In the present stage however, the transverse mouth opening is being moulded by the appearance of two longitudinal swellings which, situated anterior to the mouth, push caudally to gain a position on either side of the tongue. Such swellings represent the primordial lateral palatine processes, which subsequently become so well defined (Fig. IV.82./2).

Pouch I is now most prominent on the lateral border of the pharynx as a wing-like extension, which has inclined slightly upwards. From below it is triangular in outline (Fig. IV.82./P1). Its apex no longer makes contact with the ectoderm, although a depression is still persistent on the lateral side of the head where contact previously occurred. This depression is, of course, the remnant of the first visceral cleft and is destined to form the external opening of the ear in the adult (Fig. IV.89./9, 10). Proceeding caudally along the lateral border of the pharynx, the former position of pouch II can be distinguished only by a slight bulge situated within the apex of the obtuse angle, prescribed by the pharyngeal border in this region (Fig. IV.82./6); the strand-like connection between this point and the ectoderm, seen in earlier embryos, has disintegrated, along with the cell cords, observed in the previous stage to be associated within this pouch. The interval along the edge of the pharynx between the previous position of pouch II and pouch III has increased in length and acquired special significance in that it has become partly double-edged (Figs. IV.81./4, 5; IV.87./7, 8). Until now, the lateral pharyngeal edge separating the two pouches has consisted of a single epithelial fold. Now, however, a second lateral fold has appeared a short distance below and parallel to the original (Fig. IV.81./5). It starts

Fig. IV.83. Wax-model cast of the left half of pharyngeal lumen, embryo D 11 (11.0 mm. G.L.), (model X 100), anterolateral view.

P1 Pharyngeal pouch I (tubotympanic recess); 1 Rathke's pocket; 2 Seessel's pocket; 3 Lateral edge of oral cavity; 4 Furrow in pharyngeal floor, marking boundary between first and second visceral arches; 5 Similar furrow indicating boundary between second and third visceral arches; 6 Lateral edge of pharyngeal cavity; 7 Previous position of pouch II; 8 Position of pouch IV; 9 Trachea; 10 Esophagus.



<u>Fig. IV.83</u>. Wax-model cast of the left half of pharyngeal lumen, embryo D 11 (11.0 mm. G.L.), (model X 100), anterolateral view.

P1 Pharyngeal pouch I (tubotympanic recess); 1 Rathke's pocket; 2 Seessel's pocket; 3 Lateral edge of oral cavity; 4 Furrow in pharyngeal floor, marking boundary between first and second visceral arches; 5 Similar furrow indicating boundary between second and third visceral arches; 6 Lateral edge of pharyngeal cavity; 7 Previous position of pouch II; 8 Position of pouch IV; 9 Trachea; 10 Esophagus.

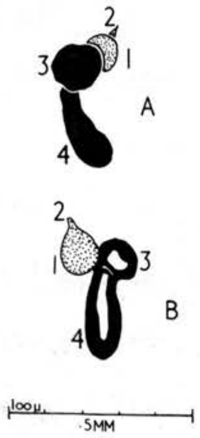


Fig. IV.84. Left complex III, (A) posterior view, (B) lateral view in section, embryo D 11 (11.0 mm. G.L.).

1 Parathyroid III; 2 Recently severed connection with pharynx; 3 Cervical vesicle; 4 Thymus.

at the junction of middle and distal thirds between the two pouches, soon becoming the more prominent of the two, before continuing caudally as the true lateral pharyngeal edge from which pouches III and IV are suspended (Fig. IV.86.). For its part, the first fold blends with the dorsal pharyngeal surface before reaching the level of the third pouch.

The third pouch is in process of being constricted off from the pharyngeal tube in this group of embryos, thus becoming the free-floating complex III (Fig. IV.86./P3). In embryos D 13, D 31 and CC 4, the connection between pouch and pharynx is now reduced to a mere strand of cells, which are arranged radially around a potential lumen. In the more developed specimens, however, the cord, being quite solid, can now be termed pharyngo-branchial stalk III (Fig. IV.85./5). The arrangement of the derivatives of pouch III approximates more or less that already observed. Thymus III is the same hollow, finger-like projection, though now its diameter has increased slightly, and it has elongated in a medial and ventral direction (Fig. IV.84./4). Parathyroid III is situated on the anterodorsal aspect of the complex, from which it is progressively freeing itself (1) (Fig. IV.85/7). It is from the parathyroid component of the complex that the connection with the pharynx is maintained until eventual separation (Fig. IV.84./2). The cervical vesicle (3) is joined caudally and laterally to parathyroid III and the proximal pole of thymus III, its former connection with the ectoderm has disintegrated. Inside its diminishing lumen, a cell cord, similar to those noted in the previous stage to be associated with the second pouch, was found to be present in embryo D 31. In the same specimen, the entire complex has an overall length of roughly 250 microns. It has severed all contact with the pharynx in embryos D 11 and CC 3, leaving but a transitory epithelial projection on the lateral edge where the break occurred.

Pouch IV (complex IV) appears as a hollow, club-shaped structure which maintains connection with the pharyngeal tube in all specimens of this group (Fig. IV.86./P4). In the less advanced embryos, this connection is tubular and can thus be termed pharyngo-branchial duct IV (Fig. IV.85./2). Through continued constriction this duct has become a cell cord in embryos CC 3 and D 11. At the same time, the minimum distance in the latter specimen between complex IV and the thyroid plates, which have migrated ventrally in company with the aortic sac, has become reduced to only 120 microns, presaging the integration of the two structures, as soon as the complex has freed itself from the pharynx. Differentiation between individual elements of the complex is now much easier, because the cells of parathyroid IV have increased sufficiently to force themselves away from the confines of the pouch wall, forming the raised parathyroid anlage on the anterolateral face of the pouch (Fig. IV.81./9). The tissues of the pouch below the parathyroid, which are tubular in some and saccular in other specimens, make up the ultimobranchial body (Fig. IV.85./3); whereas such tissues above the parathyroid bud still represent the remains of the undifferentiated pouch IV proper and are rapidly being absorbed into the complex.

The Structure of the Pharyngeal Wall

It becomes increasingly difficult in the 9 - 12 mm. embryo to regard the pharyngeal epithelium as the sole component of the wall of the pharynx. Already in previous stages certain subepithelial mesenchymal densities were recorded, which gave indication that the forerunner of the muscular envelope about the pharyngeal tube was soon to establish itself. With regard to the pharyngeal epithelium no changes which have not been previously noted can be observed in this group of embryos. Thus, the description of the epithelium in the previous stage may be consulted. Were one to examine the subepithelial tissues more critically however, significant changes, especially below the epithelium of the pharyngeal floor can be observed. From that stage in the embryonic life-cycle at which the foregut differentiated into the characteristic pouch-bearing pharynx, it has been noticed

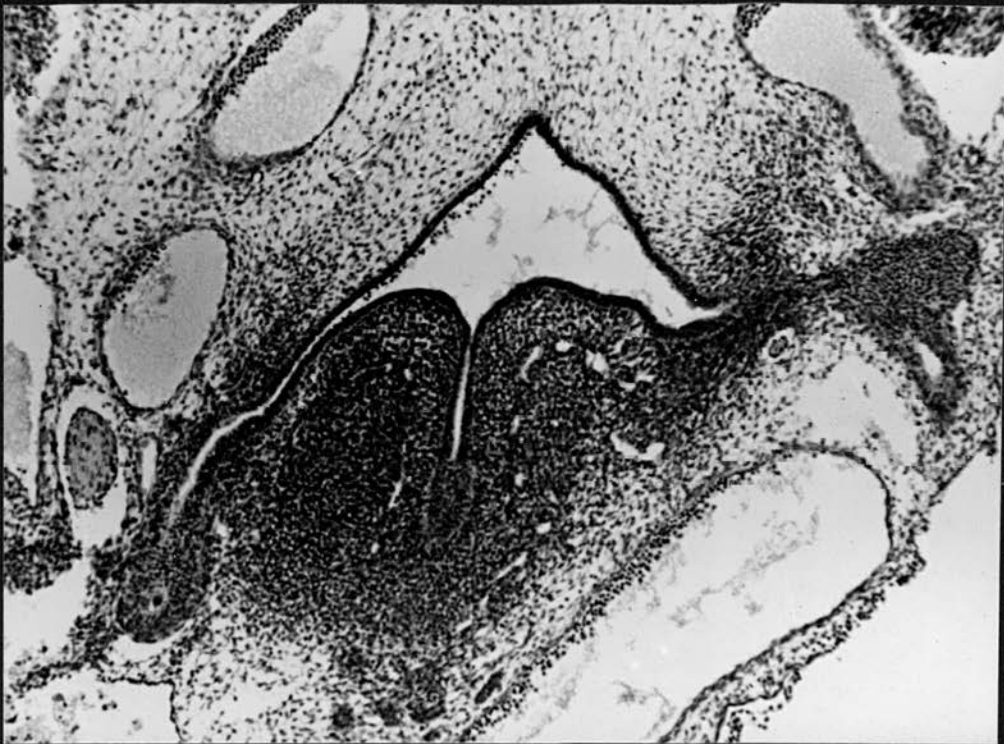
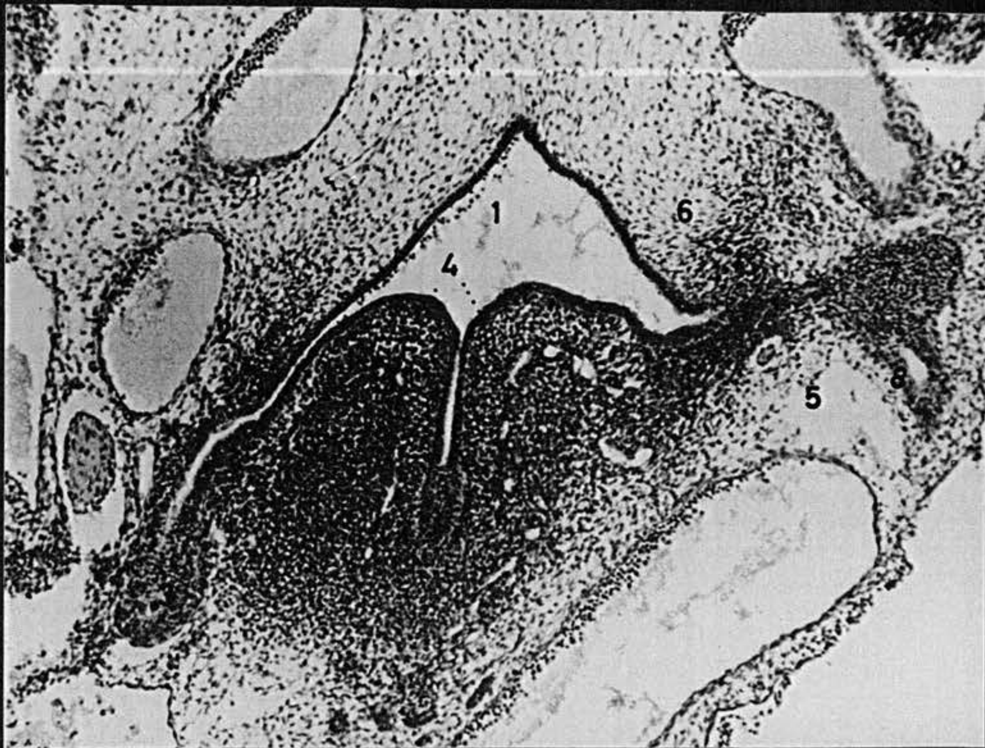


Fig. IV.85. Transverse section of posterior pharynx, passing through complex IV on one side and through complex III on the other, embryo D 36, (X 100).

1 Pharynx; 2 Pharyngobranchial duct IV; 3 Ultimobranchial body; 4 Arytenoid swelling; 5 Pharyngobranchial stalk III; 6 Primordium of pharyngeal musculature; 7 Parathyroid III; 8 Thymus III.



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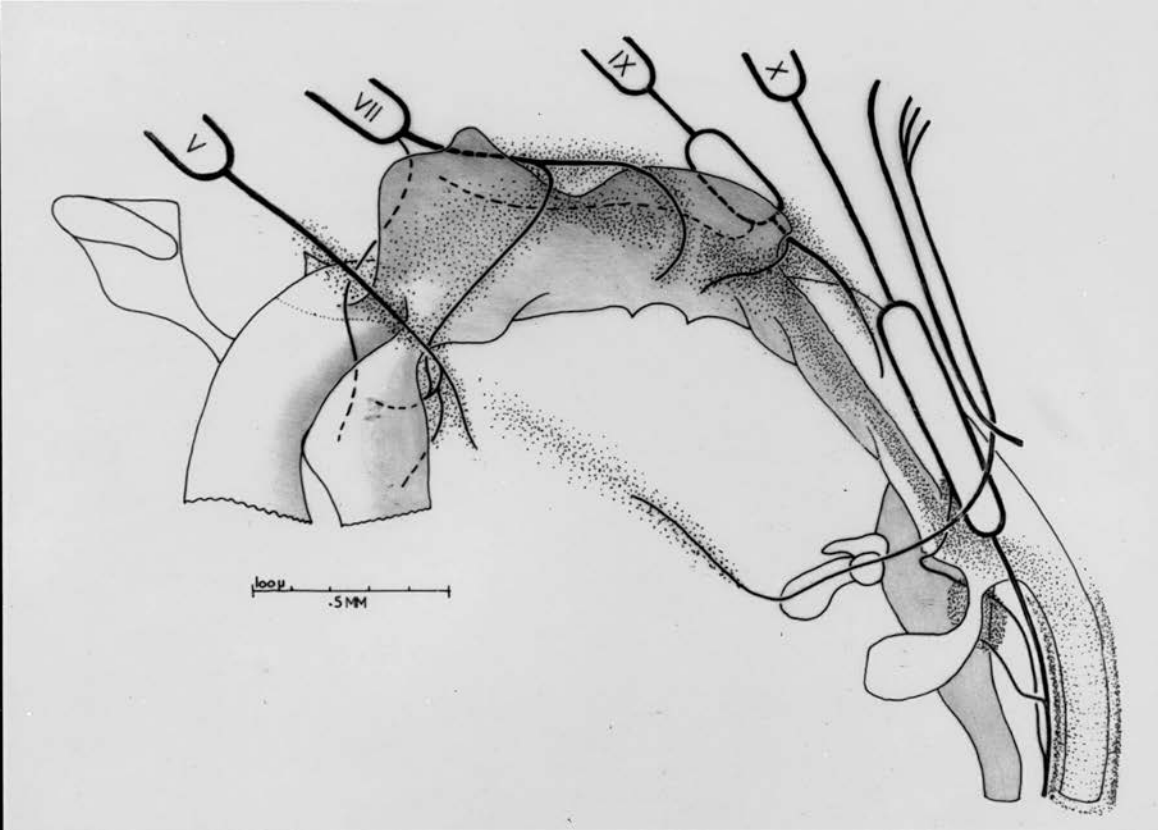
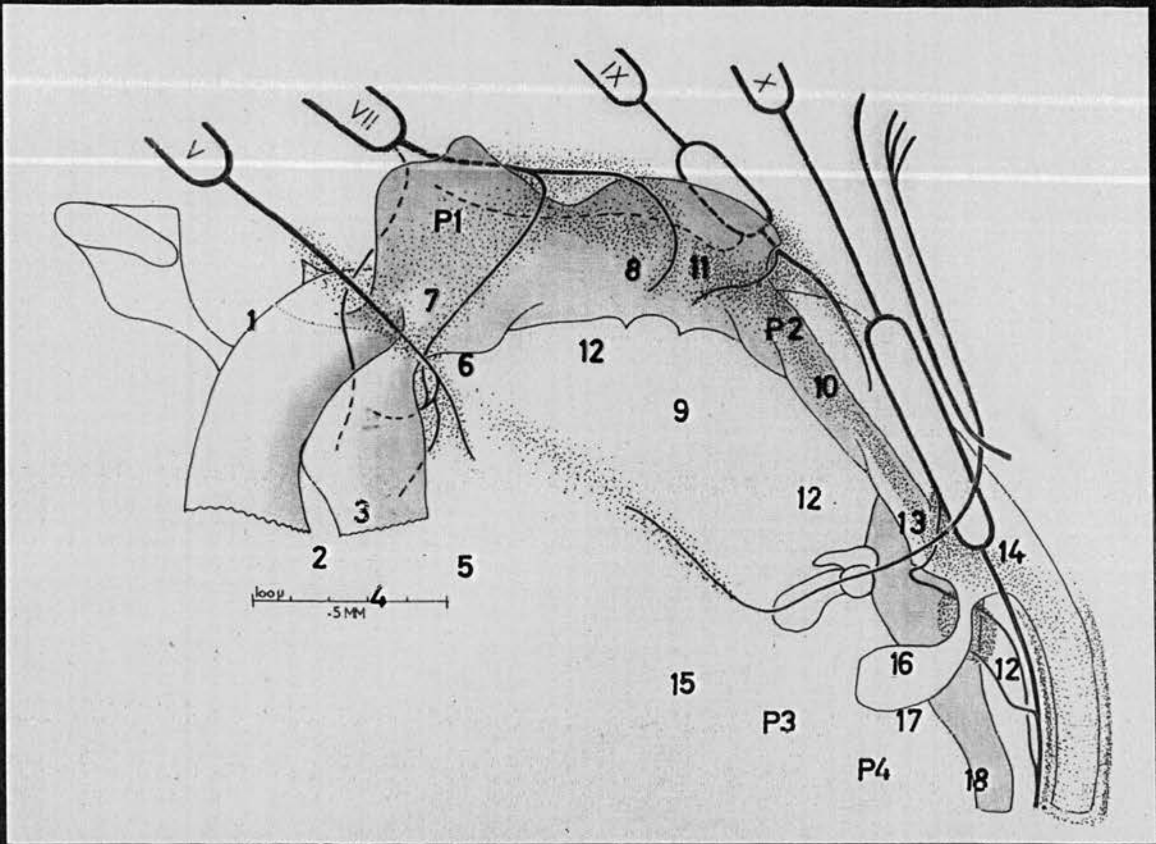


Fig. IV.86. Lateral view of pharyngeal tube with associated cranial nerves, ganglia and pre-muscle masses, 9 - 12 mm. stage, (schematic).

P1, P3, P4 Pharyngeal pouches I, III, IV; P2 Former site of pouch II; V, VII, IX, X Cranial nerves of equivalent serial number; 1 Mandibular nerve; 2 Greater superficial petrosal nerve; 3 Lingual nerve; 4 Mandibular alveolar nerve; 5 Mylohyoid nerve; 6 Chorda tympani nerve; 7 Tympanic nerve; 8 Facial nerve; 9 Glossal branch of glossopharyngeal nerve; 10 Pharyngeal branch of glossopharyngeal nerve; 11 Petrosal ganglion; 12 Cord-like mesenchymal concentration; 13 Nodose ganglion; 14 Spinal accessory nerve; 15 Hypoglossal nerve and hypoglossal pre-muscle mass; 16 Anterior laryngeal nerve; 17 Laryngeal pre-muscle mass; 18 Posterior laryngeal (recurrent) nerve; 19 Trachea; 20 Esophagus, surrounded by pre-muscle tube.



<u>Fig. IV.86</u>. Lateral view of pharyngeal tube with associated cranial nerves, ganglia and pre-muscle masses, 9 - 12 mm. stage, (schematic).

P1, P3, P4 Pharyngeal pouches I, III, IV; P2 Former site of pouch II; V, VII, IX, X Cranial nerves of equivalent serial number; 1 Mandibular nerve; 2 Greater superficial petrosal nerve; 3 Lingual nerve; 4 Mandibular alveolar nerve; 5 Mylohyoid nerve; 6 Chorda tympani nerve; 7 Tympanic nerve; 8 Facial nerve; 9 Glossal branch of glossopharyngeal nerve; 10 Pharyngeal branch of glossopharyngeal nerve; 11 Petrosal ganglion; 12 Cord-like mesenchymal concentration; 13 Nodose ganglion; 14 Spinal accessory nerve; 15 Hypoglossal nerve and hypoglossal pre-muscle mass; 16 Anterior laryngeal nerve; 17 Laryngeal pre-muscle mass; 18 Posterior laryngeal (recurrent) nerve; 19 Trachea; 20 Esophagus, surrounded by pre-muscle tube.

that the mesenchymal cushion which underlies the epithelium of the floor is of a much more dense nature than its counterpart overlying the roof. The general homogeneous density, however, is now showing evidence of becoming organised. This is characterised by the appearance of a cord-like concentration of mesenchyme which accompanies the lateral edge of the pharynx in its entire length(Fig.IV.86./12). It starts rather indistinctly below the first pouch by an equally indistinct branch from the dense mesenchyme that surrounds the mandibular nerve(1). (Such mesenchymal concentrations, it will be remembered, were for the first time seen about and chiefly medial to the course of the facial nerve in the 8 - 10 mm. stage; in the present stage however, they have become a regular feature.) This unorganised concentration of mesenchyme becomes transformed into a well-defined,cord-like structure toward the caudal extremity of the first pouch(tubotympanic recess), receiving similar tissue from the mesenchymal condensations found on the medial aspects of both Chorda tympani(6) and facial nerves(8). Where the caudally directed angle of the pharygeal edge indicates the former site of the second pouch, the cell cord thickens considerably in diameter to surround the glossal branch of the glossopharyngeal nerve(9) as it crosses the lateral edge of the pharynx immediately posterior to the angle referred to above. Thereafter, it blends again with the mesenchymal concentration surrounding the glossopharyngeal nerve (10). The cell cord maintains its situation at a level well below that of the lateral pharyngeal margin, pursuing a posteromedial course as far as the space between the previous points of attachment of pouches II and III. Here, it changes to a position above the pharyngeal edge, the transition being accomplished by its passing between the peculiar double folds, previously described, into which the lateral edge of the pharynx is thrown (Fig. IV.87./6, 7, 8). Soon afterwards, it blends with the condensation pursuing the course of the anterior laryngeal nerve (Fig. IV.86./16) which crosses the edge of the pharynx posterior to the attachment of the third pouch. Its final course is to follow on the dorsal aspect of the lateral pharyngeal edge until its eventual confluence with the developing muscular envelope of the esophagus (20). Thus, by way of recapitulation, the mesenchymal cell cord in point crosses the first and second pouches ventrally, then changes to a dorsal position to cross the pharyngo-branchial ducts III and IV on their dorsal aspects. The changeover is chanelled between the two lateral folds that occur in the margin of the pharynx between the previous sites of pouches II and III. The caudal portion of the cord can be said to be the primordium of the pharyngeal musculature (Fig. IV.91./6).

The esophageal wall in the 9 - 12 mm. embryo consists of high stratified columnar epithelium. This is succeeded by a narrow layer of loose undifferentiated

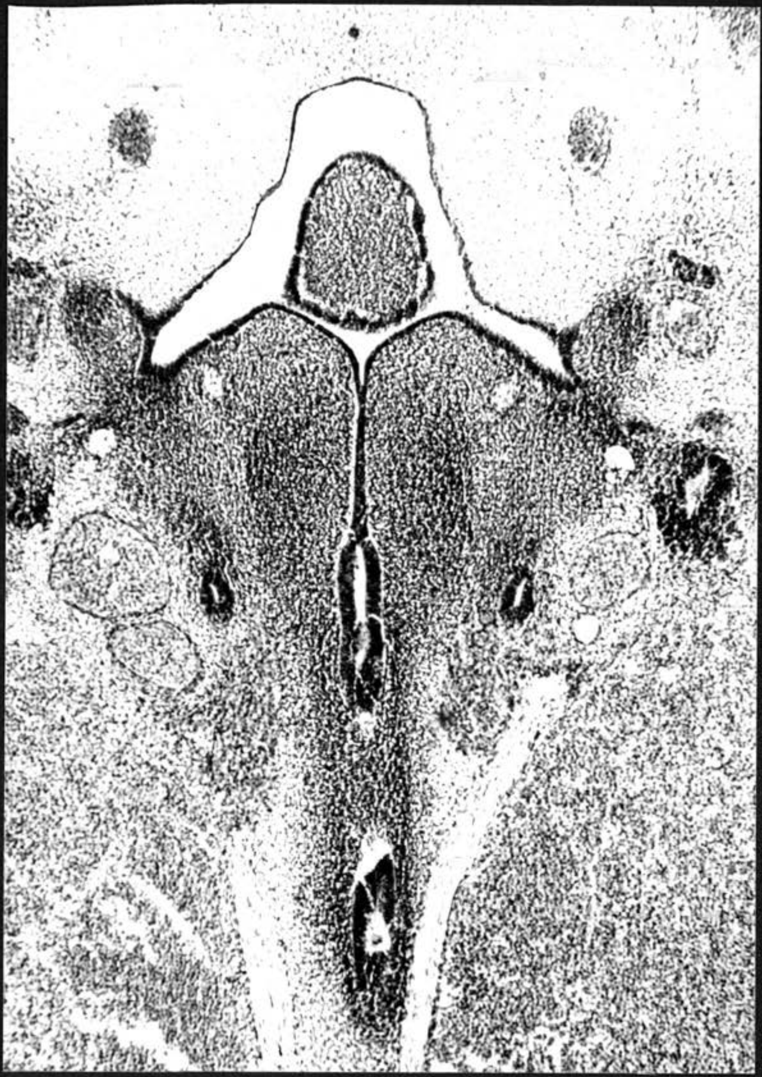
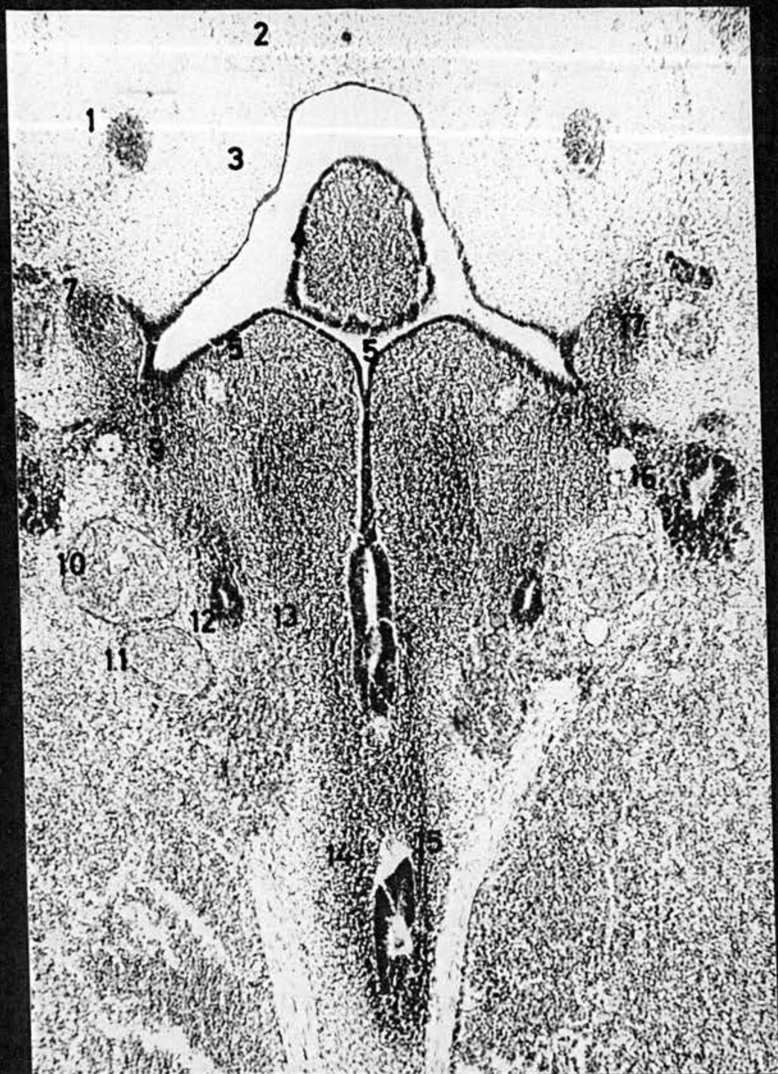


Fig. IV.87. Transverse section of posterior pharynx, embryo D 31 (8.6 mm. G.L.), (X 80).

1 Dorsal aorta; 2 Notochord; 3 Pharynx; 4 Epiglottic swelling; 5 Arytenoid swelling; 6 Cord-like density between double-edged pharynx; 7 Dorsal edge of double-edged pharynx; 8 Ventral edge of double-edged pharynx; 9 Anterior laryngeal nerve; 10 Aortic arch IV; 11 Aortic arch VI; 12 Ultimobranchial body; 13 Trachea; 14 Esophagus; 15 Vagus; 16 Cervical vesicle; 17 Aortic arch III.



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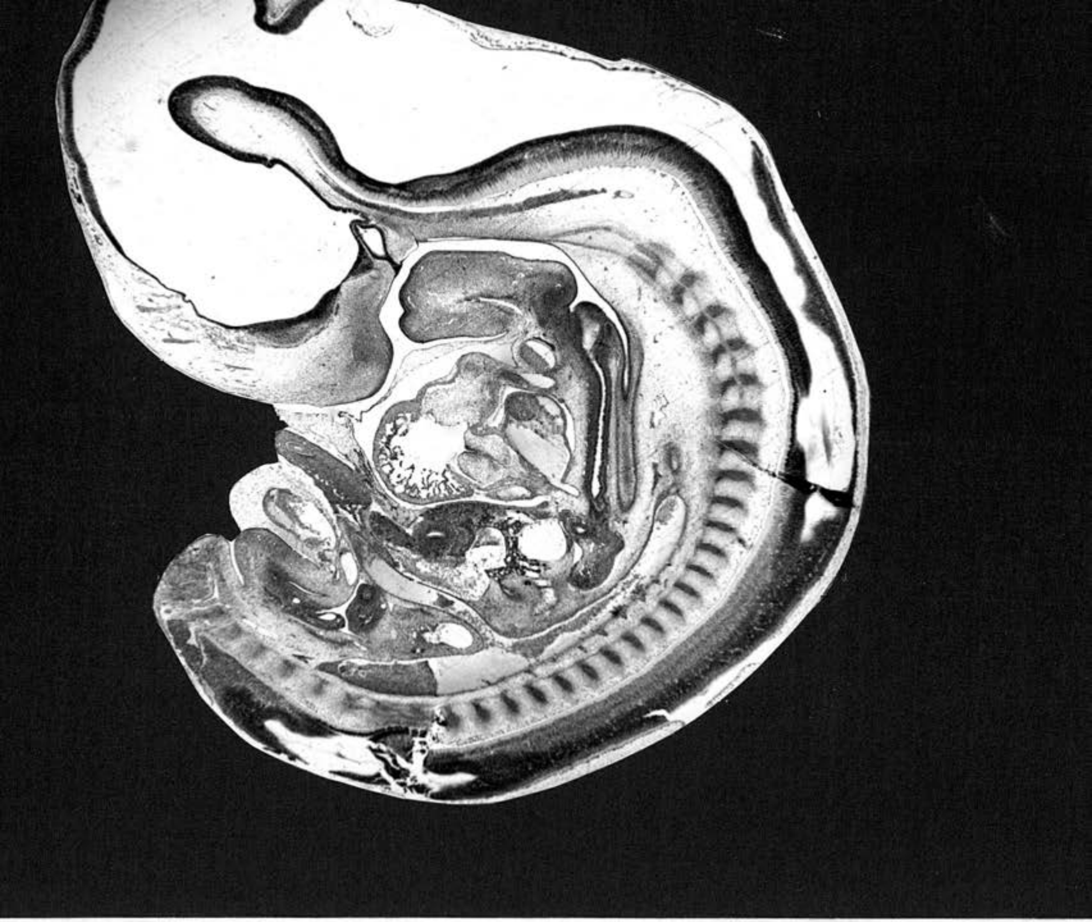
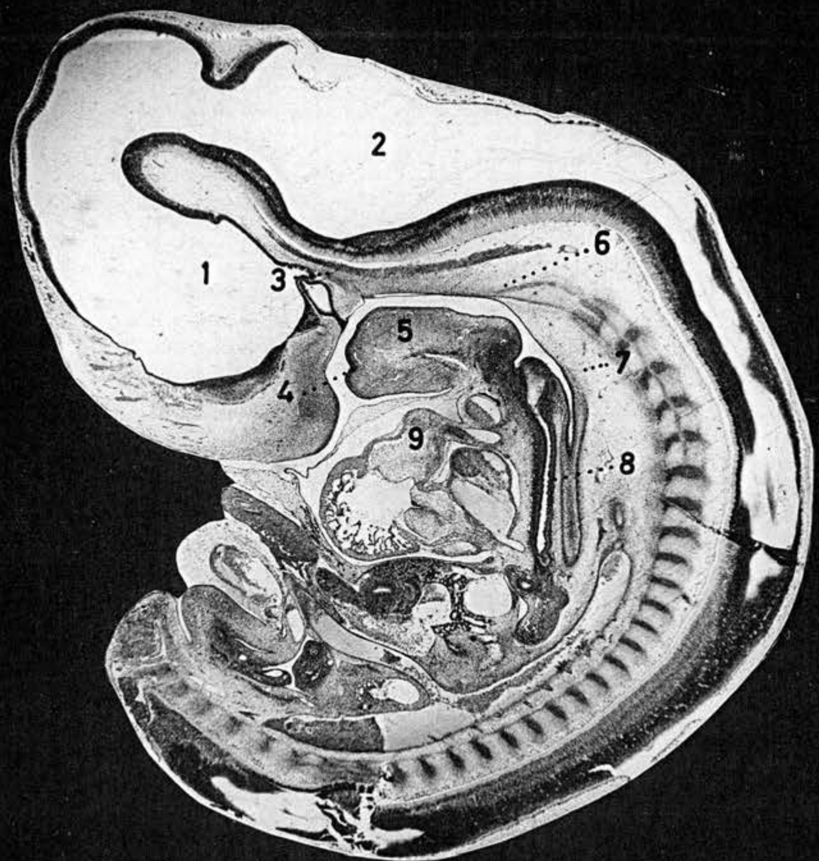


Fig. IV.88. Midsagittal section of embryo D 11 (11.0 mm. G.L.) to show position of pharynx within the embryo, (X 10).

1 Forebrain; 2 Hindbrain; 3 Rathke's pocket; 4 Mouth;
5 Primitive mandible with developing tongue; 6 Pharynx;
7 Oesophagus; 8 Trachea; 9 Heart



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1 Forebrain; 2 Hindbrain; 3 Rathke's pocket; 4 Mouth; 5 Primitive mandible with developing tongue; 6 Pharynx; 7 Esophagus; 8 Trachea; 9 Heart.

mesenchyme, about which is arranged a thin layer of denser pre-muscle tissue, the cytoplasmic elements of which have increased to the extent of balancing in amount the nuclear components (Fig. IV.88./7).

The Relationships of the Pharynx

The straight notochord overlies the flat horizontal portion of the pharynx in the median plane (Fig. IV.90./11), making contact with the dorsal surface of the pharyngeal epithelium only in embryo D 13, where the two structures come together anterior to the thyroid level. In embryo D 11 the notochord is interrupted between the thyroid level and Seessel's pocket, in which space no trace of it can be found. (A similar interruption was observed in embryo D 17 (Fig. IV.49.)). The short anterior portion of the notochord is closely applied to the dorsal surface of Seessel's pocket, from where it sweeps upwards and forwards to terminate behind the body of Rathke's pocket without however making contact with it. The caudal end of the short anterior portion is globular in contrast to the cranial end of the long posterior portion, which is thin and drawn out. The head chorda is invested by an increasing amount of mesenchyme, which arranges itself in concentric layers. This is especially noticeable anteriorly, where the chorda lifts away from the pharyngeal roof (Fig. IV.90./3). At the cervical flexure of the

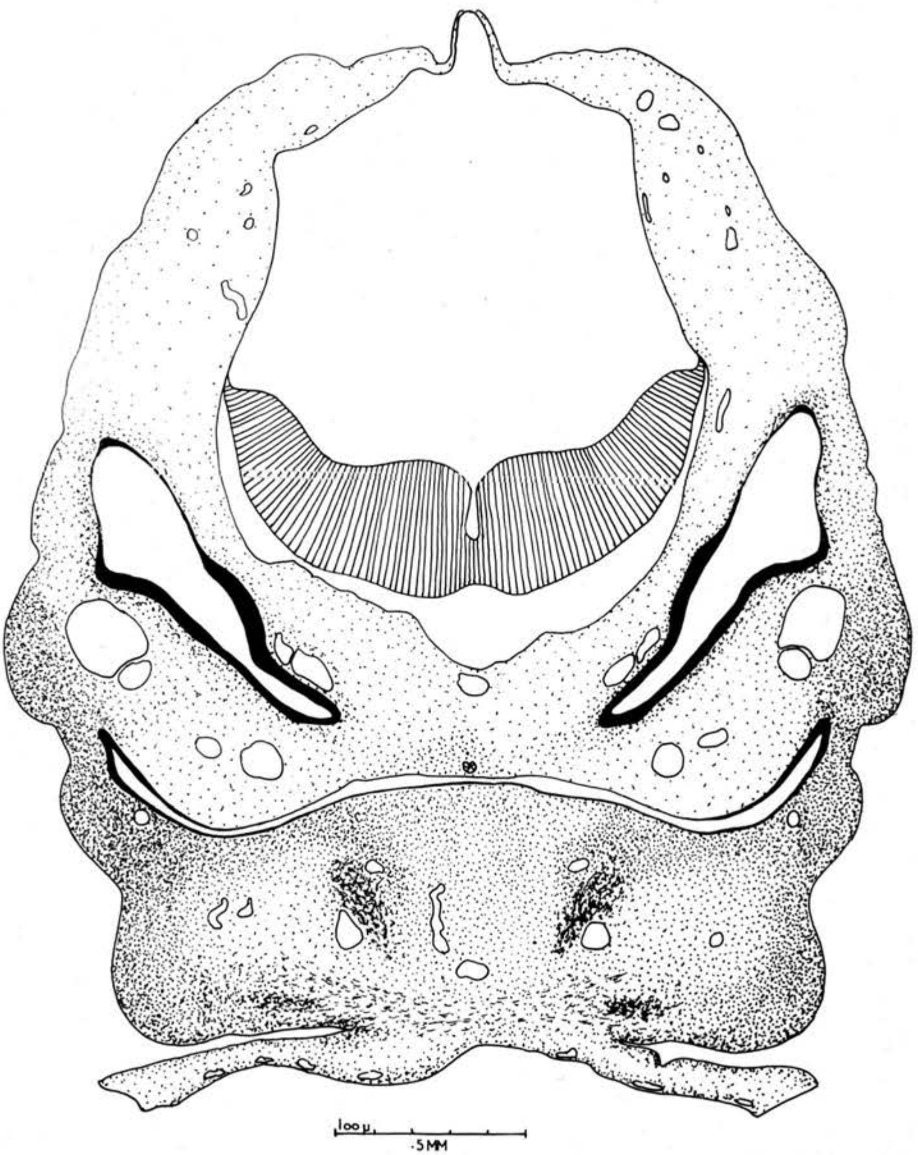
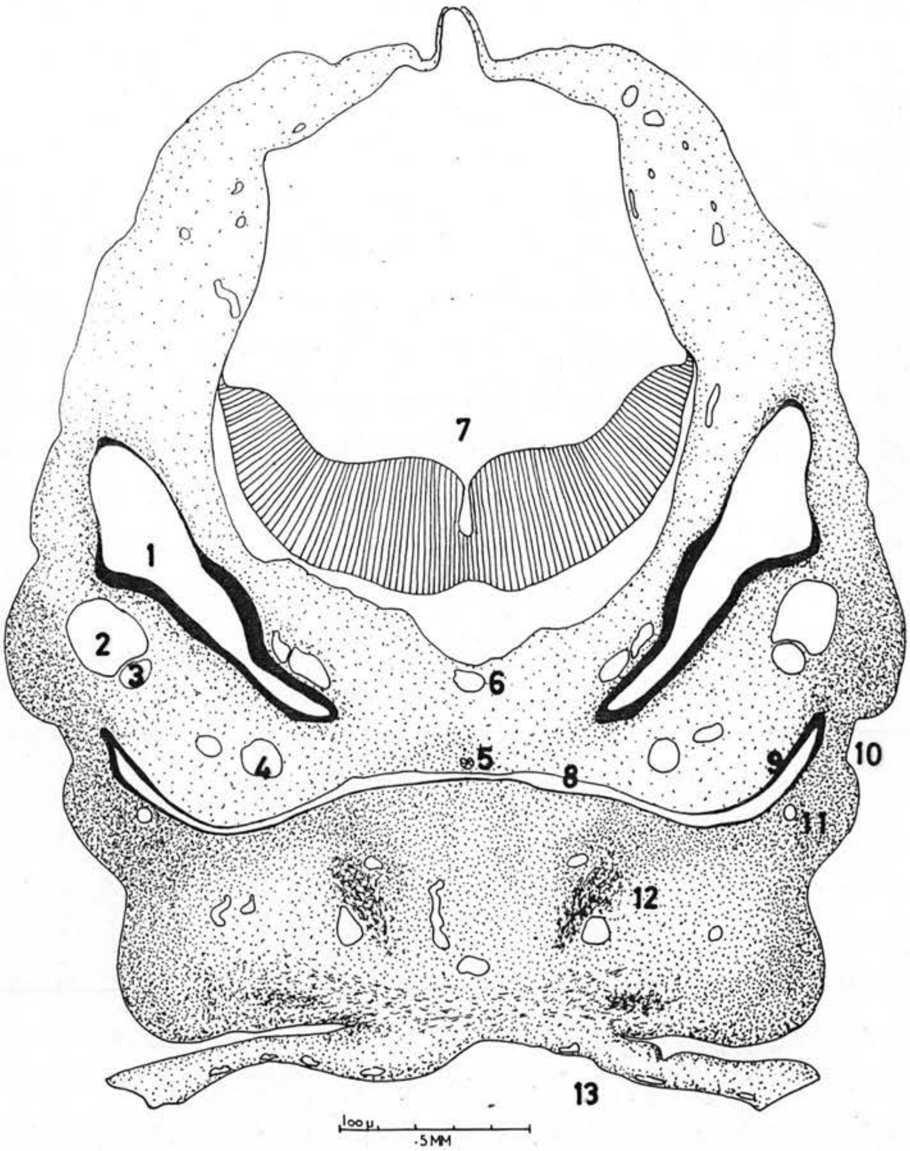


Fig. IV.89. Transverse section of the head at the level of pouch I, embryo D 31 (8.6 mm. G.L.), (semischematic).

1 Otic vesicle; 2 Anterior cardinal vein; 3 Facial nerve; 4 Dorsal aorta; 5 Notochord; 6 Basilar artery; 7 Hindbrain; 8 Pharynx; 9 Pouch I; 10 Visceral cleft I; 11 Chorda tympani nerve; 12 Hypoglossal muscle mass, and below, the hypoglossal nerve; 13 Heart.



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1 Otic vesicle; 2 Anterior cardinal vein; 3 Facial nerve; 4 Dorsal aorta; 5 Notochord; 6 Basilar artery; 7 Hindbrain; 8 Pharynx; 9 Pouch I; 10 Visceral cleft I; 11 Chorda tympani nerve; 12 Hypoglossal muscle mass, and below, the hypoglossal nerve; 13 Heart.

Fig. IV.90. Median section of pharyngeal region, embryo CC 3 (10.0 mm. G.L.), (semischematic).

1 Forebrain; 2 Rathke's pocket; 3 Dense mesenchyme stretching from pharynx into the space between forebrain and hindbrain; 4 Hindbrain; 5 Seessel's pocket; 6 Groove surrounding periphery of developing tongue; 7 Mouth; 8 Tongue; 9 Pharynx; 10 Basilar artery; 11 Notochord; 12 Dense mesenchyme stretching from pharynx to vertebra; 13 Developing cervical vertebrae; 14 Larynx; 15 Aortic sac associated dorsally with thyroid primordium; 16 Heart; 17 Trachea; 18 Esophagus.

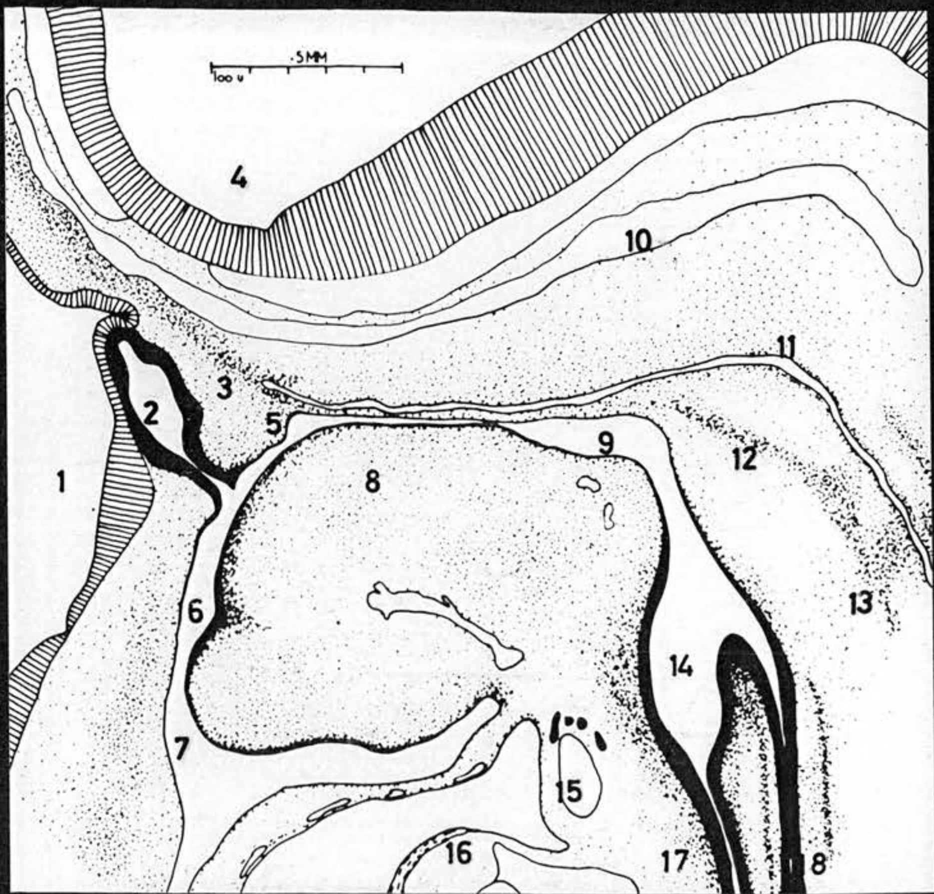

Fig. IV.90. Median section of pharyngeal region, embryo CC 3 (10.0 mm. G.L.), (semischematic).

1 Forebrain; 2 Rathke's pocket; 3 Dense mesenchyme stretching from pharynx into the space between forebrain and hindbrain; 4 Hindbrain; 5 Seessel's pocket; 6 Groove surrounding periphery of developing tongue; 7 Mouth; 8 Tongue; 9 Pharynx; 10 Basilar artery; 11 Notochord; 12 Dense mesenchyme stretching from pharynx to vertebra; 13 Developing cervical vertebrae; 14 Larynx; 15 Aortic sac associated dorsally with thyroid primordium; 16 Heart; 17 Trachea; 18 Esophagus.

pharynx, now by virtue of the disappearance of the pharyngeal recess less distinct, the head chorda parts company with the roof, and, sweeping slightly upwards at first, gains the centre of the developing cervical vertebrae in an even downward curve (11). Below, a distinct band of concentrated mesenchyme (12) accompanies the chorda, extending from the cervical flexure on the pharyngeal roof to the inferior aspect of the cervical vertebrae, without however prescribing the characteristic upward-swing of the notochord. This band of tissue, as well as its counterpart surrounding the anterior portion of the chorda and continuing upwards and forwards beyond the level of the developing hypophysis, still help impart a typical stretched appearance to the dorsum of the pharynx (Fig. IV.88.). On either side of the head chorda, the dorsal aortae (Fig. IV.89./4), following the lateral edges of the pharynx, receive at this particular developmental stage the third, fourth and sixth aortic arches, of which the fourth pair is the thickest by far, the two remaining arches being of roughly equal calibre. Those sections of dorsal aortae between the entries of the third and fourth arches have continued to diminish in diameter to the extent that they are represented by only thin connective tissue strands without apparent lumina. The right fourth and sixth aortic arches have a smaller diameter than their fellows on the left side, the sixth arch especially becoming very narrow just before it joins the right dorsal aorta. No important changes have taken place with regard to both peripheral ganglia and nerves, forming dorsal relationships with the pharynx, other than the greater superficial petrosal nerve having appeared on the geniculate ganglion to run downwards and forwards and terminate in the swellings of the future palatine processes (Fig. IV.86./2). A schematic illustration of the nerves and ganglia associated with the pharynx at this stage, incidentally, is appended in Fig. IV.86.

With the gradual disappearance of the branchial bars on the lateral aspect of the face, and the filling of the branchial clefts, it can be expected that those tissues bordering the lateral aspect of the pharynx have become much more uniform. In fact, were it not for the persistence of structures previously contained by the branchial arches, it would be quite impossible to determine their anterior and posterior boundaries now. For this reason it will be more advantageous in succeeding chapters, when describing the lateral pharyngeal relationships to include only such structures as have retained intimate association with the pharynx. Foremost among these are the branches of the mandibular (Fig. IV.86./V.), facial (VII.), glossopharyngeal (IX), the vagus (X) and hypoglossal (15) nerves, most of which pass the sharp lateral edge of the pharyngeal tube. Further mesenchymal concentrations, at this time indistinguishable from the pre-muscle masses occurring both lateral and ventral to the pharynx, can be noted chiefly on the medial aspects of the mandibular, facial, glossopharyngeal and anterior laryngeal nerves, as the latter cross the lateral pharyngeal border. Such condensations are the fore-runners of the cartilaginous branchial skeleton. That portion following the mandibular nerve assumes an anterior, medial and ventral course, after it has passed the lateral border of the pharynx, and comes to lie in the middle of the three terminal branches formed by the mandibular nerve at this stage. The first of these branches is the lingual nerve (3), which, once it has received the Chorda tympani (6) from the facial nerve trunk, continues forwards and upwards to enter from below the now slightly raised tongue swelling. The second branch is the mandibular alveolar (4) and the third the mylohyoid nerve (5). The dense mesenchyme following the trunk of the facial nerve has already been observed in embryos of the 8 - 10 mm. stage. In the present group it has proliferated further and now extends beyond the length of the facial nerve, assuming a medial course, once having crossed the pharyngeal border. Below the pharynx, contact is made with a transversely positioned bar of mesenchyme, which in all probability will prove to be the anlage of the basi-hyoid. The mesenchyme accompanying the glossopharyngeal nerve is of the same length as the nerve, reaching only the border of the pharynx. Finally, that associated with the anterior laryngeal nerve blends with the pre-muscle

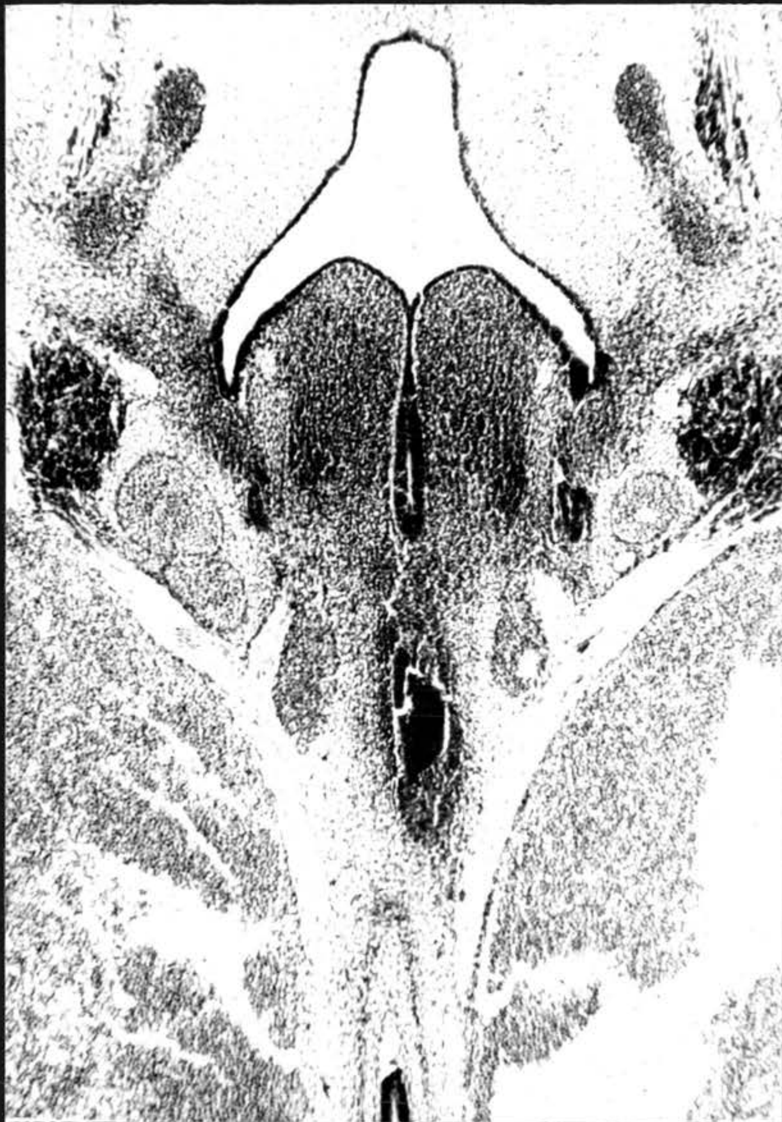

Fig. IV.91. Transverse section of posterior pharynx, about 50 microns posterior to that of Fig. IV.87., embryo D 31 (8.6 mm. G.L.), (X 80).

1 Pharynx; 2 Dorsal aorta being joined by aortic arch III; 3 Aortic arch III; 4 Arytenoid swelling; 5 Nodose ganglion; 6 Primordium of pharyngeal musculature; 7 Anterior laryngeal nerve; 8 Aortic arch IV; 9 Aortic arch VI; 10 Vagus; 11 Posterior laryngeal nerve (recurrent), note its position below the pulmonary arch artery!; 12 Trachea; 13 Pouch IV.

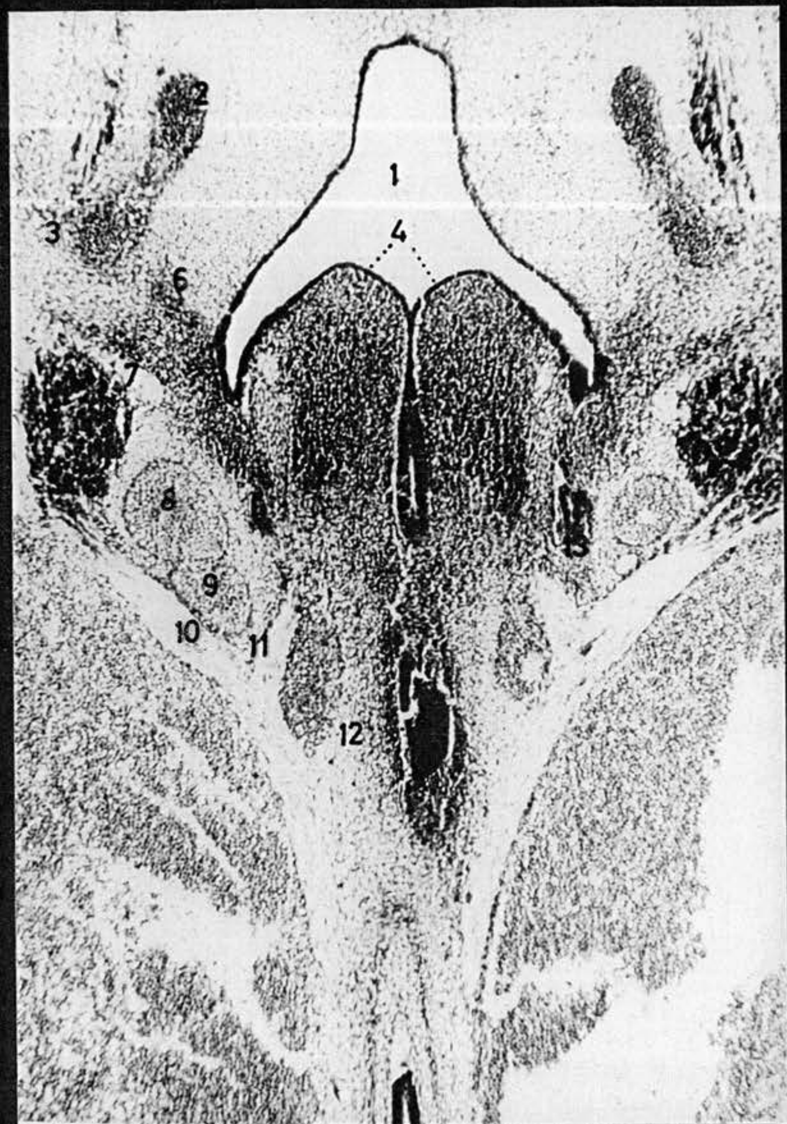

Fig. IV.91. Transverse section of posterior pharynx, about 50 microns posterior to that of Fig. IV.87., embryo D 31 (8.6 mm. G.L.), (X 80).

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mass of the pharyngeal musculature. Somewhat lateral to this region, the hypoglossal nerve (15) crosses the side of the pharynx until, having passed the lateral face of thymus III, it gains the heart bulge. From here it turns medially and anteriorly and enters the primitive jaw where it terminates within the hypoglossal pre-muscle mass previously described (Fig. IV.89./12). Small numbers of light-red staining, spindle-shaped, cytoplasmic elements have appeared in this mass foreshadowing the formation of early muscle fibre.

The ventral relations of the pharynx in the 9 - 12 mm. stage consist basically of (a) a short pre-muscle mass situated in the midline at the level of the second pouch immediately anterior to the transverse bar of mesenchyme described above, (b) the aortic sac, now split into aortic and pulmonary components and found slightly below the level of the esophageal entrance (Fig. IV.88.), and (c) the proximal end of the primitive trachea with its dense envelope of mesenchymal cells (Fig. IV.91./12).

Despite the number of minor modifications, the pharyngeal tube as a whole, apart from general overall growth, has again not appreciably changed in appearance. The pharyngeal recess so characteristic of the embryonic pharynx has begun to diminish in size, but is still present in most of the members of this group to give the pharynx the stamp of immaturity (1). The tongue and the lateral palatine processes have made their appearance and leave their impressions on the anterior portions of the pharyngeal tube. Pouch III with its now very distinct and well-differentiated derivatives is migrating from the pharynx, while pouch IV is still attached to the pharyngeal edge by its pharyngobranchial duct. A system of elongated mesenchymal densities can be observed on the lateral and ventral aspects of the pharynx, which features are the forerunners of the branchial skeleton with some of the associated muscles. Most notable of these is the long cord-like density following at first on the ventral aspect of the lateral pharyngeal edge, and, after veering dorsad between the former sites of pouches II and III, continuing along its dorsal aspect. This cord blends with similar densities which are associated with the principal nerves. Its posterior portion concerns itself in later embryonic progress with the formation of the pharyngeal musculature.

In the following stage, which covers the 15 mm. embryo group, the characteristically flexed, flat pharyngeal tube, which in effect has not changed very much from that seen in the 5 mm. stage, will finally be moulded, mainly by outside influences, into a more adult-like organ.

STAGE 9

(15.0 mm. G.L.)

The description of this stage is based on seven embryos taken from two litters:

Embryo D 42	16.0 mm. G.L.	)	
Embryo CC 2	15.0 mm. G.L.	)	
Embryo D 41	14.5 mm. G.L.	)	
Embryo D 35	15.0 mm. G.L.	)	litter 8
Embryo D 9	15.0 mm. G.L.	)	
Embryo CC 1	15.5 mm. G.L.	)	
Embryo DD 1	15.0 mm. G.L.	)	litter 8 a

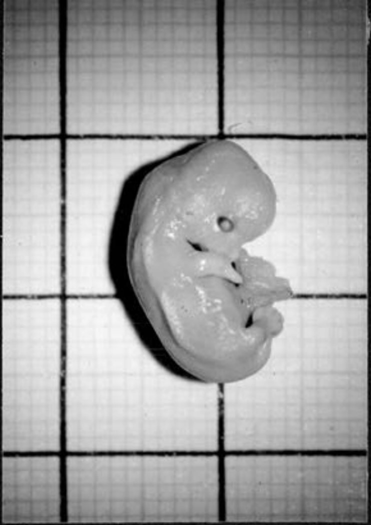


Fig. IV.92. Canine embryo D 42 (16.0 mm. G.L.).

Number of embryo:	**D 42**	Date of processing:	**April 29, 1961**
Size before fixation:	**-**	Block:	**Paraffin**
Size after fixation:	**16.0 mm. G.L.**	Stain:	**H. & E.**
Age:	**-**	Plane of section:	**Horizontal**
Position on uterus:	**-**	Thickness of section:	**10 microns**
Size of loculus:	**-**	Series made:	**D 42 (1 - 18)**
Fixed in:	**10% Form./Bouin's Fluid**		
Stored in:	**70% Alcohol**		

From litter:		**8**
Number of embryos in litter:		**9**
DAM	Breed:	**X Cocker Spaniel**
	Age:	**-**
	Weight:	**30 lb.**
SIRE	Breed:	**X Cocker Spaniel**
	Age:	**-**
	Weight:	**25 lb.**
Number of services:		**-**
Date of 1st service:		**-**
Date of 2nd service:		**-**

Date of collection: **January 18, 1960**

At time of collection Dam was alive (**X**), dead ( ).

Embryos collected hour(s) after death.

Litter received from: **McGilvray Veterinary Hospital, Toronto, Ontario.**

Remarks:

Number of embryo:	CC 2	Date of processing:	May 15, 1961
Size before fixation:	-	Block:	Paraffin
Size after fixation:	15.0 mm. G.L.	Stain:	H. & E.
Age:	-	Plane of section:	Horizontal
Position on uterus:	-	Thickness of section:	10 microns
Size of loculus:	-	Series made:	CC 2 (1 - 48)
Fixed in:	10% Form./Bouin's Fluid		
Stored in:	70% Alcohol		

From litter:		8
Number of embryos in litter:		9
DAM	Breed:	X Cocker Spaniel
	Age:	-
	Weight:	30 lb.
SIRE	Breed:	X Cocker Spaniel
	Age:	-
	Weight:	25 lb.
Number of services:		-
Date of 1st service:		-
Date of 2nd service:		-

Date of collection: January 18, 1960

At time of collection Dam was alive (X), dead ( ).

Embryos collected hour(s) after death.

Litter received from: McGilvray Veterinary Hospital, Toronto, Ontario.

Remarks: For a picture see that of litter mate D 35 (Fig.IV.94.)

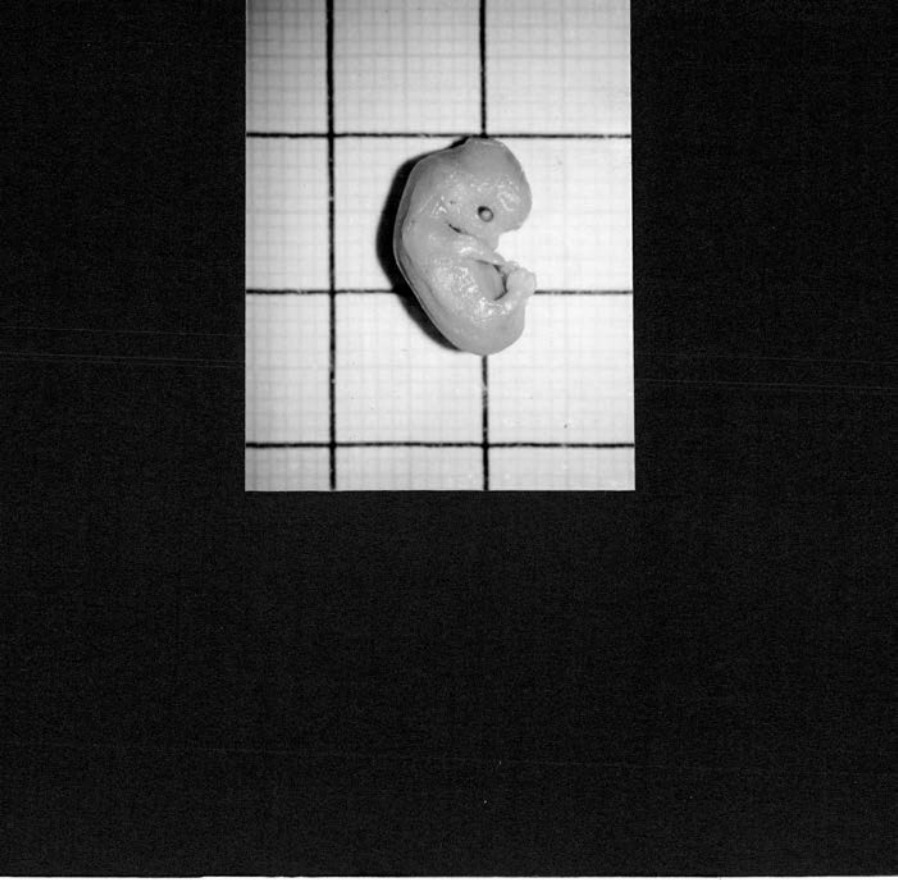


Fig. IV.93. Canine embryo D 41 (14.5 mm. G.L.).

Number of embryo:	D 41	Date of processing:	April 29, 1961
Size before fixation:	-	Block:	Paraffin
Size after fixation:	14.5 mm. G.L.	Stain:	H. & E.
Age:	-	Plane of section:	Transverse
Position on uterus:	-	Thickness of section:	10 microns
Size of loculus:	-	Series made:	D 41 (1 - 20)
Fixed in:	10% Form./Bouin's Fluid		
Stored in:	70% Alcohol		

From litter:		8
Number of embryos in litter:		9
DAM	Breed:	X Cocker Spaniel
	Age:	-
	Weight:	30 lb.
SIRE	Breed:	X Cocker Spaniel
	Age:	-
	Weight:	25 lb.
Number of services:		-
Date of 1st service:		-
Date of 2nd service:		-

Date of collection: January 18, 1960

At time of collection Dam was alive (X), dead ( ).

Embryos collected hour(s) after death.

Litter received from: McGilvray Veterinary Hospital, Toronto, Ontario.

Remarks:

Fig. IV.94. Canine embryo D 35 (15.0 mm. G.L.).

Number of embryo:	D 35	Date of processing:	May 15, 1961
Size before fixation:	-	Block:	Paraffin
Size after fixation:	15.0 mm. G.L.	Stain:	H. & E.
Age:	-	Plane of section:	Sagittal
Position on uterus:	-	Thickness of section:	10 microns
Size of loculus:	-	Series made:	D 35 (1 - 40)
Fixed in:	10% Form./Bouin's Fluid		
Stored in:	70% Alcohol		

From litter:		8
Number of embryos in litter:		9
DAM	Breed:	X Cocker Spaniel
	Age:	-
	Weight:	30 lb.
SIRE	Breed:	X Cocker Spaniel
	Age:	-
	Weight:	25 lb.
Number of services:		-
Date of 1st service:		-
Date of 2nd service:		-

Date of collection: January 18, 1960

At time of collection Dam was alive (X), dead ( ).

Embryos collected hour(s) after death.

Litter received from: McGilvray Veterinary Hospital, Toronto, Ontario.

Remarks:

Fig. IV.95. Canine embryo D 9 (15.0 mm. G.L.).

Number of embryo:	D 9	Date of processing:	August 1, 1960
Size before fixation:	-	Block:	Paraffin
Size after fixation:	15.0 mm. G.L.	Stain:	H. & E.
Age:	-	Plane of section:	Horizontal
Position on uterus:	-	Thickness of section:	10 microns
Size of loculus:	-	Series made:	D 9 (1 - 37)
Fixed in:	10% Form./Bouin's Fluid		
Stored in:	70% Alcohol		

From litter:		8
Number of embryos in litter:		9
DAM	Breed:	X Cocker Spaniel
	Age:	-
	Weight:	30 lb.
SIRE	Breed:	X Cocker Spaniel
	Age:	-
	Weight:	25 lb.
Number of services:		-
Date of 1st service:		-
Date of 2nd service:		-

Date of collection: January 18, 1960

At time of collection Dam was alive (x), dead ( ).

Embryos collected hour(s) after death.

Litter received from: McGilvray Veterinary Hospital, Toronto, Ontario.

Remarks:

Fig. IV.96. Canine embryo CC 1 (15.5 mm. G.L.).

Number of embryo:	**CC 1**	Date of processing:	**February 12, 196**
Size before fixation:	**-**	Block:	**Paraffin**
Size after fixation:	**15.5 mm. G.L.**	Stain:	**H. & E.**
Age:	**-**	Plane of section:	**Sagittal**
Position on uterus:	**-**	Thickness of section:	**10 microns**
Size of loculus:	**-**	Series made:	**CC 1 (1 - 48)**
Fixed in:	**10% Form./Bouin's Fluid**		
Stored in:	**70% Alcohol**		

From litter:		**8**
Number of embryos in litter:		**9**
DAM	Breed:	**X Cocker Spaniel**
	Age:	**-**
	Weight:	**30 lb.**
SIRE	Breed:	**X Cocker Spaniel**
	Age:	**-**
	Weight:	**25 lb.**
Number of services:		**-**
Date of 1st service:		**-**
Date of 2nd service:		**-**

Date of collection: **January 18, 1960**

At time of collection Dam was alive (**X**), dead ( ).

Embryos collected hour(s) after death.

Litter received from: **McGilvray Veterinary Hospital, Toronto, Ontario.**

Remarks:

Number of embryo:	DD 1	Date of processing:	June 16, 1961
Size before fixation:	-	Block:	-
Size after fixation:	15.0 mm. G.L.	Stain:	-
Age:	-	Plane of section:	-
Position on uterus:	-	Thickness of section:	-
Size of loculus:	-	Series made:	Used for dissection
Fixed in:	10% Form./Bouin's Fluid		
Stored in:	70% Alcohol		

From litter:		8 a
Number of embryos in litter:		7
DAM	Breed:	Springer Spaniel
	Age:	3 years
	Weight:	-
SIRE	Breed:	-
	Age:	-
	Weight:	-
Number of services:		-
Date of 1st service:		-
Date of 2nd service:		-

Date of collection: August 10, 1960

At time of collection Dam was alive ( ), dead ( ).

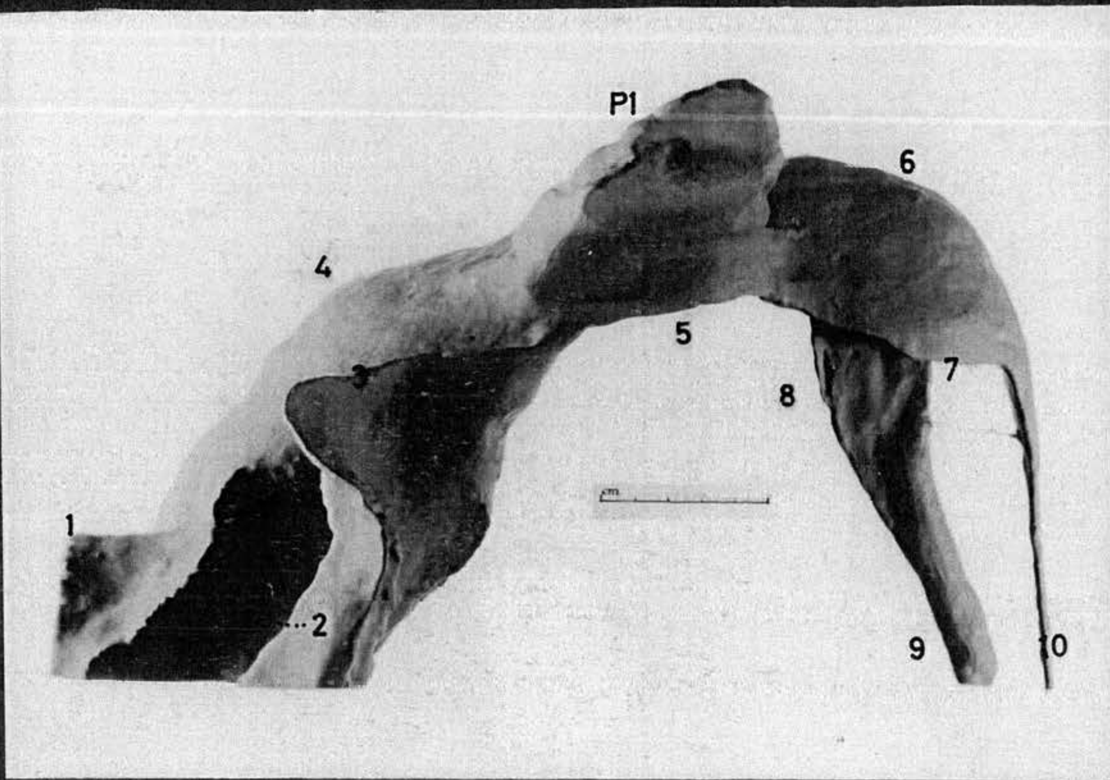
Embryos collected hour(s) after death.

Litter received from: Dr. E.A.M. Pitt, London, Ontario.

Remarks: For a photograph of the dissection see Fig.IV.121.

Fig. IV.97. Wax-model cast of the left half of the pharyngeal lumen, embryo D 9 (15 mm. G.L.), (model X 100), lateral view.

P1 Pharyngeal pouch I (tubotympanic recess); 1 Primitive choana; 2 Space occupied by lateral palatine process; 3 Lateral edge of oral cavity; 4 Cephalic flexure; 5 Newly formed caudal extension of 3; 6 Cervical flexure; 7 Original lateral edge of pharynx; 8 Larynx; 9 Trachea; 10 Esophagus.



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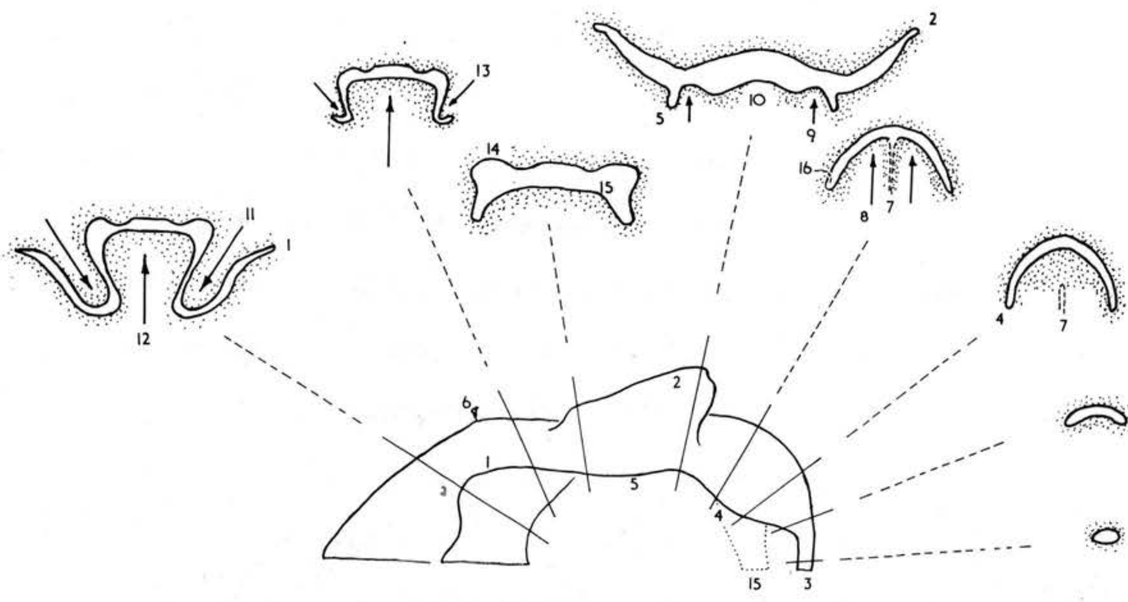
The Shape of the Pharynx

The dorsoventrally flattened, triangular pharyngeal tube, as it has been recognised since the 5 mm. stage, is gradually being moulded toward a more adult-like configuration by the growth and relative movements of the structures that surround it (Fig. IV.97.). Also the slow unbending of the embryo's long axis, facilitated perhaps in the cephalic region by the rapid forward growth of the lower jaw and the associated development of the face, have left their marks, if to a somewhat lesser degree, on the present pharyngeal pattern. Foremost among these influences are the rapidly expanding tongue and proximal portion of the larynx moving against the flat pharyngeal tube from below and transforming it into a double-walled, inverted trough (Fig. IV.98.). This trough extends from the cephalic flexure of the pharynx (6), itself now somewhat straightened by virtue of the general extension of the embryo, to the entrance into the esophagus (3). It appears fairly straight and shallow in the horizontal portion of the pharynx, before bending ventrad in an even curve at the cervical flexure. There the trough is deepest, harbouring in its interior the upper portion of the larynx (Fig. IV.97./6, 8) and assuming a shape reminiscent of that in the adult, with the exception, of course, of being bent rather than straight. Without the presence of the pharyngeal recess, this bend has lost its previous almost rectangular appearance; but the upward-



<u>Fig. IV.98.</u> Transverse sections at different levels of the pharyngeal tube at 15 mm. G.L., solid arrows indicate growth processes playing significant roles in the moulding of the primitive to more adult-like pharynx,(schematic).

1 Lateral edge of oral cavity; 2 Tubotympanic recess; 3 Esophagus; 4 Original lateral edge of pharynx; 5 Newly formed caudal extension of 1; 6 Pharyngeal hypophysis situated at cephalic flexure; 7 Entrance into larynx; 8 Arytenoid swelling; 9 Small lateral (tonsillar?) swelling on pharyngeal floor; 10 Root of tongue; 11 Lateral palatine process; 12 Body of tongue; 13 Caudal extremity of lateral palatine process; 14 Root of tubotympanic recess; 15 Floor of anterior pharynx; 16 Floor of posterior pharynx (piriform recess).

pushing epiglottic swelling, although situated in the pharyngeal floor and more rostrally placed than the pharyngeal recess, lends new emphasis to this flexure (Fig. IV.99.). Orally, the trough-like pharynx changes to the characteristic double-bent structure seen in vertebrate embryos whose lateral palatine processes have as yet not united and seem to be irreparably wedged on either side of the tongue (Figs. IV.98./11, 12; IV.121./A). Aborally, the trough-like morphology is also lost due to the contraction of the double pharyngeal wall, the posterior pharynx assuming the shape of a tube (Fig. IV.98./3). This is, of course, the esophagus which, for the first time in this study, can be noted to have migrated to the left of the median plane, where it remains throughout adult life (Fig. IV.99./22). The average pharyngeal length of the 15 mm. embryo is 1.8 mm., and the width at the first pouch is 1.0 mm. These measurements are indeed smaller than those of the 9 - 12 mm. embryos, and are due to the unbending of the head and neck flexures, and the recently accomplished turning-down of the lateral pharyngeal edges, respectively.

Rathke's pocket (3) has lost connection with the roof of the pharynx, due to the formation, and subsequent interposition between brain and horizontal pharynx, of the cartilaginous basilar plate (12). A small fraction of the connecting stalk remains however below the plate, maintaining weak contact with the pharyngeal roof. This

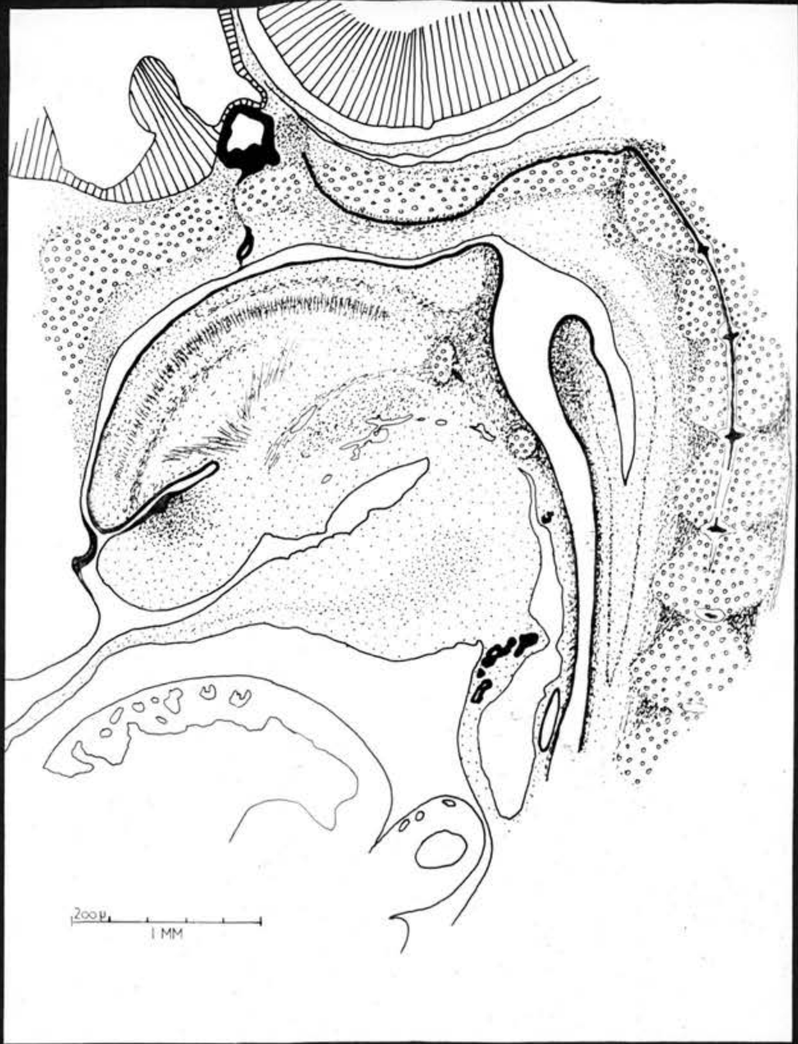
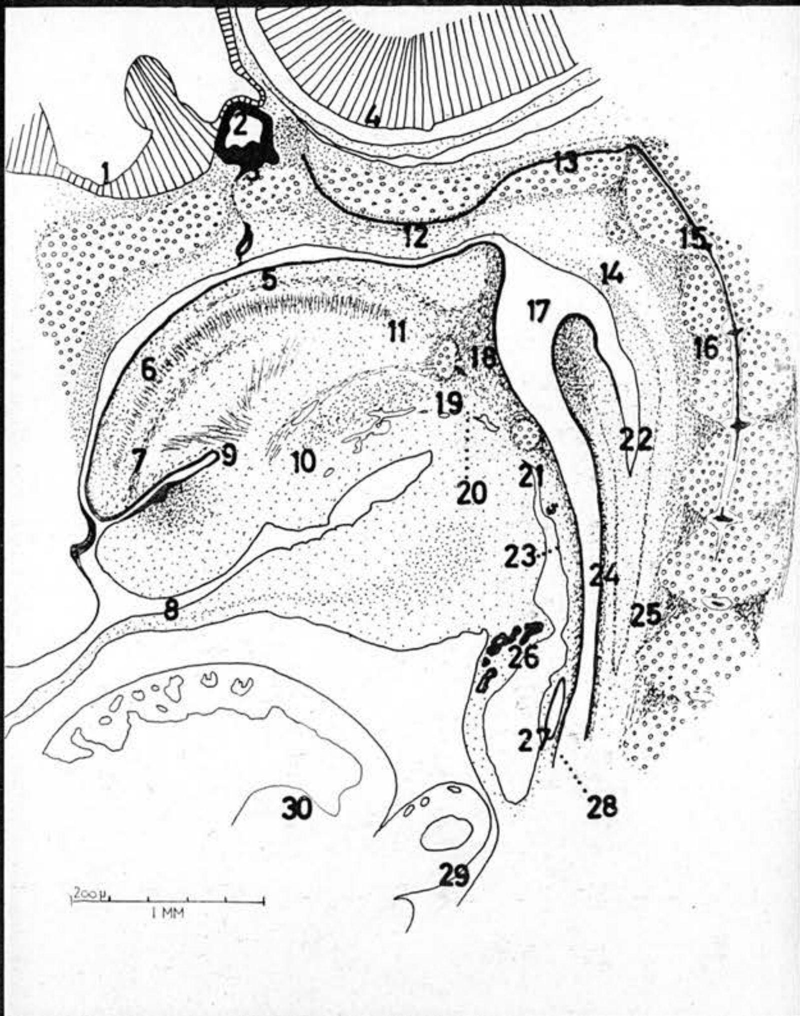


Fig. IV.99. Median section of pharyngeal region, embryo D 35, (15.0 mm. G.L.), (semischematic).

1 Forebrain; 2 Infundibulum; 3 Rathke's pocket; 4 Hindbrain; 5 Pharyngeal hypophysis; 6 Oral cavity; 7 Ventral layer of intrinsic muscles; 8 Jaw; 9 M. genioglossus; 10 M. geniohyoideus; 11 Dorsal layer of intrinsic muscles; 12 Basilar plate; 13 Basilar artery; 14 Ventral arch of atlas; 15 Notochord; 16 Centra of cervical vertebrae; 17 Pharynx; 18 M. hyoepiglotticus; 19 basihyoid; 20 Accessory thyroid tissue; 21 Cricoid cartilage; 22 Esophagus; 23 Thyroidal isthmus; 24 Trachea; 25 Muscular envelope of esophagus; 26 Thymus; 27 Internal jugular vein; 28 Right common carotid artery; 29 Left aortic arch IV; 30 Heart.



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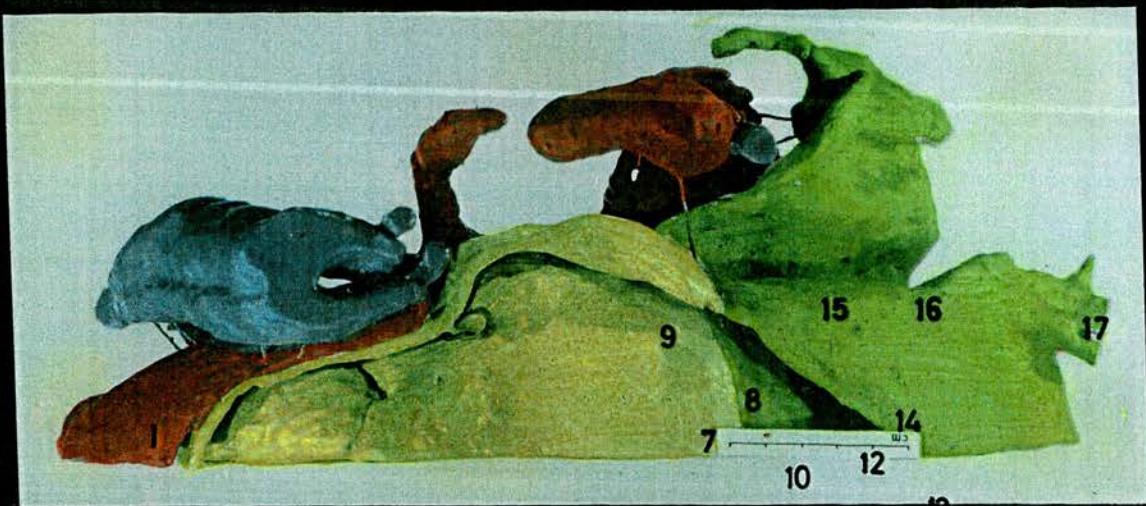
1 Forebrain; 2 Infundibulum; 3 Rathke's pocket; 4 Hindbrain; 5 Pharyngeal hypophysis; 6 Oral cavity; 7 Ventral layer of intrinsic muscles; 8 Jaw; 9 M. genioglossus; 10 M. geniohyoideus; 11 Dorsal layer of intrinsic muscles; 12 Basilar plate; 13 Basilar artery; 14 Ventral arch of atlas; 15 Notochord; 16 Centra of cervical vertebrae; 17 Pharynx; 18 M. hyoepiglotticus; 19 basihyoid; 20 Accessory thyroid tissue; 21 Cricoid cartilage; 22 Esophagus; 23 Thyroidal isthmus; 24 Trachea; 25 Muscular envelope of esophagus; 26 Thymus; 27 Internal jugular vein; 28 Right common carotid artery; 29 Left aortic arch IV; 30 Heart.

Fig. IV.100. Wax model of the left half of the pharyngeal tube with some of its major relationships, pharyngeal roof removed, embryo D 9 (15.0 mm. G.L.), (model X 100), dorsal view.

1 Primitive choana; 2 Lateral edge of oral cavity; 3 Anlage of parotid gland; 4 Mandibular nerve; 5 Horizontal bar surmounting Meckel's cartilage; 6 Reichert's cartilage; 7 Small lateral (tonsillar?) swelling; 8 Cell cord projecting into pharyngeal cavity; 9 Root of tongue (floor of pharynx); 10 Glossopharyngeal nerve; 11 Hypoglossal nerve; 12 Groove for ascending internal carotid artery; 13 Nodose ganglion; 14 Cranial cervical ganglion; 15 Epiglottic swelling; 16 Arytenoid swelling; 17 Pharyngeal muscles.



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is the so-called Pharyngeal Hypophysis (5) (Fig. IV.98./6). The previous course of the connecting stalk can still be traced through the developing cartilage of the basilar plate along a strand of embryonic connective tissue and associated capillaries (Fig. IV.101./2). It is no longer possible to identify Seessel's pocket, the barely discernible vestige of the 9 - 12 mm. stage now having been resorbed into the pharyngeal roof.

The floor of the pharynx, for the most, follows the contours and directions of the roof, the two conjoining to form a sharp, now ventrally directed, lateral edge (Fig. IV.97./5, 7). In the horizontal area of the pharynx, the floor is formed by the rapidly growing tongue and proximal laryngeal components, and furnishes these structures with their epithelial coverings. In detail, however, the conformation of the floor differs from that of the roof by the presence of a number of dorsally directed swellings, two of which having appeared laterally on either side of the root of the tongue (Figs. IV.98./9; IV.100./7). Although their united significance cannot be traced at this stage, they could perhaps be regarded as tonsillar anlagen. The epiglottic swelling (15), the highest protuberance from the floor, arises immediately behind and medial to the former. It projects above the level of the pharyngeal roof, so that in consequence a dorsal bulge is formed to accommodate for its presence (Fig. IV.99.). Posteriorly, the two arytenoid swellings are situated on either side of the median plane, somewhat overhung by the epiglottic swelling (Figs. IV.100./16; IV.106./20, 19). The characteristic T-shaped entrance to the embryonic larynx is found between the lateral arytenoid buds and behind that of the epiglottis (Fig. IV.116.).

The lateral edges of the pharynx, which were formed by the union of roof and floor, are now directed ventrally throughout their lengths, imparting to the pharyngeal cavity the inverted, trough-like appearance referred to above. It will be recalled that, up to the 9 - 12 mm. stage, the wing-like first pouch (tubotympanic recess) had been considered as forming a portion of the lateral border of the pharynx. The appearance of a secondary lateral edge below this pouch however (Fig. IV.97./P1, 5), and its union behind, with the primary border (7) precludes its being similarly described in this group of embryos. The newly appeared fold represents the caudal extremity of the lateral edge of the flat oral cavity (3), which fold has hitherto terminated either in front of, or below, the first pharyngeal pouch (Figs. IV.68./P1; IV.81./3). Due to the continued growth of the lateral palatine swellings (Fig. IV.97./2), it is driven caudad along the underside of the pouch until it unites, immediately posterior to the pouch, with the primary pharyngeal edge. A small cord of epithelial cells, about 120 microns long and 60 microns wide, arises at this point of junction and projects anteriorly into the lumen of the pharynx (Figs. IV.100./8; IV.106./7). It springs from the lateral pharyngeal edge, where the second pharyngeal pouch was noted in former stages. Similar cell cords, it will be recalled, were observed within the diminishing second pouches in the 8 - 10 mm. embryo (stage 7). None of their traces could be found in the succeeding 9 - 12 mm. embryo group however (stage 8), and for this reason it would be unwise to regard those encountered now as being identical to the ones observed previously. Unfortunately, no further 9 - 12 mm. range embryos are available at the present time, on which the disappearance of these cell cords could be definitely verified. Thus, reluctantly, those cords in the 15 mm. stage must be regarded, in the meantime, as being dissociated from those of the 8 - 10 mm. group; and whilst it is still hoped that a link-up between the features may yet be uncovered, for the moment their presence will not be used to explain some of the intricate and as yet not exactly understood problems posed by the transformation of the second mammalian pouch.

Of the original four pharyngeal pouches, at 15 mm. only pouch I (and possibly the dorsal portion of pouch II) remains associated with the pharynx, and indeed retains this relationship throughout adult life as the tympanic cavity and auditory tube (Fig. IV.97.). Pouch II (or possibly only its ventral portion) was resorbed into the wall of the pharynx, and pouches III and IV have migrated away to find new positions as the parathyroids and the thymus. The author regrets that he has to be so uncertain about the transformations of the second pouch. This study concerns itself with the development of the entire pharyngeal region, from its very beginnings to conditions as they are encountered at term. For this reason it is quite impossible to pursue at length any unsettled details, attractive though it may be, without seriously neglecting the whole work. The argument between Hammar (1902, 1903) and Frazer (1910, 1914, 1922) on the contribution of second pouch tissue to the formation of the tympanic cavity and auditory tube in the human has not brought final clarification. Since then no further attempt has been made to settle this controversy (see also page 346).

Findings in regard to the newly derived lateral pharyngeal border in the region of the first pouch help to elucidate why the pouch can no longer be considered a lateral extension of the pharyngeal cavity, but more properly should be regarded as an outpouching from the pharyngeal roof (Fig. IV.97.). The pouch otherwise presents a dorsoventrally flattened, triangular appearance, and is growing away from the roof in a lateral, caudal and dorsal direction. Its apex can be located between the ventral portion of the cochlea medially, and the recently formed ectodermal invagination of the future external acoustic meatus laterally (Fig. IV.108./4, 19, 22).

The Structure of the Pharyngeal Wall

The epithelium of the horizontal portion of the roof, having thickened, consists now of two layers of cells (Fig. IV.101./4). The basal layer is comprised of regular cuboidal cells which contain round to oval nuclei. The surface layer consists of irregular, flat to cuboidal cells which in some areas are stretched to form a fenestrated squamous sheet; in the remaining areas the superficial layer equals the basal in thickness. The epithelium rests on a loose mesenchymal cushion in which the extranuclear elements, by virtue of their growing more prominent, foreshadow the eventual maturation of the region as embryonic connective tissue.

The pharyngeal hypophysis (Fig. IV.101./3) situated at the anterior boundary of the pharynx, is an irregular segment of the stalk which connected Rathke's pocket with the pharyngeal roof. It consists of an outer, hose-like, connective tissue layer and an inner, equally tubular but much thicker, epithelial layer. The outer layer is composed of an aggregation of immature connective tissue cells with large oval nuclei, arranged circularly in two to four disordered rows around the inner epithelial core. Intertwined in the meshwork of this connective tissue sheath are four or five capillaries accompanying it throughout its length. Unlike the epithelial portion, the outer layer still maintains the connection between the pharyngeal roof and Rathke's pocket (2). In those

Fig. IV.101. Median section of pharynx in the region of cephalic flexure, embryo D 35 (15.0 mm. G.L.), (X 160).

1 Rathke's pocket; 2 Track of former connection between 1 and 3 (craniopharyngeal canal); 3 Pharyngeal hypophysis, divided into an upper tubular and a lower globular portion; 4 Pharyngeal roof; 5 Pharyngeal lumen; 6 Dorsum of tongue; 7 Pharyngobasilar fascia; 8 Basilar plate; 9 Notochord; 10 Dorsum sellae turcicae; 11 Basilar artery.

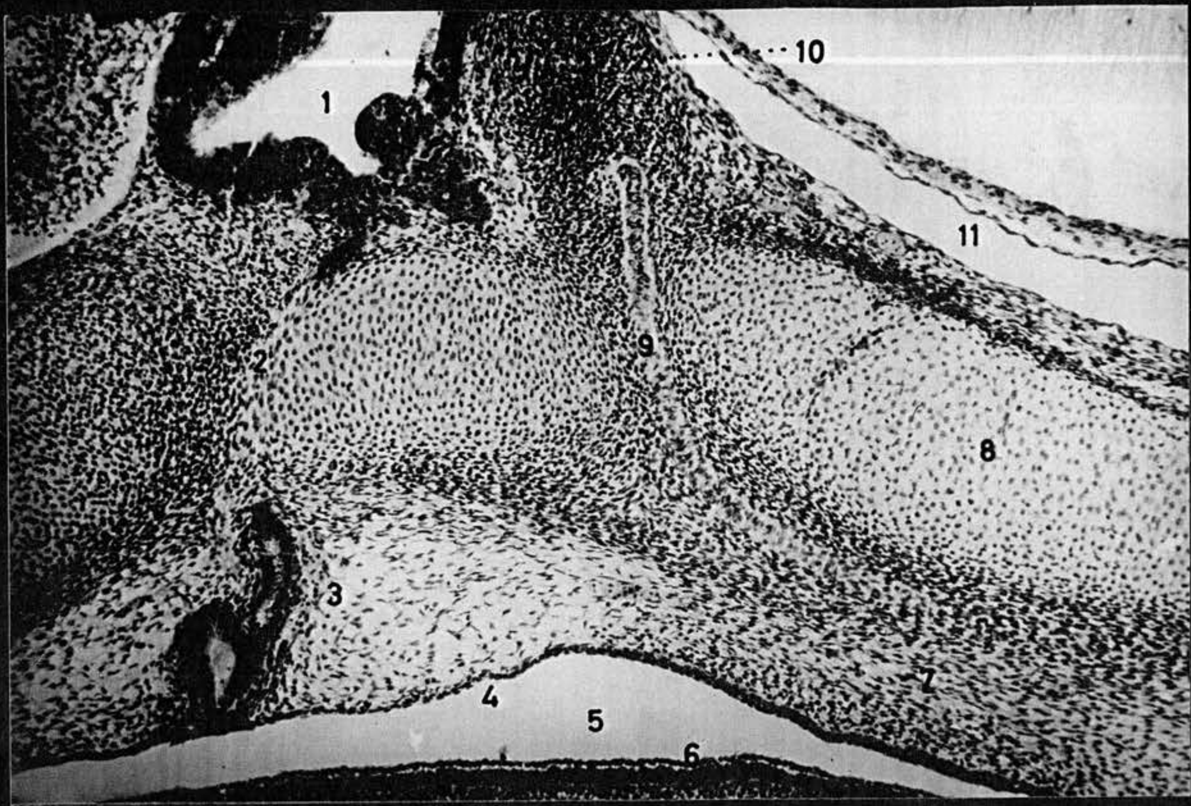

Fig. IV.101. Median section of pharynx in the region of cephalic flexure, embryo D 35 (15.0 mm. G.L.), (X 160).

1 Rathke's pocket; 2 Track of former connection between 1 and 3 (craniopharyngeal canal); 3 Pharyngeal hypophysis, divided into an upper tubular and a lower globular portion; 4 Pharyngeal roof; 5 Pharyngeal lumen; 6 Dorsum of tongue; 7 Pharyngobasilar fascia; 8 Basilar plate; 9 Notochord; 10 Dorsum sellae turcicae; 11 Basilar artery.

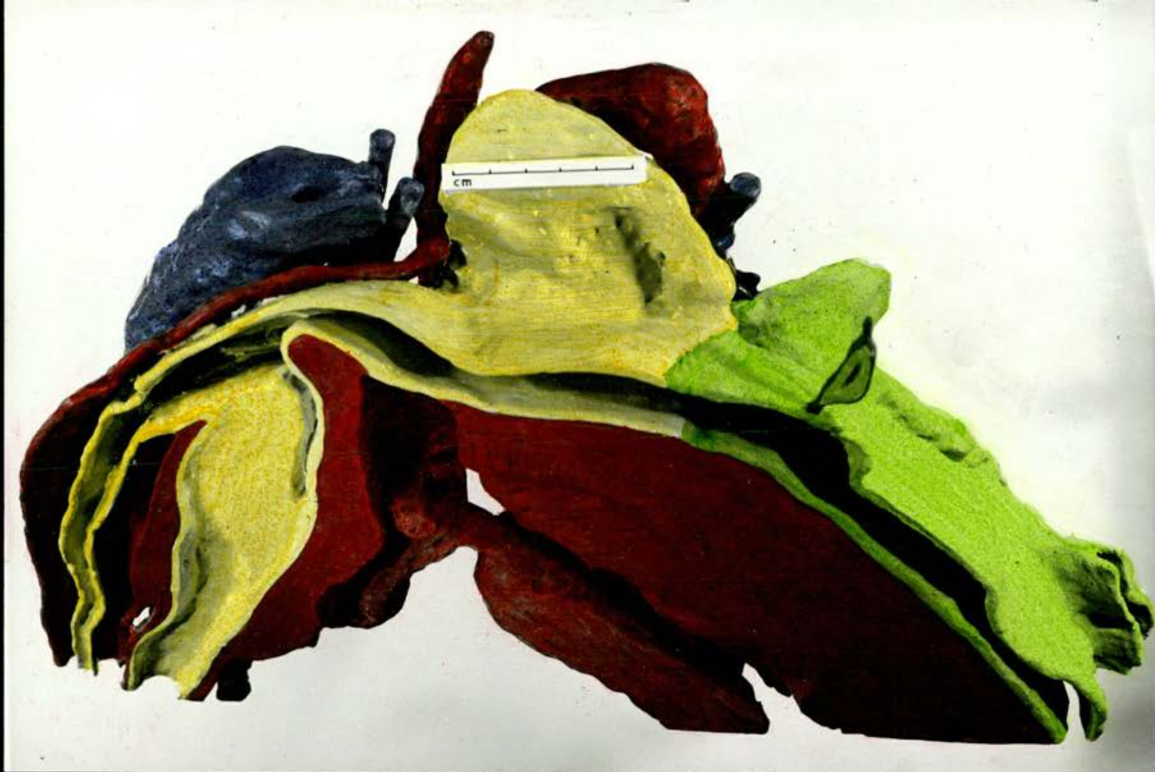

Fig. IV.102. Wax model of the left half of the pharyngeal tube with some of the major structures surrounding it, embryo D 9 (15.0 mm. G.L.), (model X 100), dorsomedial view.

P1 Tubotympanic recess; 1 Esophageal musculature; 2 Trachea; 3 Isthmus of thyroid; 4 Esophageal epithelium; 5 Larynx; 6 Basihyoid; 7 Arytenoid swelling; 8 Pharyngeal musculature; 9 Epiglottic swelling; 10 Cranial cervical ganglion; 11 Nodose ganglion; 12 Hypoglossal nerve; 13 Glossopharyngeal nerve; 14 Reichert's cartilage; 15 Meckel's cartilage; 16 Mandibular nerve; 17 Lateral edge of oral cavity; 18 Pharyngeal hypophysis; 19 Tongue; 20 M. geniohyoideus; 21 Primitive choana.

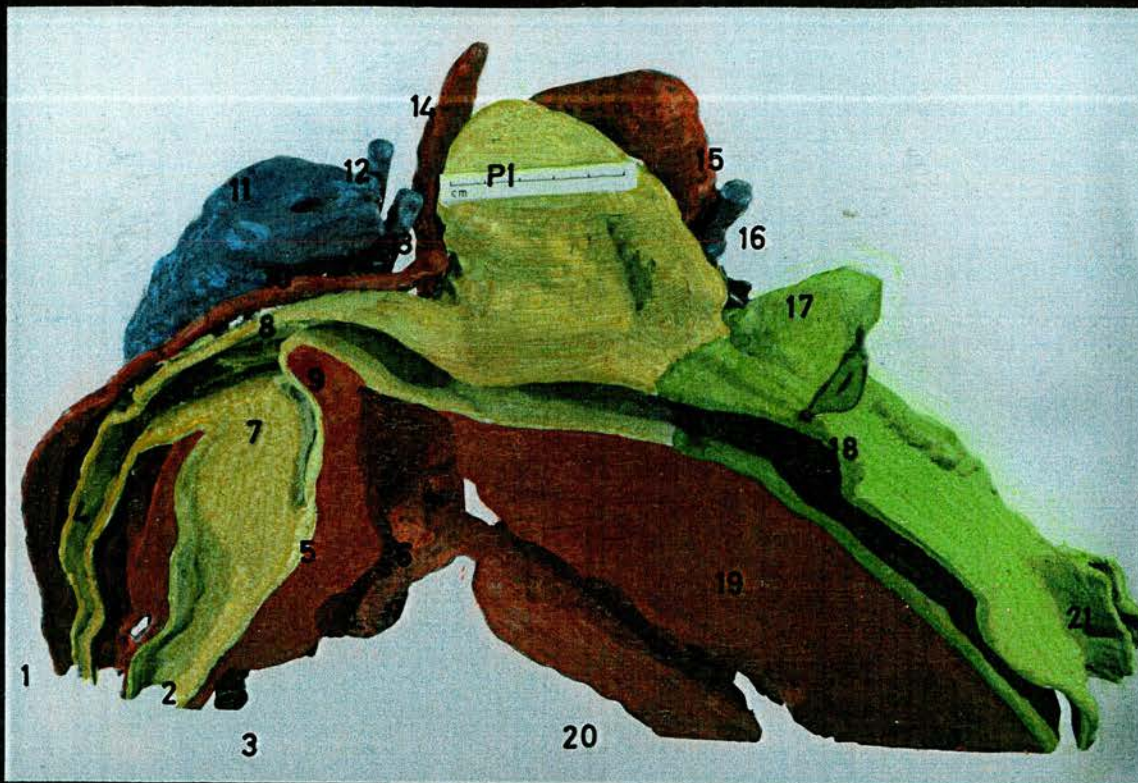

Fig. IV.102. Wax model of the left half of the pharyngeal tube with some of the major structures surrounding it, embryo D 9 (15.0 mm. G.L.), (model X 100), dorsomedial view.

P1 Tubotympanic recess; 1 Esophageal musculature; 2 Trachea; 3 Isthmus of thyroid; 4 Esophageal epithelium; 5 Larynx; 6 Basihyoid; 7 Arytenoid swelling; 8 Pharyngeal musculature; 9 Epiglottic swelling; 10 Cranial cervical ganglion; 11 Nodose ganglion; 12 Hypoglossal nerve; 13 Glossopharyngeal nerve; 14 Reichert's cartilage; 15 Meckel's cartilage; 16 Mandibular nerve; 17 Lateral edge of oral cavity; 18 Pharyngeal hypophysis; 19 Tongue; 20 M. geniohyoideus; 21 Primitive choana.

segments from which the epithelial core has disappeared, the hose-like outer layer has contracted to form a cell strand. In contrast, the inner epithelial portion is composed of stratified columnar cells arranged radially around a lumen of some 20 microns diameter and containing small amounts of debris. These cells, resting on a distinct basement membrane, which separates this portion from the outer connective tissue layer, contain oval to spindle-shaped nuclei which are found at all levels throughout the depth of this layer. This portion of the pharyngeal hypophysis, as has been mentioned above, has meantime lost all direct connection with both the pharyngeal roof and Rathke's pocket, and is now situated mid-way between the pharynx and the basilar plate.

Both medial and lateral walls of the first pharyngeal pouch in this stage consist of a single layer of low cuboidal cells, resting on an indistinct basement membrane. Towards the apex of the pouch, the epithelium increases in thickness and consists of three to four irregular rows of cuboidal cells. The underlying mesenchyme is loose and undifferentiated on the medial aspect, and in comparison, that of the lateral wall is relatively dense (Fig. IV.108./18).

The roof of the vertical portion of the pharynx is composed of epithelium, an underlying corium of primitive connective tissue and a sheet-like pre-muscle layer, which

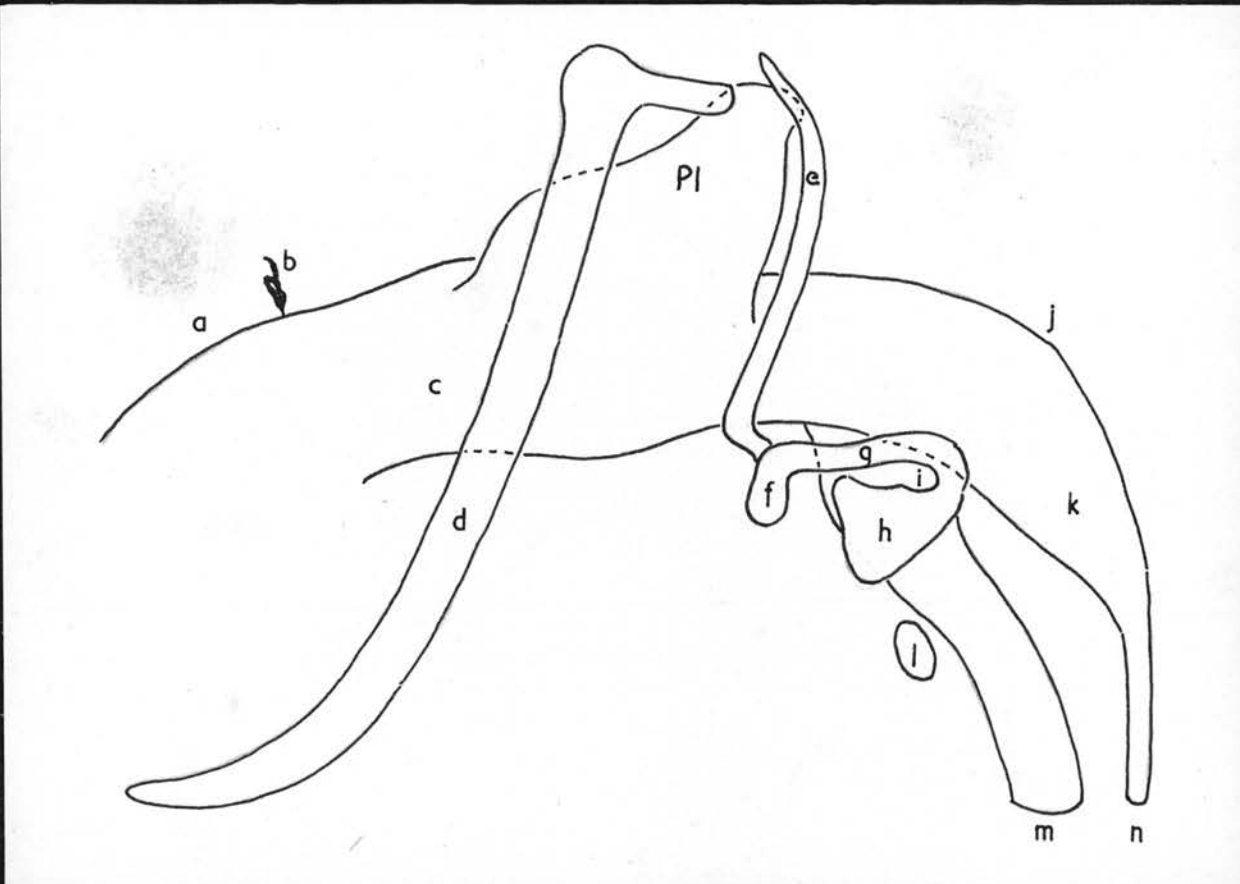

Fig. IV.103. Lateral view of pharynx and associated branchial skeleton of the 15 mm. embryo, with pharyngeal pre-muscle mass superimposed, (schematic).

1 Primordium of M. stylopharyngeus; 2 Anterior free border; 3 Primordium of M. hyopharyngeus; 4 Primordium of Mm. stylopharyngeus, pterygopharyngeus and palatopharyngeus; 5 Primordium of M. thyropharyngeus; 6 Ventral free border; 7 Primordium of Mm. thyropharyngeus and cricopharyngeus; 8 Primordium of esophageal muscle.

a Cephalic flexure; b Pharyngeal hypophysis; c Horizontal portion of pharynx; d Meckel's cartilage; e Reichert's cartilage; f Basihyoid; g Thyrohyoid; h Thyroid cartilage; i Thyroid fissure; j Cervical flexure; k Vertical portion of pharynx; l Cricoid cartilage; m Trachea; n Esophagus. PI Tubotympanic recess.

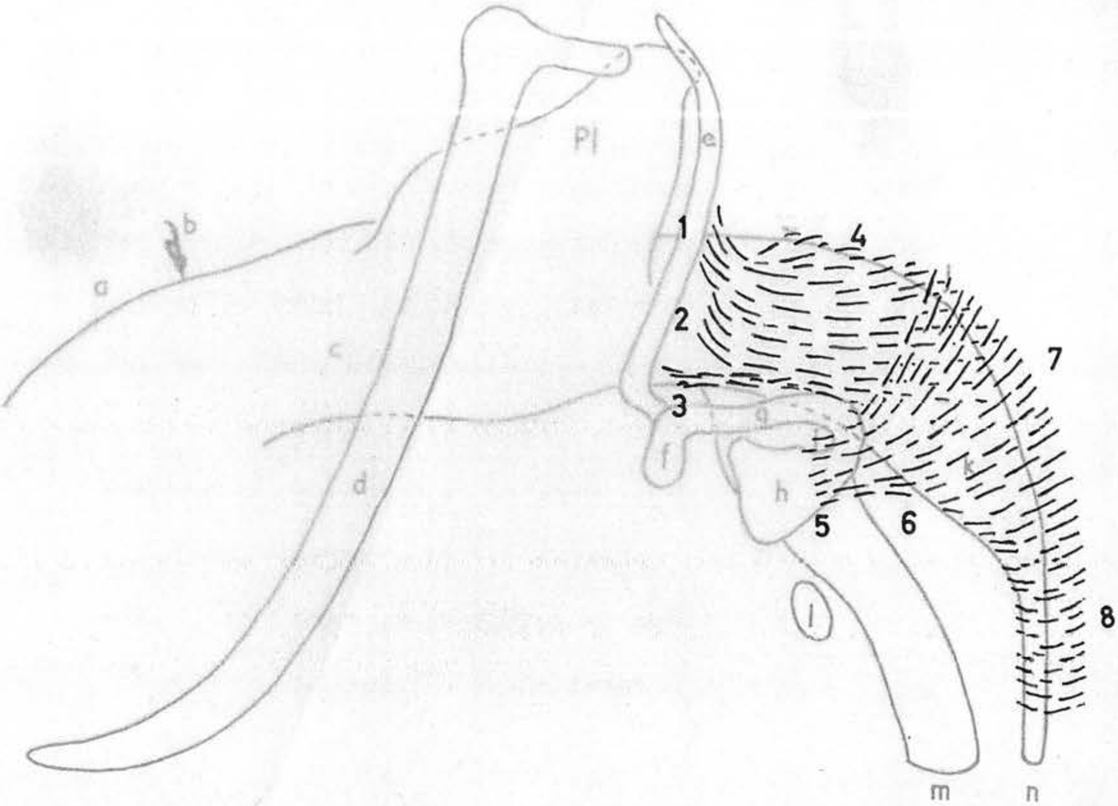

Fig. IV.103. Lateral view of pharynx and associated branchial skeleton of the 15 mm. embryo, with pharyngeal pre-muscle mass superimposed, (schematic).

1 Primordium of M. stylopharyngeus; 2 Anterior free border; 3 Primordium of M. hyopharyngeus; 4 Primordium of Mm. stylopharyngeus, pterygopharyngeus and palatopharyngeus; 5 Primordium of M. thyropharyngeus; 6 Ventral free border; 7 Primordium of Mm. thyropharyngeus and cricopharyngeus; 8 Primordium of esophageal muscle.

a Cephalic flexure; b Pharyngeal hypophysis; c Horizontal portion of pharynx; d Meckel's cartilage; e Reichert's cartilage; f Basihyoid; g Thyrohyoid; h Thyroid cartilage; i Thyroid fissure; j Cervical flexure; k Vertical portion of pharynx; l Cricoid cartilage; m Trachea; n Esophagus. PI Tubotympanic recess.

is the primordium of the pharyngeal musculature (Figs. IV.102./8; IV.104./6; IV.106./15). The epithelium is slightly modified from that of the horizontal pharynx. It is slightly taller due to a higher basal layer of cells. A thin, yet dense, layer of connective tissue, in which the nuclear elements still outweigh the cytoplasmic material, follows immediately below the basement membrane. Next to it lies a wider zone of similar, though much more loosely arranged, tissue containing a number of capillaries, which in turn is superseded by the dense sheet of pre-muscle tissue. The nuclei in this layer are of two kinds: an oval, pale-staining one and a smaller, dark, spindle-shaped type. A few strands of eosinophilic cytoplasm thread through the scattered nuclei, but in the main, the nuclear elements are still predominant in the picture.

Cap-like, the primordium of the pharyngeal musculature overlies the cervical flexure and vertical portion of the pharyngeal roof (Fig. IV.103.). It presents anterior (2) and ventral (6) free borders, of which the former run nearly parallel to Reichert's cartilage, while the latter, in general terms, follow the lateral pharyngeal rims in this region. Below the esophageal entrance, the ventral free borders of the primordium unite to form the muscular tube enveloping the esophagus (8). The two anterior borders of the primordium join in the dorsal midline somewhat behind the cervical flexure, leaving a triangular area uncovered as they do so, thus forming a notch which is open anteriorly (Fig. IV.107./13). The uncovered triangle lies opposite the pharyngeal field later to become the Fornix pharyngis. The free borders of the cap-like muscular sheet find attachment to adjacent skeletal structures by means of three muscular extensions, one for the anterior and two for the ventral borders. A strip of pre-muscle tissue sweeps upwards and outwards from the anterior border to become inserted on the medial aspect of the middle of Reichert's cartilage (Figs. IV.103./1; IV.106./10). The second, narrower muscular extension leaves the anterior portion of the ventral free border and courses forwards and inwards, towards the ventral portion of Reichert's cartilage, where it attaches on what will in due course become the kerato-hyoid (Fig. IV.103./3). The third - the widest of the pre-muscle bands - leaves the posterior portion of the ventral border to gain the lateral face of the developing thyroid cartilage (5). In their extension from the ventral border, the two last muscular bands form a notch, coincident posteriorly with the thyroid fissure (i) of the thyroid cartilage, through which the large anterior laryngeal nerve passes into the interior of the developing larynx (Fig. IV.105./15). The anterior portion of the notch is to a great extent filled by the anterior half of the thyrohyoid (Fig. IV.103./g). Although only few bundles of primitive muscle fibres are present in the primordium, it is possible to appreciate their general direction. Those in the anterior portion of the primordium are orientated horizontally, while in the posterior half they assume an almost dorsoventral course. Thus, in the vicinity of the cervical flexure, a crossing of fibres can be observed (j). Around the posterior pharynx, the fibres take on a definitely circular course, although even here and especially towards the esophageal entrance, some longitudinal strands are encountered. Within the muscular strips which project beyond the free borders of the primordium, the fibres are arranged longitudinally. Judging by the attachment of, and the fibre direction within the muscle primordium, it should be possible at this stage of development to assign correctly the names of the future pharyngeal muscles to the different regions. Thus, (I) M. stylopharyngeus to the anterior portion, passing to the middle of Reichert's cartilage (1), (II) M. hyopharyngeus to the narrow band joining the ventral portion of Reichert's cartilage (3), (III) Mm. stylopharyngeus, pterygopharyngeus and palatopharyngeus to the portion above the thyroid fissure (4), and (IV) Mm. thyropharyngeus and cricopharyngeus to the entire section between the aforementioned muscles and the esophageal muscle proper (7).

The epithelium of the pharyngeal floor consists of two layers of cells, resting on a distinct basement membrane. The basal cells are largely of low columnar type, but irregular cuboidal cells can be noted from time to time. The superficial layer is formed by a somewhat flattened cuboidal type of cell (Fig. IV.101./6). Anteriorly, the subepithelial tissue appears very dense due to a preponderance of nuclear elements, but still lacks internal organisation. Capillaries are plentiful, reaching to within 20 microns of the basement membrane. Deeper, the developing intrinsic muscle mass of the tongue extends to within 100 microns of the epithelium (Fig. IV.99./11). The subepithelial corium at the root of the tongue however, is less dense, and a thin layer of primitive, spindle-shaped connective tissue cells appears immediately below the basement membrane, leaving the deeper strata more or less undifferentiated. The pharyngeal floor proper continues caudad towards the esophageal aditus in the form of two narrow spaces, lying on either side of the anterior laryngeal structures, which have recently invaded this area from below. These spaces in fact represent the internal surfaces of the ventrally directed, lateral borders of the pharyngeal tube, postnatally being termed the Piriform Recesses (Fig. IV.98./16). Here, the epithelial lining is comprised of two to three rows of irregularly-shaped cells, presenting a rough, cobble-stone, free cell border to the pharyngeal lumen. Underlying the epithelium follows a narrow zone of embryonic connective tissue.

The esophageal epithelium at the level of the thyroid consists of two to three layers of stratified columnar cells. Enveloping this epithelial tube, there follows a wide zone of relatively undifferentiated tissue, in the subepithelial stratum of which are spindle-shaped histioblasts, lying parallel to the long axis of the esophagus. A few capillaries are also present in this layer. Circumscribing the whole, is a narrow, dense pre-muscle layer, first observed in the 8 - 10 mm. group, in which the fusiform nuclei intermingle with thin strands of acidophilic cytoplasm, running both around and parallel to the long axis of the parent tube (Figs. IV.99./22, 25; IV.102./1).

The Relationships of the Pharynx

Dorsally, the 15 mm. embryonic pharynx is related to the following structures: (a) the basilar plate and accompanying notochord, (b) the otic capsules, (c) the precursors of the Mm. longus capitis and rectus capitis ventralis, (d) the pharyngeal hypophysis, (e) the internal carotid arteries, and (f) the greater superficial petrosal and the tympanic nerves. The basilar plate is one of the first elements of the cranial skeleton to appear in the embryo, and at this particular stage it has already established itself as a cartilaginous sheet, lying between pharynx and hindbrain (Fig. IV.99./12). It is flattened dorsoventrally and fairly wide rostrally, where it gives rise later to the sphenoid. At the level of the first pharyngeal pouch (tubotympanic recess) the plate becomes narrower from side to side, thus enabling the cochlear portions of the otic capsules to expand on either side; in places, union with the plate has already occurred (Figs. IV.108./15, 22; IV.104./2, 3). Superior to the epiglottic portion of the pharynx, it remains fairly narrow, only to widen once more above the cervical flexure in the formation of the exoccipital wings. The anterior wings of the basilar plate are perforated by foramina, transmitting the internal carotid arteries, and similarly, foramina in the exoccipital wings transmit the hypoglossal nerves.

The notochord traverses the basilar plate in the manner indicated in Fig. IV.99. Originating in the dense and as yet undifferentiated tissue immediately posterior to Rathke's pocket which is destined to become the Dorsum sellae turcicae of the sphenoid (Fig. IV.101./9, 10), it penetrates the basilar plate, turns caudad, to lie within the ventral perichondrium of the plate for a distance of roughly 1.0 mm. It soon loses relationship with the pharynx however, as it again pierces the basilar plate to gain the dorsal surface this time, whereupon it pursues its characteristic, caudally directed course until it enters the bodies of the cervical vertebrae. In that area where the notochord lies closest to the pharyngeal roof, the separating mesenchyme is beginning to differentiate in the formation of the pharyngobasilar connective tissue (Fig. IV.101./7).

Meanwhile, below the posterior half of the basilar plate, the anlagen of the M. longus capitis have appeared. They ascend along the anterior face of the vertebral column, turn rostrad in conformity with the cervical flexure, and end roughly mid-way along the ventral perichondrium of the plate (Fig. IV.104./5). Dorsolaterally to either of these muscles is the much thinner and shorter M. rectus capitis ventralis, stretching from the region of the ventral arch of the atlas to a point on the basal plate immediately behind the M. longus capitis insertion (4).

The pharyngeal hypophysis (Fig. IV.99./5) is the most anterior of all the dorsal relations of the pharynx, and thus serves conveniently as a landmark, whereby the anterior boundary of the pharyngeal cavity can be defined. It will be remembered from earlier chapters that it arose immediately in front of the oral membrane, which at the time divided the oral from the pharyngeal chambers. The pharyngeal hypophysis is situated between the roof of the pharynx and the basilar plate.

The internal carotid artery ascends into the dorsal pharyngeal region associated with both nodose and cranial cervical ganglia (Fig. IV.106./12, 13, 14). It turns anteriorly under the exoccipital wing of the basilar plate, and, accompanied now by the carotid nerve, runs the full length of the pharyngeal roof, converging slightly towards the midline with its fellow from the other side. At the level of the otic capsule, it overlies the root of the tubotympanic recess (Fig. IV.108./17), giving off a single lateral branch (3). This branch runs laterally and anteriorly below the otic capsule, then crosses the seventh cranial nerve ventrally to gain a position anterior and dorsal to the external ear. At this point it breaks up into several small branches which penetrate adjacent pre-muscle masses. At the anterior boundary of the pharynx the internal carotid arteries leave the pharyngo-basilar space and pass through the anterior wings of the basilar plate as they continue their course into the cranium (Fig. IV.104./1).

The greater petrosal nerve (Fig. IV.106./1), leaving the geniculate ganglion and ending further rostrally in the sphenopalatine ganglion, forms a short dorsolateral relationship with the anterior pharynx. The nerve runs in a cranioventral direction and passes the internal carotid artery on the lateral side. Medial to its course is an elongated bar of dense tissue, which will, in later stages, give rise to the Pars sagittalis of the palatine bone, and the pterygoid bone. Further caudally, the tympanic nerve (Fig. IV.108./20), closely related to the dorsomedial aspect of the tubotympanic recess, has a similar course. It arises from the petrosal ganglion, sweeps forwards and outwards, staying always lateral to the internal carotid artery, to end in the developing otic ganglion which can be noted on the caudal face of the branching mandibular nerve immediately anterior to the recess.

The most prominent of the many structures forming lateral relationships with the pharynx, listed from before backwards, are: (a) the mandibular nerve, (b) Meckel's cartilage, (c) the external acoustic meatus, (d) Reichert's cartilage, (e) the glossopharyngeal nerve, (f) the hypoglossal nerve, and (g) the nodose ganglion. The mandibular nerve (Fig. IV.105./9) arises ventrocaudally from the semilunar ganglion and, as it approaches the lateral aspects of the embryonic pharynx, divides into three major divisions. The first fibres to leave the parent trunk enter the laterally situated pre-muscle masses of the temporal, masseter, and perhaps the buccinator muscles. Another group of nerves comes away medially and ends in the small anlage of the palatine pre-muscle mass at the root of the lateral palatine process, and in that of the pterygoid muscle slightly more caudally and ventrally. The fibres of the palatine muscle mass have a transverse course and enter the lateral palatine process from its root, but do not reach very far towards the free border of the process. Considering the direction in which the fibres run, and the fact that the M. tensor veli palatini is the only one of the palatine group of muscles to be innervated by the mandibular nerve,

Fig. IV.104. Sagittal section of pharynx, embryo D 35 (15.0 mm. G.L.), (X 55).

1 Internal carotid artery; 2 Basilar plate; 3 Cochlear portion of otocyst; 4 M. rectus capitis ventralis; 5 M. longus capitis; 6 Muscular cap; 7 Pharyngeal lumen; 8 Tongue; 9 Keratohyoid; 10 Hypoglossal nerve; 11 Thyrohyoid; 12 Thyroid cartilage; 13 Anterior laryngeal nerve; 14 M. sternohyoideus; 15 Parathyroid IV; 16 Thyroid; 17 M. sternothyroideus.

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1 Internal carotid artery; 2 Basilar plate; 3 Cochlear portion of otocyst; 4 M. rectus capitis ventralis; 5 M. longus capitis; 6 Muscular cap; 7 Pharyngeal lumen; 8 Tongue; 9 Keratohyoid; 10 Hypoglossal nerve; 11 Thyrohyoid; 12 Thyroid cartilage; 13 Anterior laryngeal nerve; 14 M. sternohyoideus; 15 Parathyroid IV; 16 Thyroid; 17 M. sternothyroideus.

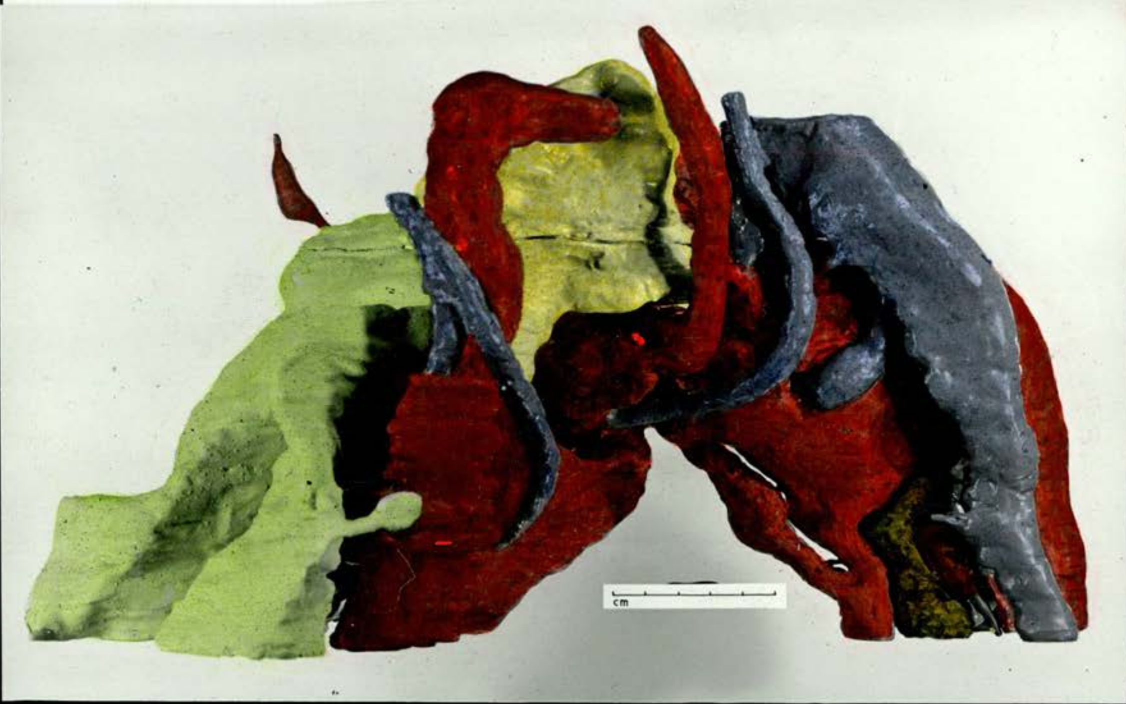
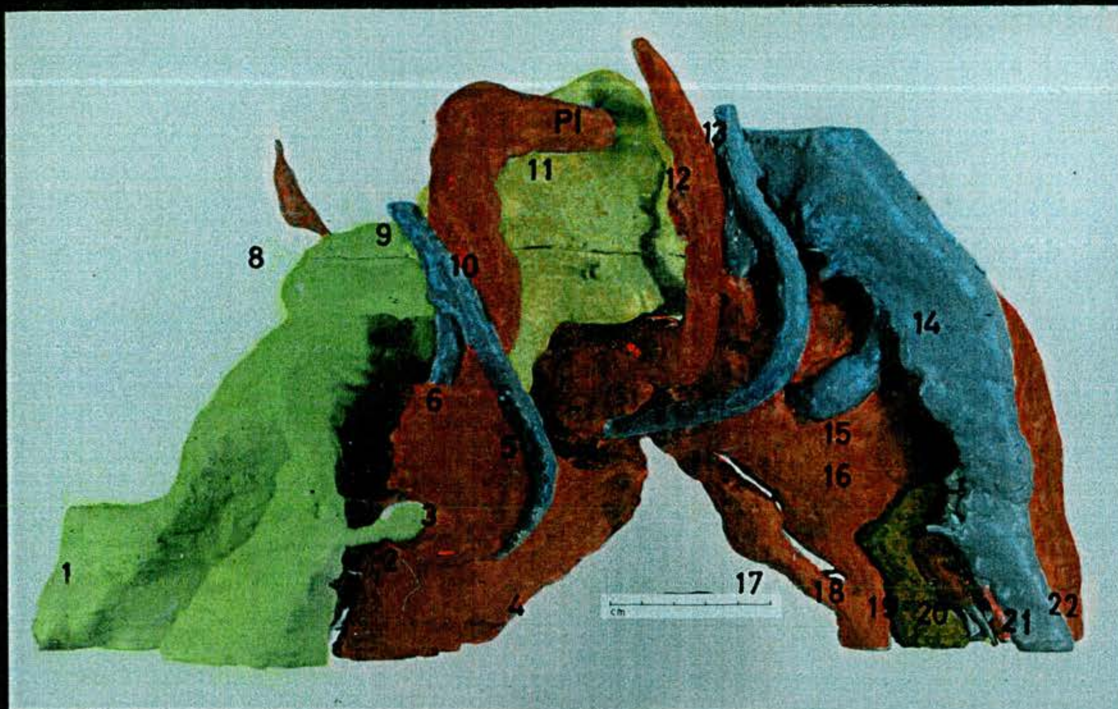


Fig. IV.105. Wax model of the left half of the pharyngeal tube with some of the major structures surrounding it, embryo D 9 (15.0 mm. G.L.), (model X 100), lateral view.

P1 Tubotympanic recess; 1 Primitive choana; 2 Bud of parotid gland; 3 Developing osseous mandible; 4 M. mylohyoideus; 5 Mylohyoid nerve; 6 Mandibular alveolar nerve; 7 Lateral rim of oral cavity; 8 Pharyngeal hypophysis; 9 Mandibular nerve; 10 Meckel's cartilage; 11 Horizontal bar at proximal extremity of Meckel's cartilage; 12 Reichert's cartilage; 13 Hypoglossal nerve; 14 Nodose ganglion; 15 Anterior laryngeal nerve; 16 Pharyngeal pre-muscle mass; 17 M. sternohyoideus; 18 M. sterno-thyroideus; 19 Thyroid; 20 Parathyroid III with thymic duct; 21 Vago-sympathetic trunk; 22 Esophageal pre-muscle mass.



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it should be safe to regard the muscle mass in question as the source of the tensor of the soft palate. The caudal portions of the palatine processes are related to the pharynx laterally at this stage and intervene between it and the more laterally placed mandibular nerve and attendant muscle masses. The main portion of the mandibular nerve continues caudoventrad and approaches Meckel's cartilage from the front (Fig. IV.105./9). At this point the nerve subdivides again into three distinct nerve trunks, of which the lingual ramus, once having received the Chorda tympani, turns mesiad to enter the body of the tongue. The mandibular alveolar branch (6) curves anteriorly and slightly laterally to enter the space between Meckel's cartilage and the developing osseous plate of the mandible (3, 10). The mylohyoid nerve (5) crosses Meckel's cartilage on its lateral aspect and, maintaining a caudoventral course, finally gains the superficial surface of the developing mylohyoid muscle (4), within the mass of which it ramifies. The otic ganglion is situated between the main trunk of the mandibular nerve in front, and the anterior border of the tubotympanic recess behind. The lateral relationships of the pharynx at the level of the recess are formed by the Chorda tympani nerve and the external acoustic meatus. The Chorda tympani, coming from the geniculate ganglion and running forwards and downwards to meet the lingual nerve, passes the apex of the recess on the lateral side (Fig. IV.108./2), and before reaching the lingual, winds around the medial aspect of Meckel's cartilage. Below the Chorda tympani nerve, the newly formed invagination of the external acoustic meatus extends towards the lateral face of the recess (19). It is however separated from the latter by a mass of dense and as yet undifferentiated cells (18) which proliferate from the region of the horizontal bar at the dorsal extremity of Meckel's cartilage (Fig. IV.105./11), later to form the malleus. At the caudal border of the recess, Reichert's cartilage (12) crosses the lateral margin of the pharynx, while behind and to the side, the cartilaginous bar is paralleled by the long and slender pre-muscle belly of the M. digastricus mandibulae (Fig. IV.106./9). Except for a small portion at the dorsal extremity, the latter muscle extends the entire length of Reichert's cartilage, until at the level of the basihyoid the two structures separate and the muscle curves anteriorly to end in the vicinity of Meckel's cartilage (Fig. IV.108./8).

Immediately behind Reichert's cartilage, the glosso-pharyngeal nerve (Fig. IV.102./13) enters the lateral pharyngeal wall from above. At the point of entrance it is joined by a twig from the nodose ganglion. Several short filaments enter the pre-muscle lamina of the pharynx from this nerve junction, some of which traverse the muscle to find distribution within the subepithelial zone of loose connective tissue. The main continuation of the nerve however pierces the muscle sheet, entering the subpharyngeal

Fig. IV.106. Horizontal section of pharynx at a level of the root of the tongue, embryo D 9 (15.0 mm. G.L.), (X 48).

1 Greater superficial petrosal nerve; 2 Maxillary division of trigeminal nerve; 3 Medial pterygoid muscle; 4 Mandibular alveolar nerve; 5 Meckel's cartilage; 6 Lateral (tonsillar?) swelling in pharyngeal floor; 7 Cell cord; 8 Reichert's cartilage; 9 M. digastricus mandibulae; 10 M. stylopharyngeus; 11 Glossal branch of glossopharyngeal nerve; 12 Internal carotid artery; 13 Nodose ganglion; 14 Cranial cervical ganglion; 15 Muscular cap; 16 M. rectus capitis ventralis; 17 M. longus capitis; 18 Vestibulum esophagi; 19 Arytenoid swellings; 20 Epiglottic swelling; 21 Piriform recess; 22 Entrance into tubotympanic recess; 23 Root of tongue; 24 Pharyngeal hypophysis; 25 Basilar plate.

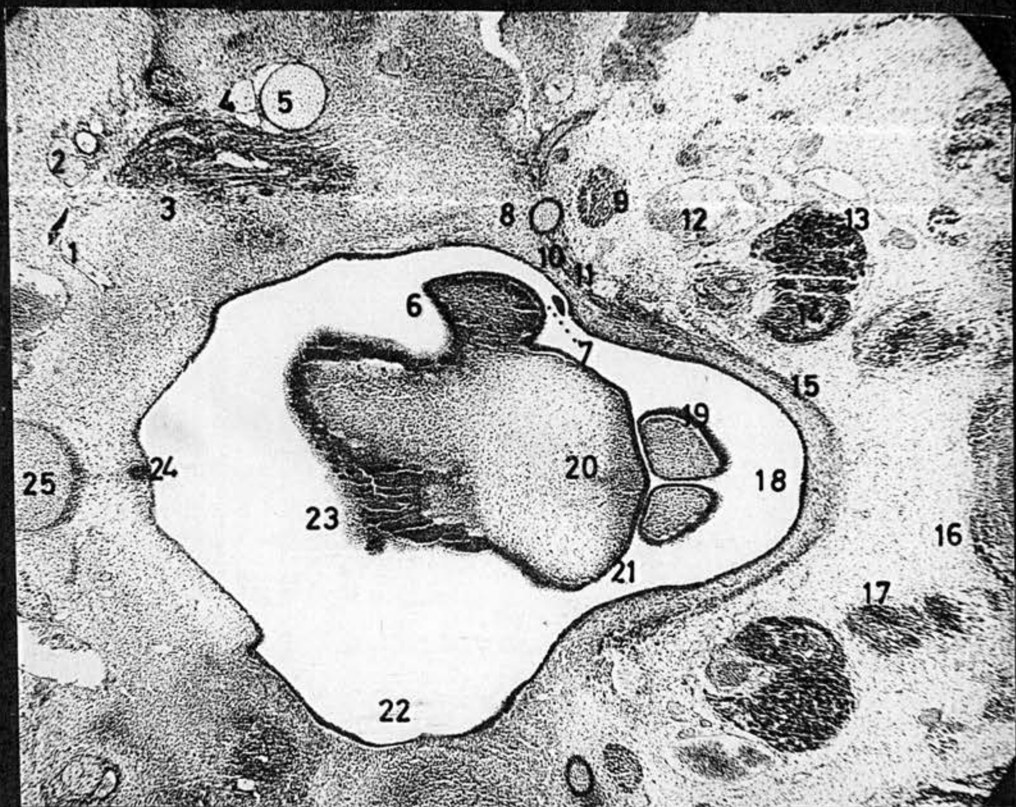


Fig. IV.106. Horizontal section of pharynx at a level of the root of the tongue, embryo D 9 (15.0 mm. G.L.), (X 48).

1 Greater superficial petrosal nerve; 2 Maxillary division of trigeminal nerve; 3 Medial pterygoid muscle; 4 Mandibular alveolar nerve; 5 Meckel's cartilage; 6 Lateral (tonsillar?) swelling in pharyngeal floor; 7 Cell cord; 8 Reichert's cartilage; 9 M. digastricus mandibulae; 10 M. stylopharyngeus; 11 Glossal branch of glossopharyngeal nerve; 12 Internal carotid artery; 13 Nodose ganglion; 14 Cranial cervical ganglion; 15 Muscular cap; 16 M. rectus capitis ventralis; 17 M. longus capitis; 18 Vestibulum esophagi; 19 Arytenoid swellings; 20 Epiglottic swelling; 21 Piriform recess; 22 Entrance into tubotympanic recess; 23 Root of tongue; 24 Pharyngeal hypophysis; 25 Basilar plate.

Fig. IV.107. Wax model of the left half of the pharyngeal tube, embryo D 9 (15.0 mm. G.L.), (model X 100), dorsal view.

P1 Tubotympanic recess; 1 Primitive choana; 2 Pharyngeal hypophysis; 3 Lateral edge of oral cavity; 4 Mandibular nerve; 5 Bud of parotid gland; 6 Horizontal bar at proximal end of Meckel's cartilage; 7 Reichert's cartilage; 8 Glossopharyngeal nerve; 9 Hypoglossal nerve; 10 Nodose ganglion; 11 Groove for ascending internal carotid artery; 12 Cranial cervical ganglion; 13 Triangular area left uncovered by pharyngeal muscles (Fornix pharyngis); 14 Pharyngeal pre-muscle mass.

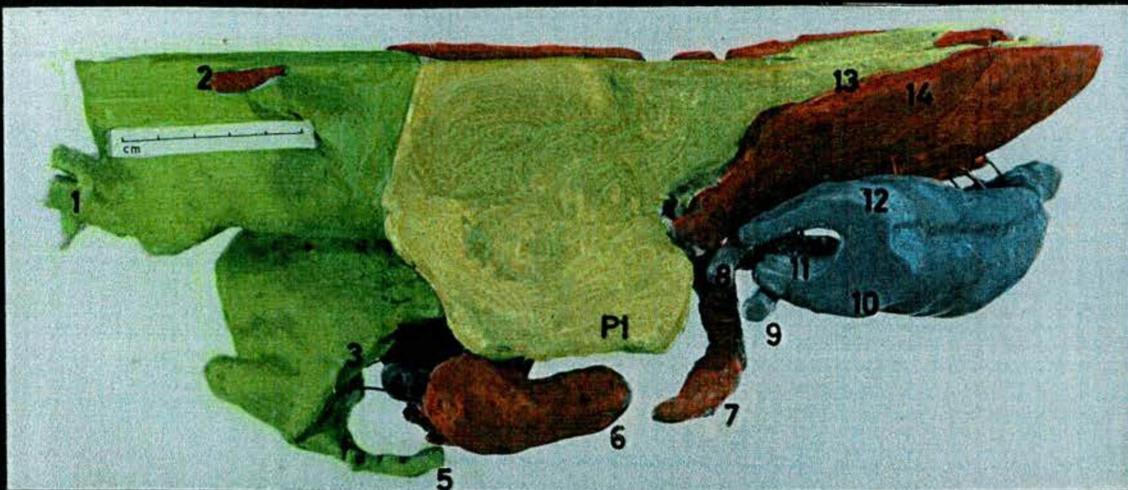


Fig. IV.107. Wax model of the left half of the pharyngeal tube, embryo D 9 (15.0 mm. G.L.), (model X 100), dorsal view.

P1 Tubotympanic recess; 1 Primitive choana; 2 Pharyngeal hypophysis; 3 Lateral edge of oral cavity; 4 Mandibular nerve; 5 Bud of parotid gland; 6 Horizontal bar at proximal end of Meckel's cartilage; 7 Reichert's cartilage; 8 Glossopharyngeal nerve; 9 Hypoglossal nerve; 10 Nodose ganglion; 11 Groove for ascending internal carotid artery; 12 Cranial cervical ganglion; 13 Triangular area left uncovered by pharyngeal muscles (Fornix pharyngis); 14 Pharyngeal pre-muscle mass.

region between the ventrally inclined pharyngeal edge above, and the keratohyoid portion of Reichert's cartilage below (Fig. IV.106./11). From here it sends a fine twig dorsally to serve the small lateral, and as yet unexplained, (tonsillar?) swelling in the pharyngeal floor (Fig. IV.108./14), thereafter terminating as five or six fibrils which ramify under the epithelium of the root of the tongue. Below and interconnected with the trunk at its terminal division, a spherical, ganglion-like structure can be observed. This could perhaps be termed the Lingual ganglion, being one of the peripheral ganglia along the glossopharyngeal nerve whose existence is recognised in the current literature (Ellenberger and Baum, 1932). The hypoglossal nerve (Figs. IV.107./9; IV.105./13) crosses the lateral border of the pharynx behind the glossopharyngeal nerve. It is closely applied to the lateral aspect of the nodose ganglion, where it curves anteriorly and slightly medially, traversing the space between ganglion and glossopharyngeal nerve before entering the body of the tongue from behind (Fig. IV.104./10).

The most caudal of the lateral pharyngeal relations is formed by the giant nodose ganglion itself (Fig. IV.105./14), reinforced at this stage of development by the anterior cervical ganglion which is applied closely to its medial aspect (Fig. IV.107./10, 12). A long groove is formed between the two elongated ganglia anteriorly (11). The common carotid artery enters this groove from below and, once having given off the external carotid artery at about the middle of the ganglia, it pursues its course through the groove as the internal carotid artery (Fig. IV.106./12, 13, 14). Somewhat below the point of emergence of the external carotid artery, the anterior laryngeal nerve (Fig. IV.105./15) leaves the nodose ganglion. It winds around the medial aspect of the common carotid and runs towards the thyroid fissure, being closely applied to the pharyngeal musculature. It passes through the fissure into the interior of the developing larynx. The external carotid artery after emerging from the groove between the nodose and the sympathetic ganglia, travels forwards, traversing the space between the ganglia and the glosso-pharyngeal nerve. The vessel gives rise to: (a) a thin pharyngeal branch at its origin, which veers caudally and medially to enter the pharyngeal muscles, (b) the occipital artery, which follows a dorsal course, paralleling that of the internal carotid, and (c) a fairly large branch passing towards the thyroid fissure which it enters in front of the anterior laryngeal nerve. Thereafter, the external carotid artery curves away from the pharynx, gives off the lingual artery, and passes between Reichert's cartilage and the vertical portion of the digastric muscle to lose relation with the pharynx.

Ventrally, the pharyngeal cavity is related to the posterior portion of the tongue, the root of the tongue, the subpharyngeal parts of the hyoid apparatus and the proximal elements of the larynx. In the 15 mm. embryo the body of the tongue is well developed and resembles adult configuration (Fig. IV.99.). Its make-up consists of pre-muscle masses for both the intrinsic as well as for the extrinsic muscles which enter the tongue from the outside. At this stage, the intrinsic muscles are represented by two dorsoventrally flattened bars, one above the other, both extending the entire length in the dorsum of the tongue. In section, the dorsal layer appears quite thin, its short pre-muscle fibres running longitudinally (11). In contrast, the ventral is thick and constituted of transversely directed fibres (7). The primordia of the extrinsic muscle as a whole are all situated below those of the intrinsics, and appear as four sagittally placed sheets. The two sheets representing the M. genioglossus (9) on either side of the median plane are flanked laterally by the hypoglossal nerve above and the lingual nerve below. Lateral to these structures is the sheet representing the primitive Mm. styloglossus and hyoglossus. A short distance caudally in the body of the tongue, this sheet bifurcates, giving rise to the flat dorsal belly of the primitive styloglossus and the equally flat ventral belly of the hyoglossus (Fig. IV.108./12). Ventral to the latter muscles, the two ducts of the sublingual and submandibular salivary glands can be observed. At the level of the anterior border of the tubotympanic recess,

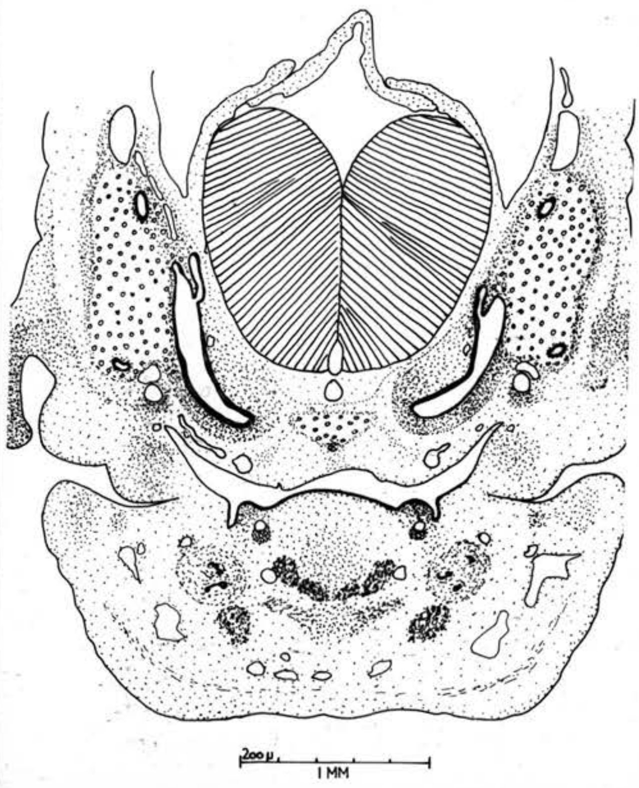


Fig. IV.108. Transverse section of ventral portion of head at the level of the first pharyngeal pouch, embryo D 41 (14.5 mm. G.L.), (semischematic).

1 Anterior cardinal vein; 2 Chorda tympani nerve; 3 Lateral branch of internal carotid artery; 4 Tubotympanic recess; 5 Small lateral (tonsillar?) swelling in pharyngeal floor; 6 Submandibular salivary gland; 7 Hypoglossal nerve; 8 M. digastricus mandibulae; 9 M. mylohyoideus; 10 Root of tongue; 11 M. geniohyoideus; 12 M. hyoglossus; 13 Pharynx; 14 Glossal branch of glossopharyngeal nerve associated ventrally with a small (lingual?) ganglion; 15 Basilar plate and notochord; 16 Branches of facial nerve; 17 Internal carotid artery; 18 Mesenchymal density between pouch I and external acoustic meatus (malleus); 19 External acoustic meatus; 20 Tympanic nerve; 21 Facial nerve; 22 Cochlear portion of otic capsule; 23 Vestibular portion of otic capsule.

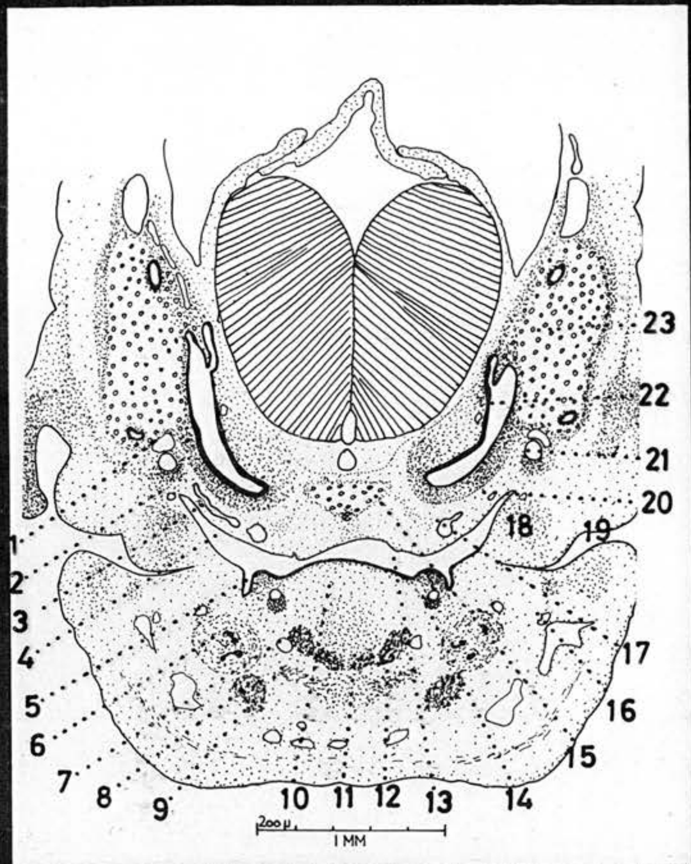


Fig. IV.108. Transverse section of ventral portion of head at the level of the first pharyngeal pouch, embryo D 41 (14.5 mm. G.L.), (semischematic).

1 Anterior cardinal vein; 2 Chorda tympani nerve; 3 Lateral branch of internal carotid artery; 4 Tubotympanic recess; 5 Small lateral (tonsillar?) swelling in pharyngeal floor; 6 Submandibular salivary gland; 7 Hypoglossal nerve; 8 M. digastricus mandibulae; 9 M. mylohyoideus; 10 Root of tongue; 11 M. geniohyoideus; 12 M. hyoglossus; 13 Pharynx; 14 Glossal branch of glossopharyngeal nerve associated ventrally with a small (lingual?) ganglion; 15 Basilar plate and notochord; 16 Branches of facial nerve; 17 Internal carotid artery; 18 Mesenchymal density between pouch I and external acoustic meatus (malleus); 19 External acoustic meatus; 20 Tympanic nerve; 21 Facial nerve; 22 Cochlear portion of otic capsule; 23 Vestibular portion of otic capsule.

the lingual nerve, accompanied by the elongated submandibular ganglion, undercuts the salivary ducts and turns laterad towards Meckel's cartilage to leave the body of the tongue. The Mm. geniohyoideus and mylohyoideus are also present in the 15 mm. embryo (11, 9). As the level of the tubotympanic recess is approached from the front, the body of the tongue diminishes in height to give way to the flattened root (10). At this level the intrinsic muscles and the genioglossus have disappeared, the hypoglossal nerve (7) has moved laterad where it now occupies the space between the styloglossus and hyoglossus, the submandibular salivary gland (6) is situated below the latter muscles, while the lingual branch of the glossopharyngeal nerve (14) has formed immediately ventral to the pharyngeal floor, being associated here with the spherical lingual ganglion. As the pharyngeal floor rises to cover the epiglottic swelling, the ventral portion of Reichert's cartilage, and the basihyoid form the ventral pharyngeal relations (Fig. IV.99./19). The geniohyoideus, mylohyoideus and hyoglossus terminate on these skeletal structures. A thin round pre-muscle belly has appeared in the median plane immediately dorsal to the basihyoid. It starts from near the apex of the epiglottis and ends just above the hyoid by splitting into two lateral portions without however fastening on to the hyoid apparatus. This is the precursor of the M. hyoepiglotticus (18). Small islands of accessory thyroid tissue, situated in the median plane and in close proximity to the basihyoid, were found in all embryos of the present group; caudoventrally to the basihyoid in four of the six specimens (20), anterodorsally in another, and in both aforementioned regions in the last. The most caudal of the structures forming ventral relationships with the pharynx, are the epiglottic and arytenoid swellings, neither of which as yet evidence any sign of cartilage formation (Fig. IV.106./19, 20).

The growth changes that have taken place in the embryo since the preceding stage can be considered to have special significance, because they transformed the hitherto quite immature pharynx into an organ much more akin to that found postnatally. Also in the surrounding regions similar major changes have occurred.

Complex IV, still attached to the pharyngeal edge in the 9 - 12 mm. stage, has broken away and has been engulfed by the thyroid primordium, which itself has reached the end of its descent from the pharyngeal floor to the proximal portion of the trachea. The first pharyngeal pouch, of course, has remained with the pharynx as the tubotympanic recess, and can be noted as now protruding from the pharyngeal roof. This is due to the appearance, below the recess, of a secondary epithelial fold, which is pushed ahead of the lateral palatine processes as they invade the pharyngeal chamber from the front. The anlage of the M. tensor veli palatini has appeared at the root of the processes as the first member of the palatine group. The esophagus has migrated to the left of the median plane where it remains from now on. It should be interesting to study the factors that bring about this shift, because, on rough examination, the tube seems to be rather isolated, being embedded in a wide zone of loose embryonic connective tissue. The tongue has been transformed from a mere wide and flat elevation on the dorsal surface of the primitive jaw, to a structure much like that of the mature animal. It is now long and narrow and well raised above the floor of the oral cavity, and keeps pace with the rapid forward growth of the face which has also begun since the previous stage. Two rounded elevations have developed on either side of the root of the tongue, just medial to the secondary folds mentioned above in connection with the progress of the lateral palatine processes. The swellings are composed of dense undifferentiated mesenchyme and will in later chapters be observed to form the palatine tonsils. The anlage for the pharyngeal musculature has flattened from the previously seen cell cord to an anteriorly notched cap-shaped layer overlying the cervical flexure of the pharynx. It is connected to adjacent skeletal structures by a number of primitive muscular bands which project beyond its rather well defined boundaries.

Posteriorly, it is confluent with the muscular envelope of the esophagus. A long cartilaginous bar, the Basilar plate, has appeared above the horizontal portion of the pharynx. It is the first part of the chondrocranium to be laid down, and concerns itself in later embryonic progress with the formation of the sphenoid and occipital bones. The basilar plate has severed the already drawn-out connection between Rathke's pocket and the pharyngeal floor. A small section of the connecting stalk remains below the plate as the Pharyngeal hypophysis.

The transformations with regard to pharyngeal morphology, as they have been portrayed in this stage, coupled with the closure of the two lateral palatine processes which will take place before the embryo reaches 30 mm. G.L., will bring the pharyngeal development to a point where the basic adult pattern is established and only further increase in size is necessary to provide for the fully matured organ.

STAGE 10

(22.0 mm. G.L.)

The observations for this stage of development are based on the following embryos:

Embryo	D 38	22.5 mm. G.L.	litter 9
Embryo	D 39	22.0 mm. G.L.	
Embryo	D 40	21.5 mm. G.L.	
Embryo	D 3	21.5 mm. G.L.	
Embryo	DD 2	23.0 mm. G.L.	

Fig. IV.109. Canine embryo D 38 (22.5 mm. G.L.).

Number of embryo:	D 38	Date of processing:	April 29, 1961
Size before fixation:	-	Block:	Paraffin
Size after fixation:	22.5 mm. C.L.	Stain:	H. & E.
Age:	-	Plane of section:	Transverse
Position on uterus:	-	Thickness of section:	10 microns
Size of loculus:	-	Series made:	D 38 (1 - 34)
Fixed in:	10% Form./Bouin's Fluid		
Stored in:	70% Alcohol		

From litter:		9
Number of embryos in litter:		5
DAM	Breed:	-
	Age:	-
	Weight:	-
SIRE	Breed:	-
	Age:	-
	Weight:	-
Number of services:		-
Date of 1st service:		-
Date of 2nd service:		-

Date of collection: April 15, 1960

At time of collection Dam was alive (X), dead ( ).

Embryos collected hour(s) after death.

Litter received from: Department of Anatomy, Ontario Veterinary College.

Remarks:

Fig. IV.110. Canine embryo D 39 (22.0 mm. G.L.).

Number of embryo:	D 39	Date of processing:	April 29, 1961
Size before fixation:	-	Block:	Paraffin
Size after fixation:	22.0 mm. G.L.	Stain:	H. & E.
Age:	-	Plane of section:	Horizontal
Position on uterus:	-	Thickness of section:	10 microns
Size of loculus:	-	Series made:	D 39 (1 - 36)
Fixed in:	10% Form./Bouin's Fluid		
Stored in:	70% Alcohol		

From litter: 9

Number of embryos in litter: 5

DAM Breed: -

Age: -

Weight: -

SIRE Breed: -

Age: -

Weight: -

Number of services: -

Date of 1st service: -

Date of 2nd service: -

Date of collection: April 15, 1960

At time of collection Dam was alive (X), dead ( ).

Embryos collected hour(s) after death.

Litter received from: Department of Anatomy, Ontario Veterinary College.

Remarks:

Fig. IV.111. Canine embryo D 40 (21.5 mm. G.L.).

Number of embryo:	D 40	Date of processing:	April 29, 1961
Size before fixation:	-	Block:	Paraffin
Size after fixation:	21.5 mm. G.L.	Stain:	H. & E.
Age:	-	Plane of section:	Sagittal
Position on uterus:	-	Thickness of section:	10 microns
Size of loculus:	-	Series made:	D 40 (1 - 25)
Fixed in:	10% Form./Bouin's Fluid		
Stored in:	70% Alcohol		

From litter:		9
Number of embryos in litter:		5
DAM	Breed:	-
	Age:	-
	Weight:	-
SIRE	Breed:	-
	Age:	-
	Weight:	-
Number of services:		-
Date of 1st service:		-
Date of 2nd service:		-

Date of collection: April 15, 1960

At time of collection Dam was alive (X), dead ( ).

Embryos collected hour(s) after death.

Litter received from: Department of Anatomy, Ontario Veterinary College.

Remarks:

Fig. IV.112. Canine embryo D 3 (21.5 mm. G.L.).

Number of embryo:	**D 3**	Date of processing:	**July 6, 1960**
Size before fixation:	**-**	Block:	**Paraffin**
Size after fixation:	**21.5 mm. G.L.**	Stain:	**H. & E.**
Age:	**-**	Plane of section:	**Horizontal**
Position on uterus:	**-**	Thickness of section:	**10 microns**
Size of loculus:	**-**	Series made:	**D 3 (1 - 58)**
Fixed in:	**10% Form./Bouin's Fluid**		
Stored in:	**70% Alcohol**		

From litter:		**9**
Number of embryos in litter:		**5**
DAM	Breed:	**-**
	Age:	**-**
	Weight:	**-**
SIRE	Breed:	**-**
	Age:	**-**
	Weight:	**-**
Number of services:		**-**
Date of 1st service:		**-**
Date of 2nd service:		**-**

Date of collection: **April 15, 1960**

At time of collection Dam was alive (**X**), dead ( ).

Embryos collected hour(s) after death.

Litter received from: **Department of Anatomy, Ontario Veterinary College.**

Remarks:

Number of embryo:	**DD 2**	Date of processing:	**June 16, 1961**
Size before fixation:	-	Block:	-
Size after fixation:	**23.0 mm. G.L.**	Stain:	-
Age:	-	Plane of section:	-
Position on uterus:	-	Thickness of section:	-
Size of loculus:	-	Series made:	**Used for dissect**-ion
Fixed in:	**10% Form./Bouin's Fluid**		
Stored in:	**70% Alcohol**		

From litter:		9
Number of embryos in litter:		5
DAM	Breed:	-
	Age:	-
	Weight:	-
SIRE	Breed:	-
	Age:	-
	Weight:	-
Number of services:		-
Date of 1st service:		-
Date of 2nd service:		-

Date of collection: **April 15, 1960**

At time of collection Dam was alive (**X**), dead ( ).

Embryos collected hour(s) after death.

Litter received from: **Department of Anatomy, Ontario Veterinary College.**

Remarks: **For a picture see that of litter mate D 40 (Fig.IV.111.), and for a picture of the dissection see Fig.IV.121.**

The Shape of the Pharynx

No major changes have taken place in the general shape of the pharyngeal tube. There has been a slight increase in size, and the pharyngeal measurements for this group of embryos are now about 2.0 mm. longitudinally, and roughly 0.9 mm. transversely just in front of the tubotympanic recess. The dorsoventral diameter in the anterior pharynx has also increased, resulting, on cross section, in a more or less rectangular lumen (Fig. IV.113./17) as compared to the flat, trough-like shape that was so obvious in younger embryos. The posterior pharynx has experienced little modification.

The roof, having formerly been convex from side to side, has reversed its bulge in the area between the pharyngeal hypophysis and the tubotympanic recess and is now concave when viewed from above. At the level of the tubotympanic recess, the roof is quite straight from side to side, to revert however to the common convexity as the cavity narrows behind to form the esophagus. From front to back the roof is quite straight, the cephalic flexure, formerly at the level of Rathke's pocket, having moved anteriorly. The cervical flexure is less marked. The tubotympanic recesses arise as laterally flattened tubes from the junction of roof and lateral wall of the pharynx (Fig. IV.113./2). They are directed upwards, backwards and outwards, and the direction of their planes is about half-way between sagittal and horizontal. If one of the pouches is sectioned transversely, it presents a rectangular lumen which comes abruptly to a point at the distal end of the recess.

Little change has taken place in the pharyngeal floor with the exception of some modification in the appearance of the so-called tonsillar swellings of the preceding stage, which have a length of 400 microns in embryo D 38. It will be recalled that embryos of the 15 mm. group showed two small swellings, pushing up from the floor anterior and lateral to the epiglottic swelling. The glossal branch of the glossopharyngeal nerve crossed below these swellings on its way to the root of the tongue, bearing at this point the spherical lingual ganglion and sending a single (tonsillar?) branch into the swelling from below (Fig. IV.108./14). In the present stage the swelling retains the same relationship to the nerve and its branch. However, an epithelial fold (alveolingual fold, Ramsay, 1935) from the root of the tongue has appeared, undercutting the swelling in a lateral direction and pushing it, as it were, to the lateral wall of the pharynx, so that what previously was a bulge arising from the pharyngeal floor and pushing dorsally, has by this transition become a bulge in the lateral pharyngeal wall and is directed medially (Fig. IV.113./21). Consequently, the floor of the pharynx, being in its anterior portion the root of the tongue, is quite smooth until it rises to cover first the median epiglottic and then the two arytenoid swellings, before it descends to form the anterior wall of the esophagus. The true floor of the posterior pharynx, represented by the two piriform recesses on either side of the arytenoid swellings, is unchanged (Fig. IV.116./18). Short cell cords were again observed springing from the lateral walls of the piriform recesses and protruding into the pharyngeal lumen.

Up to the 15 mm. stage it was expedient to consider the sharp lateral fold in which floor and roof joined as being the lateral wall of the pharyngeal tube. This fold received a great deal of attention especially in the previous stage, because it had turned ventrally throughout the length of the pharynx, causing the latter to look like an inverted trough. In this group of embryos, however, the fold has disappeared in the anterior portion but remains as the piriform recess in the posterior pharynx. Aided also by the fact that the height of the lumen in the anterior pharynx has increased, it is now possible to talk of a true lateral wall of the pharyngeal tube (Fig. IV.113.). A feature in the lateral pharyngeal wall is the lateral palatine process protruding medially (16). In the oral cavity these processes are wide and seem to be suspended from the roof to hang on either side of the body of the tongue, which arises from the floor (Figs. IV.114./1; IV.121./B). In the anterior pharynx they become very much narrower and appear as longitudinal swellings on the lateral walls, situated between the tonsillar swellings below and the openings into the tubotympanic recesses above (Fig. IV.113./16). Decreasing gradually in height, the palatine swellings taper away in the posterior pharynx, where they can be made out just above the piriform recesses as slight epithelial folds. It should be quite safe to regard them as the forerunners of the palatopharyngeal folds.

The Structure of the Pharyngeal Wall

No changes can be reported with regard to the epithelial lining of the pharyngeal cavity and its various extensions. The epithelia, in general, are still quite embryonic in nature and bear little resemblance to their adult counterparts. The inner epithelial core of the pharyngeal hypophysis has transformed into an upper tubular portion in which the lumen is irregular and ill-defined, and a lower globular part containing an ordinary lumen (Fig. IV.115./2). In two embryos there was a single cell strand connection between this portion and the epithelium of the pharyngeal roof, a feature not present in the 15 mm. stage. The connection between the pharyngeal hypophysis and Rathke's pocket above the basal plate has become very vague and can barely be traced along the course of one or two capillaries. For a more detailed description of the cellular make-up of the pharyngeal hypophysis the reader is referred to the previous stage (page 181).

The circum-vallate papillae have made their appearance on the pharyngeal floor just anterior to the root of the tongue (Fig. IV.116./22). They are round epithelial evaginations protruding well above the level of the tongue, their core being filled with fairly loose mesenchyme which is just starting to differentiate toward embryonic connective tissue. In embryo D 3 and D 39 two vallate papillae were found on either side of the midline; whereas in embryo D 38 there were two sets of three. The median lingual sulcus is present for the first time in this group of embryos. It is well developed along the body of the tongue and fades out opposite the tonsillar swellings (21)(Fig. IV.114./21). The epithelium covering the tonsillar swellings is not different from that over the root of the tongue. It consists of a fairly heavy basal layer of columnar cells and a thin superficial layer of irregular cells bearing round nuclei. Below the epithelium is a dense mass of as yet undifferentiated mesenchymal cells, which appear to be almost devoid of any cytoplasmic material. This dense tissue fills the entire swelling and blends into the much looser mesenchyme at its base.

The muscular cap (Fig. IV.116./11) covering the cervical flexure of the pharynx has not undergone significant changes. A new extension, however, situated

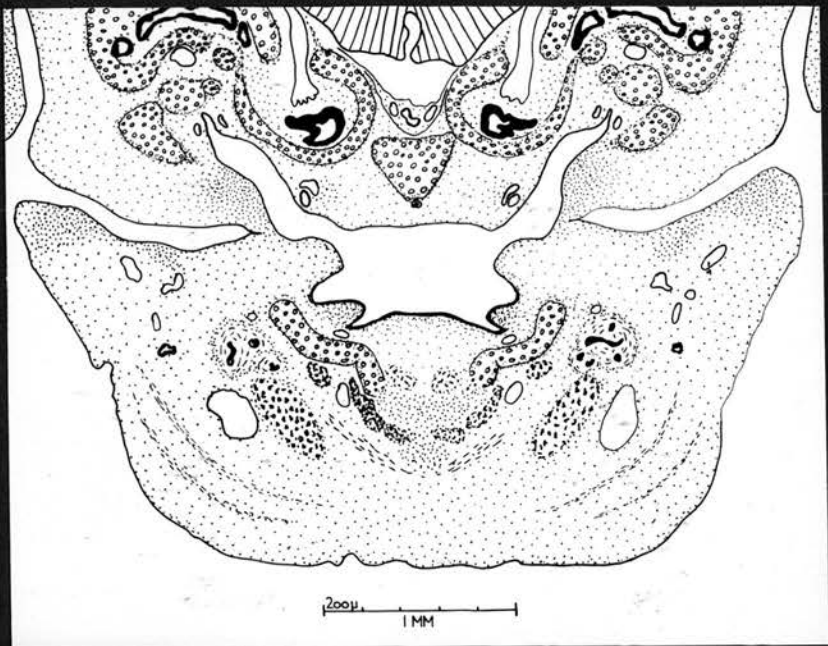


Fig. IV.113. Transverse section of pharynx and lower jaw at the level of the tubotympanic recess, embryo D 38 (22.5 mm. G.L.), (semischematic).

1 External acoustic meatus; 2 Tubotympanic recess; 3 Chorda tympani; 4 Dorsal extremity of Meckel's cartilage; 5 Incus; 6 Stapes; 7 Cochlea; 8 Basilar artery; 9 Basal plate; 10 Head chorda; 11 Cochlear nerve; 12 Facial nerve; 13 Tympanic nerve; 14 Density of future malleus; 15 Internal carotid artery and carotid nerve; 16 Palatine swellings; 17 Pharyngeal lumen; 18 Parotid duct; 19 Submandibular salivary gland; 20 Reichert's cartilage; 21 Tonsillar swelling; 22 Glossal branch of glossopharyngeal nerve; 23 M. digastricus mandibulae; 24 M. hyoglossus; 25 Caudal portion of deep intrinsic muscle of tongue; 26 M. geniohyoideus; 27 M. mylohyoideus; 28 hypoglossal nerve; 29 M. styloglossus; 30 Branches of facial nerve.

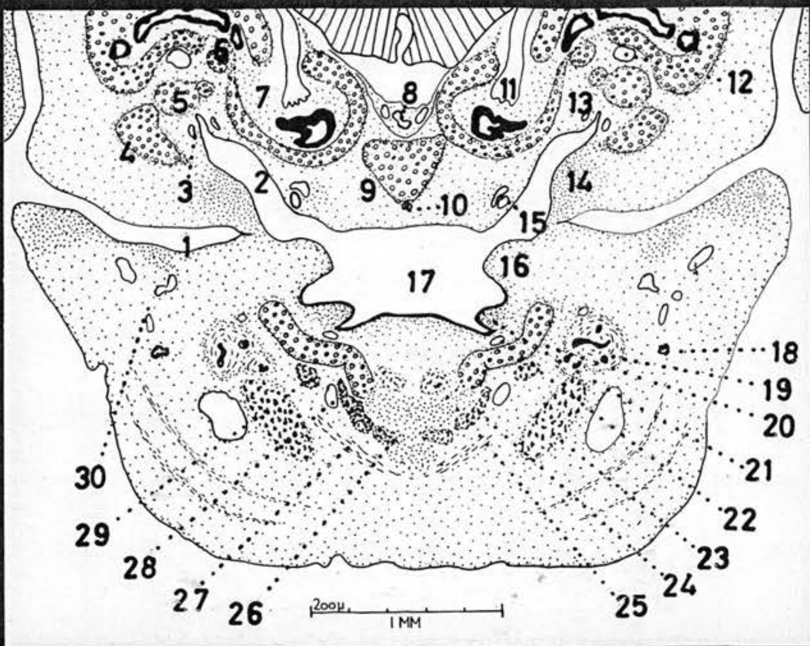
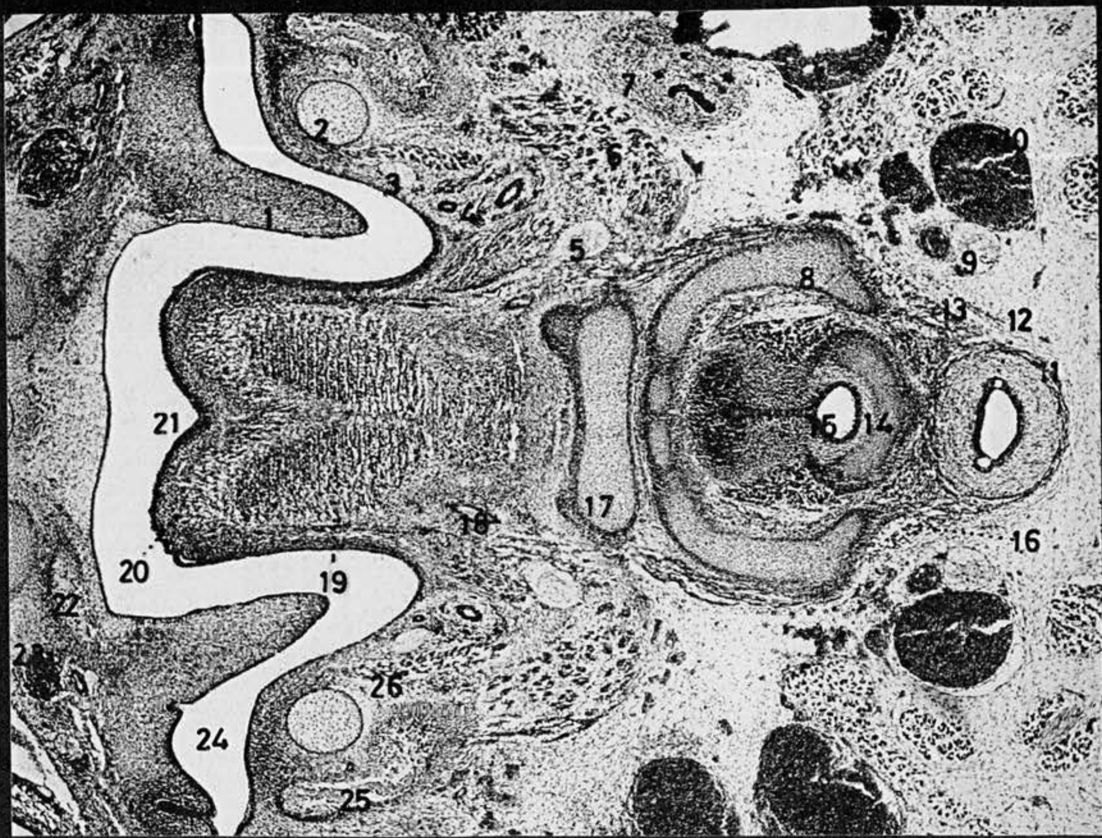


Fig. IV.113. Transverse section of pharynx and lower jaw at the level of the tubotympanic recess, embryo D 38 (22.5 mm. G.L.), (semischematic).

1 External acoustic meatus; 2 Tubotympanic recess; 3 Chorda tympani; 4 Dorsal extremity of Meckel's cartilage; 5 Incus; 6 Stapes; 7 Cochlea; 8 Basilar artery; 9 Basal plate; 10 Head chorda; 11 Cochlear nerve; 12 Facial nerve; 13 Tympanic nerve; 14 Density of future malleus; 15 Internal carotid artery and carotid nerve; 16 Palatine swellings; 17 Pharyngeal lumen; 18 Parotid duct; 19 Submandibular salivary gland; 20 Reichert's cartilage; 21 Tonsillar swelling; 22 Glossal branch of glossopharyngeal nerve; 23 M. digastricus mandibulae; 24 M. hyoglossus; 25 Caudal portion of deep intrinsic muscle of tongue; 26 M. geniohyoideus; 27 M. mylohyoideus; 28 hypoglossal nerve; 29 M. styloglossus; 30 Branches of facial nerve.

Fig. IV.114. Horizontal section of oral cavity, somewhat ventral to the plane of section in Fig. IV.116, embryo D 3 (21.5 mm. G.L.), (X 37).

1 Lateral palatine process; 2 Meckel's cartilage; 3 Lingual nerve; 4 Sublingual and submandibular salivary ducts; 5 Hypoglossal nerve; 6 M. digastricus mandibulae; 7 Submandibular salivary gland; 8 Thyroid cartilage; 9 Vagosympathetic trunk; 10 Internal jugular vein; 11 Esophagus; 12 Intraepithelial spaces; 13 M. cricoesophageus; 14 Cricoid cartilage; 15 Laryngeal lumen; 16 Caudal border of M. thyropharyngeus; 17 Basihyoid; 18 Lingual artery; 19 Deep intrinsic muscle of tongue; 20 Superficial intrinsic muscle of tongue; 21 Median sulcus of tongue; 22 Osseous bar of palatine (Pars sagittalis) and pterygoid bones; 23 Sphenopalatine ganglion; 24 Oral cavity; 25 Developing mandible; 26 M. mylohyoideus.



<u>Fig. IV.114</u>. Horizontal section of oral cavity, somewhat ventral to the plane of section in Fig. IV.116, embryo D 3 (21.5 mm. G.L.), (X 37).

1 Lateral palatine process; 2 Meckel's cartilage; 3 Lingual nerve; 4 Sublingual and submandibular salivary ducts; 5 Hypoglossal nerve; 6 M. digastricus mandibulae; 7 Submandibular salivary gland; 8 Thyroid cartilage; 9 Vagosympathetic trunk; 10 Internal jugular vein; 11 Esophagus; 12 Intraepithelial spaces; 13 M. cricoesophageus; 14 Cricoid cartilage; 15 Laryngeal lumen; 16 Caudal border of M. thyropharyngeus; 17 Basihyoid; 18 Lingual artery; 19 Deep intrinsic muscle of tongue; 20 Superficial intrinsic muscle of tongue; 21 Median sulcus of tongue; 22 Osseous bar of palatine (Pars sagittalis) and pterygoid bones; 23 Sphenopalatine ganglion; 24 Oral cavity; 25 Developing mandible; 26 M. mylohyoideus.

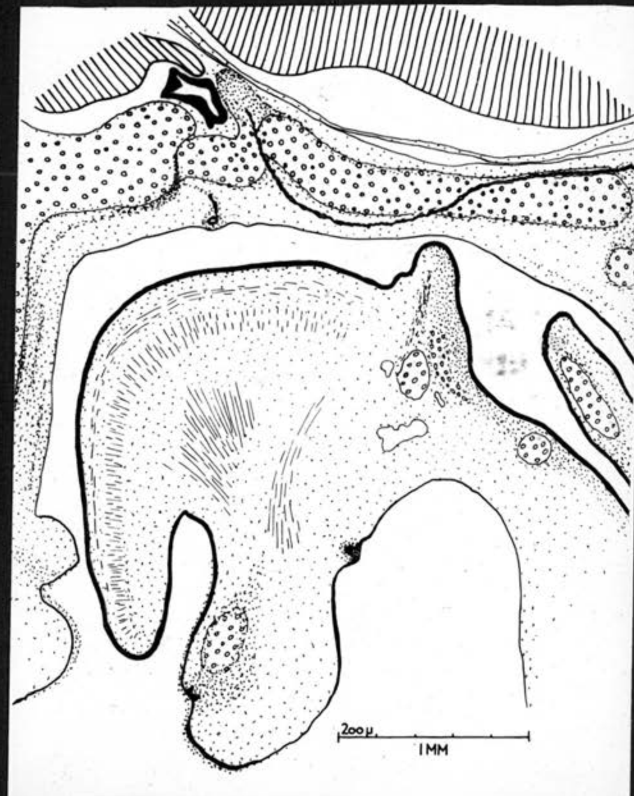
above the primordium of the stylopharyngeus is growing away from the cap in an anterior direction, passing Reichert's cartilage on the medial side. It does not as yet give any evidence where it may attach, but by virtue of its position it should be safe to conclude that it is the primordium of the pterygopharyngeus. In the space between the posterior larynx and the esophagus the muscle mass of the crico-esophageus has appeared (Fig. IV.114./13). The cricoid cartilage (14) is well established in the 22 mm. embryo but the cricopharyngeus muscle has not yet developed. The muscular envelope of the esophagus (11) is still thin but shows good muscle fibre formation mostly of the circular kind.

At the level of the cricoid cartilage where the esophagus is freeing itself from the muscular confines of the developing pharyngeal constrictors, there are some peculiar intercellular spaces in the epithelium of the esophagus (12). The esophageal lumen at this level has an oval cross section (300 microns greatest diameter) and the intra-epithelial spaces are located at the poles of this oval. They are tubular and run parallel to the long axis of the esophagus. Usually there is only one space to a side but two or three which communicate with each other have been observed. The spaces never open into the esophageal lumen despite the fact that the wall dividing them from the esophageal lumen is often only one cell thick. These spaces are present at this level for a length of about 300 microns; and it is difficult to establish their significance at this time (see also pages 245 and 262).

The Relationships of the Pharynx

Overlying the roof of the pharynx is a cushion of embryonic connective tissue in which the number of capillaries is increasing. It separates the roof from the basal plate, on either side of which and at the level of the tubotympanic recesses are the now cartilaginous cochlear portions of the otic capsules (Fig. IV.113./7). The notochord is present and continuous, starting in the dense tissue which later is to become the Dorsum sellae turcicae. It runs through the basal plate and along its ventral surface for a time, then piercing it once more to gain the dorsal surface, from where it enters the centre of the cervical vertebrae (Fig. IV.115./3, 4, 6). At the level where the notochord passes ventrally through the basal plate, the internal carotid arteries, having formed dorsal relations with most of the anterior pharynx along their subbasal course and given off the single small lateral branch, pass into the cranium via the carotid foramina. They are accompanied by the carotid nerve (Fig. IV.113./15). The most anterior of the dorsal relations is the medially placed pharyngeal hypophysis which serves as a landmark for the anterior extent of the pharynx (Figs. IV.115./2; IV.116./2). The greater superficial petrosal nerve (24), leaving the seventh cranial nerve just below the geniculate ganglion, takes an anterior and ventral course along the dorsolateral face of the pharynx and ends farther forwards in the sphenopalatine ganglion which lies in the root of the lateral palatine processes in this group of embryos (Fig. IV.114./23). A bar of osseous tissue has appeared just medial to the course of this nerve which gives rise, in later stages, to the perpendicular portion of the palatine bone and the pterygoid bone (Fig. IV.116./23). It is the first osseous tissue encountered in the head. In the previous 15 mm. stage, a bar of very dense mesenchymal tissue was found in the same location. As the tubotympanic recess passes upwards below the cochlea, the tympanic nerve comes to lie between the two structures on its way from the petrosal ganglion behind to the otic ganglion in front of the recess (Fig. IV.113./13). At the level of the posterior border of the cochlea, the M. longus capitis is inserted on the ventral surface of the basal plate. The paired muscle runs caudally and slightly ventrally and covers the insertion of the M. rectus capitis ventralis which lies immediately caudal to the insertion of the former muscle. At this point the pharynx bends ventrally at the cervical flexure to become the esophagus, which continues the relationship to the M. longus capitis and, later, to the M. longus colli.

Anterior to the level of the tubotympanic recess, the structures forming lateral relations with the pharynx are the various derivatives of the first visceral arch. Notable among these is Meckel's cartilage which crosses the lateral face of the pharynx in an anteroventral direction (Figs. IV.116./5; IV.114./2). On its medial side run first the lingual nerve (3) and then, higher up, the Chorda tympani, while the main structures on its lateral side are the alveolar nerve and the developing osseous mandible (25). The two muscles associated with Meckel's cartilage are the medial pterygoid (Fig. IV.116./6) joining it from in front and above, and the mylohyoid, leaving it ventrally (Fig. IV.114./26). Anterior and medial to Meckel's cartilage is the previously observed pre-muscle mass of the M. tensor veli palatini, lying in the root of the palatine process and sending its fibres ventrally and medially towards the free edge of the palatine process. It is innervated by a branch of the mandibular nerve. Between the primordia of the medial pterygoid and the Tensor veli palatini muscles, there is an epithelial cell cord running parallel to the lateral edge of the primitive mouth cavity. This cord is peculiar in that it does not make contact with the oral or pharyngeal mucosa, as certainly the anlagen of the salivary ducts do. In its anterior course it splits to form up to three parallel running strands which reunite or end blindly. At various levels this cord has produced a lumen and by so doing has transformed itself into a duct, giving it the appearance of embryonic salivary ducts, of which the parotid, the sublingual and the submandibular are already present in the 22 mm. embryo. Ruling out the possibility of it being a transitory salivary duct, it is, quite likely, an epithelial remnant of the obliterated lateral edge in the area of junction between the oral and the pharyngeal cavities. As the laterally flattened tubotympanic recess arises from the dorsolateral angle of the pharynx, the tensor primordium moves dorsally and comes to lie in front of the narrow dorsal face of the recess. A little further caudally, Reichert's cartilage, similar to Meckel's, crosses the lateral surface of the pharynx (Fig. IV.116./9). It is accompanied behind by the M. digastricus mandibulae which attaches itself to the cartilage a little higher up (17). The external acoustic meatus forms lateral relations with the tubotympanic recess at the level at which Reichert's cartilage is passing the ventral portion of the pharyngeal cavity (Fig. IV.113./1, 20). Between the external acoustic meatus and the primitive auditory tube is the cellular density of the future malleus (14), which in this group of embryos is continuous with the enlarged dorsal portion of Meckel's cartilage (4). Immediately behind Reichert's cartilage and in the space between the lateral pharyngeal wall and the M. digastricus mandibulae are the glossopharyngeal and the hypoglossal nerves (Fig. IV.116./8, 16), the former being accompanied for a short distance by the pharyngeal



<u>Fig. IV.115</u>. Median section of pharyngeal region, embryo D 40 (21.5 mm. G.L.), (semischematic).

1 Rathke's pocket; 2 Pharyngeal hypophysis; 3 Head chorda; 4 Dorsum sellae turcicae; 5 Basilar artery; 6 Basal plate; 7 Epiglottis; 8 M. hyoepiglotticus; 9 Deep intrinsic muscle of tongue; 10 Superficial intrinsic muscle of tongue; 11 M. genioglossus; 12 M. geniohyoideus; 13 Basihyoid; 14 Thyroid cartilage; 15 Laryngeal lumen; 16 Ventral arch of atlas; 17 Vestibulum esophagi; 18 Cricoid cartilage; 19 Trachea.

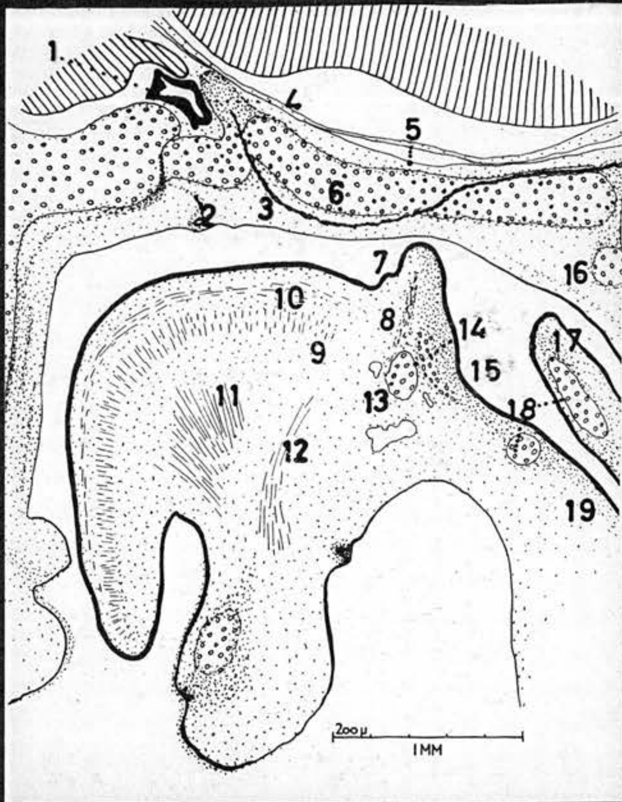


Fig. IV.115. Median section of pharyngeal region, embryo D 40 (21.5 mm. G.L.), (semischematic).

1 Rathke's pocket; 2 Pharyngeal hypophysis; 3 Head chorda; 4 Dorsum sellae turcicae; 5 Basilar artery; 6 Basal plate; 7 Epiglottis; 8 M. hyoepiglotticus; 9 Deep intrinsic muscle of tongue; 10 Superficial intrinsic muscle of tongue; 11 M. genioglossus; 12 M. geniohyoideus; 13 Basihyoid; 14 Thyroid cartilage; 15 Laryngeal lumen; 16 Ventral arch of atlas; 17 Vestibulum esophagi; 18 Cricoid cartilage; 19 Trachea.

Fig. IV.116. Horizontal section of pharynx, embryo D 3 (21.5 mm. G.L.), (X 37).

1 Basal plate; 2 Pharyngeal hypophysis; 3 Pharyngeal lumen; 4 Palatine swelling; 5 Meckel's cartilage; 6 M. pterygoideus medialis; 7 Tonsillar swelling; 8 Glossal branch of glossopharyngeal nerve; 9 Reichert's cartilage; 9A External carotid artery; 10 Thyrohyoid; 11 Pharyngeal muscles; 12 Anterior portion of larynx; 13 Vagosympathetic trunk; 14 Internal jugular vein; 15 Internal carotid artery; 15A Anterior laryngeal nerve; 16 Hypoglossal nerve; 17 M. digastricus mandibulae; 18 Piriform recess; 19 M. hyoepiglotticus; 20 Root of tongue; 21 Median sulcus of tongue; 22 Circumvallate papilla; 23 Osseous bar of palatine (Pars sagittalis) and pterygoid bones; 24 Greater superficial petrosal nerve.

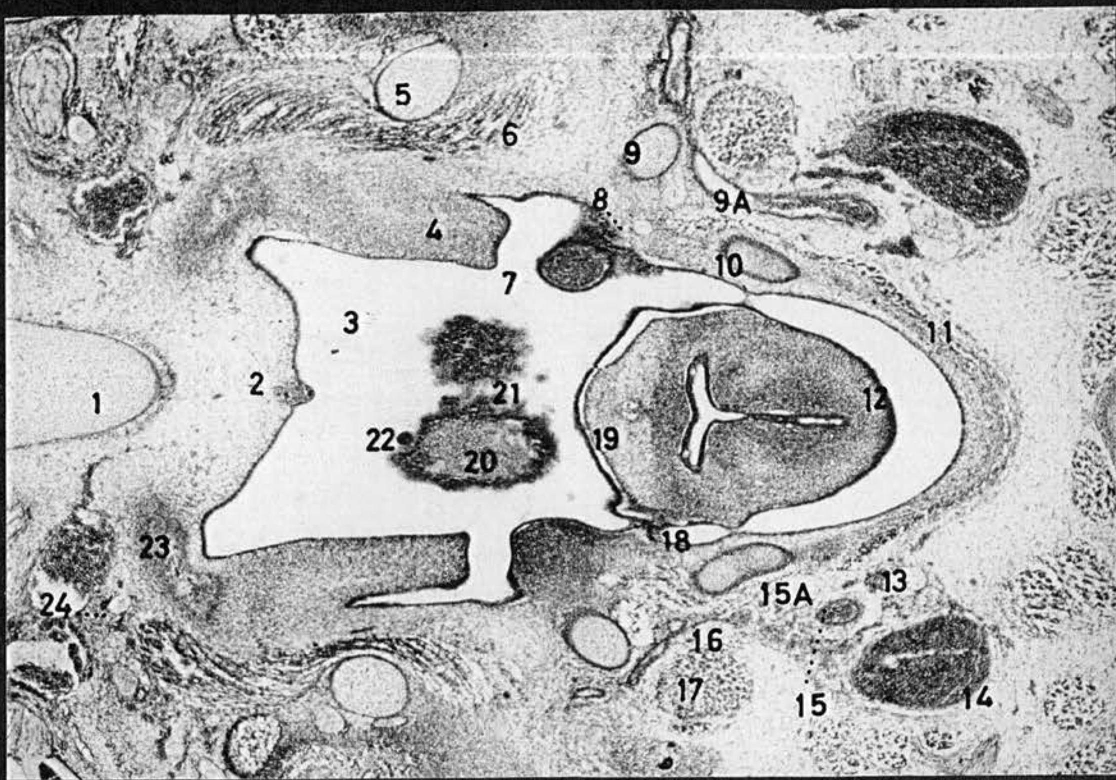


Fig. IV.116. Horizontal section of pharynx, embryo D 3 (21.5 mm. G.L.), (X 37).

1 Basal plate; 2 Pharyngeal hypophysis; 3 Pharyngeal lumen; 4 Palatine swelling; 5 Meckel's cartilage; 6 M. pterygoideus medialis; 7 Tonsillar swelling; 8 Glossal branch of glossopharyngeal nerve; 9 Reichert's cartilage; 9A External carotid artery; 10 Thyrohyoid; 11 Pharyngeal muscles; 12 Anterior portion of larynx; 13 Vagosympathetic trunk; 14 Internal jugular vein; 15 Internal carotid artery; 15A Anterior laryngeal nerve; 16 Hypoglossal nerve; 17 M. digastricus mandibulae; 18 Piriform recess; 19 M. hyoepiglotticus; 20 Root of tongue; 21 Median sulcus of tongue; 22 Circumvallate papilla; 23 Osseous bar of palatine (Pars sagittalis) and pterygoid bones; 24 Greater superficial petrosal nerve.

branch of the vagus. Behind the level of the tubotympanic recess, where the pharynx turns ventrally at the cervical flexure, short lateral relations are formed by (a) the thyrohyoid coming up from below and joining the anterior cornu of the thyroid cartilage (10), (b) the anterior laryngeal nerve emerging from the thyroid fissure and running dorsally towards the nodose ganglion (15A), and (c) the initial section of the external carotid artery (9A).

The principal ventral relation of the anterior pharynx is formed by the posterior portion of the body of the tongue. Immediately below its epithelial covering are the two layers of intrinsic pre-muscle masses, observed for the first time in the 15 mm. stage (Fig. IV.115./9, 10). Lateral to the musculature of the tongue the sub-lingual and submandibular salivary ducts underlie the floor of the pharynx for some distance (Fig. IV.114./4). The lingual nerve loops around them ventrally as it leaves the body of the tongue to ascend alongside Meckel's cartilage (3). At the level where the body of the tongue gives way to the root, the terminal branches of the glossal division of the glossopharyngeal nerve come together under the epithelium to form the glossal nerve which runs backwards and outwards to cross under the tonsillar swellings where it enlarges in the formation of the lingual ganglion (Fig. IV.116./7, 8). Immediately behind the level of the tonsillar swellings, the basihyoid with its anterior and posterior extensions is the principal ventral relation to the pharyngeal chamber (Fig. IV.115./13). The future keratohyoids leave the basihyoid in an anterolateral direction and after a short distance turn laterad as the epihyoids and then upwards along the lateral surface of the pharynx as Reichert's cartilages (Fig. IV.113./20). Below the basihyoid and close enough to it to be embedded in its primitive perichondrium is a small island of accessory thyroid tissue in embryos D 38, D 40 and D 39, but none in embryo D 3, which must be regarded as an exception, since so far in this study in every embryo some thyroid tissue had been encountered in the vicinity of the basihyoid. Above the basihyoid arises the short but quite distinct primordium of the hyoepiglotticus muscle (Fig. IV.116./19). The thyrohyoids leave the posterior aspects of the basihyoid and are directed upwards and backwards, embracing the upper portion of the developing larynx and forming ventral relations to the piriform recesses. A little further caudally, the anterior laryngeal nerve crosses below the piriform recess on its way from the interior of the larynx to the thyroid fissure, where it emerges to turn dorsally along the lateral face of the posterior pharynx (15A).

The transformation of the pharynx from a typical embryonic structure to one more akin to that of the adult that has taken place in the preceding stage, is continued in the 22 mm. stage. But despite these modifications the pharynx is still a simple organ, considering that it is as yet undivided into an upper respiratory and a lower digestive portion. The anlagen for the soft palate which could bring about such a division are present in the shape of the lateral palatine swellings. The tonsillar swellings have, by the appearance of an epithelial groove, been pushed, as it were, from their position in the floor to the lateral walls of the pharynx. Of the structures surrounding the pharynx, the cricoid cartilage and the cartilaginous capsule of the cochlea have formed, while the medial pterygoid muscle, a major lateral relation of the pharyngeal tube from now on, has made its appearance. In a very confined zone of the esophageal mucosa, rather peculiar intraepithelial spaces have been observed. An attempt will be made, during the succeeding stages, to find a reason for their presence.

S T A G E 11

(29.0 mm. G.L.)

The observations for this stage were carried out on seven embryos taken from two litters as follows:

Embryo	D 43	29.0 mm. G.L.	litter 9 a
Embryo	D 44	25.0 mm. (!) G.L.	litter 9 a
Embryo	D 45	29.0 mm. G.L.	litter 9 a
Embryo	CC 7	28.0 mm. G.L.	litter 9 b
Embryo	CC 8	29.0 mm. G.L.	litter 9 b
Embryo	DD 3	30.0 mm. G.L.	litter 9 b
Embryo	DD 4	28.0 mm. G.L.	litter 9 b

Embryo D 44 is 4 mm. shorter than its litter mates due to more pronounced body flexion.

With the appearance of the eye lids and their subsequent closure, mammalian embryos are considered to become fetuses. This transition is taking place in the dog in this group of embryos, and it will therefore be appropriate to use the term fetus from the next stage onwards.

Fig. IV.117. Canine embryo D 43 (29.0 mm. G.L.).

Number of embryo:	D 43	Date of processing:	April 28, 1961
Size before fixation:	-	Block:	Paraffin
Size after fixation:	29.0 mm. G.L.	Stain:	H. & E.
Age:	-	Plane of section:	Horizontal
Position on uterus:	-	Thickness of section:	10 microns
Size of loculus:	65 x 45 mm. (fixed)	Series made:	D 43 (1 - 47)
Fixed in:	10% Formalin		
Stored in:	10% Formalin		

From litter: 9 a

Number of embryos in litter: 8

DAM Breed: -

Age: -

Weight: -

SIRE Breed: -

Age: -

Weight: -

Number of services: -

Date of 1st service: -

Date of 2nd service: -

Date of collection: -

At time of collection Dam was alive ( ), dead ( ).

Embryos collected hour(s) after death.

Litter received from: Department of Anatomy, Ontario Veterinary College.

Remarks:

Fig. IV.118. Canine embryo D 44 (25.0 mm. (!) G.L.).

Note pronounced flexion of the spine.

Number of embryo:	D 44	Date of processing:	April 28, 1961
Size before fixation:	-	Block:	Paraffin
Size after fixation:	25.0 mm. G.L.(!)	Stain:	H. & E.
Age:	-	Plane of section:	Transverse
Position on uterus:	-	Thickness of section:	10 microns
Size of loculus:	65 x 45 mm. (fixed)	Series made:	D 44 (1 - 42)
Fixed in:	10% Formalin		
Stored in:	10% Formalin		

From litter:		9 a
Number of embryos in litter:		8
DAM	Breed:	-
	Age:	-
	Weight:	-
SIRE	Breed:	-
	Age:	-
	Weight:	-
Number of services:		-
Date of 1st service:		-
Date of 2nd service:		-
Date of collection:		-

At time of collection Dam was alive ( ), dead ( ).

Embryos collected hour(s) after death.

Litter received from: Department of Anatomy, Ontario Veterinary College.

Remarks:

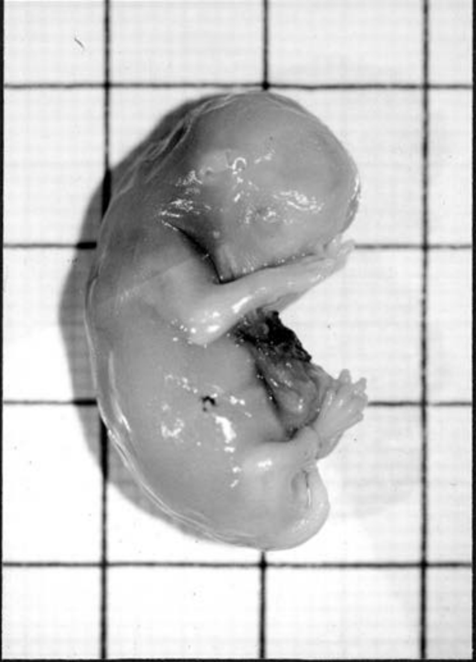


Fig. IV.119. Canine embryo D 45 (29.0 mm. G.L.).

Number of embryo:	D 45	Date of processing:	April 28, 1961
Size before fixation:	-	Block:	Paraffin
Size after fixation:	29.0 mm. G.L.	Stain:	H. & E.
Age:	-	Plane of section:	Sagittal
Position on uterus:	-	Thickness of section:	10 microns
Size of loculus:	65 x 45 mm. (fixed)	Series made:	D 45 (1 - 41)
Fixed in:	10% Formalin		
Stored in:	10% Formalin		

From litter:		9 a
Number of embryos in litter:		8
DAM	Breed:	-
	Age:	-
	Weight:	-
SIRE	Breed:	-
	Age:	-
	Weight:	-
Number of services:		-
Date of 1st service:		-
Date of 2nd service:		-
Date of collection:		-

At time of collection Dam was alive ( ), dead ( ).

Embryos collected hour(s) after death.

Litter received from: Department of Anatomy, Ontario Veterinary College.

Remarks:

Number of embryo:	CC 7	Date of processing:	June 14, 1961
Size before fixation:	-	Block:	Paraffin
Size after fixation:	28.0 mm. G.L.	Stain:	H. & E.
Age:	-	Plane of section:	Sagittal
Position on uterus:	-	Thickness of section:	10 microns
Size of loculus:	-	Series made:	CC 7 (1 - 155)
Fixed in:	10% Form./Bouin's Fluid		
Stored in:	70% Alcohol		

From litter:		9 b
Number of embryos in litter:		7
DAM	Breed:	German Shepherd
	Age:	-
	Weight:	50 lb.
SIRE	Breed:	X Collie
	Age:	-
	Weight:	50 lb.
Number of services:		-
Date of 1st service:		-
Date of 2nd service:		-

Date of collection: July 28, 1960

At time of collection Dam was alive (X), dead ( ).

Embryos collected hour(s) after death.

Litter received from: Dr. O.G. Smith, Hamilton, Ontario.

Remarks: For a picture see sketch of litter mate CC 8 overleaf (Fig.IV.120.)

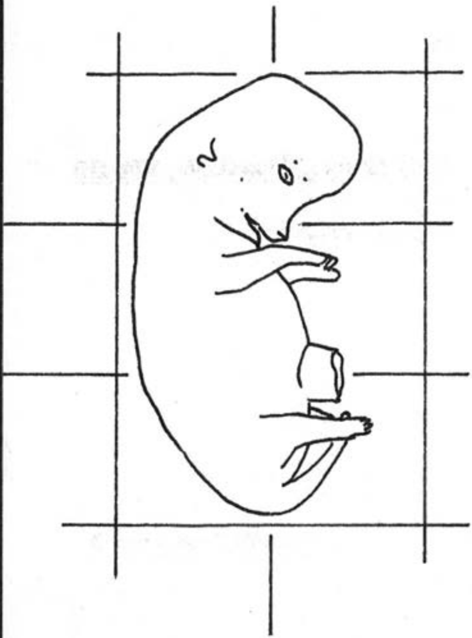


Fig. IV.120. Sketch of canine embryo CC 8 (29.0 mm. G.L.).

Number of embryo:	CC 8	Date of processing:	June 14, 1962
Size before fixation:	-	Block:	Paraffin
Size after fixation:	29.0 mm. G.L.	Stain:	H. & E.
Age:	-	Plane of section:	Horizontal
Position on uterus:	-	Thickness of section:	10 microns
Size of loculus:	-	Series made:	CC 8 (1 - 129)
Fixed in:	10% Form./Bouin's Fluid		
Stored in:	70% Alcohol		

From litter:		9 b
Number of embryos in litter:		7
DAM	Breed:	German Shepherd
	Age:	-
	Weight:	50 lb.
SIRE	Breed:	X Collie
	Age:	-
	Weight:	50 lb.
Number of services:		-
Date of 1st service:		-
Date of 2nd service:		-

Date of collection: July 28, 1960

At time of collection Dam was alive (X), dead ( ).

Embryos collected hour(s) after death.

Litter received from: Dr. O.G. Smith, Hamilton, Ontario.

Remarks:

Number of embryo:	DD 3	Date of processing:	June 16, 1961
Size before fixation:	-	Block:	-
Size after fixation:	30.0 mm. G.L.	Stain:	-
Age:	-	Plane of section:	-
Position on uterus:	-	Thickness of section:	-
Size of loculus:	-	Series made:	Used for dissecti
Fixed in:	10% Form./Bouin's Fluid		
Stored in:	70% Alcohol		

From litter:		9 b
Number of embryos in litter:		7
DAM	Breed:	German Shepherd
	Age:	-
	Weight:	50 lb.
SIRE	Breed:	X Collie
	Age:	-
	Weight:	50 lb.
Number of services:		-
Date of 1st service:		-
Date of 2nd service:		-

Date of collection: July 28, 1960

At time of collection Dam was alive (X), dead ( ).

Embryos collected hour(s) after death.

Litter received from: Dr. O.G. Smith, Hamilton, Ontario.

Remarks: For a picture see sketch of litter mate CC 8 (Fig.IV.120.)

Number of embryo:	DD 4	Date of processing:	June 16, 1961
Size before fixation:	-	Block:	-
Size after fixation:	26.0 mm. G.L.	Stain:	-
Age:	-	Plane of section:	-
Position on uterus:	-	Thickness of section:	-
Size of loculus:	-	Series made:	Used for dissectio-n
Fixed in:	10% Form./Bouin's Fluid		
Stored in:	70% Alcohol		

From litter:		9 b
Number of embryos in litter:		7
DAM	Breed:	German Shepherd
	Age:	-
	Weight:	50 lb.
SIRE	Breed:	X Collie
	Age:	-
	Weight:	50 lb.
Number of services:		-
Date of 1st service:		-
Date of 2nd service:		-

Date of collection: July 28, 1960

At time of collection Dam was alive (X), dead ( ).

Embryos collected hour(s) after death.

Litter received from: Dr. O.G. Smith, Hamilton, Ontario.

Remarks: For a picture see sketch of litter mate CC 8 (Fig.IV.120.)

Fig. IV.121. Ventral views of heads of different developmental stages, the lower jaws and tongues were removed to show the closing of the palate.

A Embryo DD 1 (15.0 mm. G.L.) of stage 9; B Embryo DD 2 (23.0 mm. G.L.) of stage 10; C Embryo DD 3 (30.0 mm. G.L.) of stage 11; D Embryo DD 4 (28.0 mm. G.L.) of stage 11.

1 Lateral palatine processes growing towards the midline; 2 Nasal septum; 3 Pharyngeal isthmus; 4 Recently closed palate.

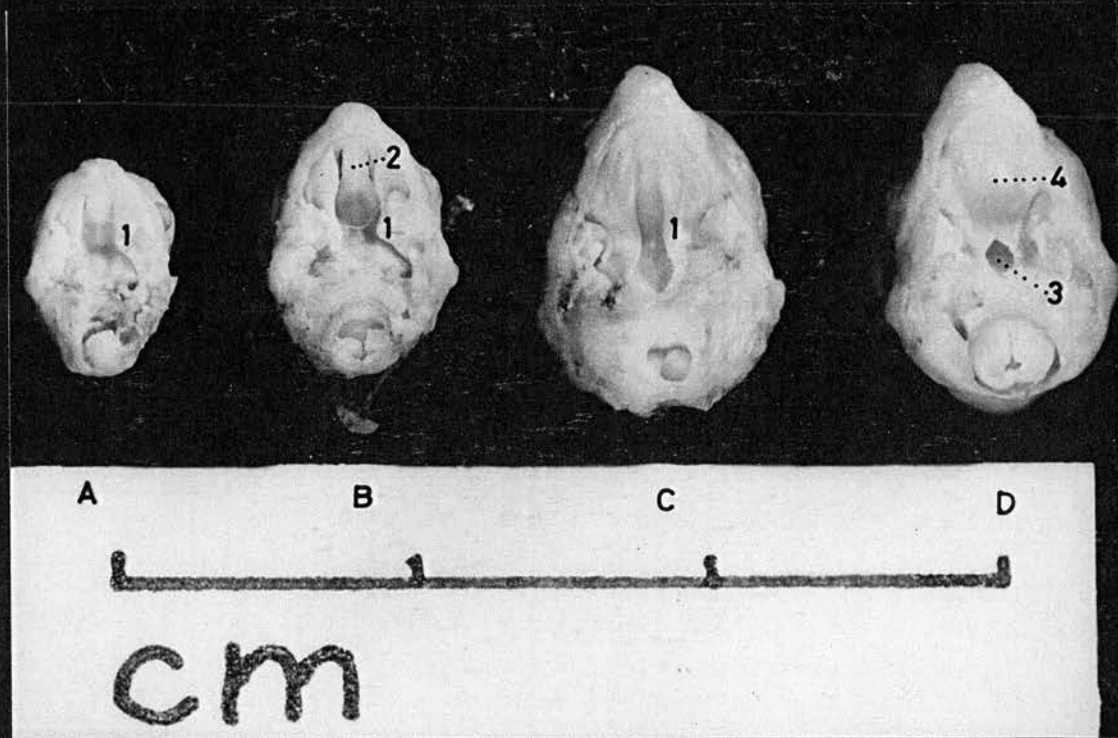


Fig. IV.121. Ventral views of heads of different developmental stages, the lower jaws and tongues were removed to show the closing of the palate.

A Embryo DD 1 (15.0 mm. G.L.) of stage 9; B Embryo DD 2 (23.0 mm. G.L.) of stage 10; C Embryo DD 3 (30.0 mm. G.L.) of stage 11; D Embryo DD 4 (28.0 mm. G.L.) of stage 11.

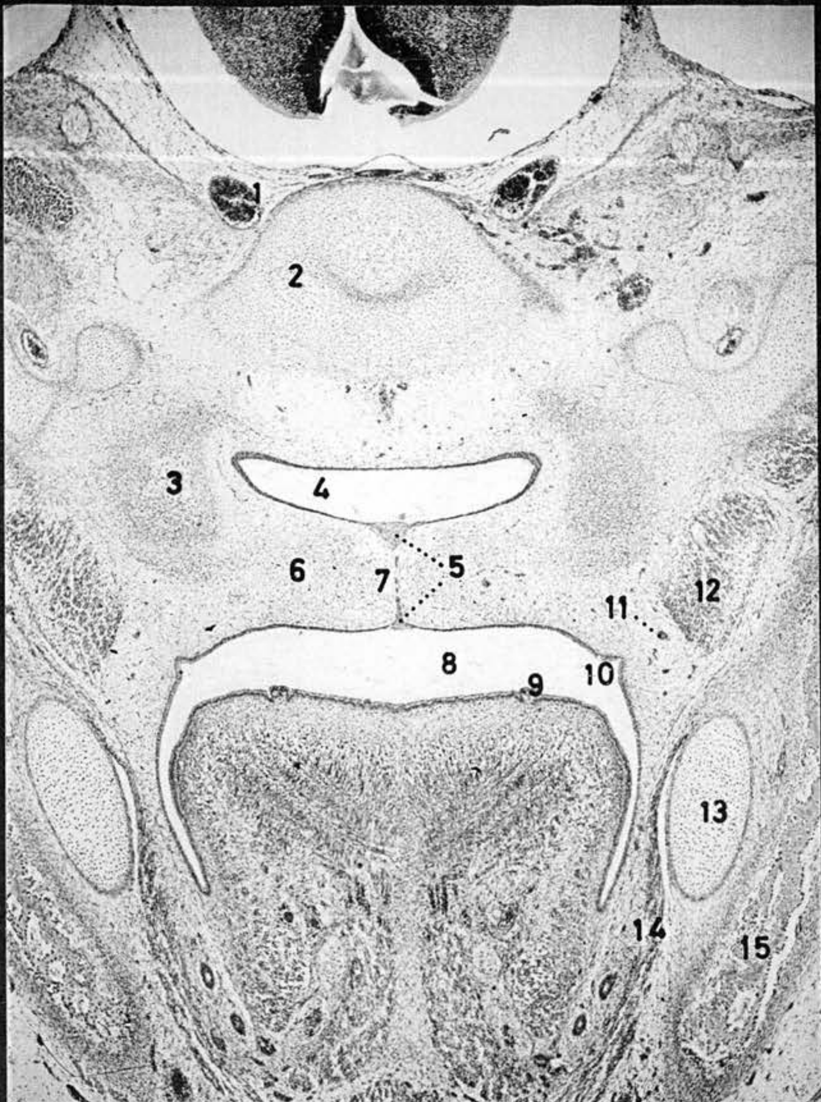
1 Lateral palatine processes growing towards the midline; 2 Nasal septum; 3 Pharyngeal isthmus; 4 Recently closed palate.

The Shape of the Pharynx

The pharyngeal chamber in the 29 mm. embryo has reached a length of roughly 2.4 mm., and a width of 1.2 mm., just in front of the tubotympanic recesses. The lateral palatine processes, which have been in evidence in the oral cavity since the 9 - 12 mm. stage, have finally joined in the midline, separating the primitive nasal cavity from the primitive oral cavity, and forming the future palate (Figs. IV.121.; IV.122./6). In the region of the common pharyngeal cavity, this fusion proceeds in a posterior direction, but has not reached completion in this group. Consequently, the soft palate is as yet imperfect. The fusion extends to the level of the entrance into the auditory tubes, and from here backwards the palatine processes are separate and continue as the previously seen palatine swellings along the lateral walls of the pharyngeal chamber (Fig. IV.125./6). Despite this imperfection the pharyngeal tube can already be divided into its two main adult compartments, the one above the soft palate being the nasopharynx, and the one below forming the oropharynx (Fig. IV.122./4, 8). These two chambers communicate in the adult through the round pharyngeal isthmus (Foramen intrapharyngicum)(Fig. IV.121./3) which is bounded by the posterior border of the soft palate anteriorly, and the two palatopharyngeal folds on either side, which meet each other in the midline above the esophageal entrance. In the 29 mm. embryo this foramen has a roughly triangular shape

Fig. IV.122. Transverse section of anterior pharynx, embryo D 44 (25.0 mm. (!) G.L.), (X 38).

1 Internal carotid artery; 2 Basal plate; 3 Developing sagittal portion of palatine and pterygoid bones; 4 Nasopharynx; 5 Cell proliferation filling longitudinal groove on either side of recently fused soft palate; 6 Soft palate; 7 Epithelial islands trapped during fusion of soft palate; 8 Oropharynx; 9 Circumvallate papilla; 10 Transient longitudinal groove indicating position of former lateral edge of posterior oral cavity; 11 Longitudinally orientated cell cord, which resulted from the obliteration of the lateral edge of posterior oral cavity (see also 10); 12 M. pterygoideus medialis; 13 Meckel's cartilage; 14 M. mylohyoideus; 15 Developing mandible.



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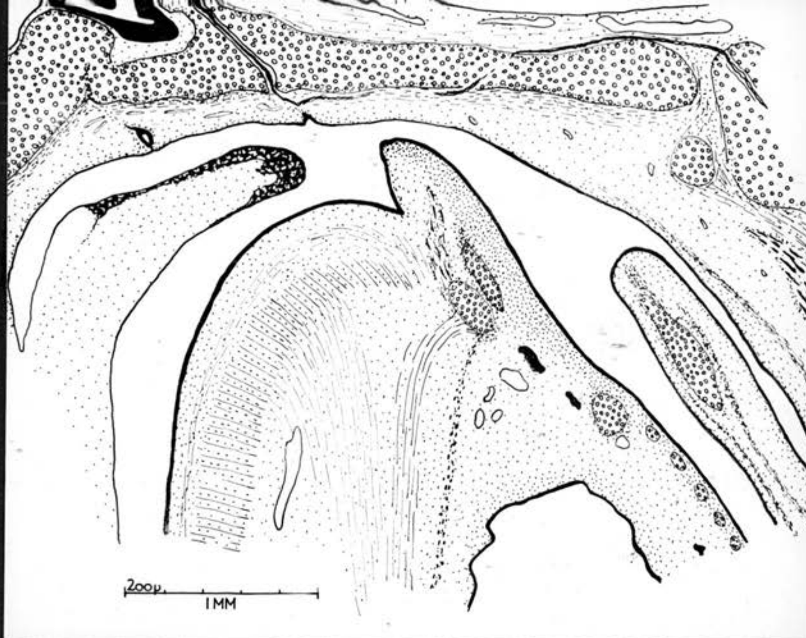


Fig. IV.123. Median section of pharyngeal region, embryo D 45 (29.0 mm. G.L.), (semischematic).

1 Nasopharyngeal duct; 2 Soft palate; 3 Oral cavity; 4 Epithelial sheet trapped during fusion of palatine processes; 5 Pharyngeal hypophysis; 6 Persistent Seessel's pocket; 7 Epiglottic swelling; 8 Notochord; 9 Basal plate; 10 Ventral arch of atlas; 11 M. longus capitis; 12 Cricoid cartilage; 13 Esophagus; 14 Trachea; 15 Isthmus of the thyroid gland; 16 Tracheal rings; 17 Accessory thyroid tissue; 18 M.mylohyoideus; 19 M. geniohyoideus; 20 M. genioglossus; 21 Ventral portion of intrinsic lingual musculature; 22 Dorsal portion of intrinsic lingual musculature; 23 M. hyoepiglotticus; 24 Basihyoid; 25 Thyroid cartilage.

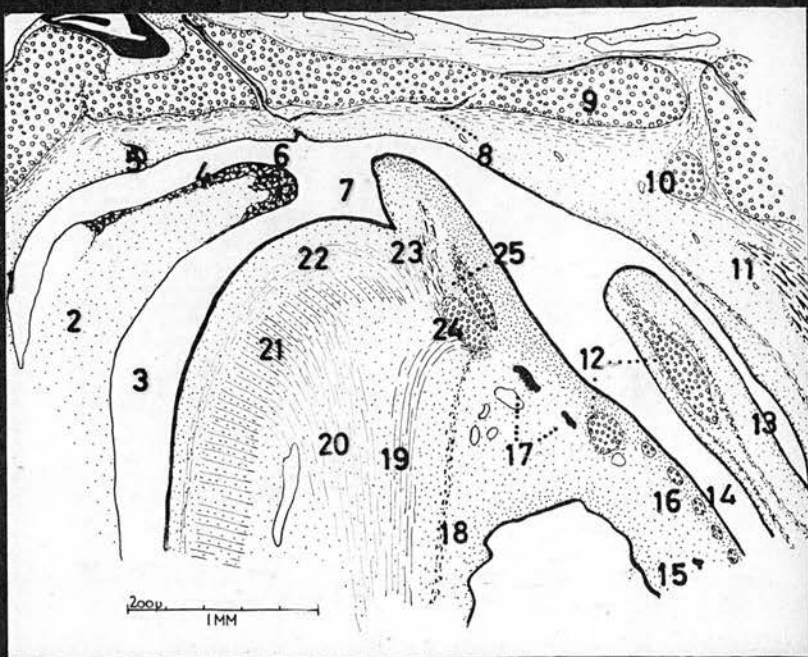


Fig. IV.123. Median section of pharyngeal region, embryo D 45 (29.0 mm. G.L.), (semischematic).

1 Nasopharyngeal duct; 2 Soft palate; 3 Oral cavity; 4 Epithelial sheet trapped during fusion of palatine processes; 5 Pharyngeal hypophysis; 6 Persistent Seessel's pocket; 7 Epiglottic swelling; 8 Notochord; 9 Basal plate; 10 Ventral arch of atlas; 11 M. longus capitis; 12 Cricoid cartilage; 13 Esophagus; 14 Trachea; 15 Isthmus of the thyroid gland; 16 Tracheal rings; 17 Accessory thyroid tissue; 18 M.mylohyoideus; 19 M. geniohyoideus; 20 M. genioglossus; 21 Ventral portion of intrinsic lingual musculature; 22 Dorsal portion of intrinsic lingual musculature; 23 M. hyoepiglotticus; 24 Basihyoid; 25 Thyroid cartilage.

with its rounded base in the posterior pharynx and its apex anteriorly between the two diverging palatine processes.

The nasopharynx extends from a level through the pharyngeal hypophysis to the pharyngeal isthmus. Anteriorly, it is continued by the nasopharyngeal duct, through which it is in communication with the nasal cavity (Fig. IV.123./1); posteriorly, it joins the ventral chamber of the pharynx through the pharyngeal isthmus. At the level of the pharyngeal hypophysis, the nasopharynx is a dorsoventrally flattened tube, the roof and floor of which are straight from side to side and run parallel to each other. In two embryos of this group (D 44 and CC 8), the epithelium overlying the pharyngeal hypophysis is drawn upwards by the epithelial connection between the pharyngeal roof and the epithelial core of the pharyngeal hypophysis, forming a small infundibulum at this point, a feature observed for the first time since the rupture of the connecting stalk to Rathke's pocket. Seessel's pocket, last observed in the 9 - 12 mm. stage, has persisted in embryos D 45 and CC 8. In both specimens this evagination is situated below the emergence of the notochord from the basal plate, about 800 microns behind the level of the pharyngeal hypophysis. In embryo CC 8 it takes the form of an epithelial tube about 250 microns in length, extending in front of the notochord from the pharyngeal roof well into the basal plate. In embryo D 45 it is only a small conical evagination in the roof (6). Just behind the evagination in the same embryo there is a peculiar, very faintly red-staining, mass (not shown in the drawing) not much larger than the evagination itself. It resembles in structure developing bone, but true bone on the same section stains much darker. There is a possibility that this mass of tissue has played a role in the retention of Seessel's pocket, although it was not present in embryo CC 8 in which, in fact, the persisting pocket was much larger. In the latter specimen, apart from the retained Seessel's pocket, there is a third midline evagination about 400 microns caudal to Seessel's pocket. It is also small and conical, and, since there are no other structures in its vicinity, one could perhaps postulate that it is a manifestation of a previous contact between the notochord and the pharyngeal roof, of which a fair number were observed in earlier stages.

At the anterior border of the entrance into the tubotympanic recesses, both roof and floor of the nasopharynx become concave from side to side when viewed from above (Fig. IV.122./4). This concavity is carried upwards and outwards into the recesses and gives them their general direction (Fig. IV.125.). At the same level the floor of the nasopharynx divides in the midline and the now separate lateral palatine swellings, as they continue posteriorly, retain the same slope they had when they were still united (6). Just behind the auditory openings, the roof of the nasopharynx straightens, and, continuing posteriorly,

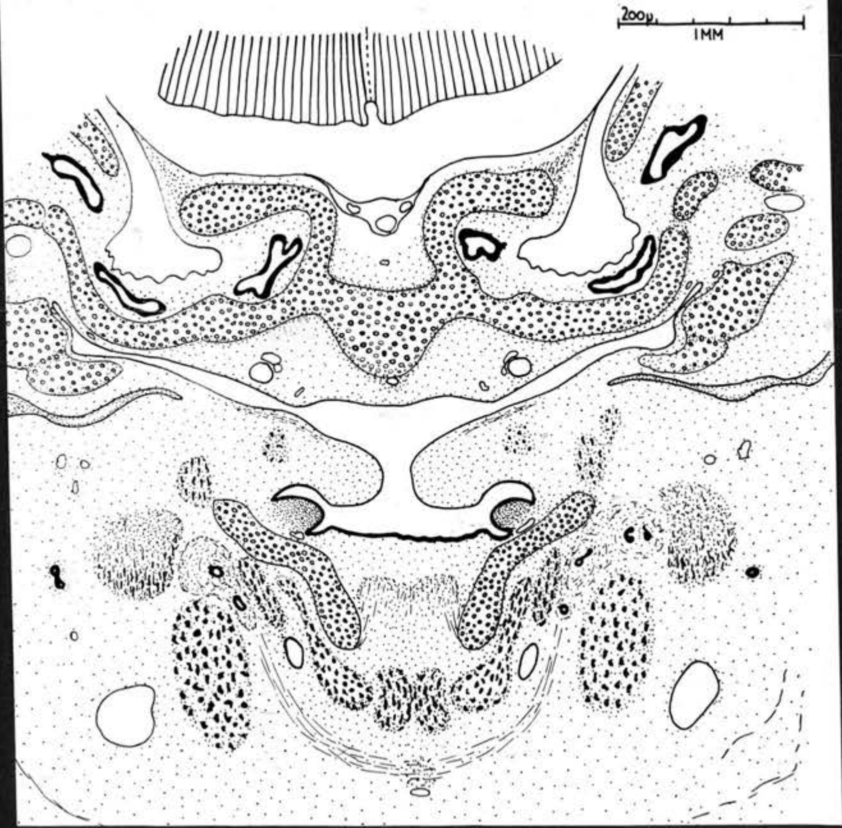


Fig. IV.124. Transverse section of pharyngeal region, embryo D 44 (25.0 mm. (!) G.L.), part reconstruction, (semischematic).

1 Cochlear nerve; 2 Cochlea; 3 Basilar artery; 4 Basal plate; 5 Notochord; 6 Internal carotid artery and nerve; 7 Tubotympanic recess (auditory tube); 8 Ventral sacculation of tympanic cavity opposite malleus; 9 Tympanic nerve; 10 Chorda tympani nerve; 11 Stapes; 12 Incus; 13 Facial nerve; 14 Dorsal extremity of Meckel's cartilage; 15 External acoustic meatus; 16 M. pterygoideus medialis; 17 M. pterygopharyngeus; 18 M. levator veli palatini; 19 Palatine swelling; 20 Tonsillar sinus; 21 Tonsillar swelling; 22 Glossal branch of glossopharyngeal nerve; 23 Submandibular salivary gland; 24 Posterior portion of masseter muscle; 25 Parotid duct; 26 M. digastricus mandibulae; 27 M. mylohyoideus; 28 Hypoglossal nerve; 29 M. hyoglossus; 30 M. geniohyoideus; 31 Posterior extremity of intrinsic muscle of tongue; 32 Reichert's cartilage; 33 M. styloglossus.

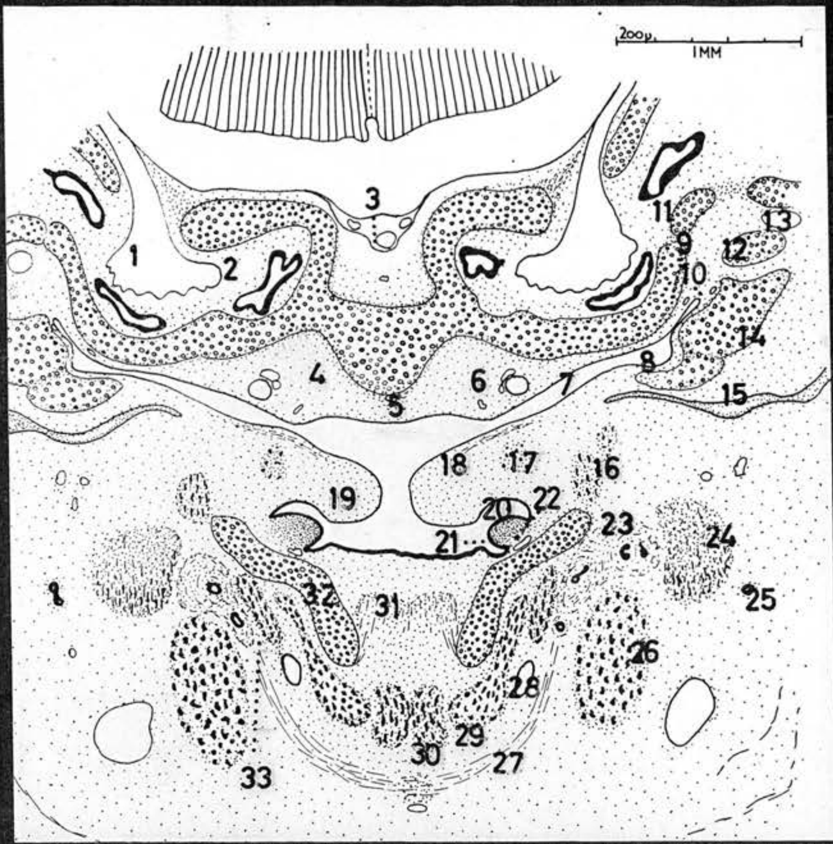


Fig. IV.124. Transverse section of pharyngeal region, embryo D 44 (25.0 mm. (!) G.L.), part reconstruction, (semischematic).

1 Cochlear nerve; 2 Cochlea; 3 Basilar artery; 4 Basal plate; 5 Notochord; 6 Internal carotid artery and nerve; 7 Tubotympanic recess (auditory tube); 8 Ventral sacculation of tympanic cavity opposite malleus; 9 Tympanic nerve; 10 Chorda tympani nerve; 11 Stapes; 12 Incus; 13 Facial nerve; 14 Dorsal extremity of Meckel's cartilage; 15 External acoustic meatus; 16 M. pterygoideus medialis; 17 M. pterygopharyngeus; 18 M. levator veli palatini; 19 Palatine swelling; 20 Tonsillar sinus; 21 Tonsillar swelling; 22 Glossal branch of glossopharyngeal nerve; 23 Submandibular salivary gland; 24 Posterior portion of masseter muscle; 25 Parotid duct; 26 M. digastricus mandibulae; 27 M. mylohyoideus; 28 Hypoglossal nerve; 29 M. hyoglossus; 30 M. geniohyoideus; 31 Posterior extremity of intrinsic muscle of tongue; 32 Reichert's cartilage; 33 M. styloglossus.

returns to the convexity that it retains into adult life. The nasopharynx is slightly convex from front to back when seen from above (Fig. IV.123.). The tubotympanic recesses have changed little from the 22 mm. to the 29 mm. stage. For the first time however, the distal portion of the recess has become moulded between the malleus ventrally and laterally and the cartilaginous capsule of the cochlea above (Fig. IV.127.). This results in an upward turn of the end portion of the tube and the development, at the bend, of an enlarged ventrolaterally directed saccule (Fig. IV.124./7, 8).

The oropharynx in the 29 mm. embryo is a similarly compressed tube with the tongue crowding into it from below and flattening the lumen still further, but allowing it to extend ventrally on either side (Fig. IV.122./8). At the same level as the auditory tubes leave the nasopharynx, the primordia of the palatine tonsils are situated in the lateral walls of the oropharynx (Fig. IV.124./21). When compared in size to the tonsillar swellings of the last stage, they were found to have doubled their bulk, measuring now nearly 150 microns on cross section and about 800 microns in length. So, already in the 29 mm. stage, their shape resembles very much their adult counterparts. Between the palatine processes above and the root of the tongue below, a tonsillar sinus has formed in which the tonsillar swellings lie well protected (20). The dorsal portion of the sinus is formed by the widening posterior extremity of a longitudinal groove in the dorsolateral region of the oropharynx, which has been recognised earlier as being the remnant of the obliterated original lateral edge into which the roof and the floor of the primitive oral cavity converged (Fig. IV.122./10). The cell cord in the overlying tissue trapped by this obliteration can still be made out (11). The portion of the sinus below the tonsillar swelling is the same epithelial fold that, in the 22 mm. stage, undercut, in a ventrolateral direction, the original tonsillar swelling when it arose from the floor of the pharyngeal tube; it has also enlarged. The dorsal portion of the tonsillar sinus continues backwards and downwards to form the narrow floor of the piriform recess. Here, a ventrally directed longitudinal fold is also being obliterated by epithelial apposition and loss of the distal edge as a discarded cell cord, in the same manner as it occurred in the previous stage to the same fold, but in front of the tonsillar swelling.

<u>The Structure of the Pharyngeal Wall</u>

On the whole, the epithelia in the different regions of the pharynx are similar. Regional differentiation has not taken place, and, judging by the quite embryonic character of the epithelia, will have to wait for some time. They consist of a basal layer of columnar cells and a superficial layer of cuboidal cells. In areas where the epithelia are somewhat thinner, such as in the nasopharynx, the thicker areas being the floor of the pharynx anteriorly and the entire posterior pharynx, the basal layer is made up of cuboidal cells and the top layer of a squamous sheet. In general, as the epithelial linings approach crevices or form folds, they become thicker and seem to lose their otherwise conspicuous cell organisation. Underlying the epithelia of the pharynx in the 29 mm. embryo is a thin layer of embryonic connective tissue.

The pharyngeal hypophysis consists of two portions: a proximal globular one, which as a rule is connected to the epithelium of the pharynx in this group of embryos, and a distal tubular part (Fig. IV.123./5). The latter is directed forwards and upwards along the previous path of the S-shaped stalk that connected Rathke's pocket with the pharyngeal roof. In embryo CC 7 this connection is still present through a canal in the basal plate (craniopharyngeal canal), and in embryo D 43 only a blood vessel marks the course of the former stalk. It will be remembered that the communication between the pharyngeal roof and the body of Rathke's pocket broke in the 15 mm. embryo with the appearance of the cartilaginous basal plate (9); therefore, finding a continuous epithelial strand between the two structures is surely an exception to the rule. The tubular portion of the pharyngeal hypophysis is now devoid of a lumen and is rapidly transforming into an unorganised cell cord. Also in the globular part below, where hitherto the epithelial cells had radially surrounded a quite regular lumen, cell organisation is being lost. Surrounding the epithelial core is a meshwork of connective tissue that arises from the subepithelial connective tissue of the pharyngeal roof and extends to the primitive perichondrium of the basal plate. Interlaced with this connective tissue sheath are a number of blood vessels of larger than capillary size which are connected to a pair of recently formed longitudinally running vessels just below the basal plate.

The epithelial lining of the tubotympanic recesses consists of a single layer of squamous cells; but the ventral surface of the dorsoventrally flattened tubes is somewhat thicker, the lining cells being almost of the low cuboidal type. In the edges formed by the confluence of the dorsal and ventral surfaces around the periphery of the recess (Fig. IV.127.), the epithelium again is thicker, consisting of two or three layers of irregular cells.

Although not strictly belonging to the confines of the pharynx, an interesting development in the recently formed roof of the oral cavity should be mentioned. About ten transversely running epithelial thickenings have appeared, foreshadowing the formation of the transverse ridges of the hard palate (Rugae palati) (Fig. IV.126./18). These thickenings become less developed and are more closely spaced as the oropharynx is approached. The tallness of the epithelium forming the ridges is due to proliferation in the basal layer, where the columnar cells form two or three rows and crowd the unchanged superficial layer into the lumen of the oral chamber.

The wall structure of the pharyngeal floor has changed little. The basal layer of the epithelium has grown considerably taller, adding to the increase in overall thickness of the epithelium over the root of the tongue. The nuclei in these cells are equally tall and are found at all levels, mostly however away from the basement membrane. The superficial layer is made up of cuboidal cells containing round nuclei. Embedded in this relatively high epithelium are the circumvallate papillae (Fig. IV.122./9). They do not protrude from the free border as much as they did in the last stage. The lingual epithelium, by dipping ventrally to the root of each papilla, has allowed for the formation of a ring-like space (moat). The tissue that fills the core of the papillae is a little denser than the subepithelial tissues surrounding them. The epithelium covering the papillae has lost the clear-cut organisation of the surrounding areas, although in height it is the same. The circumvallate papillae were found to be roughly 85 microns in diameter in the 29 mm. embryo.

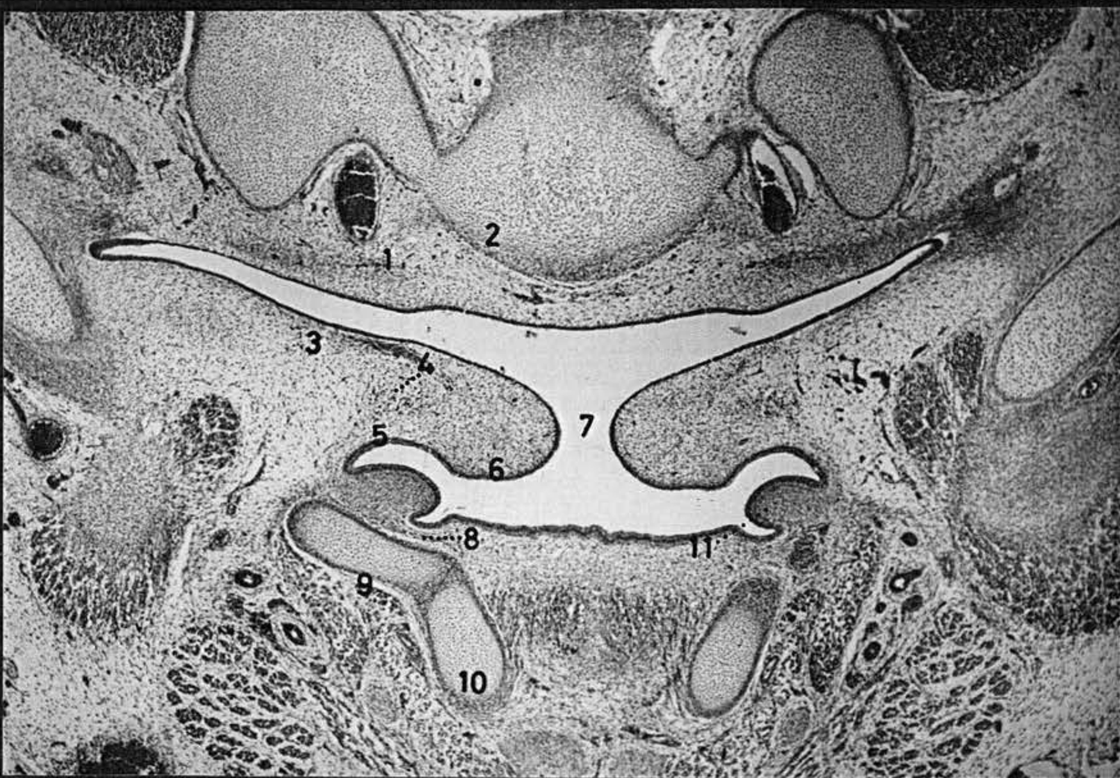
A narrow zone of darkly staining round cells was observed in the region of the root of the tongue between the basement membrane of the epithelium and the subepithelial connective tissue layer. Subsequent stages will have to be examined before this round-cell concentration can be regarded as the primordium of the lingual tonsils. The epithelium covering the tonsillar swellings is of the same type as that found over the root of the tongue, but is slightly lower. Under it is an almost homogeneous mass of round nuclei with a mere sprinkling of the more spindle-shaped ones, indicating a preponderance of the non-connective tissue elements at this time.

Several changes can be reported for the 29 mm. stage with regard to the development of the pharyngeal and palatine muscles. The pre-muscle mass for the <u>pterygo-pharyngeus</u>, observed in the 22 mm. embryo to push forward from the muscular cap along the lateral face of the pharynx, has increased in length and ends now in the vicinity of the anlage for the hamulus of the pterygoid bone. This is about at the level of the tonsils (Fig. IV.125./5). Somewhat ventral to this muscle and more closely applied to the pharyngeal chamber, is a faint indication of the <u>palatopharyngeus</u> pre-muscle mass. The <u>stylopharyngeus</u>, first seen in the 15 mm. stage, shows now a different fibre distribution. Previously, the pre-muscle strands would leave the medial surface of Reichert's cartilage and run mesiad and slightly ventrad towards the pharyngeal chamber. Arrived there, all the fibres turned posteriorly around

the glossal branch of the glossopharyngeal nerve and fanned out on the lateral wall of the posterior pharynx. In this stage, however, not all the bundles perform this posterior turn, some course ventrally and others even anteriorly.
At the caudal end of the pharyngeal constrictors, a small bundle of fibres has separated from the main bulk of the muscle and attached itself to the posterior border of the now well established cricoid cartilage. This bundle represents the primordium of the cricopharyngeus muscle.
The Tensor veli palatini has been seen for some time in the previous stages. In the 29 mm. embryo it has developed into a much more distinct entity which takes its origin from the vicinity of the otic ganglion and ends while passing around the ventral face of the hamulus of the pterygoid bone. The hamulus at this stage is a dense bar of tissue and is the posterior continuation of the already ossifying pterygoid plate (Fig. IV.122./3). The fibres of the tensor turn medially around the ventral border of the hamulus and end shortly after by fanning out in a horizontal plane. Associated with the origin of the tensor and with the ventral border of the opening into the auditory tube is a new small strip of pre-muscle tissue, running medially, ventrally and posteriorly under the dorsal surface of the palatine process, which at this level has not quite united with its fellow in the midline. It crosses the pterygopharyngeus on the dorsal side. This small pre-muscle spindle develops into the Levator veli palatini (Fig. IV.125./4).

Fig. IV.125. Transverse section of pharynx, embryo D 44 (25.0 mm. (!) G.L.), (X 40).

1 Internal carotid artery; 2 Basal plate; 3 Tubotympanic recess; 4 M. levator veli palatini; 5 M. pterygopharyngeus; 6 Palatine swellings; 7 Pharyngeal lumen; 8 Tonsillar swelling; 9, 10 Reichert's cartilage; 9 Epihyoid; 10 Keratohyoid; 11 Tonsillar sinus.



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1 Internal carotid artery; 2 Basal plate; 3 Tubotympanic recess; 4 M. levator veli palatini; 5 M. pterygopharyngeus; 6 Palatine swellings; 7 Pharyngeal lumen; 8 Tonsillar swelling; 9, 10 Reichert's cartilage; 9 Epihyoid; 10 Keratohyoid; 11 Tonsillar sinus.

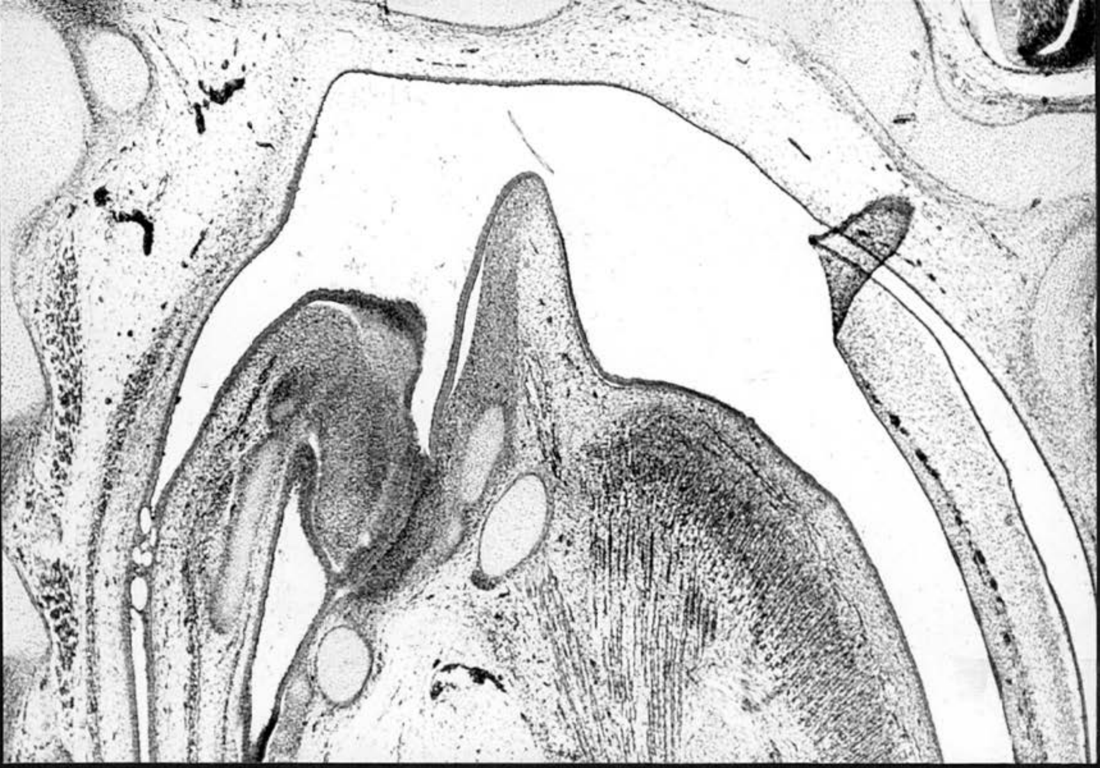


Fig. IV.126. Paramedian section of pharyngeal region, embryo CC 7 (28.0 mm. G.L.), (X 40).

1 M. longus capitis; 2 Muscular cap; 3 Vestibulum esophagi; 4 Intraepithelial spaces in esophagus; 5 Esophagus surrounded by esophageal muscle; 6 Cricoid lamina; 7 Laryngeal lumen; 8 Arch of cricoid cartilage; 9 Accessory thyroid tissue; 10 M. genioglossus; 11 Basihyoid; 12 Thyroid cartilage; 13 M. hyoepiglotticus; 14 Epiglottic swelling; 15 Isthmus faucium; 16 Soft palate; 17 Nasopharynx; 18 Developing rugae palati.

Fig. IV.126. Paramedian section of pharyngeal region, embryo CC 7 (28.0 mm. G.L.), (X 40).

1 M. longus capitis; 2 Muscular cap; 3 Vestibulum esophagi; 4 Intraepithelial spaces in esophagus; 5 Esophagus surrounded by esophageal muscle; 6 Cricoid lamina; 7 Laryngeal lumen; 8 Arch of cricoid cartilage; 9 Accessory thyroid tissue; 10 M. genioglossus; 11 Basihyoid; 12 Thyroid cartilage; 13 M. hyoepiglotticus; 14 Epiglottic swelling; 15 Isthmus faucium; 16 Soft palate; 17 Nasopharynx; 18 Developing rugae palati.

The intraepithelial spaces, observed for the first time in the previous stage, are still present in the wall of the esophagus (Fig. IV.126./4). They have enlarged, possibly by the confluence of several smaller ones. Similar spaces have been observed in this group of embryos in the apposing epithelia between the arytenoid swellings. Therefore, these spaces are not a feature peculiar to the developing esophagus, although limited in all embryos only to a very well defined area, but may be growth reactions wherever embryonic epithelia lie in apposition. It must be recorded however, that such spaces had not been observed while the dorsal and ventral surfaces of the primitive posterior oral cavity opposed each other before being resorbed in the 22 mm. stage, or, in the present group of specimens, in the piriform recesses where similar obliterations by epithelial apposition are taking place.

The esophageal wall structure has changed little, with the exception perhaps of its epithelium having lost its previous clear-cut cellular organisation. The epithelium consists now of three or four irregular layers of roundish cells. The underlying, hitherto quite loose, zone of connective tissue is denser, with the primitive connective tissue fibres in longitudinal orientation. The muscle coat on the outside is thin, composed mostly of circular fibres (5).

In the median plane of the newly fused palate there is a fenestrated sheet of epithelial tissue. It is composed of the two coverings of the free medial borders of the palatine shelves, which were trapped in the process of fusion (Figs. IV.123./4; IV.122./7). These cellular islands have lost their two-layered organisation and have started to disintegrate, as can be observed from their rather degenerating cellular appearance. At the level where the palatine processes have not reached each other and the two epithelia lie in apposition before being trapped, cell proliferation has taken place, filling this transient space. Also on the dorsal and ventral surfaces of the palate, where in the midline the opposing rounded free borders have left a shallow groove, similar epithelial cell proliferations occupy these spaces (5). It can be assumed that these latter proliferations have been lodged at first between the opposing epithelia, and, as fusion progressed, have been pushed upwards and downwards to fill the transient grooves. Clearly, here is a third way in which pharyngeal epithelia opposing each other can react. In the first instance, alluded to in the preceding stage, epithelial apposition, especially in the esophagus and larynx, produced intraepithelial spaces. Secondly, in the same stage, as in the case of the obliteration of the lateral edge of the anterior pharynx, epithelia appose each other and disintegrate, leaving perhaps only a cord of trapped cells. Thirdly, in the present stage, epithelia by apposing each other promote the proliferation of cells which in further fetal life disappear again.

The Relationships of the Pharynx

The structures overlying the pharynx are the same as have been enumerated for the previous two stages. In some areas of the basal plate the chondral cells are degenerating, foreshadowing the beginning of ossification. The intramembranous ossification centres for the sagittal portion of the palatine, and pterygoid bones have enlarged and have approached the basal plate on either side, forming new dorsal relations with the nasopharynx (Fig. IV.122./2, 3). The notochord weaves through the basal plate in the manner which has been described before. It is however no longer continuous in all embryos of this stage, one break having occurred in its subbasilar course and a second one, observed in embryo D 45, as it passes through the basal plate somewhat further caudally (Fig. IV.123./8). The cochlea overlies the widened posterior portion of the tubotympanic recess, which moulds itself around its convex underside and other surrounding structures (see contour lines in Fig. IV.127.). Between the entrance into the auditory tube and the pharyngeal hypophysis, the plate-like ossification centre of the pterygoid bone has isolated itself from the common osseous bar described above. It is connected anteriorly to the ossification centre of the sagittal portion of the palatine bone by a mass of dense tissue. Posteriorly, it is superseded by more dense tissue, the future hamulus, around which the ventral portion of the Tensor veli palatini turns mesiad.

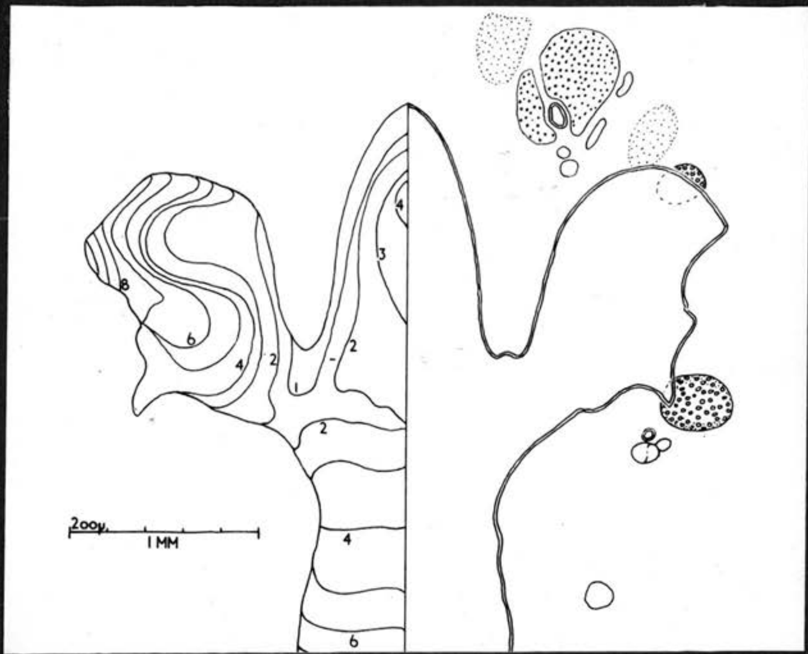
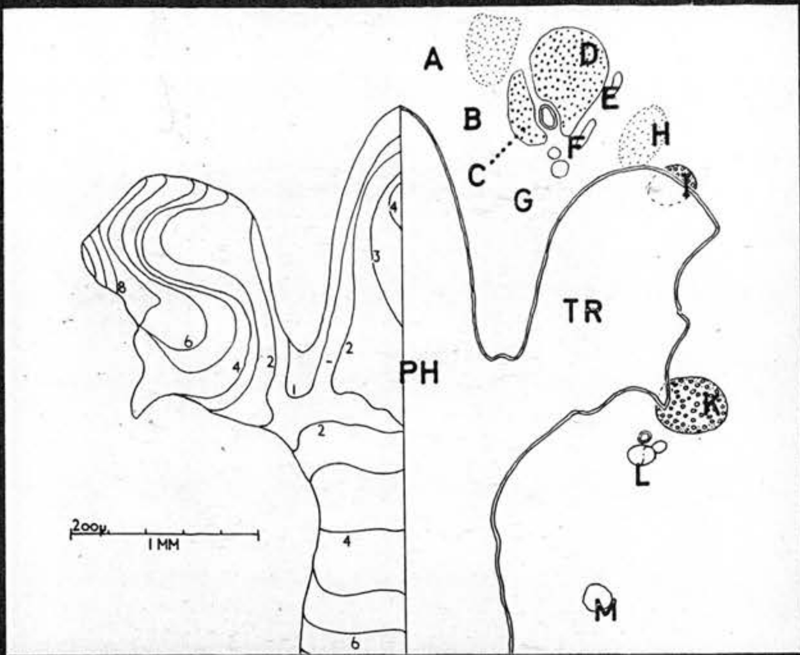


Fig. IV.127. Graphic reconstruction of posterior pharynx, showing the extent of the tubotympanic recesses and major lateral relations, embryo CC 8 (29.0 mm. G.L.), dorsal view.

The left half represents the dorsal surface in relief. Arabic figures indicate comparative heights in ascending order.

PH Pharynx; TR Tubotympanic recess; A M. longus capitis; B Anterior cervical ganglion; C Internal carotid artery; D Nodose ganglion; E Spinal accessory nerve; F Hypoglossal nerve; G Glossopharyngeal nerve; H M. digastricus mandibulae; I Reichert's cartilage; K Meckel's cartilage; L Mandibular alveolar artery and nerve; M Maxillary nerve.



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The medial pterygoid muscle (Fig. IV.122./12), being better developed in this group of embryos, forms now a major lateral relation with the pharynx. It runs almost horizontally in the root of the recently closed palatine processes and interposes itself between the pharynx and Meckel's cartilage (13), as the latter crosses the pharynx laterally in its ascent to the area of the middle ear. A new small nerve, originating from the maxillary division of the trigeminal nerve, curves around the ventral aspect of the muscle at a level somewhat anterior to the pharyngeal hypophysis and passes posteriorly and slightly medially to enter the palatine process. It can be traced in the 29 mm. embryo to about the level of the tonsils where it fades out in the centre of the as yet ununited palatine swellings. The medial extremity of the external acoustic meatus (Fig. IV.124./15) underlies the widened portion of the tubotympanic recess. Due to recent epithelial cell proliferation, these flattened ducts have nearly been occluded and only retain lumina where the original lumen had been quite wide. The remaining lateral relations of the pharyngeal chamber are the same as have been described for the 15 and 22 mm. stages. An indication of their position is given in a drawing designed to show the extent of the tubotympanic recesses (Fig. IV.127.).

Ventrally, the relations are also unchanged. Accessory thyroid tissue has been found in the vicinity of the basihyoid in all specimens (Fig. IV.126./9).

Several structures within the narrow spectrum of observation on the head have changed from embryonic conformation to a distinctly adult pattern in this stage. The most obvious is the development and closure of the eye lids. By this external sign, embryologists, according to established convention, fix the state of development after which an embryo should be called a fetus. It seems that at this juncture most of the organs have, in general, attained a gross morphology akin to that of the adult, and that from this point forward only an increase in size and bulk seems to be necessary to produce an exact replica of the species.

Most important, of course, in an investigation into the evolution of the pharynx is the closing of the palatine processes, which provides, by the formation of the soft palate, a horizontal shelf that divides the hitherto common pharyngeal cavity into an upper respiratory and a lower digestive channel. Because the soft palate is as yet insufficiently fused and still relatively short, the opening through which the two compartments communicate (pharyngeal isthmus) is proportionately large. Cartilaginous rings have formed at regular intervals along the tracheal tube, providing this organ also with a more adult appearance. Increased rate of growth in the epithelium adjacent to the tonsillar swellings has produced a tonsillar sinus, inside of which the tonsillar swellings lie well protected, as indeed they do in the adult. The tubotympanic recess has been moulded especially by the rapid enlargement of the second and third visceral arches in such manner as to make its proximal portion, the future auditory tube, progressively narrower. The distal portion however, flattened between the cochlea above and the developing malleus below, has continued to grow and is now wider than the proximal part, reversing the previous relationship. The wider portion is destined to form the middle ear (Fig. IV.127.).

Now that a recognisable adult pattern has been attained by the pharyngeal tube, the structures comprising its wall begin to figure more prominently, and already the anlagen of three new muscles, the levator of the palate, the palatopharyngeus and the cricopharyngeus have appeared.

S T A G E 12

(40 mm. C.R.L.)

The observations for this stage were made on the following fetuses:

Fetus D 46	41 mm.	C.R.L.	litter 10
Fetus D 47	33 mm. (!)	G.L.	

Fetus D 47 was received in an unnaturally bent condition which, although it was otherwise similar to its litter mate, reduced its crown-rump length considerably. Because the nead-neck segment of this fetus was straight and well preserved, it was decided to include it, rather than base the 40 mm. stage on only one specimen.

Fig. IV.128. Canine fetus D 46 (41 mm. C.R.L.).

Number of embryo:	D 46	Date of processing:	April 28, 1961
Size before fixation:	-	Block:	Paraffin
Size after fixation:	41 mm. C.R.L.	Stain:	H. & E.
Age:	-	Plane of section:	Sagittal
Position on uterus:	-	Thickness of section:	10 microns
Size of loculus:	-	Series made:	D 46 (1 - 30)
Fixed in:	10% Form./Bouin's Fluid		
Stored in:	70% Alcohol		

From litter:		10
Number of embryos in litter:		5
DAM	Breed:	Mixed Breed
	Age:	-
	Weight:	-
SIRE	Breed:	-
	Age:	-
	Weight:	-
Number of services:		-
Date of 1st service:		-
Date of 2nd service:		-

Date of collection: April 1, 1960

At time of collection Dam was alive ( ), dead ( ).

Embryos collected hour(s) after death.

Litter received from: Dr. A.G. Misener, Chicago, Illinois.

Remarks:

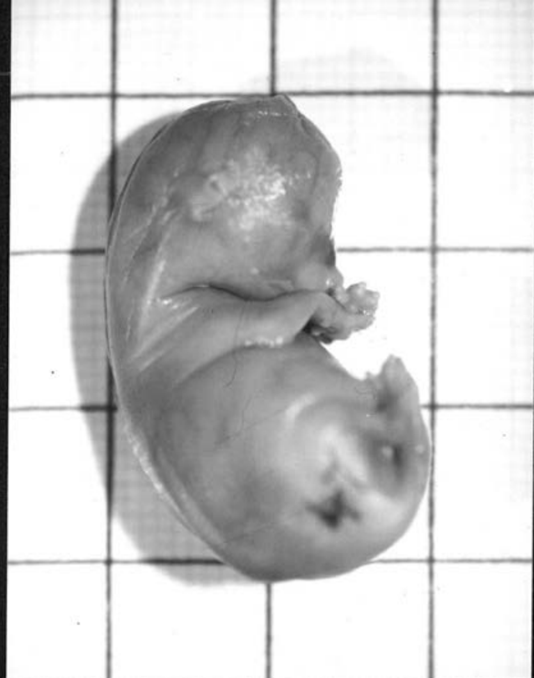


Fig. IV.129. Canine fetus D 47 (33 mm. (!) G.L.).

This specimen was bent accidentally during fixation.

Number of embryo:	D 47	Date of processing:	April 28, 1961
Size before fixation:	-	Block:	Paraffin
Size after fixation:	33 mm. G.L. (!)	Stain:	H. & E.
Age:	-	Plane of section:	Transverse
Position on uterus:	-	Thickness of section:	10 microns
Size of loculus:	-	Series made:	D 47 (1 - 29)
Fixed in:	10% Form./Bouin's Fluid		
Stored in:	70% Alcohol		

From litter:		10
Number of embryos in litter:		5
DAM	Breed:	Mixed Breed
	Age:	-
	Weight:	-
SIRE	Breed:	-
	Age:	-
	Weight:	-
Number of services:		-
Date of 1st service:		-
Date of 2nd service:		-

Date of collection: April 1, 1960

At time of collection Dam was alive ( ), dead ( ).

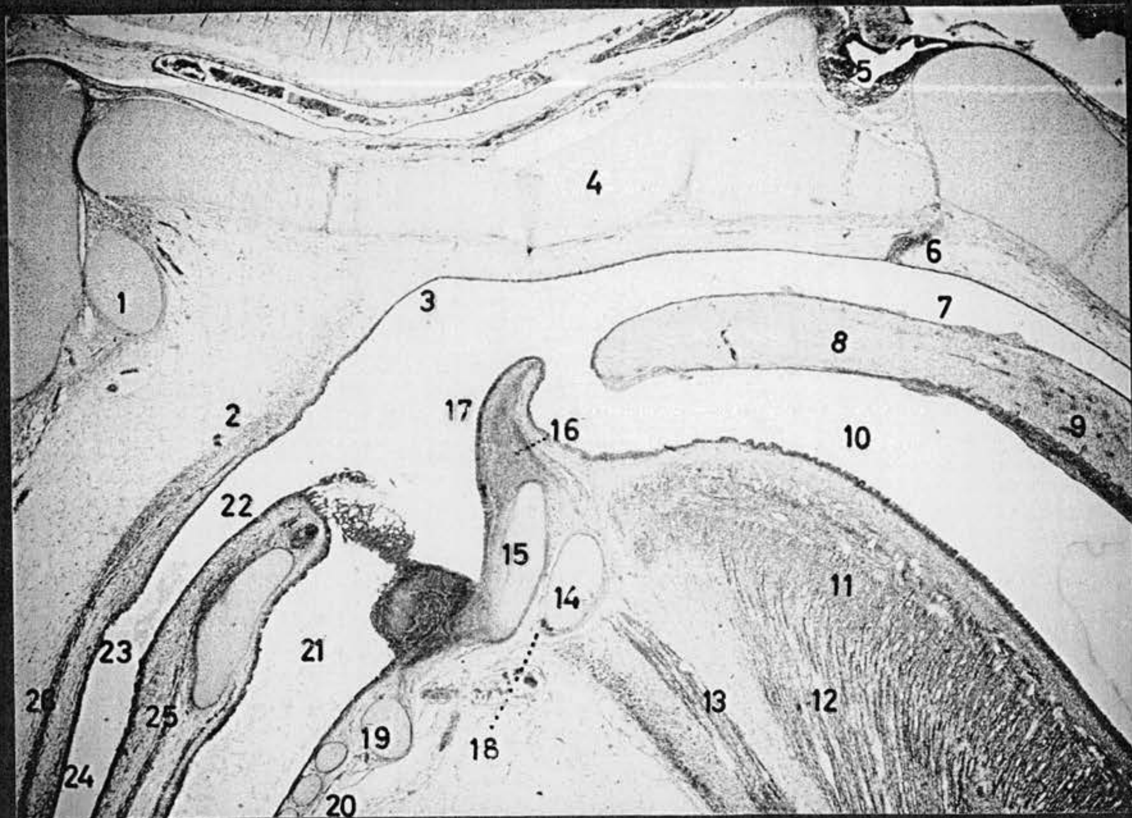
Embryos collected hour(s) after death.

Litter received from: Dr. A.G. Misener, Chicago, Illinois.

Remarks:

Fig. IV.130. Median section of pharynx, embryo D 46 (41 mm. C.R.L.), (X 25).

1 Atlas; 2 Cap of pharyngeal musculature; 3 Fornix pharyngis; 4 Basal plate; 5 Hypophysis; 6 Pharyngeal hypophysis; 7 Nasopharynx; 8 Soft palate; 9 Fenestrated epithelial sheet; 10 Isthmus faucium; 11 Intrinsic muscles of the tongue; 12 M. genioglossus; 13 M. geniohyoideus; 14 Basihyoid; 15 Thyroid cartilage; 16 M. hyoepiglotticus; 17 Epiglottis; 18 Accessory thyroid tissue; 19 Cricoid cartilage; 20 Tracheal rings; 21 Larynx; 22 Vestibulum esophagi; 23 Collar of roughened epithelium; 24 Esophagus; 25 M. cricoesophageus; 26 Esophageal muscle.



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The Shape of the Pharynx

Further general enlargement of the pharyngeal tube has taken place. Its longitudinal measurement for this stage is 3.2 mm., and transversely, just in front of the entrance into the auditory tubes, 1.5 mm. With the appearance of the soft palate during the last stage and the closing of its two lateral components in this stage, the pharyngeal tube of the 40 mm. fetus greatly resembles that of the adult. The notable difference is that the soft palate does not reach to the epiglottis and ends roughly opposite the caudal extremity of the tonsillar swellings (Fig. IV.130./8, 17). In the adult, the caudal end of the soft palate easily reaches the level of the epiglottis, and projects well beyond the region of the palatine tonsils (Fig. II.2./SP). There is still some evidence of the recent fusion of the two lateral palatine processes in the form of a triangular indentation in the centre of the Arcus veli palatini. The two palatopharyngeal folds, continuing the Arcus veli palatini on either side, have become more distinct and stronger at their posterior extremities, resulting in a slight, horizontally placed constriction of the posterior pharynx, which divides the nasopharynx from the oropharynx in this region. Owing to this constriction, a slight bulge has appeared above the pharyngeal isthmus, which is likely to persist as the Fornix pharyngis of the adult (Fig. IV.130./3). Judging by this remoulding, one could assume that the posterior extremities of the palatine swellings have united over the entrance into the Vestibulum esophagi, completing the circle of the pharyngeal isthmus (Foramen intrapharyngicum) which is formed by the Arcus veli palatini in front and the Arcus palatopharyngei on either side.

In the oropharynx only changes in the palatine tonsils have occurred. The tonsillar sinus has enlarged, creating the impression that the tonsillar swellings have not grown, or have even decreased, in size (Fig. IV.131./14). This illusion however, is soon dispelled by measurements, which show that the tonsils have indeed increased in thickness by about 80 microns, whereas in length they have remained at 800 microns. The Plica semilunaris, hanging down from the ventral surface of the soft palate and covering the adult tonsils medially, has started to take on form, although it is still rather short and does not yet afford much protection for the tonsil (13). The entire tonsillar complex seems to have moved upwards on the lateral pharyngeal wall and is found in the 40 mm. fetus more nearly in the region of the soft palate than in that of the root of the tongue, from where, in fact, it arose.

The Structure of the Pharyngeal Wall

As was previously seen, the epithelial lining of the pharyngeal tube consists of two layers: (a) a tall basal layer mostly of columnar cells and (b) a low superficial layer of cells. In areas, such as the flat dorsal and ventral surfaces of the soft palate and the roof of the nasopharynx, where the epithelium is low, the basal layer is made up of cuboidal cells covered by a superficial squamous sheet. Over the root of the tongue, along the lateral walls and piriform recesses and in the posterior pharynx the lining is taller, and in these regions the basal cell layer is of the columnar and the superficial one of the cuboidal type. A zone of embryonic connective tissue underlies all the epithelia and replaces the undifferentiated mesenchyme of earlier stages. Where the connective tissue layer separates epithelium from pharyngeal musculature, it is denser than in the non-muscular areas.

There is no difference between the epithelium of the dorsal, or nasal, surface and that of the ventral, or oral, surface of the soft palate. It is thin on both sides, growing slightly taller laterally as it blends with the lateral walls of the naso- and oropharynx respectively. On both dorsal and ventral surfaces there is a low ridge of epithelium running along the midline. Beneath these ridges lie epithelial cell cords, which are the remnants of the previously noted cell masses, that filled the transient longitudinal troughs created there by the fusion of the soft palate. Along the median plane in the interior of the soft palate, very little of the trapped, fenestrated cell sheet of the preceding stage is left; only a few cell cords descending from the epithelium remain. Anterior to the level of the pharyngeal hypophysis however, much more of the fenestrated cell sheet is present, although here also it shows signs of degeneration (Fig. IV.130./9). The embryonic connective tissue inside the soft palate shows a circular arrangement to either side of the midline, which perhaps could be interpreted as the laying-down of the palatinus muscle, although as yet no myoblasts have appeared. The underside of the palatopharyngeal folds is thrown into two or three deep folds, the most prominent of which is the posterior continuation of the Plica semilunaris.

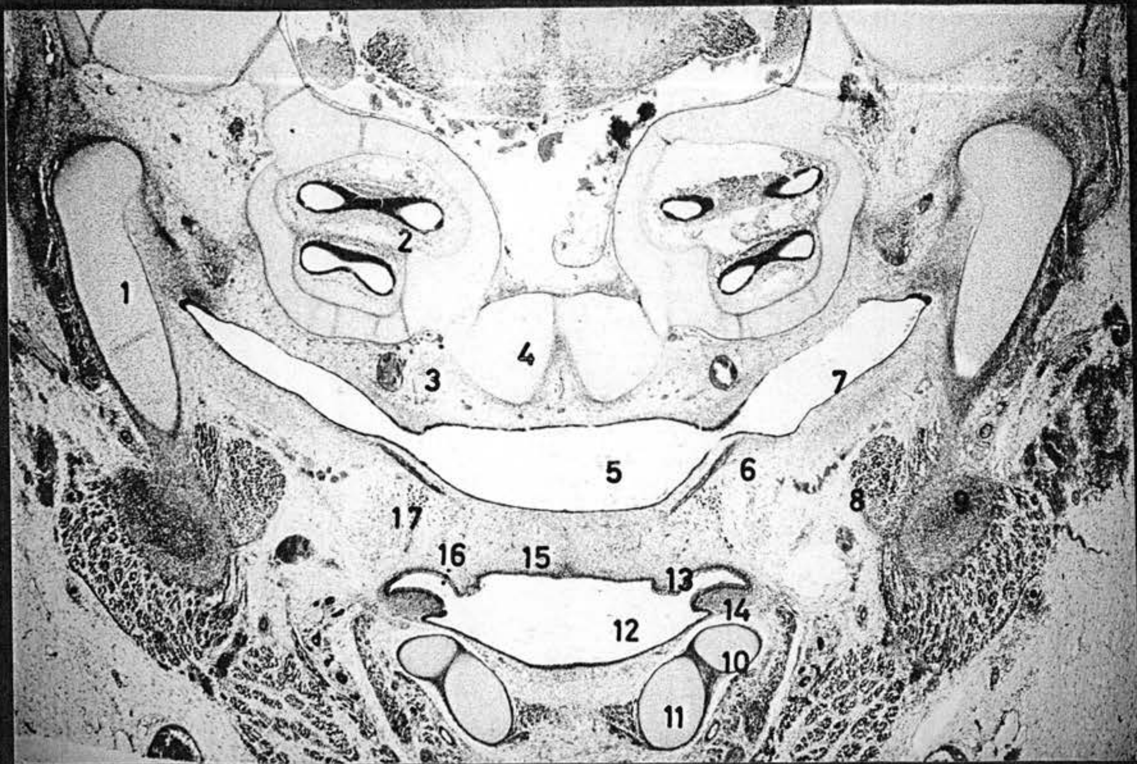
The floor of the pharynx consists of the root of the tongue anteriorly, and the two piriform recesses, which embrace the anterior structures of the larynx, posteriorly. The lingual epithelium of the 40 mm. fetus is the first of the linings to show some evidence of papillary body formation. Especially over the root of the tongue, the fairly tall epithelium forms numerous rounded cobble-stone elevations. The underlying connective tissue, being now well supplied with capillaries, fills the newly created spaces from below. More anteriorly over the body of the tongue, only the superficial layer is undulated in section, leaving the basement membrane and the subepithelial connective tissue unchanged. Between the superficial and the basal layers of cells in the epithelium especially over the root of the tongue, a non-staining space has appeared. It is more conspicuous in the vicinity of the median lingual sulcus, but spreads out over much of the dorsum of the tongue. The cell membranes of the superficial cuboidal cell layer can be made out crossing this space. Thus it would appear that the cytoplasm of these cells has concentrated around the nuclei which lie immediately under the free border of the epithelium. Toward the lateral walls of the pharynx and especially in the tonsillar areas, the superficial cell layer has started to proliferate. The circumvallate papillae are about 100 microns in diameter at this stage and do not project as much above the surface of the epithelium as they did previously. Their exposed surface is covered by a sheet of single squamous cells under which is a layer of very densely packed, spindle-shaped columnar cells. The nuclei, also spindle-shaped, are crowded away from the basement membrane, leaving a wide eosin-staining zone composed of cytoplasm and cell membranes exposed to the underlying connective tissues. Due to the contraction of the moat at the base of the papilla, the connective tissue core, which previously resembled a cylinder, has now taken on the shape of an inverted cone.

The tonsillar swelling, when compared to its counterpart in the 30 mm. embryo, has become somewhat flattened dorsoventrally. It springs from the lateral pharyngeal wall like a shelf, roughly 800 microns long, being overhung for the most of its length by the plica semilunaris which has recently appeared on the underside of the soft palate (Fig. IV.131./13, 14, 16). The epithelium covering the entire tonsillar complex, although similar in structure, is taller than that of the root of the tongue by virtue of cellular proliferation that has taken place in its superficial cell layer. The tonsillar swelling is filled with a dense mass of cells almost devoid of cytoplasmic material. Most of the nuclei are oval and pale staining, but a number of round darkly staining ones are encountered at this stage. The ventral portion of the swelling is being invaded by an extension of connective tissue from the lateral pharyngeal wall, carrying in a few capillaries as well. The tonsillar branch of the glossopharyngeal nerve also enters through this portal of connective tissue.

The muscular cap overlying the posterior pharynx distinguishes itself from that of the 30 mm. embryo by the lengthening of the existing muscular extensions and the appearance of a new one. The different pre-muscle masses making up the muscular wall of the posterior pharynx have differentiated sufficiently to permit the description of separate muscles by name, save perhaps for the muscular band running toward the future thyro-, epi- and kerato-hyoids. The <u>pterygopharyngeus</u> (Figs. IV.132./D; IV.131./17), observed for the first time in the 22 mm. embryo, has elongated in a rostral direction and ends now a short distance behind the hamulus of the pterygoid bone. It is a flat band of loose muscle fibres which mingle posteriorly with the fibres of the hyopharyngeus and thyropharyngeus. It passes below the primordium of the <u>Levator veli palatini</u> (6) (Fig. IV.132./C), a small muscular spindle situated

Fig. IV.131. Transverse section of pharynx at the level of the openings into the tubotympanic recesses, embryo D 47 (33 mm. (!) G.L.), (X 25).

1 Meckel's cartilage; 2 Cochlea; 3 Internal carotid artery; 4 Basal plate; 5 Nasopharynx; 6 Levator veli palatini; 7 Tubotympanic recess; 8 M. pterygoideus medialis; 9 Osseous mandible; 10 Epihyoid; 11 Keratohyoid; 12 Oropharynx; 13 Plica semilunaris; 14 Tonsillar swelling; 15 Soft palate; 16 Supratonsillar fossa; 17 M. pterygopharyngeus.



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1 Meckel's cartilage; 2 Cochlea; 3 Internal carotid artery; 4 Basal plate; 5 Nasopharynx; 6 Levator veli palatini; 7 Tubotympanic recess; 8 M. pterygoideus medialis; 9 Osseous mandible; 10 Epihyoid; 11 Keratohyoid; 12 Oropharynx; 13 Plica semilunaris; 14 Tonsillar swelling; 15 Soft palate; 16 Supratonsillar fossa; 17 M. pterygopharyngeus.

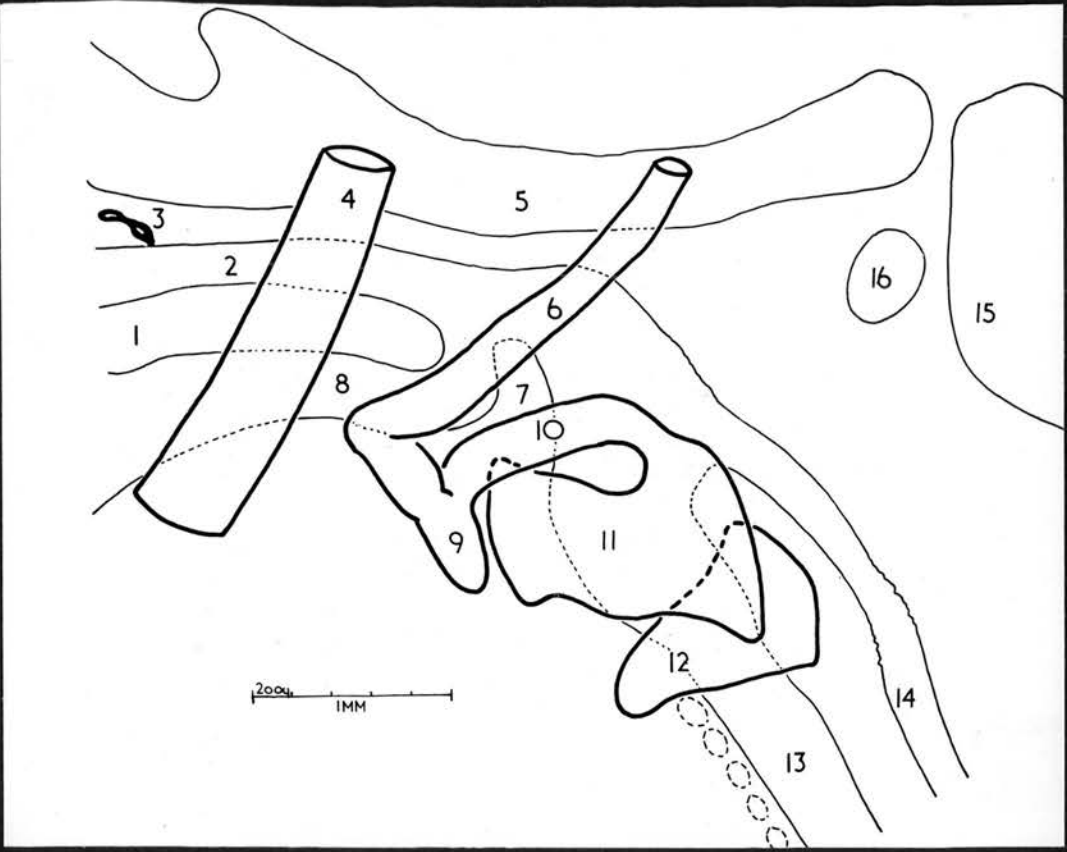
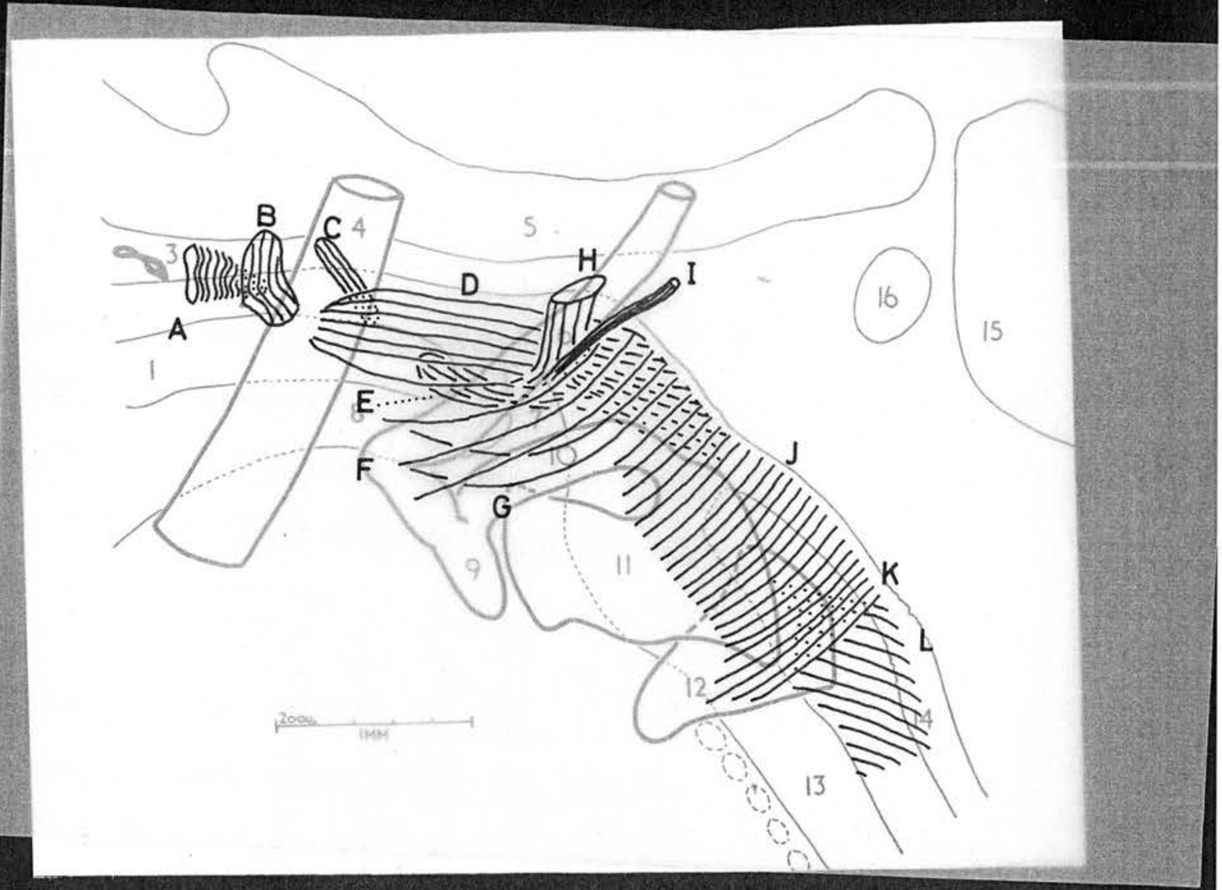


Fig. IV.132. Lateral view of pharyngeal region in the 40 mm. fetus. Thin lines: outline of the pharynx and basal plate in median section. Heavy lines: skeletal structures flanking pharynx. Superimposed lines: pharyngeal musculature.

A Pterygoid bone; B Tensor veli palatini; C Levator veli palatini; D Pterygopharyngeus; E Palatopharyngeus; F Dorsal portion of hyopharyngeus; G Ventral portion of hyopharyngeus; H Stylopharyngeus; I Glossopharyngeal nerve; J Thyropharyngeus; K Cricopharyngeus; L Cricoesophageus.

1 Soft palate; 2 Nasopharynx; 3 Pharyngeal hypophysis; 4 Meckel's cartilage; 5 Basal plate; 6 Reichert's cartilage; 7 Epiglottis; 8 Isthmus faucium; 9 Basihyoid; 10 Thyrohyoid; 11 Thyroid cartilage; 12 Cricoid cartilage; 13 Trachea; 14 Esophagus; 15 Epistropheus; 16 Atlas.



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1 Soft palate; 2 Nasopharynx; 3 Pharyngeal hypophysis; 4 Meckel's cartilage; 5 Basal plate; 6 Reichert's cartilage; 7 Epiglottis; 8 Isthmus faucium; 9 Basihyoid; 10 Thyrohyoid; 11 Thyroid cartilage; 12 Cricoid cartilage; 13 Trachea; 14 Esophagus; 15 Epistropheus; 16 Atlas.

below the entrance into the tubotympanic recess and running transversely. The palatopharyngeus (E) is a new extension of the muscular cap. It is confluent with the fibres of the pterygopharyngeus posteriorly and extends forwards along the lateral face of the pharynx, inside the palato-pharyngeal folds, fading out just before reaching the free edge of the soft palate. It lies medioventral to the pterygopharyngeus and does not reach the level of the levator of the palate. The hyopharyngeus extension (F, G) from the muscular cap is still vague. It originates from the cap between the stylopharyngeus and the thyropharyngeus muscles and extends anteriorly towards the angle formed between the thyrohyoid, keratohyoid and epihyoids of the hyoid apparatus, where it seems to divide into two muscular bands, the ventral one of which passes medial to the dorsal. The stylopharyngeus (H) is quite distinct, leaving the medial aspect of the stylohyoid and entering the muscular cap where the pterygopharyngeal and hyopharyngeal extensions come together. The fibres of this muscle on approaching the lateral pharyngeal wall, spread out in all directions, save dorsally, with a preponderance of the fibres going backwards. The glossopharyngeal nerve (I) enters the pharyngeal wall through the fibres of this muscle. The thyropharyngeus (J) is the largest of the pharyngeal muscles at this stage. It extends from the muscular cap to the lateral face of the fairly well developed thyroid cartilage. A small slip of muscle at its caudal border is longer and attaches itself to the posterior aspect of the cricoid cartilage; this is the primordium of the cricopharyngeus (K). Below the last named muscles are fibres that extend from the cricoid cartilage to the muscular coat of the esophagus and which can be called the cricoesophageus (L). The median pharyngeal raphe has not yet formed and the fibres of fellow-muscles still cross over the midline. The esophageal muscle has separated into a deep and a superficial layer. These are both obliquely circular and cross each other at right angles.

The epithelial lining of the initial portion of the esophagus is thicker than that of the pharyngeal chamber, consisting of three to four irregular layers of cuboidal cells. The free border of the epithelium is quite smooth in the Vestibulum esophagi (Fig. IV.130./22) and in the initial portion of the esophagus. Opposite the cricoid lamina however, at the level where the esophagus is freeing itself from the confines of the pharyngeal constrictors, the epithelial lining appears very rough and pitted (23). This collar of coarse epithelium has a length of about 600 microns. The fact that intraepithelial spaces exist in its lateral walls, suggests that this area is identical with that seen in the preceding two stages where similar intraepithelial spaces were present at the pharyngo-esophageal junction. The transverse diameter of the esophageal lumen in embryo D 3 of the 22 mm. stage was

300 microns; the portion of esophagus in which the intra-epithelial spaces appeared was similarly 300 microns in length. In the 40 mm. fetuses both these measurements have roughly doubled, which one would consider reasonable, because the outside dimensions of the embryos have done approximately the same. Consequently, one can argue that the appearance of these intraepithelial spaces is associated with the creation, or increase, of lumen, which, as has been shown, has actually doubled in that portion of esophagus since the spaces were first observed. Furthermore one can assume that, by virtue of the probable confluence of these spaces, the epithelial growth process originates in the lateral walls of the esophagus, and, as the lumen gradually expands, leaves traces of this process, such as the pitting of the free border, in the antero-lateral and posterolateral walls of the tube but not in the midsagittal plane of the esophagus, as indeed can be observed in the 40 mm. fetus. Because this central zone of epithelium was smooth in the 22 mm. embryo, one should have expected it to remain like that, if the above assumption is correct, since it then would never be involved in the changes that are going on to either side of it. One has also to take into account that this narrow section of the esophagus is not the only place where luminal increase is taking place. Measurements 1 mm. below the collar in the 22 mm. embryo and 2 mm. below the same collar in the 40 mm. fetus, although approximately

only one half the values for the collar area, also show a 100% increase in diameter despite the absence of intraepithelial spaces, pitting and roughness. Clearly, there are other factors involved which could explain these changes in such a delimited area. At the pharyngoesophageal junction of the adult appears a narrow collar-like epithelial elevation, marking the boundary between the finer longitudinal folds in the Vestibulum esophagi and the coarser folds of the same direction in the esophagus proper; the term Limen pharyngoesophagicum is given to it (Fig. II.2./LE). In view of the fact that the esophageal epithelium is the only one in the pharyngeal area at this state of development which remotely resembles adult mucosa, and could therefore perhaps be considered the most advanced, it might be possible that the roughened collar is the primordium of this anatomical borderline. Johnson (1910) observed similar vacuoles in the esophagus of the human, pig, rat and rabbit embryo. They seem to be more numerous in the thoracic section of the esophagus in these species, but, as in the dog, confined primarily to the lateral walls of the tube. He also had difficulty in explaining their presence and possible function, but came however to the conclusion that they take part in enlarging the esophageal lumen.

The embryonic connective tissue immediately underlying the esophageal epithelium is dense and its fibres are orientated longitudinally; further outwards, towards the double muscular layers, it is looser in arrangement.

Small blood vessels and capillaries seem to be concentrated in the outer zone. In addition to that, a larger number of blood vessels was observed to surround the roughened collar of epithelium opposite the cricoid lamina.

The Relationships of the Pharynx

The cartilage cells in the centre of the cartilaginous basal plate are starting to degenerate, and their lacunae have become larger in preparation for ossification. The notochord follows the same course as was shown in Fig. IV.123. of the preceding stage, a third rupture having appeared in its anterior intrabasal course. The connective tissue zone intervening between the pharynx and the basal plate is denser now and shows more fibre formation close to the perichondrium (Fig. IV.130.). On the whole, the fibres are longitudinally orientated. In the region of the cervical flexure the direction of the fibres in the syncytium is anteroventral with relation to the basal plate. The fibres stretch from the area of the atlas to the now almost flattened cervical flexure of the pharyngeal tube, without however reaching its epithelium. So they are unable, at this stage at any rate, to have a modifying effect on the posterior portion of the naso-pharynx (Fig. IV.130.). The pharyngeal hypophysis has undergone further degenerative changes and represents an irregular tubular organ attached to the roof of the pharynx and extending anterodorsally to the ventral surface of the basal plate (6).

One of the principal lateral relations of the pharynx is the medial pterygoid muscle (Fig. IV.131./8). It lies in the same plane as the soft palate and extends progressively backwards as the mandible, to which it is attached, expands. The Tensor veli palatini, originating from below the tubotympanic recess in the neighbourhood of the otic ganglion and lying slightly dorsal to the pterygoid muscle, has grown stronger and longer at its caudal end. Here, the fibres which, after passing around the hamulus of the pterygoid bone, extend in a mediocaudal direction, have elongated, whereas the ones destined to pass directly medial still reach only to the ventral aspect of the hamulus. The Levator veli palatini is still only a slender bundle, crossing ventral to the tubotympanic recess and dorsal to the pterygopharyngeus muscle (Fig. IV.131./6). It does not give as yet any indication as to its final attachments. A curved bar of osseous tissue has appeared below the expanded portion of the tubotympanic recess which later becomes the tympanic cavity. The bar is roughly 100 microns in diameter and lies just anterior to the anteromedial edge of the flattened external acoustic meatus. It lies in a nearly horizontal plane and the convexity of its curve is pointing anteriorly. This bar is the anlage of the tympanic portion of the temporal bone and is termed the Tympanic ring (Fig. IV.138./2).

The remaining structures bounding the pharynx dorsally, laterally and ventrally have changed neither in development nor in position to an appreciable degree, and the appropriate sections of earlier stages where they have been described may be consulted.

The continuing fusion of the two palatine processes in the formation of the soft palate have brought the pharyngeal tube closer to adult configuration, although the soft palate does yet lack the measure of posterior growth notable in the adult. The epithelia are still quite embryonic in the 40 mm. fetus. The pharyngeal and palatine muscles have made the most rapid progress in this stage of development.

STAGE 13

(50 mm. C.R.L.)

The observations for the 50 mm. stage are based on seven fetuses taken from two litters as follows:

Fetus D 48	48 mm. C.R.L.	litter 11
Fetus D 49	43 mm. C.R.L.	litter 11
Fetus D 2	50 mm. C.R.L.	litter 12
Fetus D 50	50 mm. C.R.L.	litter 12
Fetus D 51	53 mm. C.R.L.	litter 12
Fetus DS 3	50 mm. C.R.L.	litter 12
Fetus DS 4	52 mm. C.R.L.	litter 12

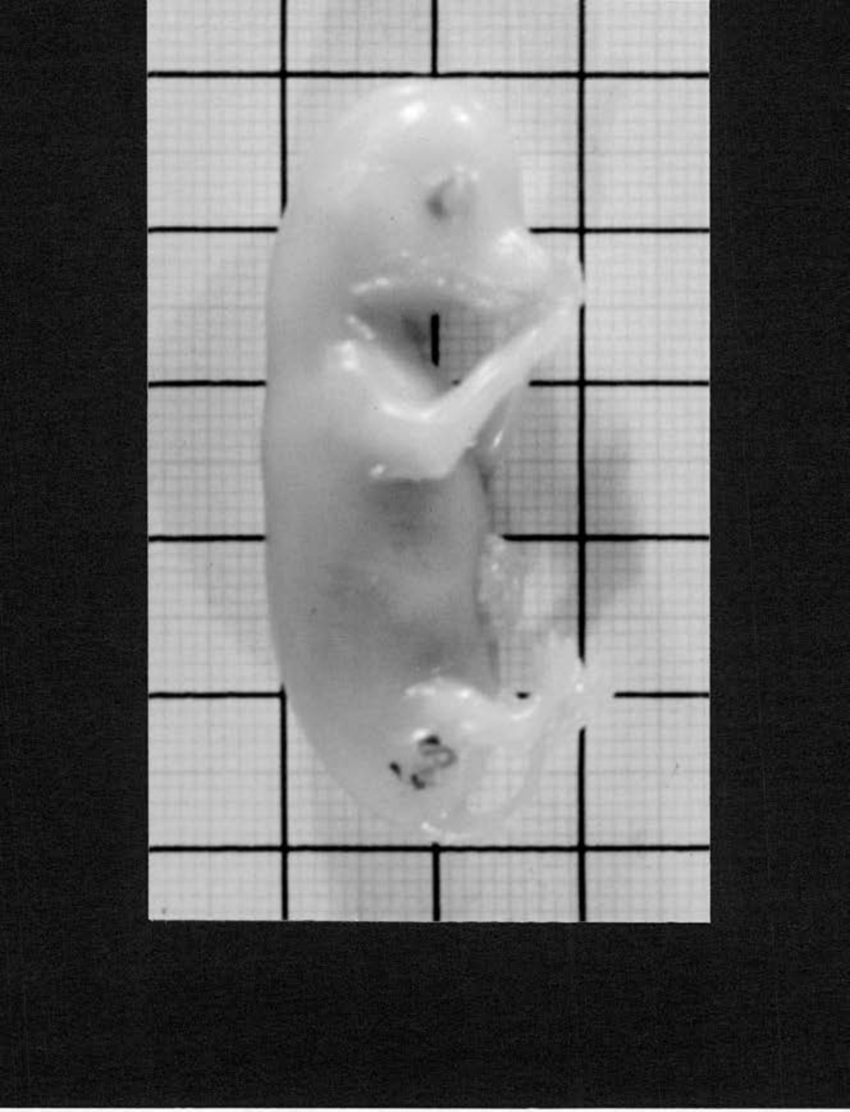


Fig. IV.133. Canine fetus D 48 (48 mm. C.R.L.).

Number of embryo:	D 48	Date of processing:	April 28, 1961
Size before fixation:	-	Block:	Paraffin
Size after fixation:	48 mm. C.R.L.	Stain:	H. & E.
Age:	-	Plane of section:	Transverse
Position on uterus:	-	Thickness of section:	10 microns
Size of loculus:	-	Series made:	D 48 (1 - 44)
Fixed in:	10% Formalin		
Stored in:	10% Formalin		

From litter:		11
Number of embryos in litter:		4
DAM	Breed:	-
	Age:	-
	Weight:	-
SIRE	Breed:	-
	Age:	-
	Weight:	-
Number of services:		2
Date of 1st service:		-
Date of 2nd service:		-

Date of collection: April, 1960

At time of collection Dam was alive ( ), dead (X).

Embryos collected 2 hour(s) after death.

Litter received from: Edinburgh Clinic

Remarks:

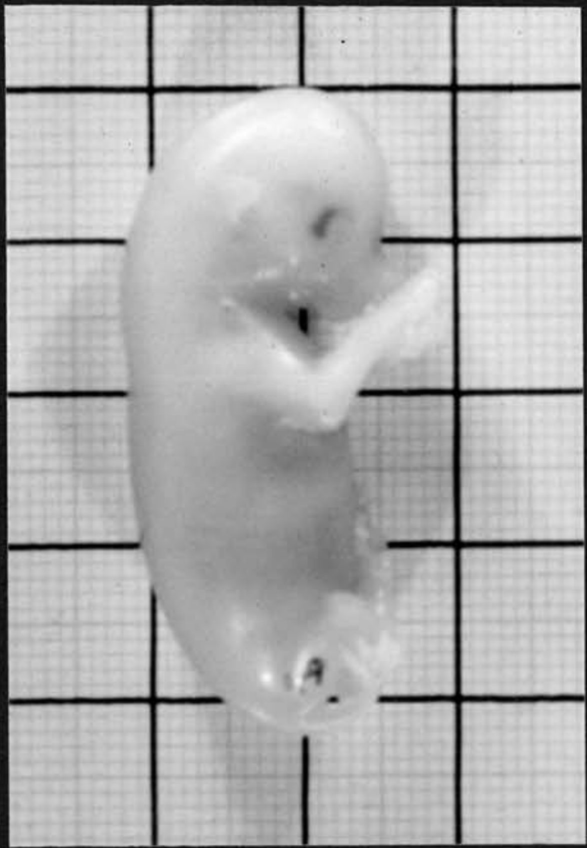


Fig. IV.134. Canine fetus D 49 (43 mm. C.R.L.).

Number of embryo:	D 49	Date of processing:	April 28, 1961
Size before fixation:	-	Block:	Paraffin
Size after fixation:	43 mm. C.R.L.	Stain:	H. & E.
Age:	-	Plane of section:	Sagittal
Position on uterus:	-	Thickness of section:	10 microns
Size of loculus:	-	Series made:	D 49 (1 - 37)
Fixed in:	10% Formalin		
Stored in:	10% Formalin		

From litter:		11
Number of embryos in litter:		4
DAM	Breed:	-
	Age:	-
	Weight:	-
SIRE	Breed:	-
	Age:	-
	Weight:	-
Number of services:		2
Date of 1st service:		-
Date of 2nd service:		-

Date of collection: April, 1960

At time of collection Dam was alive ( ), dead (X).

Embryos collected 2 hour(s) after death.

Litter received from: Edinburgh Clinic

Remarks:

Fig. IV.135. Canine fetus D 2 (50 mm. C.R.L.).

Number of embryo:	**D 2**	Date of processing:	**June 21, 1960**
Size before fixation:	**-**	Block:	**Paraffin**
Size after fixation:	**50 mm. C.R.L.**	Stain:	**H. & E.**
Age:	**-**	Plane of section:	**Horizontal**
Position on uterus:	**-**	Thickness of section:	**10 microns**
Size of loculus:	**-**	Series made:	**D 2 (1 - 27)**
Fixed in:	**10% Form./Bouin's Fluid**		
Stored in:	**70% Alcohol**		

From litter: **12**

Number of embryos in litter: **9**

DAM	Breed:	**X German Shepherd**
	Age:	**-**
	Weight:	**55 lb.**
SIRE	Breed:	**-**
	Age:	**-**
	Weight:	**-**

Number of services: **-**

Date of 1st service: **-**

Date of 2nd service: **-**

Date of collection: **January 12, 1960**

At time of collection Dam was alive (**X**), dead ( ).

Embryos collected hour(s) after death.

Litter received from: **Dr. L. Laforet, Windsor, Ontario.**

Remarks:

Fig. IV.136. Canine fetus D 50 (50 mm. C.R.L.).

Number of embryo:	**D 50**	Date of processing:	**May 3, 1961**
Size before fixation:	**-**	Block:	**Paraffin**
Size after fixation:	**50 mm. C.R.L.**	Stain:	**H. & E.**
Age:	**-**	Plane of section:	**Transverse**
Position on uterus:	**-**	Thickness of section:	**10 microns**
Size of loculus:	**-**	Series made:	**D 50 (1 - 41)**
Fixed in:	**10% Form./Bouin's Fluid**		
Stored in:	**70% Alcohol**		

From litter:		**12**
Number of embryos in litter:		**9**
DAM	Breed:	**X German Shepherd**
	Age:	**-**
	Weight:	**55 lb.**
SIRE	Breed:	**-**
	Age:	**-**
	Weight:	**-**
Number of services:		**-**
Date of 1st service:		**-**
Date of 2nd service:		**-**

Date of collection: **January 12, 1960**

At time of collection Dam was alive (**X**), dead ( ).

Embryos collected hour(s) after death.

Litter received from: **Dr. L. Laforet, Windsor, Ontario.**

Remarks:

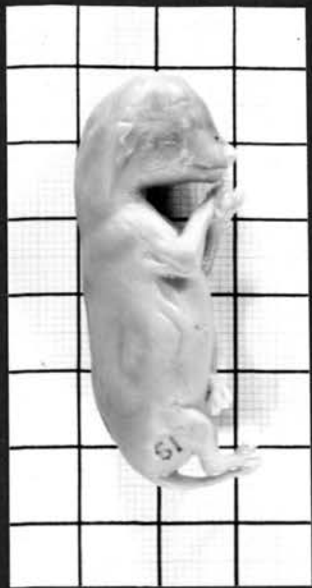


Fig. IV.137. Canine fetus D 51 (53 mm. C.R.L.).

Number of embryo:	**D 51**	Date of processing:	**May 3, 1961**
Size before fixation:	**-**	Block:	**Paraffin**
Size after fixation:	**53 mm. C.R.L.**	Stain:	**H. & E.**
Age:	**-**	Plane of section:	**Transverse**
Position on uterus:	**-**	Thickness of section:	**10 microns**
Size of loculus:	**-**	Series made:	**D 51 (1 - 24)**
Fixed in:	**10% Form./Bouin's Fluid**		
Stored in:	**70% Alcohol**		

From litter:		**12**
Number of embryos in litter:		**9**
DAM	Breed:	**X German Shepherd**
	Age:	**-**
	Weight:	**55 lb.**
SIRE	Breed:	**-**
	Age:	**-**
	Weight:	**-**
Number of services:		**-**
Date of 1st service:		**-**
Date of 2nd service:		**-**

Date of collection: **January 12, 1960**

At time of collection Dam was alive (**X**), dead ( ).

Embryos collected hour(s) after death.

Litter received from: **Dr. L. Laforet, Windsor, Ontario.**

Remarks:

Number of embryo:	**DS 3**	Date of processing:	**June 8, 1960**
Size before fixation:	**-**	Block:	**Paraffin**
Size after fixation:	**50 mm. C.R.L.**	Stain:	**H. & E.**
Age:	**-**	Plane of section:	**Sagittal**
Position on uterus:	**-**	Thickness of section:	**10 microns**
Size of loculus:	**-**	Series made:	**DS 3 (1 - 18)**
Fixed in:	**10% Form./Bouin's Fluid**		
Stored in:	**70% Alcohol**		

From litter:		**12**
Number of embryos in litter:		**9**
DAM	Breed:	**X German Shepherd**
	Age:	**-**
	Weight:	**55 lb.**
SIRE	Breed:	**-**
	Age:	**-**
	Weight:	**-**
Number of services:		**-**
Date of 1st service:		**-**
Date of 2nd service:		**-**

Date of collection: **January 12, 1960**

At time of collection Dam was alive (**X**), dead ( ).

Embryos collected hour(s) after death.

Litter received from: **Dr. L. Laforet, Windsor, Ontario.**

Remarks: **For a picture see that of litter mate D 2 (Fig.IV.135.)**

Number of embryo:	**DS 4**	Date of processing:	**June 8, 1960**
Size before fixation:	**-**	Block:	**Paraffin**
Size after fixation:	**52 mm. C.R.L.**	Stain:	**H. & E.**
Age:	**-**	Plane of section:	**Sagittal**
Position on uterus:	**-**	Thickness of section:	**10 microns**
Size of loculus:	**-**	Series made:	**DS 4 (1 - 15)**
Fixed in:	**10% Form./Bouin's Fluid**		
Stored in:	**70% Alcohol**		

From litter:		**12**
Number of embryos in litter:		**9**
DAM	Breed:	**X German Shepherd**
	Age:	**-**
	Weight:	**55 lb.**
SIRE	Breed:	**-**
	Age:	**-**
	Weight:	**-**
Number of services:		**-**
Date of 1st service:		**-**
Date of 2nd service:		**-**

Date of collection: **January 12, 1960**

At time of collection Dam was alive (**X**), dead ( ).

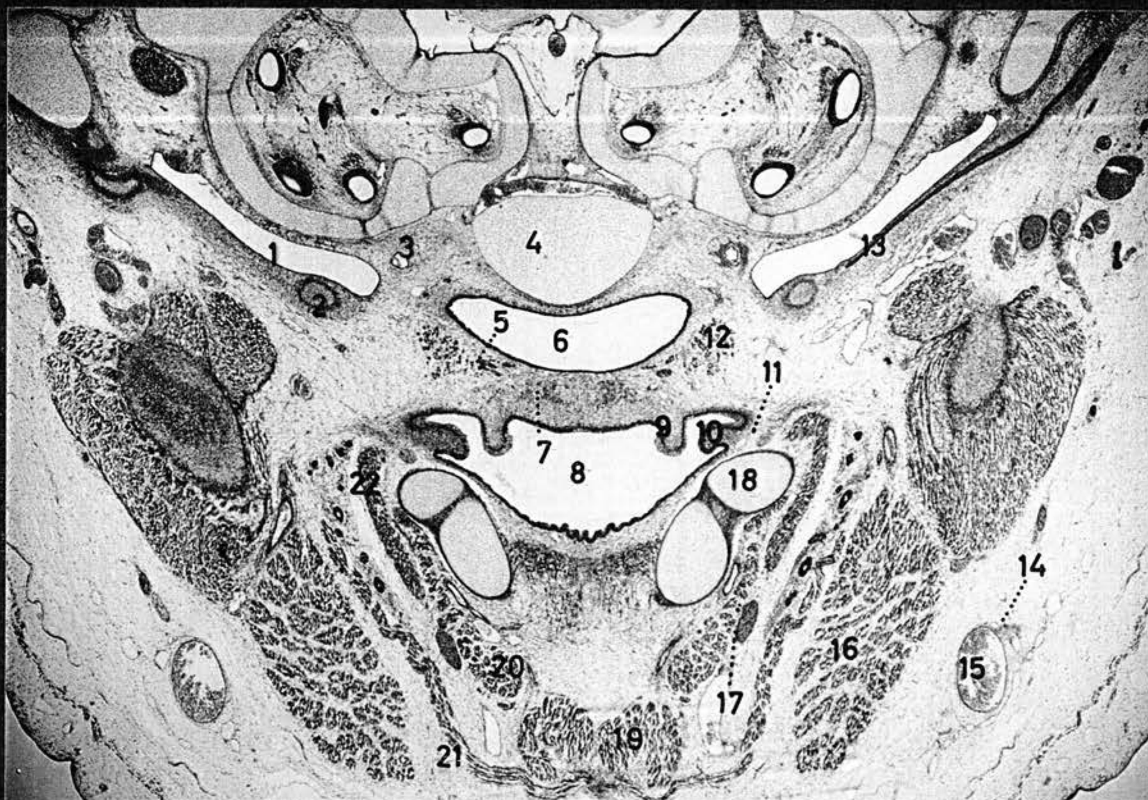
Embryos collected hour(s) after death.

Litter received from: **Dr. L. Laforet, Windsor, Ontario.**

Remarks: **For a picture see that of litter mate D 2 (Fig.IV.135.)**

Fig. IV.138. Transverse section of pharynx, fetus D 51 (53 mm. C.R.L.), (X 25).

1 Tubotympanic recess; 2 Tympanic ring; 3 Internal carotid artery; 4 Basilar plate; 5 M. levator veli palatini; 6 Nasopharynx; 7 M. palatinus; 8 Oropharynx; 9 Plica semilunaris; 10 Tonsillar swelling; 11 Glossal branch of glossopharyngeal nerve; 12 M. pterygopharyngeus; 13 External acoustic meatus applied to the ventral surface of tubotympanic recess to form tympanic membrane; 14 Primordium of submandibular lymph node; 15 External maxillary vein; 16 M. digastricus mandibulae; 17 Hypoglossal nerve; 18 Epihyoid; 19 M. geniohyoideus; 20 M. hyoglossus; 21 M. mylohyoideus; 22 M. styloglossus.



<u>Fig. IV.138</u>. Transverse section of pharynx, fetus D 51 (53 mm. C.R.L.), (X 25).

1 Tubotympanic recess; 2 Tympanic ring; 3 Internal carotid artery; 4 Basilar plate; 5 M. levator veli palatini; 6 Nasopharynx; 7 M. palatinus; 8 Oropharynx; 9 Plica semilunaris; 10 Tonsillar swelling; 11 Glossal branch of glossopharyngeal nerve; 12 M. pterygopharyngeus; 13 External acoustic meatus applied to the ventral surface of tubotympanic recess to form tympanic membrane; 14 Primordium of submandibular lymph node; 15 External maxillary vein; 16 M. digastricus mandibulae; 17 Hypoglossal nerve; 18 Epihyoid; 19 M. geniohyoideus; 20 M. hyoglossus; 21 M. mylohyoideus; 22 M. styloglossus.

The Shape of the Pharynx

As can be seen from the accompanying illustrations, the pharyngeal tube of the 50 mm. fetus resembles adult configuration a great deal (Figs. IV.138.; IV.139.). It is obvious that from now on no further major changes in the shape of the pharynx can be expected. Therefore, the observations on this and the following stages, although continuing to be subdivided as before, will concentrate most on the structure of the pharyngeal wall, and less on the shape of the pharynx or its relationships, since these have for the most part been established. Mention will be made however of any significant developmental changes not only of the pharyngeal region but also of the embryo as a whole. Such ancillary information helps to identify given developmental stages of the specimens included in this study. In this context it should be recorded that anlagen for hair follicles have appeared for the first time in this group of embryos, while the primordia of the retropharyngeal and submandibular lymph nodes have also made their appearance (Fig. IV.138./14).

The soft palate, showing little evidence in histological sections of its recent fusion, has elongated towards the epiglottis and divides the pharynx into the dorsal respiratory and the ventral digestive compartments (Fig. IV.139./11, 7). These two dorsoventrally flattened elongated cavities communicate with each other through the pharyngeal isthmus, an opening situated just above the entrance into the larynx (Figs. IV.140./17; IV.139.). The oropharynx, being longer than the nasopharynx, is continued posteriorly beyond the pharyngeal isthmus as the Vestibulum esophagi (16). This chamber overlies the larynx and is moulded over the laryngeal cartilages, notably the cricoid and the arytenoids. The length of the pharynx in the 50 mm. fetus ranges from 3.4 to 3.7 mm. and its width, taken just in front of the opening into the tubotympanic recesses, ranges from 1.4 to 2 mm. Compared with the same measurements in the 40 mm. stage a steady and even increase has again taken place.

The section of the nasopharynx anterior to the auditory tubes has been transformed from a dorsoventrally flattened shape to a narrower but thicker tube by the expansion of the pterygoid bones to either side of it (Fig. IV.140./4). In fetus D 48 a number of longitudinal folds have appeared which could have resulted from the compression exerted by the two lateral pterygoid plates. The roof between the auditory tubes is flat, to be so as it passes backwards parallel with the basal plate to about the level of the epiglottis. Here it begins to narrow, and soon ends in a point. From this point onwards the much narrowed roof of the pharynx by turning ventrad loses its relation with the overlying basal plate and descends to the horizontal constriction in the posterior pharynx which is created by the pull of the two lateral palatine swellings (palatopharyngeal folds) and which, incidentally, also marks the caudal extent of the nasopharynx (Fig. IV.139./14). From here on, the roof of the pharynx, once again wide, is shaped like a vault, forming the dorsal limits of the Vestibulum esophagi (16), and then, gradually narrowing, continues as the dorsal half of the esophagus.

The shape of the oropharynx has also changed slightly due to the further development of the hyoid apparatus. Just in front of the epiglottis, in which a sheet of dense tissue foreshadows the laying-down of the epiglottic cartilage, the hitherto rather flat lumen has taken on a triangular shape. The base stretches between the two tonsillar complexes along the underside of the soft palate, with the apex pointing ventrally to the median sulcus of the tongue (Fig. IV.138./8). The epihyoids (18) are pushing upwards on either side of the root of the tongue, giving the lateral walls of the oropharynx the inclination which identifies them as the two lateral sides of the triangle. The tonsillar swellings have increased to an average length of 1 mm. and a greatest diameter of nearly 300 microns. The epithelial folds (Plicae semilunares) which originate from the ventral surface of the soft palate and which later cover the tonsils from the medial side, extend now farther ventrally into the oropharyngeal chamber (9).

The Structure of the Pharyngeal Wall

The proliferation in the superficial layer of the pharyngeal epithelium that was observed in the 40 mm. fetus to have begun in and around the tonsillar complexes, has spread over almost all the surfaces of the oropharynx. This gives this portion of the epithelium, for the first time, a resemblance to the future stratified squamous mucosa. In the postlaryngeal part of the pharynx the epithelium has grown a little taller, but still consists of several irregular layers of cells, as also does the lining of the esophagus, but which does not as yet bear as much similarity to the stratified squamous type. The epithelium in the anterior portion of the oropharynx now consists of the familiar darker staining basal layer, of either columnar or cuboidal cells, and a superficial zone of several layers of quite lightly staining irregular cells, mostly of the cuboidal type. At a glance, this arrangement is quite similar to stratified squamous epithelium. The formation of a papillary body is confined only to the dorsum of the tongue, especially to that of its root. The picture in the nasopharynx is still the same as has been noted during the last few stages. The epithelium consists of a basal and a superficial layer of cells, between which, in this group of fetuses, an irregular space has appeared in various regions, occasionally containing single cells. The lining of the auditory tubes is now very thin and consists of a single layer of squamous cells. Along both the dorsal and the ventral midline of the soft palate the trapped masses of epithelial cells from the previous fusion seem to have been squeezed into the lumina above and below, and form, on cross section, club-shaped appendages consisting of masses of degenerating cells with karyolytic nuclei. There is no more trapped epithelium to be seen in the centre of the soft palate along the median plane. The intraepithelial spaces in the esophagus opposite the cricoid cartilage, which by virtue of their obscure nature have received much attention previously, seem to have vanished, leaving only a very small number of such spaces in the lateral and anterior walls of the tube. The roughness in the epithelium of this collar-like section also seems to have disappeared. The lumen of the initial portion of the esophagus is transforming from the smooth oval shape observed earlier to that of a rectangle which no doubt marks the first step towards the formation of longitudinal folds.

The palatinus muscle, although weak, can definitely be made out in horizontal sections as two slender pre-muscle bellies, lying on either side of the median plane in the centre of the soft palate (Figs. IV.138./7; IV.139./9). They have as yet no anterior attachment, because the horizontal portion of the palatine bones, though present in anlage, has not advanced far enough caudally. At their posterior extremity however, the

Fig. IV.139. Median section of pharynx, fetus DS 4 (52 mm. C.R.L.), (X 25).

1 Nasal septum; 2 Pharyngeal hypophysis; 3 Nasopharyngeal duct; 4 Horizontal portion of palatine bone; 5 Oral cavity; 6 Tongue; 7 Isthmus faucium; 8 Soft palate; 9 M. palatinus; 10 M. palatopharyngeus; 11 Nasopharynx; 12 Basilar plate; 13 Fornix pharyngis; 14 Arcus palatopharyngeus; 15 Pharyngeal musculature; 16 Vestibulum esophagi; 17 Cricoid cartilage; 18 Larynx; 19 Thyroid cartilage; 20 Epiglottis; 21 M. hyoepiglotticus; 22 Basihyoid; 23 Accessory thyroid tissue.

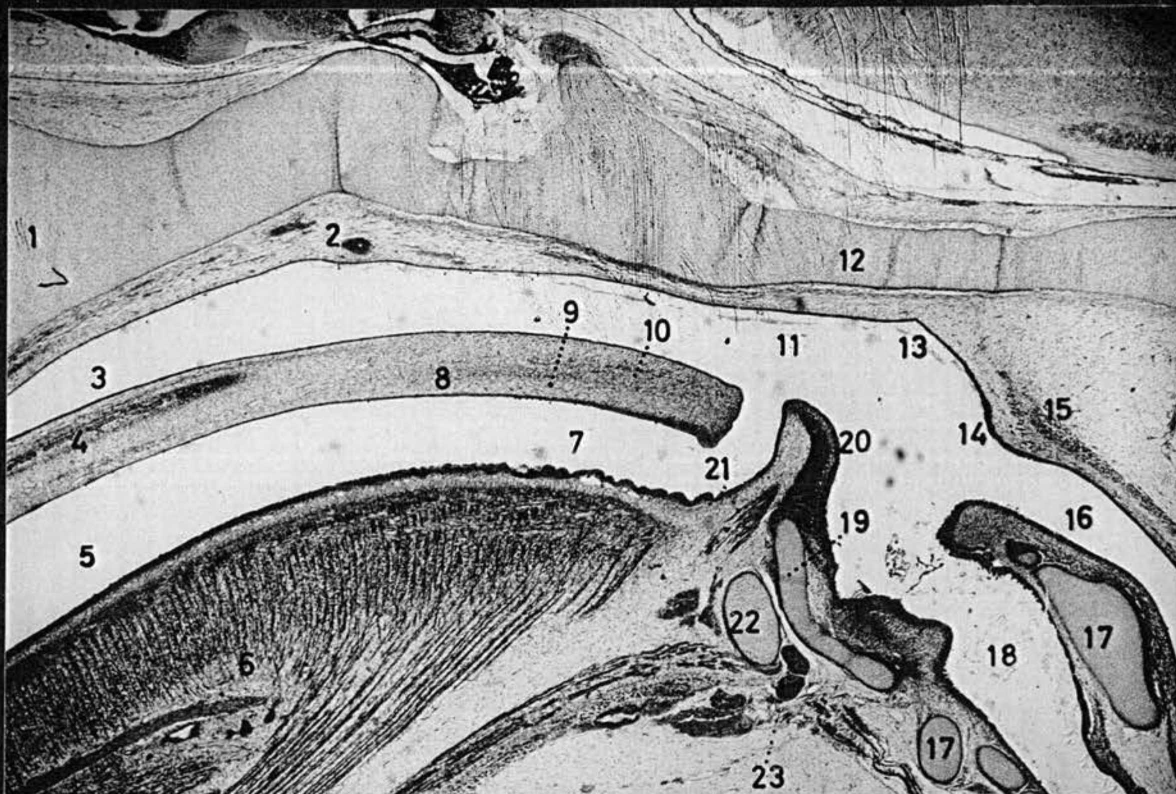


Fig. IV.139. Median section of pharynx, fetus DS 4 (52 mm. C.R.L.), (X 25).

1 Nasal septum; 2 Pharyngeal hypophysis; 3 Nasopharyngeal duct; 4 Horizontal portion of palatine bone; 5 Oral cavity; 6 Tongue; 7 Isthmus faucium; 8 Soft palate; 9 M. palatinus; 10 M. palatopharyngeus; 11 Nasopharynx; 12 Basilar plate; 13 Fornix pharyngis; 14 Arcus palatopharyngeus; 15 Pharyngeal musculature; 16 Vestibulum esophagi; 17 Cricoid cartilage; 18 Larynx; 19 Thyroid cartilage; 20 Epiglottis; 21 M. hyoepiglotticus; 22 Basihyoid; 23 Accessory thyroid tissue.

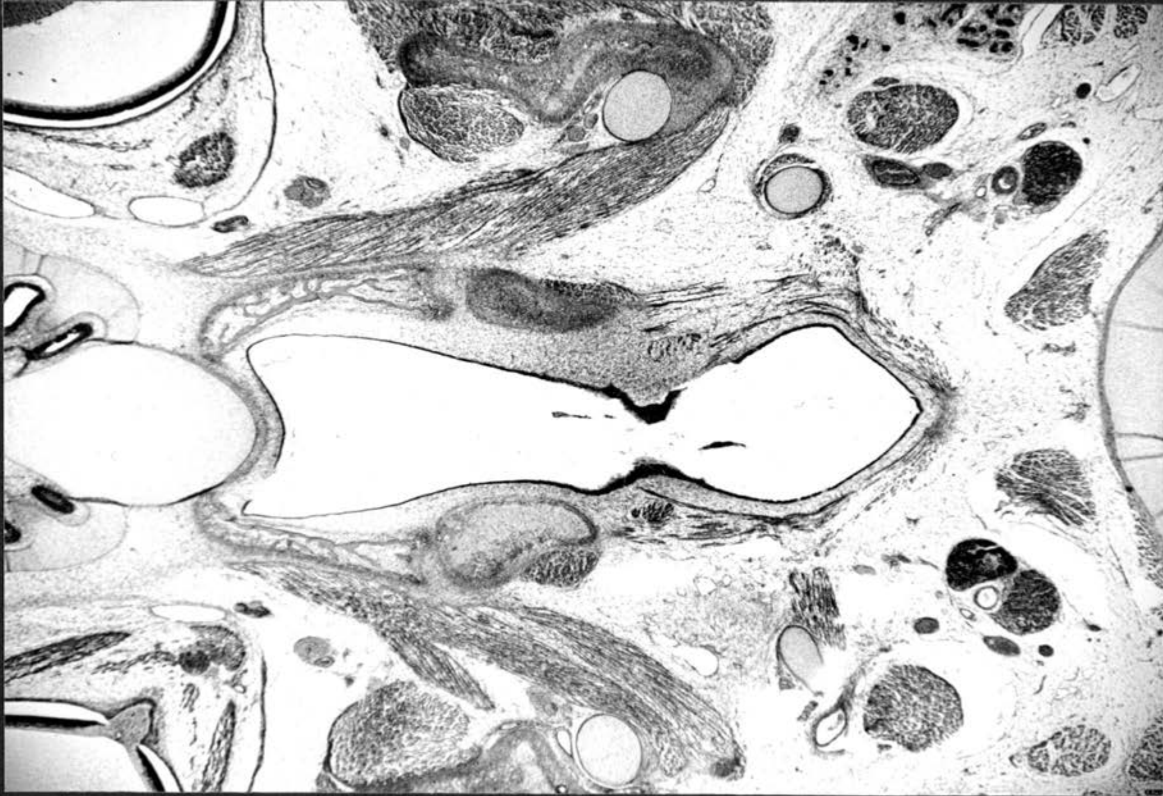
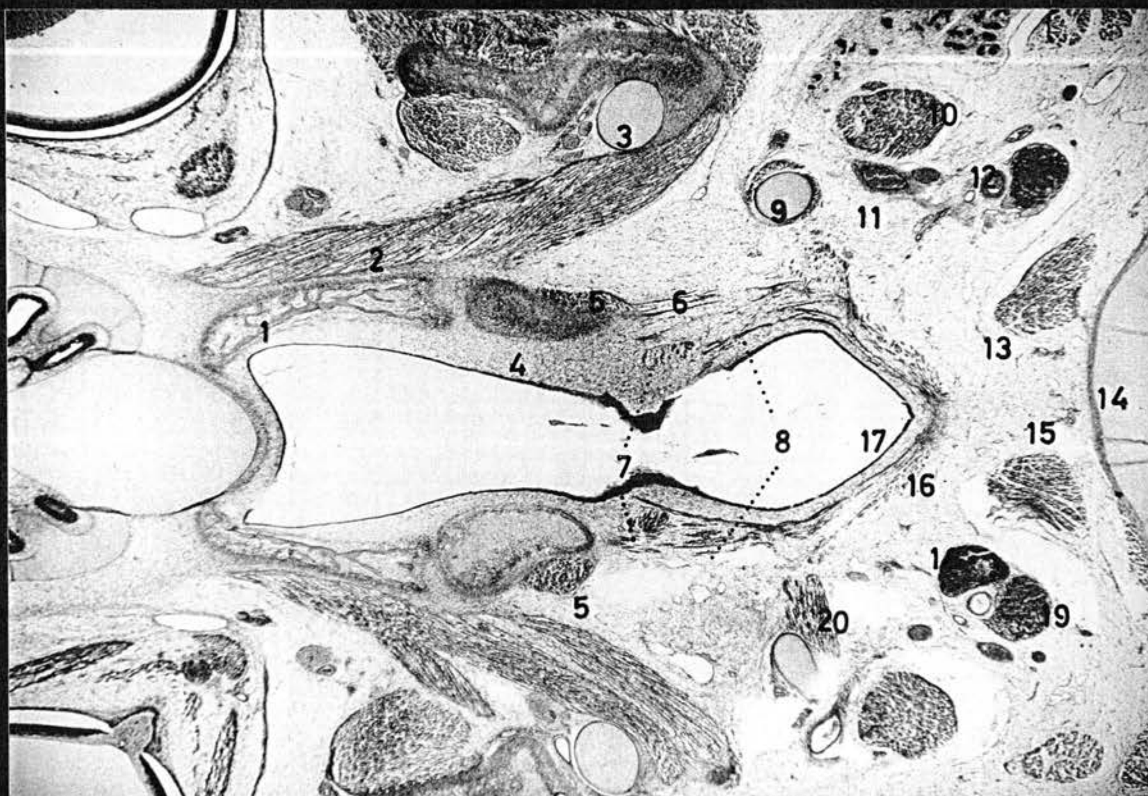


Fig. IV.140. Horizontal section of pharynx, fetus D 2 (50 mm. C.R.L.), (X 20).

1 Vertical portion of palatine bone; 2 M. pterygoideus medialis; 3 Meckel's cartilage; 4 Pterygoid hamulus; 5 M. tensor veli palatini; 6 M. pterygopharyngeus; 7 M. levator veli palatini; 8 M. palatopharyngeus; 9 Reichert's cartilage; 10 M. digastricus mandibulae; 11 External carotid artery; 12 Internal carotid artery; 13 M. longus capitis; 14 Ventral arch of atlas; 15 M. rectus capitis ventralis; 16 Muscular cap; 17 Pharyngeal isthmus; 18 Anterior cervical ganglion; 19 Nodose ganglion; 20 M. stylopharyngeus.



<u>Fig. IV.140</u>. Horizontal section of pharynx, fetus D 2 (50 mm. C.R.L.), (X 20).

1 Vertical portion of palatine bone; 2 M. pterygoideus medialis; 3 Meckel's cartilage; 4 Pterygoid hamulus; 5 M. tensor veli palatini; 6 M. pterygopharyngeus; 7 M. levator veli palatini; 8 M. palato-pharyngeus; 9 Reichert's cartilage; 10 M. digastricus mandibulae; 11 External carotid artery; 12 Internal carotid artery; 13 M. longus capitis; 14 Ventral arch of atlas; 15 M. rectus capitis ventralis; 16 Muscular cap; 17 Pharyngeal isthmus; 18 Anterior cervical ganglion; 19 Nodose ganglion; 20 M. stylopharyngeus.

palatinus muscles extend to within a short distance of the free border of the soft palate. The pterygopharyngeus, by continuing its forward growth, has now made contact with the hamulus of the pterygoid bone (Fig. IV.140./6); it has also become wider both anteriorly where it sends a number of separate muscle strands into the substance of the soft palate, and along the lateral wall of the nasopharynx. A short distance caudal to the level through the free edge of the soft palate it combines and forms a single sheet with the palatopharyngeus muscle which runs forwards and inwards inside the palatopharyngeal folds, almost meeting its fellow from the other side in the centre of the Arcus veli palatini (8). The levator of the palate (7), fanning out towards the midline, can be seen passing between the two preceding muscles; however, from both of these, small muscle strands pass it on the alternate side from the one on which the parent muscle passes. In general it can be said that the portions of both the pterygopharyngeus and palatopharyngeus muscles which lie in front of Reichert's cartilage have become less distinct, and it is rather difficult to interpret their present state of development. As can be gleaned from the illustrations, these muscles have not formed regular bodies as yet, and it is often difficult to tell to which muscle the separate strands belong (6, 8, 16). The tensor of the palate (5) has grown longer and lies now for the most part below the entrance and the proximal portion of the auditory tube.

Its fibres, after having passed around the hamulus, fan out and are superseded by a sheet of dense non-muscular tissue, perhaps the forerunner of the palatine aponeurosis. There is little change with regard to the middle and posterior constrictors of the pharynx which have been illustrated in detail for the 40 mm. fetus. The cricoesophageus, although present as a pre-muscle mass in the previous stages, can now be made out to originate from the caudal end of the median ridge of the cricoid lamina and to spread laterally, caudally and dorsally, forming the inner layer of the two oblique esophageal muscles.

The Relationships of the Pharynx

The medially placed pharyngeal hypophysis (Fig. IV.139./2) is showing signs of degeneration. It forms the most anterior dorsal relation of the pharynx and serves, at the same time, as an undeviating landmark for the anterior limit of the pharynx. This view will gradually have to be revised, however, as the anlagen of the generally recognised landmarks for the anterior extent in the adult are slowly carried forward by the lengthening of the oral cavity. The internal carotid arteries, accompanied along their subbasal course by the carotid nerves, are moving more and more laterally, away from the pharyngeal roof, and can no longer be regarded as dorsal relations. They cross over the tubotympanic recesses, maintaining dorsal relationships with these outgrowths of the pharynx however (Fig. IV.138./3).

Lateral to the nasopharynx lie the rapidly enlarging pterygoid bones, which until now have been formed by intramembranous ossification. In all specimens of the 50 mm. group a cartilaginous core, on the periphery of which ossification continues to take place, has developed in the bones (Fig. IV.140./4). The posterior extremities of the thyrohyoids, which were observed previously to lie below the piriform recesses, have moved upwards and outwards and form now lateral relations with the posterior pharynx. On the other hand, structures, such as the internal and external carotid arteries and the nodose and the anterior cervical ganglia, that used to be affiliated with the posterior pharynx laterally, have slowly moved out of reach of the pharynx, caused, no doubt, by the slow unbending of the head that has taken place during the last few stages (11, 12, 18, 19). These same structures, or at least their ventral continuations, have, however, retained their lateral relationships to the proximal portion of the œsophagus.

The continuous spread of epithelial proliferation, which originated in the vicinity of the palatine tonsils, creates for the first time in this study a condition, whereby the oropharynx can be differentiated from the nasopharynx by its epithelial lining. The pharyngeal tube and its adjacent structures have increased in linear fashion with perhaps again, as was the case in the previous stage, the muscles both of the pharynx and of the soft palate making the greatest advance.

STAGE 14

(70 mm. C.R.L.)

The observations for this developmental stage are based on five fetuses taken from three litters:

Fetus D 53	66 mm. C.R.L.	litter 13
Fetus D 54	68 mm. C.R.L.	litter 14
Fetus D 55	68 mm. C.R.L.	
Fetus D 58	70 mm. C.R.L.	litter 15
Fetus D 59	-	

No measurements are given for the last specimen listed, because it was decapitated when received by the author.

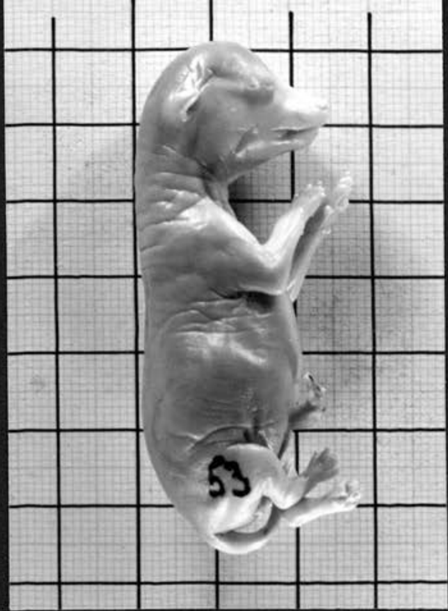


Fig. IV.141. Canine fetus D 53 (66 mm. C.R.L.).

Number of embryo:	**D 53**
Size before fixation:	**-**
Size after fixation:	**66 mm. C.R.L.**
Age:	**-**
Position on uterus:	**-**
Size of loculus:	**-**
Fixed in:	**Bouin's Fluid**
Stored in:	**70% Alcohol**
Date of processing:	**May 3, 1961**
Block:	**Paraffin**
Stain:	**H. & E.**
Plane of section:	**Transverse**
Thickness of section:	**10 microns**
Series made:	**D 53 (1 - 59)**

From litter:		**13**
Number of embryos in litter:		**7**
DAM	Breed:	**Cocker Spaniel**
	Age:	**3 - 4 years**
	Weight:	**33 lb.**
SIRE	Breed:	**-**
	Age:	**-**
	Weight:	**-**
Number of services:		**-**
Date of 1st service:		**-**
Date of 2nd service:		**-**

Date of collection: **May 11, 1960**

At time of collection Dam was alive ( ), dead (**x**).

Embryos collected **2** hour(s) after death.

Litter received from: **Ontario Veterinary College**

Remarks:

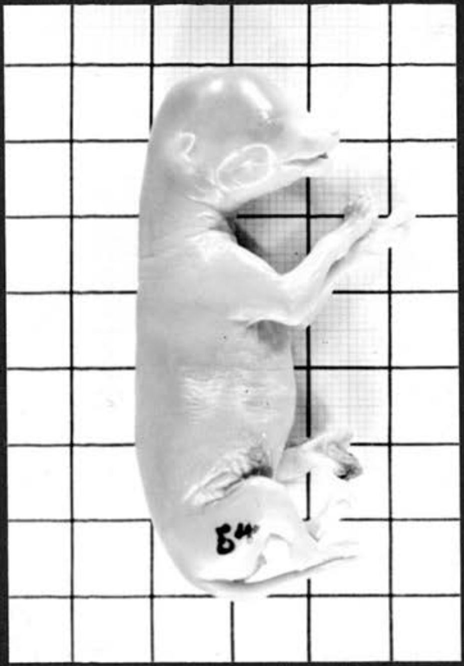


Fig. IV.142. Canine fetus D 54 (68 mm. C.R.L.).

Number of embryo:	**D 54**	Date of processing:	**May 11, 1961**
Size before fixation:	**-**	Block:	**Paraffin**
Size after fixation:	**68 mm. C.R.L.**	Stain:	**H. & E.**
Age:	**-**	Plane of section:	**Sagittal**
Position on uterus:	**-**	Thickness of section:	**10 microns**
Size of loculus:	**-**	Series made:	**D 54 (1 - 15)**
Fixed in:	**Bouin's Fluid**		
Stored in:	**70% Alcohol**		

From litter:		**14**
Number of embryos in litter:		**6**
DAM	Breed:	**X Corgi**
	Age:	**-**
	Weight:	**-**
SIRE	Breed:	**-**
	Age:	**-**
	Weight:	**-**
Number of services:		**-**
Date of 1st service:		**-**
Date of 2nd service:		**-**

Date of collection: **January 12, 1960**

At time of collection Dam was alive ( ), dead (**X**).

Embryos collected **1** hour(s) after death.

Litter received from: **Dr. L. Cobb, Ontario Veterinary College.**

Remarks:

Fig. IV.143. Canine fetus D 55 (68 mm. C.R.L.).

Number of embryo:	D 55	Date of processing:	May 11, 1962
Size before fixation:	-	Block:	Paraffin
Size after fixation:	68 mm. C.R.L.	Stain:	H. & E.
Age:	-	Plane of section:	Transverse
Position on uterus:	-	Thickness of section:	10 microns
Size of loculus:	-	Series made:	D 55 (1 - 62)
Fixed in:	Bouin's Fluid		
Stored in:	70% Alcohol		

From litter:		14
Number of embryos in litter:		6
DAM	Breed:	X Corgi
	Age:	-
	Weight:	-
SIRE	Breed:	-
	Age:	-
	Weight:	-
Number of services:		-
Date of 1st service:		-
Date of 2nd service:		-

Date of collection: January 12, 1960

At time of collection Dam was alive ( ), dead (X).

Embryos collected 1 hour(s) after death.

Litter received from: Dr. L. Cobb, Ontario Veterinary College.

Remarks:

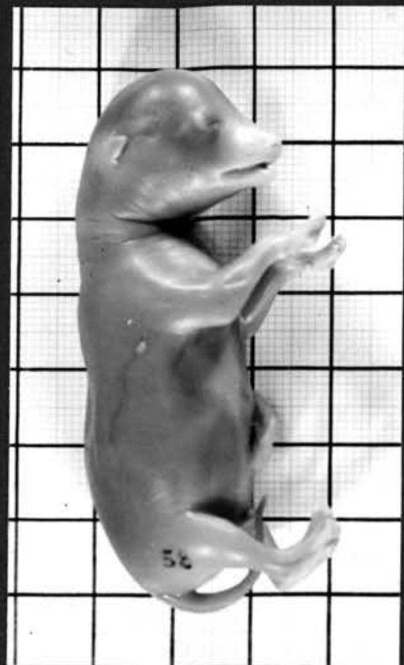


Fig. IV.144. Canine fetus D 58 (70 mm. C.R.L.).

Number of embryo:	**D 58**	Date of processing:	**May 11, 1960**
Size before fixation:	**-**	Block:	**Paraffin**
Size after fixation:	**70 mm. C.R.L.**	Stain:	**H. & E.**
Age:	**-**	Plane of section:	**Sagittal**
Position on uterus:	**-**	Thickness of section:	**10 microns**
Size of loculus:	**-**	Series made:	**D 58 (1 - 26)**
Fixed in:	**10% Form./Bouin's Fluid**		
Stored in:	**70% Alcohol**		

From litter:		**15**
Number of embryos in litter:		**4**
DAM	Breed:	**X Collie and Terrier**
	Age:	**-**
	Weight:	**30 lb.**
SIRE	Breed:	**German Shepherd**
	Age:	**-**
	Weight:	**-**
Number of services:		**-**
Date of 1st service:		**-**
Date of 2nd service:		**-**

Date of collection: **February 7, 1960**

At time of collection Dam was alive (**X**), dead ( ).

Embryos collected hour(s) after death.

Litter received from: **Dr. E. Cucuel, Chicago, Illinois.**

Remarks:

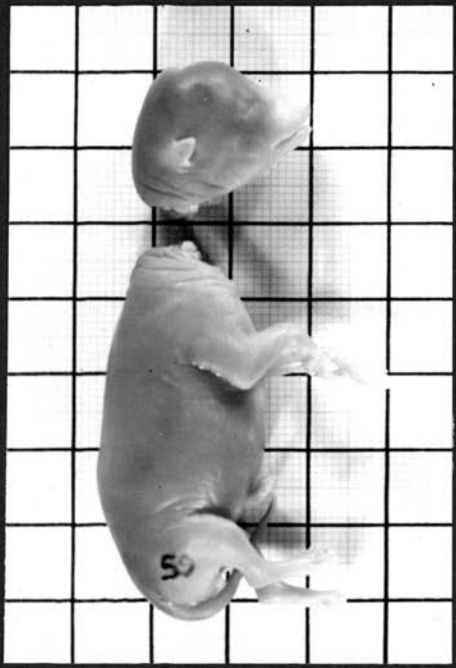


Fig. IV.145. Canine fetus D 59.

Number of embryo:	**D 59**	Date of processing:	**May 11, 1960**
Size before fixation:	**-**	Block:	**Paraffin**
Size after fixation:	**-**	Stain:	**H. & E.**
Age:	**-**	Plane of section:	**Transverse**
Position on uterus:	**-**	Thickness of section:	**10 microns**
Size of loculus:	**-**	Series made:	**D 59 (1 - 41)**
Fixed in:	**10% Form./Bouin's Fluid**		
Stored in:	**70% Alcohol**		

From litter: **15**

Number of embryos in litter: **4**

DAM Breed: **X Collie and Terrier**

Age: **-**

Weight: **30 lb.**

SIRE Breed: **German Shepherd**

Age: **-**

Weight: **-**

Number of services: **-**

Date of 1st service: **-**

Date of 2nd service: **-**

Date of collection: **February 7, 1960**

At time of collection Dam was alive (**X**), dead ( ).

Embryos collected hour(s) after death.

Litter received from: **Dr. E. Cucuel, Chicago, Illinois.**

Remarks:

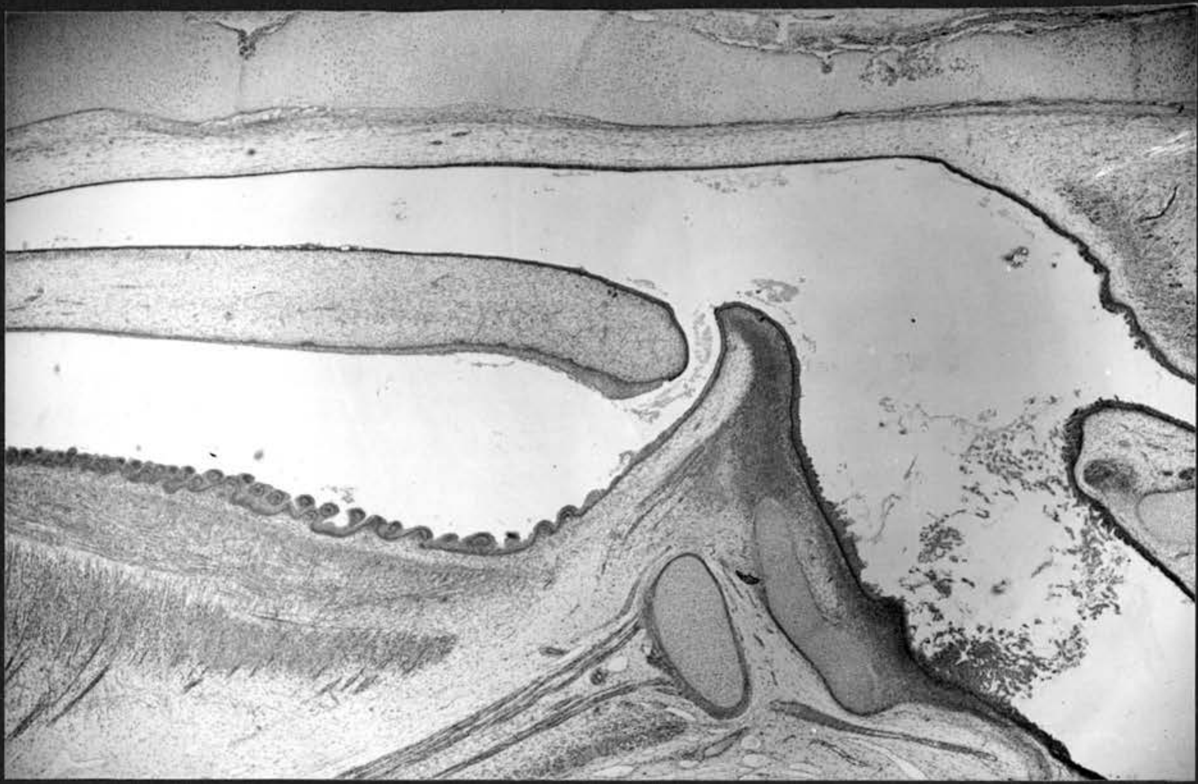
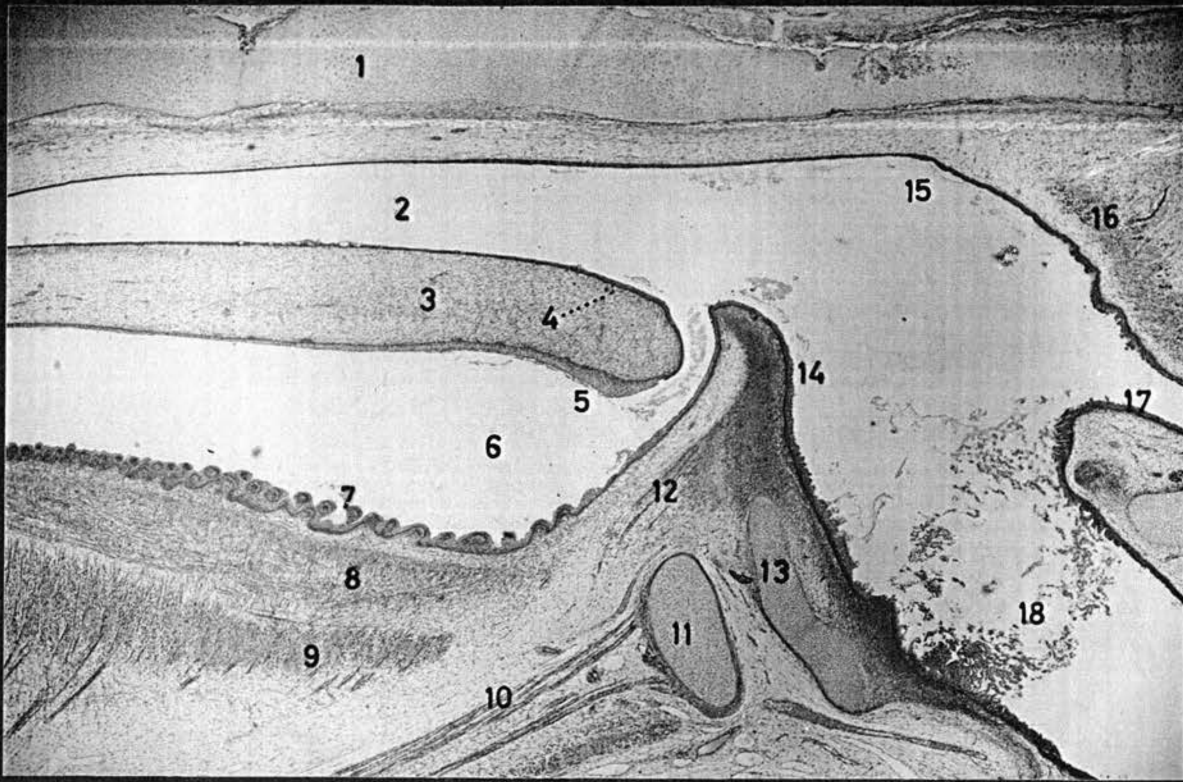


Fig. IV.146. Median section of pharynx, fetus D 54 (68 mm. C.R.L.), (X 20).

1 Basilar plate; 2 Nasopharynx; 3 Soft palate; 4 Anlagen of pharyngeal glands; 5 Epithelial proliferation caused by the fusion of lateral palatine processes; 6 Isthmus faucium; 7 Filiform papillae on root of tongue; 8 Dorsal layer of intrinsic lingual muscle; 9 Ventral layer of intrinsic lingual muscle; 10 M. geniohyoideus; 11 Basihyoid; 12 M. hyoepiglotticus; 13 Thyroid cartilage; 14 Epiglottis; 15 Fornix pharyngis; 16 Pharyngeal musculature; 17 Vestibulum esophagi; 18 Laryngeal lumen.



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1 Basilar plate; 2 Nasopharynx; 3 Soft palate; 4 Anlagen of pharyngeal glands; 5 Epithelial proliferation caused by the fusion of lateral palatine processes; 6 Isthmus faucium; 7 Filiform papillae on root of tongue; 8 Dorsal layer of intrinsic lingual muscle; 9 Ventral layer of intrinsic lingual muscle; 10 M. geniohyoideus; 11 Basihyoid; 12 M. hyoepiglotticus; 13 Thyroid cartilage; 14 Epiglottis; 15 Fornix pharyngis; 16 Pharyngeal musculature; 17 Vestibulum esophagi; 18 Laryngeal lumen.

The Shape of the Pharynx

The conformation of the pharyngeal tube has remained constant, although it has increased in size. The longitudinal pharyngeal measurements for the fetuses in this group range from 4.5 to 4.7 mm., and the transverse measurements of the nasopharynx, taken just in front of the openings into the auditory tubes, range from 1.9 to 2.4 mm. The soft palate is longer in the 70 mm. fetus and reaches to within 200 microns of the epiglottis (Fig. IV.146.). The spaces on either side of the tongue, at the level of junction between its body and root, are being obliterated by epithelial apposition. Two such appositions are present on either side, and between each pair a fold is being produced. It is possible that these folds will later transform into the palatoglossal arches, although in the present state of events they cannot be regarded as such, because they are not long enough to reach to the underside of the soft palate. The tonsillar complex, which consists of the long and narrow tonsillar swelling and the Plica semilunaris suspended from the soft palate, has also increased in length relative to the surrounding structures. The tonsillar swellings measure 1.8 mm. in length and have a greatest diameter of 330 microns. Posteriorly, they fade out into a fold, which, running parallel to the Plica semilunaris, extends beyond the level of the palatine arch (Fig. IV.147./13). The lumen of the initial portion of the esophagus, having been observed in the preceding stage to resemble a dorsoventrally flattened rectangle, has become irregularly stellate with five points at its beginning but soon changing to six a little further down the tube.

The Structure of the Pharyngeal Wall

The epithelial lining of the nasopharynx, which was seen to be still undifferentiated in the 50 mm. fetus, has at last changed from the embryonic type to one resembling its adult counterpart. (It will be recalled that similar transformations with regard to the oropharyngeal epithelium had taken place earlier, in the 40 mm. stage.) Its basal layer is unchanged but the superficial layer has become distinctly columnar, showing a heavy free border over most of the surfaces. These changes do not extend into the flat auditory tubes where the dorsal surface is still lined by a single layer of squamous cells and the ventral surface by a layer of cuboidal cells.

In the 70 mm. fetus the ubiquitous pharyngeal glands appear. They are mainly seen in the dorsal and ventral surfaces of the soft palate, being less concentrated in the vicinity of the median plane (Fig. IV.146./4). They are solid, bud-like outgrowths from the basal layer of the epithelium, pushing into the underlying primitive connective tissue. The buds have an average diameter from 20 to 25 microns, the longest ones extend over

100 microns into the substratum, and they are all relatively straight. The superficial layer of the epithelium overlying these outgrowths is unchanged. They are found in the floor and the lateral walls of the naso-pharynx, but not in the auditory tubes, and none are visible in the roof at this stage. On the other hand, in the oropharynx they occupy the roof and also extend down the lateral walls, avoiding the region of the tonsillar complex. At the present stage of development there are more anlagen for the pharyngeal glands in the ventral surface of the soft palate than there are in the dorsal. None are seen below the esophageal epithelium.

With the exception of the anlagen for the pharyngeal glands, the epithelium lining the oropharynx is largely unchanged. The only manifestation remaining of the fusion of the soft palate is a longitudinal epithelial thickening along its mid-ventral line (Fig. IV.146./5). Starting again from the tonsillar complex, a proliferation of the darker staining basal layer of epithelium is taking place. This has spread to the surface of the tongue, but other areas are unaffected. The epithelium over the root of the tongue has in addition been modified by a deepening of the hitherto observed shallow papillary body and a proliferation of the superficial layers of cells opposite the interpapillary evaginations, producing what resembles filiform papillae which all point in a dorsocaudal direction (7). They are especially well formed around the median sulcus but less pronounced laterally towards the tonsillar complexes. The remainder of the dorsum of the tongue is quite smooth, although the undersurface of the epithelium is beginning to be moulded over a fine papillary body.

The proliferation of the basal layer of the oro-pharyngeal epithelium, mentioned above as having originated in and around the tonsillar complexes, is especially heavy in the tonsillar swellings. Here, the former single basal layer consists of three to four irregular layers of cells. Inside the tonsillar swellings the densely packed mass of round cells that has been observed ever since the swellings arose, has been replaced by connective tissue; and if it was not for a central core of small darkly staining cells, among which only small amounts of connective tissue fibres are present, it would indeed be difficult to distinguish between the tonsillar swelling and the overhanging Plica semilunaris, which in this group of fetuses has almost the same outline on cross section (Fig. IV.147./12). There is a dense network of capillaries inside the tonsillar swellings, the supplying vessels and nerves still entering along the bottom of these shelf-like structures.

The epithelium lining the Vestibulum esophagi, which in the 50 mm. fetus was quite undifferentiated and consisted, like the lining of the esophagus itself, of several irregular rows of cells, has also modified and is composed now of a darker staining basal layer and two to three rows of lighter cuboidal cells. The superficial layers are grooved longitudinally, especially in the roof of this cavity. This appearance gives way to an area of roughness as the esophagus leaves the confines of the pharyngeal constrictors, and is superseded by a smooth superficial layer. The intraepithelial spaces observed to be present in younger specimens have disappeared.

Many of the palatine and pharyngeal muscles are well developed and distinct, but it is still difficult to gain a clear picture of their arrangement in the posterior division of the soft palate and with regard to the hyo-pharyngeus muscle. Even in the mature animal the latter muscle is a very elusive structure to study, because it seems to vary in thickness and attachments in every specimen. Therefore, the apparent lack of design with respect to this section of the pharyngeal constrictors in the 70 mm. fetus need not cause too much concern. For the posterior portion of the soft palate however, Dyce (1957) has described a constant adult muscular plan. It is perhaps because of the many strands into which the levator, the pterygopharyngeus and the palatopharyngeus in this group of fetuses divide that confusion still reigns. The median pharyngeal raphe, which serves in the adult to a greater or lesser extent as an insertion for all pharyngeal muscles, has started to form. It is present in this group of fetuses as a plaque of dense connective tissue, very rich in fibres, immediately behind the Fornix pharyngis. Only the fibres of the superficial muscles are inserted in the raphe, the deeper ones cross the midline as before. The primitive raphe only extends backwards to about opposite the posterior limit of the pharyngeal isthmus. From here on, the fibres of the pharyngeal constrictors still continue across the midline. It appears, therefore, that the pharyngeal raphe develops in an anterior to posterior direction and starts first superficially and later in the deeper strata. In the 70 mm. fetus it furnishes attachment only to the hyopharyngeus and the anterior portion of the thyropharyngeus muscles.

The palatinus muscle consists of two indistinct long bellies, lying in the substance of the soft palate on either side of the midline. They do not reach the full length of the soft palate, and for this reason have not formed any attachments. The tensor of the soft palate (Fig. IV.148./A) has a well formed belly and originates from the underside of the auditory tube in the vicinity of the otic ganglion. Solid structures for its origin have not yet appeared. The muscle extends downwards and inwards until it reaches the ventral surface of the hamulus. Here, the bulk of the fibres terminate and are continued by a sheet of dense tissue (palatine aponeurosis) which extends medially and anteriorly into the soft palate. This sheet, although not reaching the vicinity of the palatinus, let alone the midline, lies in the dorsal stratum of the soft palate, and, if continued medially in the same direction, would pass dorsal to the palatinus muscle. A few muscle fibres of the tensor incline medially and slightly posteriorly, after turning around the caudal tip of the hamulus, and end below the levator of the palate (B). This muscle also originates under the auditory tube, but a little caudal to the tensor, and runs medially, posteriorly and ventrally into the substance of the soft palate. As it passes the caudal edge of the pterygoid bone, it spreads into a horizontal sheet, crosses the pterygopharyngeus dorsally, and, before terminating in the vicinity of the palatinus, underlies that portion of the palatopharyngeus which passes forward into the body of the soft palate. The pterygopharyngeus (C) (Fig. IV.147./2) originates from the midline behind the Fornix pharyngis. Being a deep muscle in this area, it does not make contact with the newly formed pharyngeal raphe. It runs forwards and is joined by the palatopharyngeus for a short distance. At the level where the stylopharyngeus enters, the palatopharyngeus parts company with the pterygopharyngeus and the latter continues forwards in a straight line to attach itself at the hamulus of the pterygoid bone. Before it reaches its destination however, it detaches two or three strands of muscle fibres which turn slightly medial to end either in the substance of the soft palate or on the pterygoid bone above the hamulus. On its way

Fig. IV.147. Transverse section of pharynx, fetus D 55 (68 mm. C.R.L.), (X 20).

1 Tubotympanic recess; 2 M. pterygopharyngeus; 3 M. longus capitis; 4 Basilar plate; 5 Cochlea; 6 Internal carotid artery and carotid nerve; 7 Nasopharynx; 8 Tip of epiglottis; 9 M. palatopharyngeus; 10 Soft palate; 11 Oropharynx; 12 Posterior extremity of Plica semilunaris; 13 Posterior extremity of tonsillar swelling; 14 Stylohyoid; 15 Epihyoid; 16 M. styloglossus; 17 Keratohyoid; 18 Dorsal layer of intrinsic lingual muscle; 19 M. hyoepiglotticus; 20 M. hyoglossus; 21 Hypoglossal nerve; 22 M. mylohyoideus.

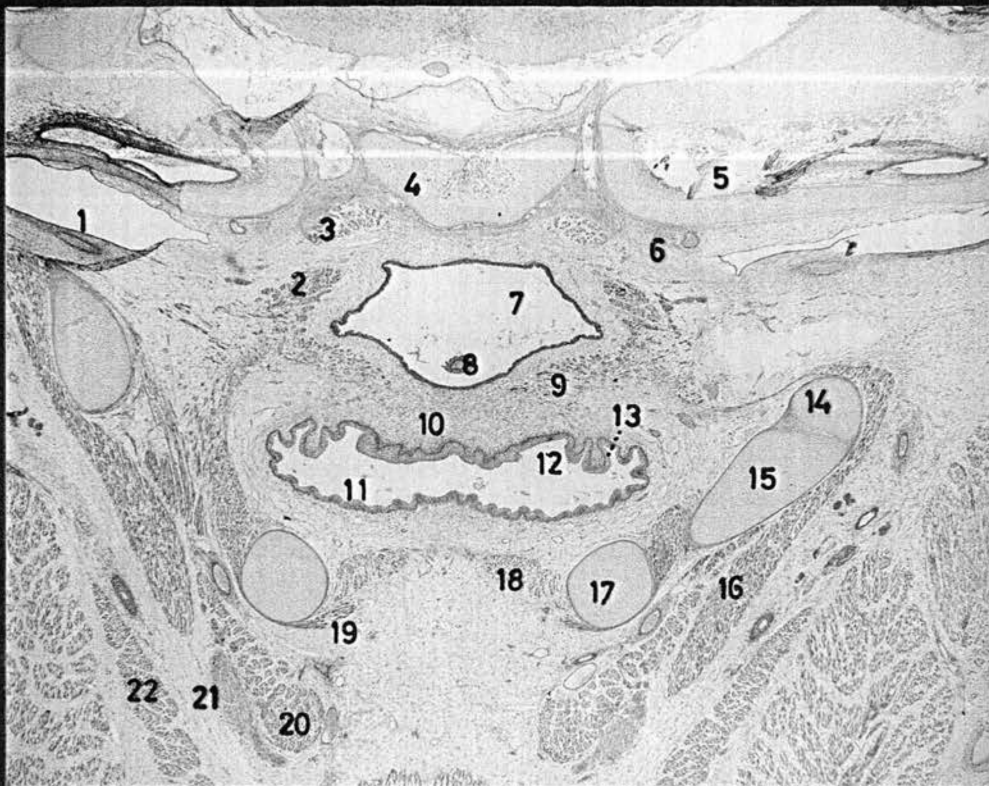


Fig. IV.147. Transverse section of pharynx, fetus D 55 (68 mm. C.R.L.), (X 20).

1 Tubotympanic recess; 2 M. pterygopharyngeus; 3 M. longus capitis; 4 Basilar plate; 5 Cochlea; 6 Internal carotid artery and carotid nerve; 7 Nasopharynx; 8 Tip of epiglottis; 9 M. palatopharyngeus; 10 Soft palate; 11 Oropharynx; 12 Posterior extremity of Plica semilunaris; 13 Posterior extremity of tonsillar swelling; 14 Stylohyoid; 15 Epihyoid; 16 M. styloglossus; 17 Keratohyoid; 18 Dorsal layer of intrinsic lingual muscle; 19 M. hyoepiglotticus; 20 M. hyoglossus; 21 Hypoglossal nerve; 22 M. mylohyoideus.

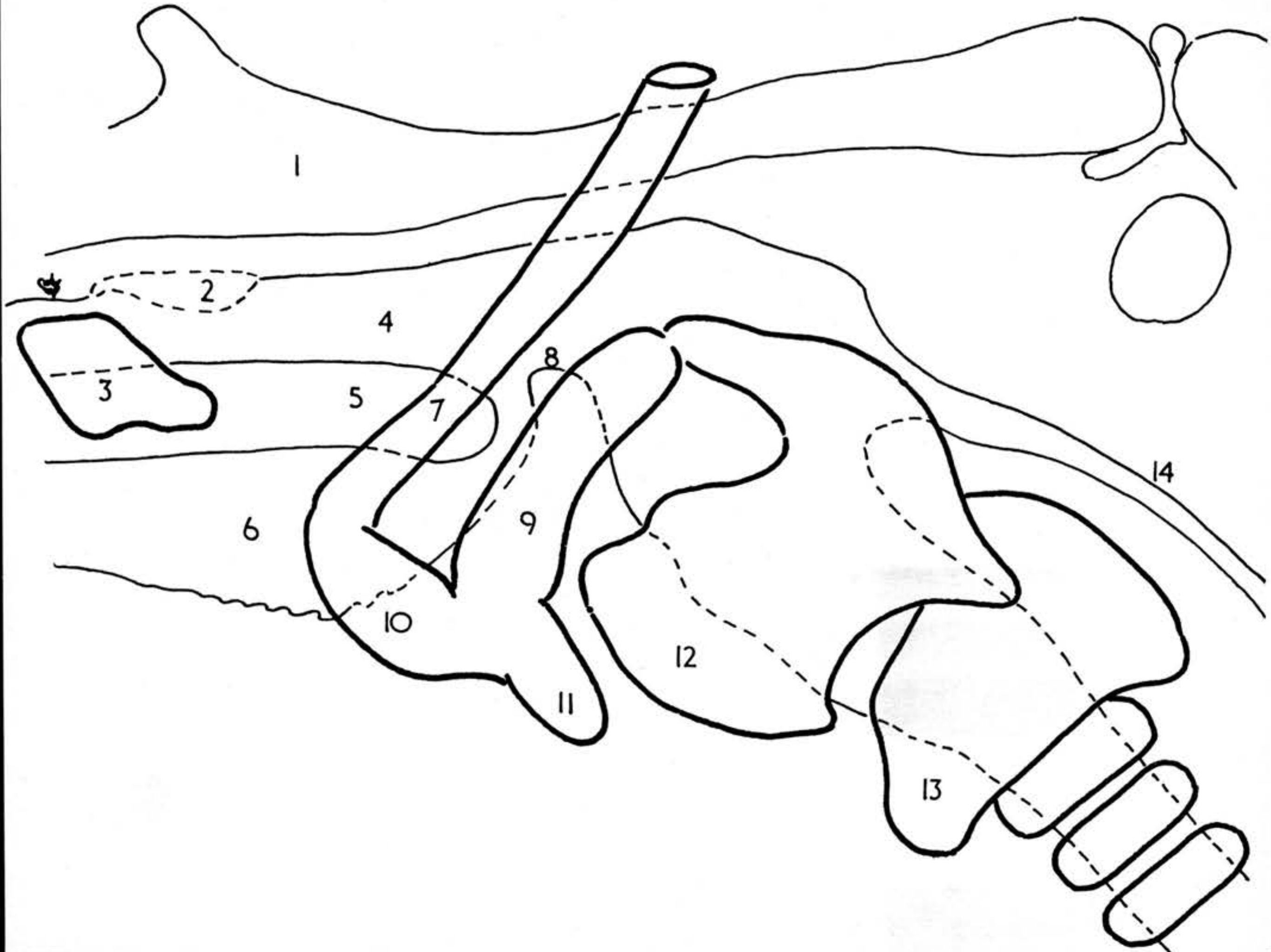


Fig. IV.148. Lateral view of pharynx and associated skeletal structures, the developing palatine and pharyngeal muscles are superimposed, based on fetuses D 54 and D 55 (both 68 mm. C.R.L.), (X 25), (schematic).

1 Basal plate; 2 Position of entrance into tubotympanic recess; 3 Anlage of pterygoid bone; 4 Nasopharynx; 5 Soft palate; 6 Isthmus faucium; 7 Stylohyoid; 8 Epiglottis; 9 Thyrohyoid; 10 Keratohyoid; 11 Basihyoid; 12 Thyroid cartilage; 13 Cricoid cartilage; 14 Vestibulum esophagi.

A M. tensor veli palatini; B M. levator veli palatini; C M. pterygopharyngeus; D M. stylopharyngeus; E M. palatopharyngeus; F M. hyopharyngeus; G M. thyropharyngeus; H M. cricopharyngeus; I M. cricoesophageus; J Esophageal muscle.

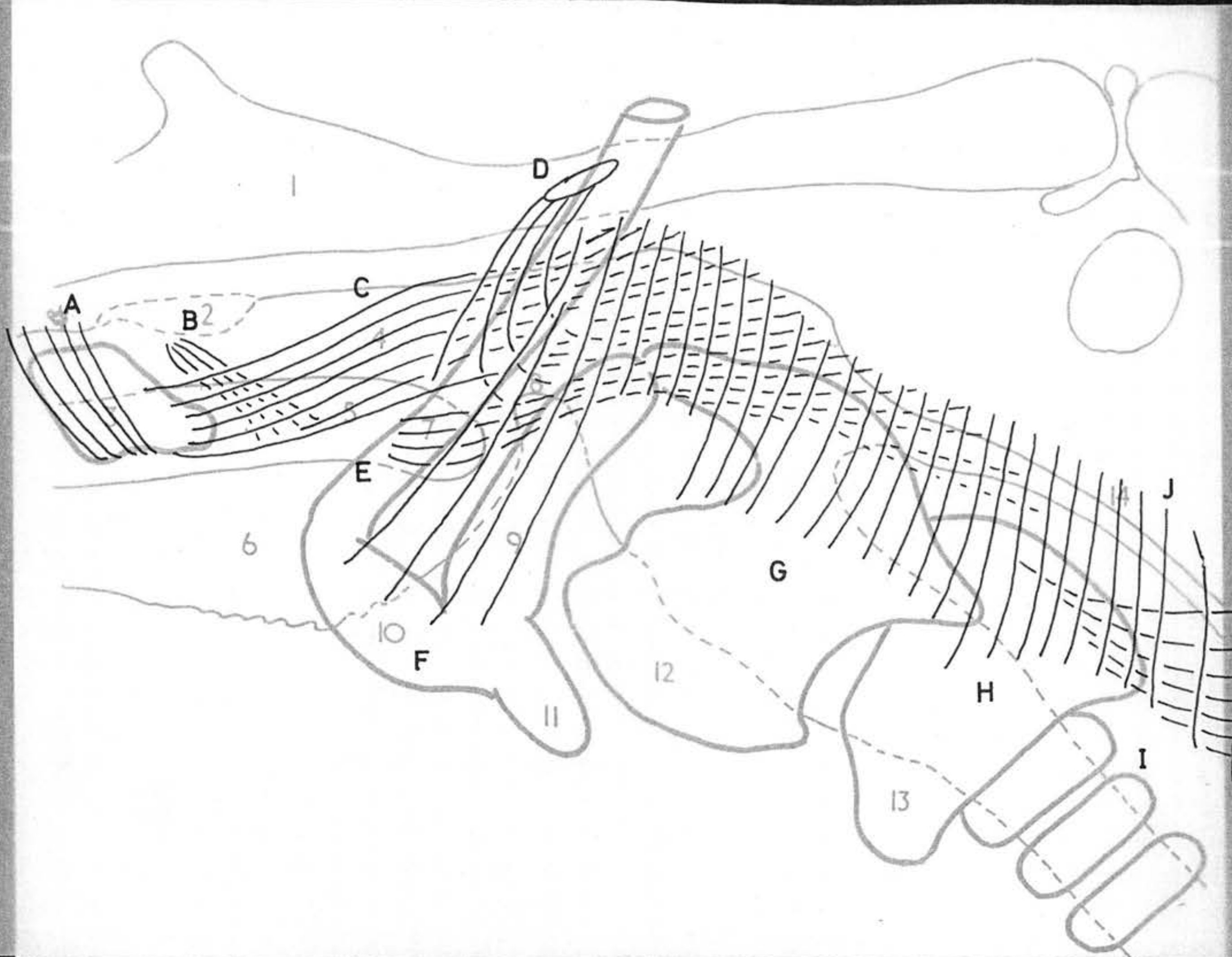


Fig. IV.148. Lateral view of pharynx and associated skeletal structures, the developing palatine and pharyngeal muscles are superimposed, based on fetuses D 54 and D 55 (both 68 mm. C.R.L.), (X 25), (schematic).

1 Basal plate; 2 Position of entrance into tubotympanic recess; 3 Anlage of pterygoid bone; 4 Nasopharynx; 5 Soft palate; 6 Isthmus faucium; 7 Stylohyoid; 8 Epiglottis; 9 Thyrohyoid; 10 Keratohyoid; 11 Basihyoid; 12 Thyroid cartilage; 13 Cricoid cartilage; 14 Vestibulum esophagi.
A M. tensor veli palatini; B M. levator veli palatini; C M. pterygopharyngeus; D M. stylopharyngeus; E M. palatopharyngeus; F M. hyopharyngeus; G M. thyropharyngeus; H M. cricopharyngeus; I M. cricoesophageus; J Esophageal muscle.

forward along the lateral face of the pharynx, some of its ventral fibres intermingle with the fibres of the stylopharyngeus. Further along, the bulk of the fibres of the pterygopharyngeus pass lateral to the levator as the latter enters the soft palate from above, but some of the fibres that do not attach themselves on the pterygoid bone pass the levator medially. The palatopharyngeus muscle (9) (Fig. IV.148./E) also arises from the midline posterior to the fornix. Its fibres, being ventral to those of the pterygopharyngeus, reach farther backwards however. It runs forwards on the dorsolateral face of the Vestibulum esophagi under cover of the transversely running fibres of the thyropharyngeus. Up to about the level where the stylopharyngeus enters, it forms a flat sheet with the pterygopharyngeus. Then it gradually turns medially inside the palatopharyngeal folds and loses contact with the latter muscle. The bulk of its fibres, curving anteromedially, follow the palatopharyngeal folds and, entering the palatine arch, proceed towards the midline, without however making contact with the palatopharyngeus from the other side. As this division turns medially into the arch, a smaller division of the palatopharyngeus is detached which passes forwards into the substance of the soft palate, where it intermingles with the fibres of the levator, although some strands of this division pass the levator on the dorsal side. The stylopharyngeus muscle (D) originates from the medial aspect of Reichert's cartilage and passes towards the lateral aspect of the pharynx in a ventral, medial and slightly anterior direction. Most of its fibres terminate in the connective tissue on the lateral face of the pharynx, after having intermingled with the fibres of the pterygopharyngeus and palatopharyngeus muscles. A considerable portion of the fibres however turn caudally and run along the lateral face of the pharynx, contained between the caudal portion of the palatopharyngeus medially and the anterior division of the thyropharyngeus laterally. Although its fibres extend farther backwards than those of the palatopharyngeus, they do not reach the midline, but fade out on the lateral aspect of the Vestibulum esophagi. The hyopharyngeus (F) is a very thin sheet of muscle, originating from the primitive raphe,and covers the caudal portions of the pterygopharyngeus, the palatopharyngeus and the stylopharyngeus, the fibres of which muscles it crosses at right angles. It fans out to end on the lateral face of the thyrohyoid and keratohyoid cartilages. The thyropharyngeus (G) is the constrictor overlying the Vestibulum esophagi. Its fibres come from the mid-dorsal line and terminate on the lateral face of the thyroid cartilage. It is continued caudally without demarcation by the cricopharyngeus (H), whose fibres have the same origin and course, but are somewhat longer and end on the lateral face and the caudal border of the cricoid cartilage. This muscle in turn is followed, again without division, by the outer layer of the esophageal muscle (J). The cricoesophageus muscle (I) lies deep to the cricopharyngeus. It originates from the median dorsal ridge of the cricoid lamina and passes backwards, fanning out and embracing the esophageal tube from below. Its fibres form the deeper of the two oblique layers of the esophageal muscle coat.

The Relationships of the Pharynx

The remnant of the stalk to Rathke's pocket is still present as the pharyngeal hypophysis. It is a cord-like aggregation of epithelial cells surrounded by a thin capsule of connective tissue. Its previous lumen is filled with debris and detached cells. A vague connection to the roof of the nasopharynx is still being maintained, at which point the epithelium of the pharyngeal roof is slightly thickened. The pharyngeal hypophysis seems to be slowly degenerating. The basal plate, overlying both the pharyngeal hypophysis and the horizontal portion of the pharynx, is beginning to ossify in two centres (Fig. IV.146./1). One of these is at the level of Rathke's pocket and the other just above the Fornix pharyngis where the pharynx inclines ventrally and loses relation with the basal plate. At these two locations an osseous collar has formed which surrounds the basal plate. Capillary buds are pushing into the centre of the plate from its dorsal surface (Fig. IV.147./4). Ossification has not yet begun in the centre of the cartilage. These observations confirm the findings of Drews (1933) who, using the alizarin technique, describes ossification in the base of the cranium to appear in the 70 mm. (40 day) embryo. At the caudal end of the basilar plate where the cartilaginous precursors of the occipital bone and the atlas come together to form the atlanto_occipital joint, a joint space and faint outline of a joint capsule have developed (Fig. IV.148.). The recently appeared cartilaginous centre in the pterygoid bone which flanks the nasopharynx on either side just in front of the entrance into the auditory tubes (3) has enlarged and extends now into the hamulus as well. Formerly, the hamulus was made up of dense tissue only. The glossal branch of the glossopharyngeal nerve, as it leaves the root of the tongue forms immediate lateral relations with the posterior portions of the tonsillar swellings. As has been mentioned in several of the preceding stages, accessory thyroid tissue is regularly present at the ventral aspect of the basihyoid. The thyroid cells are arranged in small circles, foreshadowing the formation of follicles. The interstices are filled with blood vessels.

Other than for an increase in size, the only advance toward adult pattern was made by the epithelium covering the soft palate, in the appearance of the anlagen for the pharyngeal glands. The palatine and pharyngeal muscles have also advanced, but, notwithstanding this, the muscular pattern in the caudal portion of the soft palate is still not too apparent.

STAGE 15

(90 mm. C.R.L.)

The observations for this stage were carried out on three fetuses taken from two litters:

Fetus D 60	92 mm. C.R.L.	litter 16
Fetus D 61	91 mm. C.R.L.	litter 16
Fetus D 63	93 mm. C.R.L.	litter 17

Fig. IV.149. Canine fetus D 60 (92 mm. C.R.L.).

Number of embryo:	**D 60**	Date of processing:	**November 16, 1961**
Size before fixation:	**-**	Block:	**Paraffin**
Size after fixation:	**92 mm. C.R.L.**	Stain:	**H. & E.**
Age:	**-**	Plane of section:	**Sagittal**
Position on uterus:	**-**	Thickness of section:	**10 microns**
Size of loculus:	**-**	Series made:	**D 60 (1 - 18)**
Fixed in:	**Bouin's Fluid**		
Stored in:	**70% Alcohol**		

From litter:		**16**
Number of embryos in litter:		**9**
DAM	Breed:	**X Collie**
	Age:	**-**
	Weight:	**-**
SIRE	Breed:	**-**
	Age:	**-**
	Weight:	**-**
Number of services:		**-**
Date of 1st service:		**-**
Date of 2nd service:		**-**

Date of collection: **March 28, 1960**

At time of collection Dam was alive ( ), dead (**X**).

Embryos collected **1** hour(s) after death.

Litter received from: **Ontario Veterinary College**

Remarks:

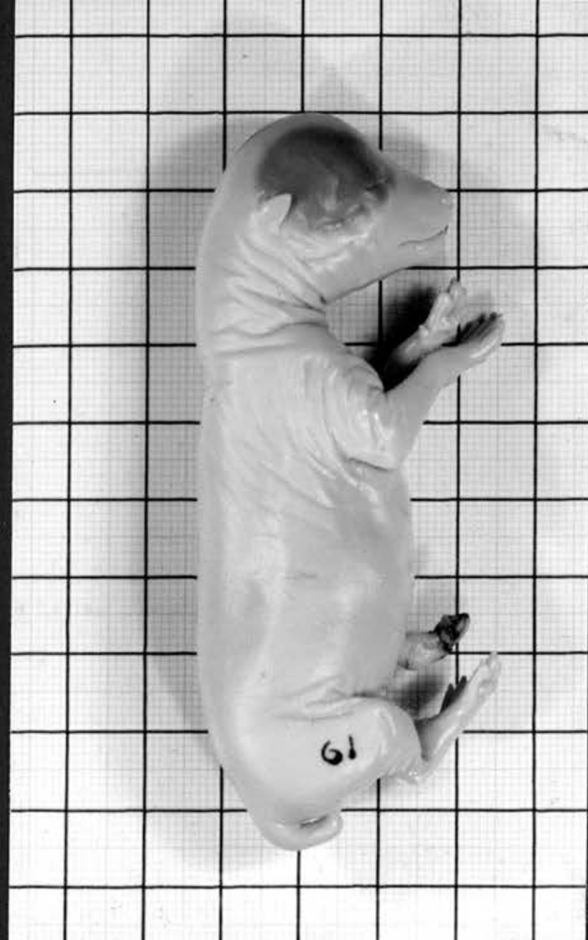


Fig. IV.150. Canine fetus D 61 (91 mm. C.R.L.).

Number of embryo:	**D 61**	Date of processing:	**November 16, 1961**
Size before fixation:	**-**	Block:	**Paraffin**
Size after fixation:	**91 mm. C.R.L.**	Stain:	**H. & E.**
Age:	**-**	Plane of section:	**Transverse**
Position on uterus:	**-**	Thickness of section:	**10 microns**
Size of loculus:	**-**	Series made:	**D 61 (1 - 39)**
Fixed in:	**Bouin's Fluid**		
Stored in:	**70% Alcohol**		

From litter:		**16**
Number of embryos in litter:		**9**
DAM	Breed:	**X Collie**
	Age:	**-**
	Weight:	**-**
SIRE	Breed:	**-**
	Age:	**-**
	Weight:	**-**
Number of services:		**-**
Date of 1st service:		**-**
Date of 2nd service:		**-**

Date of collection: **March 28, 1960**

At time of collection Dam was alive ( ), dead (**X**).

Embryos collected **1** hour(s) after death.

Litter received from: **Ontario Veterinary College**

Remarks:

Fig. IV.151. Canine fetus D 63 (93 mm. C.R.L.).

Number of embryo:	**D 63**	Date of processing:	**November 16, 1961**
Size before fixation:	**-**	Block:	**Paraffin**
Size after fixation:	**93 mm. C.R.L.**	Stain:	**H. & E.**
Age:	**-**	Plane of section:	**Horizontal**
Position on uterus:	**-**	Thickness of section:	**10 microns**
Size of loculus:	**-**	Series made:	**D 63 (1 - 27)**
Fixed in:	**10% Formalin**		
Stored in:	**10% Formalin**		

From litter:		**17**
Number of embryos in litter:		**3**
DAM	Breed:	**-**
	Age:	**-**
	Weight:	**-**
SIRE	Breed:	**-**
	Age:	**-**
	Weight:	**-**
Number of services:		**-**
Date of 1st service:		**-**
Date of 2nd service:		**-**
Date of collection:		**-**

At time of collection Dam was alive ( ), dead ( ).

Embryos collected hour(s) after death.

Litter received from: **Edinburgh Surgery**

Remarks:

Fig. IV.152. Median section of pharynx, fetus D 60 (92 mm. C.R.L.), (X 20).

1 Basilar plate; 2 Pharyngobasilar fascia; 3 Nasopharynx; 4 M. palatopharyngeus; 5 M. palatinus; 6 M. levator veli palatini; 7 Epiglottis with M. hyoepiglotticus; 8 Intrinsic lingual muscle; 9 M. genioglossus; 10 M. geniohyoideus; 11 M. mylohyoideus; 12 Ramus communicans of lingual veins; 13 Accessory thyroid; 14 Basihyoid; 15 Thyroid cartilage; 16 Cricoid cartilage; 17 Laryngeal lumen; 18 Vestibulum esophagi; 19 Fornix pharyngis; 20 Pharyngeal musculature.

Fig. IV.152. Median section of pharynx, fetus D 60 (92 mm. C.R.L.), (X 20).

1 Basilar plate; 2 Pharyngobasilar fascia; 3 Nasopharynx; 4 M. palatopharyngeus; 5 M. palatinus; 6 M. levator veli palatini; 7 Epiglottis with M. hyoepiglotticus; 8 Intrinsic lingual muscle; 9 M. genioglossus; 10 M. geniohyoideus; 11 M. mylohyoideus; 12 Ramus communicans of lingual veins; 13 Accessory thyroid; 14 Basihyoid; 15 Thyroid cartilage; 16 Cricoid cartilage; 17 Laryngeal lumen; 18 Vestibulum esophagi; 19 Fornix pharyngis; 20 Pharyngeal musculature.

The Shape of the Pharynx

With the exception of some minor changes and a general increase in size, the shape of the pharyngeal chamber has remained constant in the 90 mm. fetus and resembles that of the adult. Its longitudinal measurement was found to be 5.3 mm. in fetus D 60, and the width of the pharynx, just in front of the entrance into the auditory tubes, 2.5 mm. in fetus D 61. The soft palate extends now beyond the level of the epiglottis, the latter having adapted itself to this new situation by contraction and by forward flexion of its apex (Fig. IV.152./7). It is conceivable that the hyoepiglottic muscle played a role in this contraction. Previously, the soft palate was seen to push against the anterior face of the epiglottis. It appears that, with the lengthening of the soft palate, the epiglottis, rather than yielding to the pressure from the front and flattening out over the Aditus laryngis (Fig. II.2./E), did the opposite to get out of the way of the expanding soft palate. This peculiar position of the epiglottis was found in all specimens of this group; peculiar, because the recognised adult respiratory or swallowing positions of the epiglottis are quite different. It would appear, however, that the present position of the epiglottis could best facilitate any flow of fluid from the amniotic cavity into either esophagus or trachea. Such flow would have to pass through the oro-pharynx, because the anterior nares are still closed by a loose plug of desquamated cells and debris. It is possible, therefore, that the anteriorly bent position of the epiglottis poses less resistance to such a flow (Becker et al., 1939) than the previous upright position in which the Isthmus faucium, now that the soft palate is longer, would have been sealed off, except perhaps for the two small lateral channels of the piriform recesses.

An expanding connective tissue density above the entrance into the auditory tubes causes (a) the pharyngeal openings of the tubes to be compressed dorsally, and (b) the entire entrance to be moved slightly ventrally to a new position on the lateral wall of the nasopharynx and away from the one formerly held in the dorsolateral angle of the chamber. This density can perhaps be interpreted as the forerunner of the cartilage of the auditory tube. Further back in the nasopharynx, the fornix has taken on the shape of a three-sided pyramid, of which one side lies in the horizontal plane and is parallel to the basal plate of the skull, and the other two sides form the lateral walls of the caudal portion of the nasopharynx (Fig. IV.152./19).

The Structure of the Pharyngeal Wall

The epithelia lining the pharyngeal chamber are of three types: stratified columnar epithelium in the nasopharynx; stratified squamous epithelium in the rest of the chamber and the esophagus; and a modified stratified squamous epithelium over the dorsum and the root of the tongue. The stratified columnar type of the nasopharynx extends now well into the auditory tubes, although as the tympanic cavity is approached it reverts to the previously noted single or double cuboidal layer especially on the roof. The nasopharyngeal epithelium is still quite irregular in thickness, that covering the floor being thicker than that lining the roof. Numerous light-staining cells, which perhaps represent primordial goblet cells, have appeared in the superficial layer of this epithelium.

The epithelium covering the dorsal surface of the tongue is of the stratified squamous type, much the same as is found throughout the remainder of the pharyngeal cavity. It differs, however, in that it rests on a well developed papillary body, which imparts a distinct waviness, like cobble-stones, to the free surface of the epithelium. Until the 70 mm. fetus of the preceding stage, the free border of the epithelium over the dorsum of the tongue had been found to be quite smooth, but now for the first time these cobble-stone elevations have appeared opposite the connective tissue papillae which extend upwards into the epithelium from below. The dorsocaudally directed filiform papillae over the root of the tongue have grown more distinct and are also longer (Fig. IV.153./11). The moats surrounding the circumvallate papillae, which are embedded in the epithelium of the tongue, have been caused to disappear by the apposition of the inner and outer walls. The papillae, when seen in horizontal section, have taken on an oval shape at this stage, their shortest and longest diameters measuring 200 microns and 250 microns respectively.

The stratified squamous epithelium lining the rest of the pharyngeal chamber and the esophagus has typical adult pattern. It is smooth along the underside of the soft palate where it consists of a double layer of cuboidal cells at the base (Stratum germinativum) which is followed by three to four rows of irregular cells and topped by three to four rows of very flat cells. On the free border some of the flat cells are thrown off and are found as debris in the pharyngeal lumen. The average thickness of the stratified squamous epithelium on the ventral surface of the soft palate is 50 microns. The epithelium covering the proximal parts of the larynx, the Vestibulum esophagi and the initial portion of the esophagus is also of the stratified squamous type, but it is not as smooth and regular as that on the soft palate. In the Vestibulum esophagi it is thrown into fine longitudinal folds. All the epithelia rest on a cushion of embryonic connective tissue of varying thickness in which the extranuclear material (fibres) greatly outweigh the nuclear elements.

The buds of the pharyngeal glands are better developed in the 90 mm. fetus and extend from the basal layer of the epithelium to a depth of about 180 microns into the substratum (10). Their bulbous end portions show the formation of primitive lumina, and in some of the larger glandular buds they have already divided into two branches. The glands are most numerous on the dorsal surface and lateral walls of the oro-pharynx where an exceptionally large one (450 microns long and well branched) was observed close to the Plica semilunaris of the tonsillar complex. In the nasopharynx the buds are

<u>Fig. IV.153</u>. Transverse section of pharynx, fetus D 61 (91 mm. C.R.L.), (X 20).

1 Tympanic ring; see also same structure on other side; 2 Tubotympanic recess; 3 Cochlea; 4 Basilar plate; 5 Internal carotid artery and carotid nerve; 6 Nasopharynx; 7 M. levator veli palatini; 8 M. pterygopharyngeus; 9 M. palatinus; 10 Pharyngeal gland buds; 11 Root of tongue and filiform papillae in section; 12 Plica semilunaris; 13 Tonsillar swelling; 14 Sub-lingual gland; 15 M. styloglossus; 16 M. mylohyoideus; 17 Hypo-glossal nerve; 18 M. hyoglossus; 19 Intrinsic lingual muscle; 20 Mandible with associated masticatory muscles; 21 External maxillary vein.

<u>Fig. IV.153</u>. Transverse section of pharynx, fetus D 61 (91 mm. C.R.L.), (X 20).

1 Tympanic ring; see also same structure on other side; 2 Tubotympanic recess; 3 Cochlea; 4 Basilar plate; 5 Internal carotid artery and carotid nerve; 6 Nasopharynx; 7 M. levator veli palatini; 8 M. pterygopharyngeus; 9 M. palatinus; 10 Pharyngeal gland buds; 11 Root of tongue and filiform papillae in section; 12 Plica semilunaris; 13 Tonsillar swelling; 14 Sublingual gland; 15 M. styloglossus; 16 M. mylohyoideus; 17 Hypoglossal nerve; 18 M. hyoglossus; 19 Intrinsic lingual muscle; 20 Mandible with associated masticatory muscles; 21 External maxillary vein.

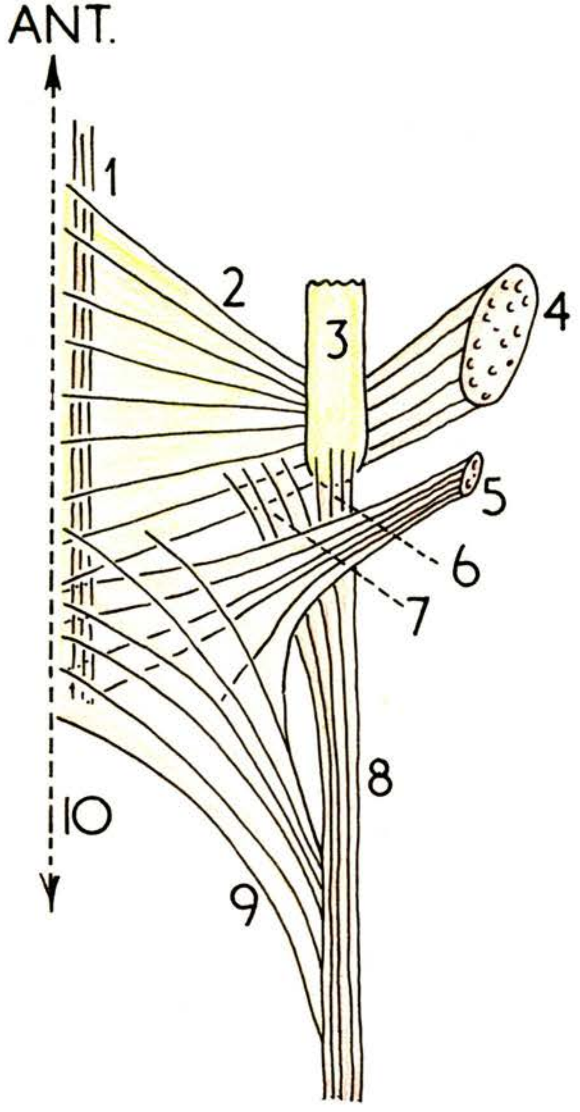


Fig. IV.154. Schematic representation of the muscular pattern in the soft palate of the 90 mm. embryo, dorsal view.

1 Palatinus; 2 Tensor aponeurosis; 3 Hamulus; 4 Tensor; 5 Levator; 6 Hamular division of pterygopharyngeus; 7 Palatine division of pterygopharyngeus; 8 Pterygopharyngeus; 9 Palato-pharyngeus; 10 Midline.

smaller and are only present on its floor and lateral walls, but have not appeared in the auditory tubes. Similar glandular buds are present for the first time in the lateral walls of the esophagus in the region opposite the first few tracheal rings.

The muscular arrangement in the soft palate is now a little less confusing. The palatinus (Figs. IV.154./1; IV.153./9; IV.152./5) runs as a double-bellied muscle along the long axis of the soft palate on either side of the midline. It originates just posterior to the recently laid-down horizontal portion of the palatine bone but does not make actual contact with it. Towards the free edge of the soft palate, the muscle fans out a little and its individual strands interdigitate with the transversely running fibres of the palatopharyngeus. The most anterior of the extrinsic muscles of the soft palate is the Tensor veli palatini (Fig. IV.154./4) which originates from the anterior surface of the auditory tube where it has made contact with the newly formed connective tissue density above the initial portion of the tube. From here it passes ventromesiad along the lateral face of the pterygoid bone to the hamular process where it flattens to turn medially. Its continuation into the soft palate is not muscular but a progressively widening sheet of primitive connective tissue (palatine aponeurosis) which, lying in the horizontal plane, stretches to the midline to make contact with its fellow from the other side. It is inclined slightly upwards so that it comes to lie dorsal to the palatinus muscle

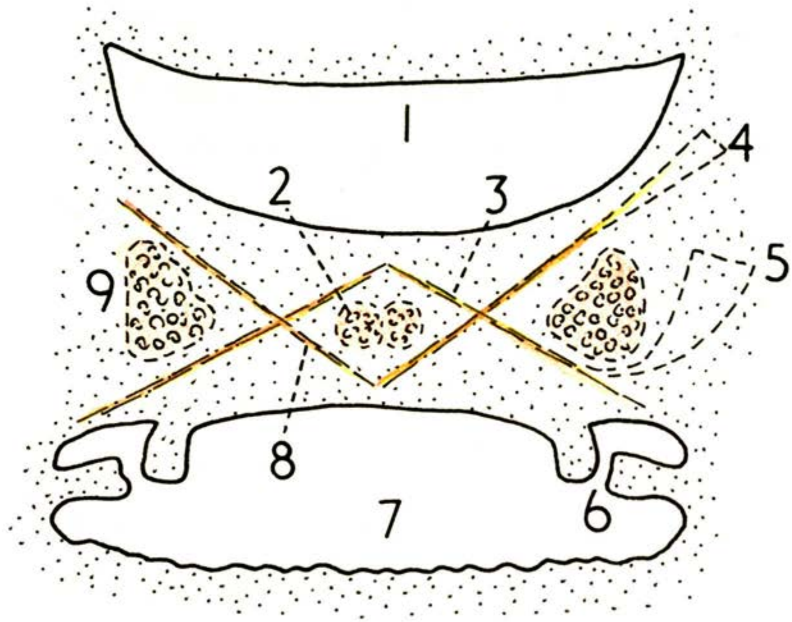


Fig. IV.155. Transverse section of pharynx to show muscular pattern within the soft palate, based on fetus D 61 (91 mm. C.R.L.), (schematic), (see also Fig. IV.153.).

1 Nasopharynx; 2 Palatinus; 3 Plane of tensor aponeurosis anteriorly and palatopharyngeus posteriorly; 4 Levator; 5 Tensor; 6 Tonsillar complex; 7 Oropharynx; 8 Plane of levator; 9 Pterygopharyngeus.

(Fig. IV.155./5, 3). Its anterior angle is attached to the palatine bone. The next muscle entering the soft palate from the lateral side is the Levator veli palatini (Figs. IV.154./5; IV.153./7). It arises under the initial portion of the auditory tube just posterior to the origin of the preceding muscle. It has not made contact with any hard structure in the vicinity of the auditory tube, but has grown towards the tympanic ring (1) that was described earlier (stage 12) to have appeared below the tubotympanic recess. It is possible that in later development this osseous bar will furnish the attachment for the muscle. From this position, the slender muscle passes ventrally, medially and posteriorly and, fanning out as well, enters the soft palate behind the aponeurosis of the tensor. The bulk of the fibres of the levator retain this course and reach the midline below the palatinus muscle, forming, as it were, a cross with the aponeurosis of the tensor (Fig. IV.155.). Some of the more caudally positioned fibres of the levator however change their direction to an almost posterior course and become continuous or intermingled with those of the palatopharyngeus muscle that enters the soft palate inside the palatopharyngeal fold (Fig. IV.154.). Between the two muscles that enter the soft palate from the lateral side (tensor and levator) runs the pterygopharyngeus muscle (8) which, together with the palatopharyngeus (9), approaches the soft palate from behind. This muscle originates on the posterior tip of the hamular process and passes as a relatively discreet bundle backwards along the lateral face of

the pharyngeal cavity (Fig. IV.152./8). A few fibres join the main muscle shortly behind the hamulus. They have originated from just above the tensor aponeurosis inside the soft palate (Fig. IV.154./7). The pterygopharyngeus ends in the median pharyngeal raphe in the vicinity of the pharyngeal fornix.
In that part of the pharyngeal chamber where the palato-pharyngeal folds blend with the lateral wall of the nasopharynx, the palatopharyngeus muscle (9) (Fig. IV.152./4), which has run forwards on the lateral face of the pharynx together with the pterygopharyngeus, leaves the latter muscle and turns medially into the soft palate along the Arcus veli palatini. Its medial fibres follow the curvature of the free border of the soft palate and meet corresponding fibres from the other side in the midline. The fibres more laterally placed in the muscle enter the substance of the soft palate, always staying above the level of the palatinus muscle, and thus assume the same upward direction as the tensor aponeurosis (Fig. IV.155./3). They meet with their fellows from the other side in the midline at about the level of the hamulus. Some fibres of this division intermingle or even join with the posteriorly directed ones of the levator (Fig. IV.154.). The posterior extremity of the palatopharyngeus ends by being inserted into the pharyngeal raphe a little below the insertion of the pterygopharyngeus muscle. At a level where these two muscles part company, the stylopharyngeus enters the muscular wall on the lateral face of the pharynx. Its fibres originate from the medial aspect of the stylohyoid in which an ossification

centre has appeared. Upon reaching the wall, the fibres spread out and run in posterior, anterior and ventral directions. The anterior fibres mingle with those of the palatopharyngeus and pterygopharyngeus and some of them, no doubt, reach the soft palate. The posterior fibres run close to the pharyngeal epithelium under cover of the middle and posterior constrictors of the pharynx and fade out over the Vestibulum esophagi without however reaching the median raphe. They are situated ventral to the fibres of the palatopharyngeus and for the most part run parallel to them. The ventral portions of these fibres are vaguely attached to the posterior cornu of the thyroid cartilage, while others of this division bypass the hard structures of the larynx and mingle with the fibres of the cricoesophageus and so are lost in the inner muscular coat of the esophagus. The hyopharyngeus, thyropharyngeus and cricopharyngeus muscles have not changed appreciably, and their description in previous stages may be consulted.

The Relationships of the Pharynx

The main dorsal relation is the basal plate which is separated from the roof of the nasopharynx by the pharyngobasilar fascia (Fig. IV.152./1, 2, 3). The pharyngeal hypophysis is embedded in this fascia and, attached to the epithelium of the pharyngeal roof, appears in this group of embryos as an irregular epithelial structure with several small lumina. Earlier in this study, it served as a welcome landmark for the anterior extent of the embryonic pharynx, but has now lost its importance in this respect because the fully developed pharyngeal chamber reaches much farther rostrally (to the last molar, in fact) than to the level of the hypophysis. The only useful guide it provides at this juncture is that, of its immediate surroundings at any rate, those parts anterior to it were derived from ectoderm and those posterior to it from endoderm. The two ossification centres of the basal plate have grown in size and bone spicules can now be observed also in the centre of the plate and not just along its periphery as was the case in earlier stages. The four straight flexors of the head almost fill the previous connective tissue space below the atlanto-occipital joint and to either side of the fornix. The vagosympathetic trunk and the common carotid artery pass into the neck parallel to these muscles between the initial portion of the esophagus medially and the well established retropharyngeal lymph nodes laterally.

The principal lateral relations of the pharyngeal chamber are, as before, the vertical rami of the mandible and the medial pterygoid muscle (Fig. IV.153./20). Meckel's cartilage, which until now has always been a major landmark in transverse sections of the pharyngeal region, has been resorbed into the expanding osseous mandible in its central portion, although still present at the base of the skull and in the anterior extremity of the horizontal ramus of the mandible. A new immediate lateral relation to the tonsillar complex is provided by the emerging elongated sublingual salivary gland (14) which lies dorsal to the submandibular and to its own ducts, both of which have been observed for some time.

Ossification has not yet begun in the ventral components of the hyoid apparatus. Accessory thyroid tissue, consisting now of an aggregation of distinct thyroid follicles filled with homogeneous eosin-staining material, was again observed in all specimens of this group immediately below the basihyoid (Fig. IV.152./13).

The elongation of the soft palate and the peculiar forward bending of the epiglottis can be regarded as the only structural changes that have occurred in the 90 mm. fetus. Microscopically, the appearance of primordial goblet cells in the nasopharynx and the lengthening and initial branching of the pharyngeal glands are the most striking innovations. Also a more lucid muscular pattern inside the soft palate has been accomplished by the growth and consolidation of the extrinsic muscles of this structure.

S T A G E 16

(120 mm. C.R.L.)

The following three fetuses, all of the same litter, were examined for the description of this stage:

Fetus D 64	120 mm. C.R.L.	litter 20
Fetus D 65	118 mm. C.R.L.	
Fetus D 71	118 mm. C.R.L.	

The pharyngeal regions of fetuses D 64 and D 65 were sectioned serially, while fetus D 71 was used for gross dissection. For the first time in this study the examined specimens were covered by a very fine coat of hair (Fig. IV.156.).

Fig. IV.156. Canine fetus D 64 (120 mm. C.R.L.).

Number of embryo:	**D 64**	Date of processing:	**November 14, 1961**
Size before fixation:	**-**	Block:	**Paraffin**
Size after fixation:	**120 mm. C.R.L.**	Stain:	**H. & E.**
Age:	**-**	Plane of section:	**Transverse**
Position on uterus:	**-**	Thickness of section:	**10 microns**
Size of loculus:	**-**	Series made:	D **64 (1 - 16)**
Fixed in:	**10% Formalin**		
Stored in:	**10% Formalin**		

From litter:		**20**
Number of embryos in litter:		**5**
DAM	Breed:	**X Terrier**
	Age:	**-**
	Weight:	**25 lb.**
SIRE	Breed:	**-**
	Age:	**-**
	Weight:	**-**
Number of services:		**-**
Date of 1st service:		**-**
Date of 2nd service:		**-**

Date of collection: **October 29, 1960**

At time of collection Dam was alive (**X**), dead ( ).

Embryos collected hour(s) after death.

Litter received from: **Edinburgh Surgery**

Remarks:

Fig. IV.157. Canine fetus D 65 (118 mm. C.R.L.).

Number of embryo:	**D 65**	Date of processing:	**November 14, 1961**
Size before fixation:	**-**	Block:	**Paraffin**
Size after fixation:	**118 mm. C.R.L.**	Stain:	**H. & E.**
Age:	**-**	Plane of section:	**Sagittal**
Position on uterus:	**-**	Thickness of section:	**10 microns**
Size of loculus:	**-**	Series made:	**D 65 (1 - 27)**
Fixed in:	**10% Formalin**		
Stored in:	**10% Formalin**		

From litter:		**20**
Number of embryos in litter:		**5**
DAM	Breed:	**X Terrier**
	Age:	**-**
	Weight:	**25 lb.**
SIRE	Breed:	**-**
	Age:	**-**
	Weight:	**-**
Number of services:		**-**
Date of 1st service:		**-**
Date of 2nd service:		**-**

Date of collection: **October 29, 1960**

At time of collection Dam was alive (**X**), dead ( ).

Embryos collected hour(s) after death.

Litter received from: **Edinburgh Surgery**

Remarks: **Due to lateral flexion of the body this specimen measures only 115 mm. C.R.L. on the accompanying photograph (Fig.IV.157.)**

Number of embryo:	**D 71**	Date of processing:	**January 16, 1962**
Size before fixation:	**-**	Block:	**-**
Size after fixation:	**118 mm. C.R.L.**	Stain:	**-**
Age:	**-**	Plane of section:	**-**
Position on uterus:	**-**	Thickness of section:	**-**
Size of loculus:	**-**	Series made:	**Used for dissectio**
Fixed in:	**10% Formalin**		
Stored in:	**10% Formalin**		

From litter:		**20**
Number of embryos in litter:		**5**
DAM	Breed:	**X Terrier**
	Age:	**-**
	Weight:	**25 lb.**
SIRE	Breed:	**-**
	Age:	**-**
	Weight:	**-**
Number of services:		**-**
Date of 1st service:		**-**
Date of 2nd service:		**-**

Date of collection: **October 29, 1960**

At time of collection Dam was alive (**X**), dead ( ).

Embryos collected hour(s) after death.

Litter received from: **Edinburgh Surgery**

Remarks: **For a picture see that of litter mate D 64 (Fig.IV.156.)**

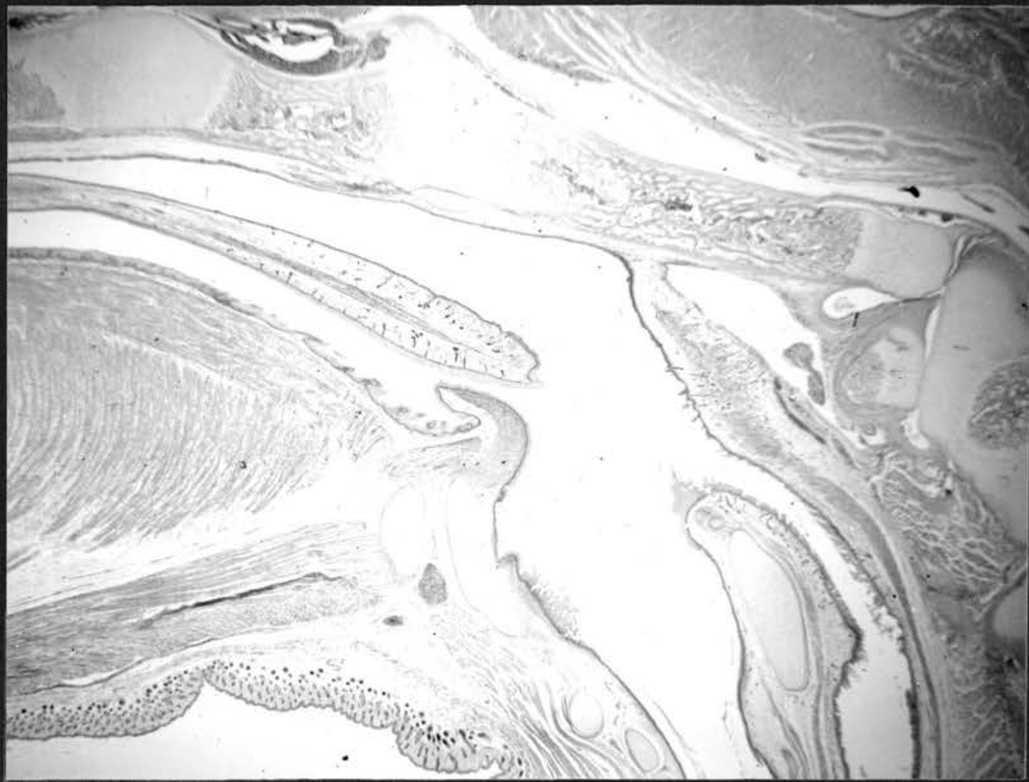


Fig. IV.158. Median section of pharynx, fetus D 65 (118 mm. C.R.L.), (X 10).

1 Hypophysis; 2 Base of skull; 3 Fornix pharyngis; 4 Joint capsule of atlantooccipital joint; 5 Ventral arch of atlas; 6 Body of epistropheus; 7 Pharyngeal musculature; 8 Vestibulum esophagi; 9 Limen pharyngo-esophagicum; 10 Cricoid cartilage; 11 Laryngeal lumen; 12 Soft palate; 13 Epiglottis; 14 Basihyoid; 15 Accessory thyroid; 16 Tongue; 17 M. palatinus.

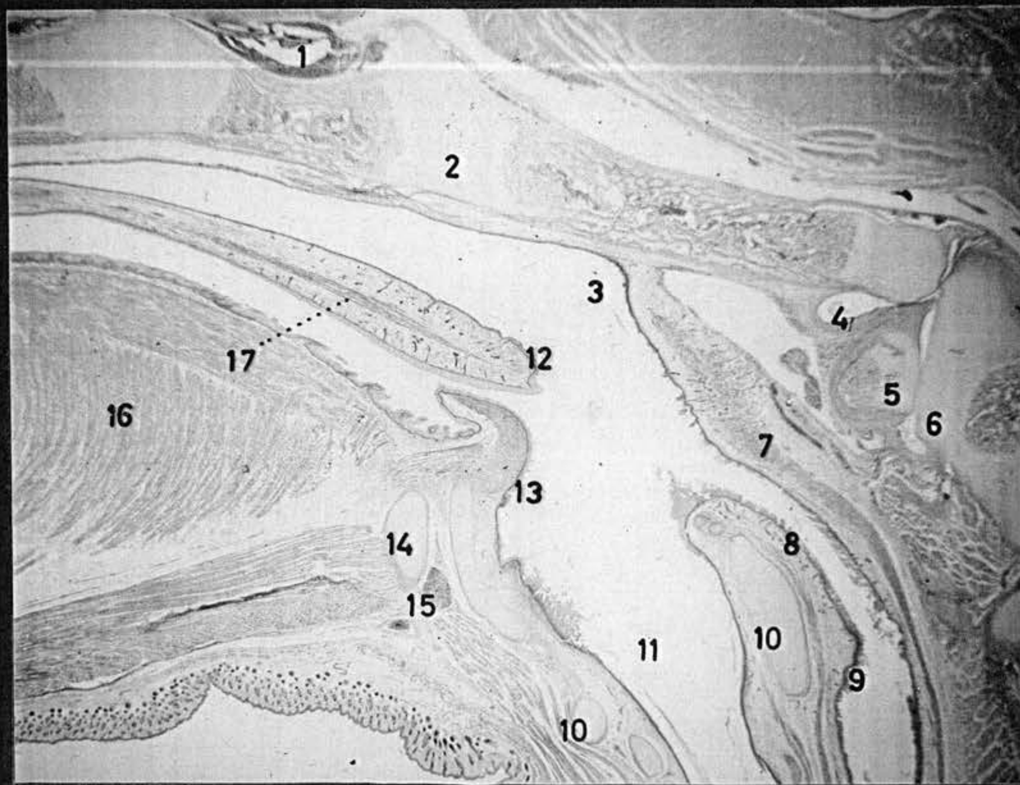


Fig. IV.158. Median section of pharynx, fetus D 65 (118 mm. C.R.L.), (X 10).

1 Hypophysis; 2 Base of skull; 3 Fornix pharyngis; 4 Joint capsule of atlantooccipital joint; 5 Ventral arch of atlas; 6 Body of epistropheus; 7 Pharyngeal musculature; 8 Vestibulum esophagi; 9 Limen pharyngo-esophagicum; 10 Cricoid cartilage; 11 Laryngeal lumen; 12 Soft palate; 13 Epiglottis; 14 Basihyoid; 15 Accessory thyroid; 16 Tongue; 17 M. palatinus.

The Shape of the Pharynx

No structural changes can be reported for the 120 mm. fetus. The pharyngeal chamber has increased in size and now measures 6.5 mm. longitudinally and 2.8 mm. transversely. The epiglottis is again found in the peculiar forward-bent position in all specimens of this group (Fig. IV.158./13). Grossly, the pharyngeal cavity of the dissected fetus looks little different from that of an adult animal. The soft palate, however, still gives evidence of its recent closure by a faint line, running in the median plane along its ventral surface, and a small anteriorly directed indentation in the centre of its free posterior edge. The wall of the opened Vestibulum esophagi appears to be thicker and also lighter in colour than that of the esophagus. The transition from the thicker to the thinner wall can be observed by the unaided eye as a narrow, collar-like swelling which has formed at this junction. This is referred to as the Limen pharyngoesophagicum and is considered to represent the caudal extent of the pharyngeal chamber in the dog (Zietzschmann, 1939) (Fig. IV.158./9). The tonsillar swellings have a diameter of 0.9 mm. and their length was found to be about 3 mm. (Fig. IV.159./10).

The Structure of the Pharyngeal Wall

A fairly thick acidophilic band has appeared along the free border of the now pseudostratified columnar epithelium that is lining the nasopharynx, and looks like a condensation of the exposed cell membrane. Presumably, the ciliated border found on top of respiratory epithelium develops from this band. A number of large, very faintly staining cell clusters have appeared in the epithelium of the lateral wall of the nasopharynx, especially surrounding the pharyngeal portion of the auditory tubes. They are equivalent in size to the bulk of about ten to twelve columnar cells and replace the original epithelium from its free border to the basement membrane. These spaces are subdivided into smaller polyhedral units by what seem to be very pale cell membranes. It is difficult in the second last developmental stage of this study to predict into which adult structures they may develop. They are not associated with buds of the pharyngeal glands, but look very much like glandular acini. The only explanation that comes to mind is that they perhaps represent local cell degeneration at points where excretory ducts of pharyngeal glands burrow through the epithelium, although, as stated above, glandular buds were not seen in the vicinity.

New glandular buds have appeared for the first time in the roof of the nasopharynx, in the root of the tongue and in the anterior and posterior walls of the initial portion of the esophagus. Those glands that have been observed for some time, especially the ones along the ventral surface of the soft palate, are well branched now and appear like small clusters at the commencement of their excretory ducts (Fig. IV.159.). The glandular lumen seems to be continuous from the free border

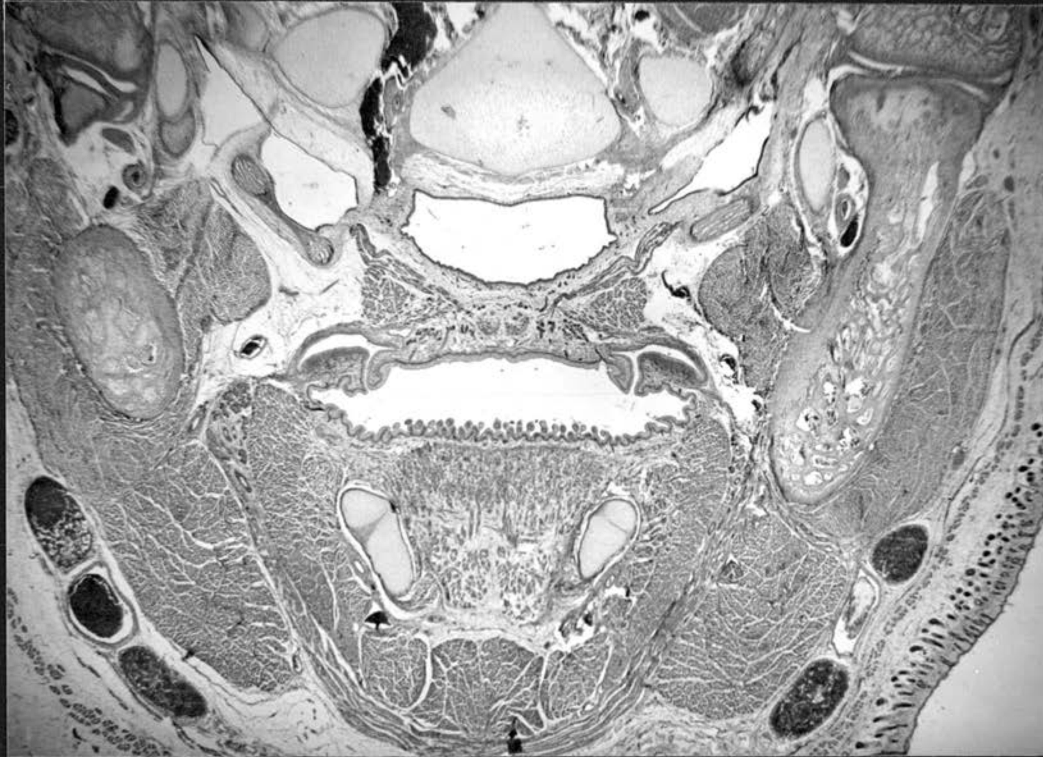


Fig. IV.159. Transverse section of pharynx, fetus D 64 (120 mm. C.R.L.), (X 10).

1 Tympanic cavity; 2 Anlage of pharyngeal tonsil; 3 Meckel's cartilage; 4 Vertical ramus of mandible; 5 M. pterygoideus medialis; 6 M. levator veli palatini close to tympanic ring; 7 M. pterygopharyngeus; 8 M. palatinus; 9 M. palatopharyngeus; 10 Palatine tonsil; 11 Root of tongue; 12 Keratohyoid; 13 M. geniohyoideus; 14 M. hyoglossus; 15 M. styloglossus; 16 M. mylohyoideus; 17 M. digastricus mandibulae; 18 External maxillary vein; 19 Mandibular lymph nodes.

Fig. IV.159. Transverse section of pharynx, fetus D 64 (120 mm. C.R.L.), (X 10).

1 Tympanic cavity; 2 Anlage of pharyngeal tonsil; 3 Meckel's cartilage; 4 Vertical ramus of mandible; 5 M. pterygoideus medialis; 6 M. levator veli palatini close to tympanic ring; 7 M. pterygopharyngeus; 8 M. palatinus; 9 M. palatopharyngeus; 10 Palatine tonsil; 11 Root of tongue; 12 Keratohyoid; 13 M. geniohyoideus; 14 M. hyoglossus; 15 M. styloglossus; 16 M. mylohyoideus; 17 M. digastricus mandibulae; 18 External maxillary vein; 19 Mandibular lymph nodes.

of the epithelium to the ends of the branches, which, on cross section, have already the characteristic appearance of mucous glands. In the soft palate these glandular clusters mingle freely with the fibres of the muscles (Fig. IV.158.).

The first evidence of pharyngeal lymphoid tissue appears in this stage. It shows itself in the accumulation of free, round cells along the dorsal border of the flat tonsillar swellings and extending, first dorsally and then medially, for a short distance along the wall of the supratonsillar fossa (Fig. IV.159./10). The cells do not as yet exhibit any organisation into nodules, nor have they invaded the epithelium which protects the tonsillar complex. Another aggregation of free cells is under the epithelium on either side of the Fornix pharyngis. By virtue of their position it can be assumed that these cell densities are the forerunners of the pharyngeal tonsils (2). No trace can be seen at this stage of development of the other lymphoid elements recognised to form the "tonsillar ring" of the pharynx in the mature animal (Nickel, Schummer, Seiferle, 1960). As regards the muscular elements of the pharyngeal wall and the soft palate, no significant changes have occurred since the description of the 90 mm. fetus. The origin of the levator has again moved a little closer to the tympanic ring, the latter being noticeable below the tubotympanic recess (6).

The Relationships of the Pharynx

No important modifications in the structures surrounding the pharynx have taken place. In all the sectioned specimens of this group accessory thyroid tissue was again found below the basihyoid (Fig. IV.158./15).

The appearance of lymphoid tissue in the tonsillar swellings and in the roof of the nasopharynx can be regarded as the only significant innovation at this stage of development. More attention will be given to the further development of the tonsillar ring of the pharynx in the following chapter, especially to the development of the palatine tonsils.

STAGE 17

(Full term)

The last stage of this study is based on seven full-term fetuses all of the same litter.

Fetus	D 72	183 mm. C.R.L.	litter 21
Fetus	D 73	180 mm. C.R.L.	
Fetus	DD 5	180 mm. C.R.L.	
Fetus	DD 6	185 mm. C.R.L.	
Fetus	DD 7	180 mm. C.R.L.	
Fetus	DD 8	178 mm. C.R.L.	
Fetus	DD 9	184 mm. C.R.L.	

The pharyngeal region of the first two fetuses listed were sectioned serially, the others were dissected under the dissecting microscope.

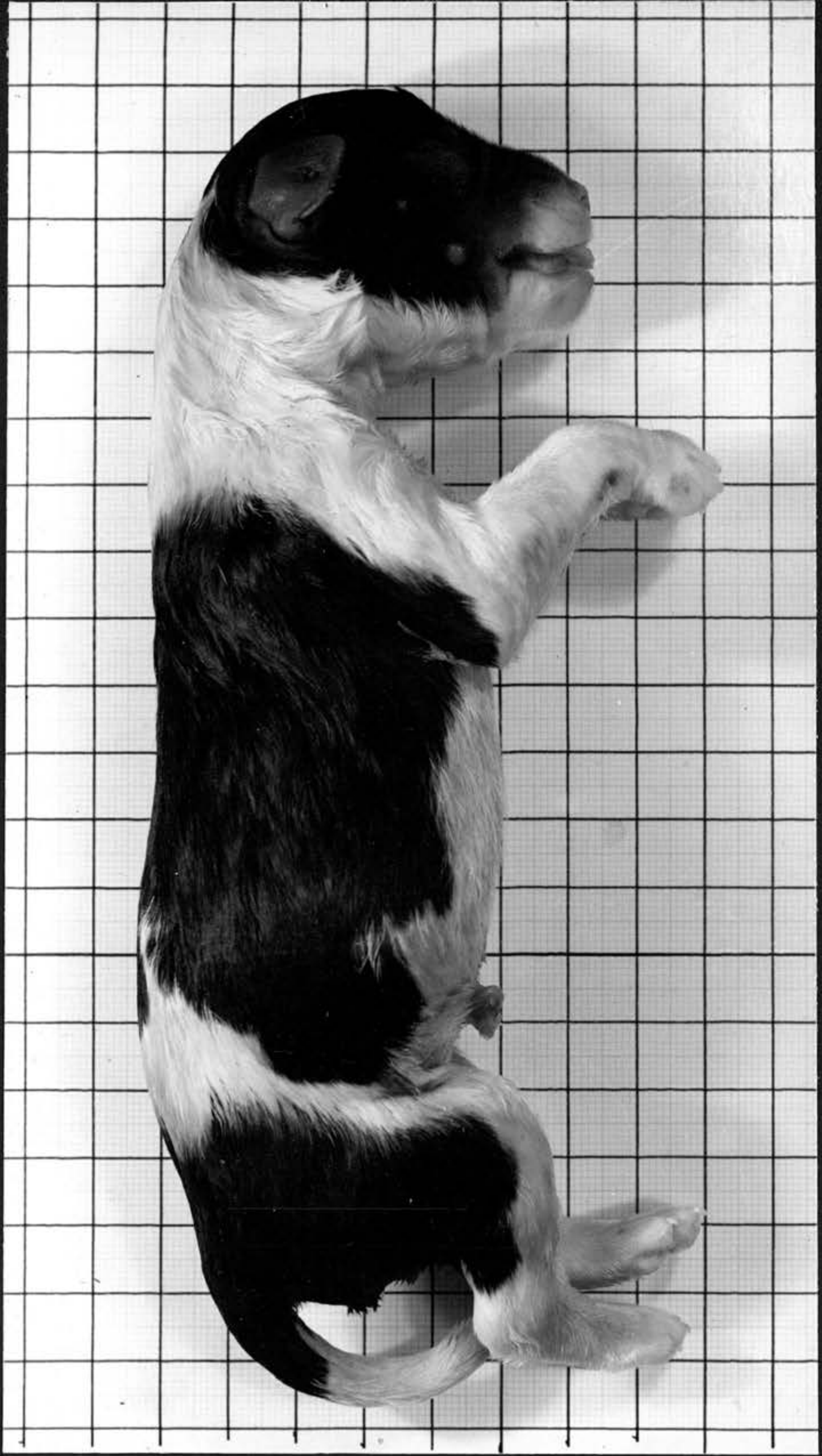


Fig. IV.160. Canine term fetus D 72 (183 mm. C.R.L.).

Number of embryo:	**D 72**	Date of processing:	**January 13, 1962**
Size before fixation:	**-**	Block:	**Paraffin**
Size after fixation:	**183 mm. C.R.L.**	Stain:	**H. & E.**
Age:	**-**	Plane of section:	**Transverse**
Position on uterus:	**-**	Thickness of section:	**10 microns**
Size of loculus:	**-**	Series made:	**D 72 (1 - 34)**
Fixed in:	**10% Formalin**		
Stored in:	**10% Formalin**		

From litter: **21**

Number of embryos in litter: **7**

DAM Breed: **-**

Age: **-**

Weight: **-**

SIRE Breed: **-**

Age: **-**

Weight: **-**

Number of services: **-**

Date of 1st service: **-**

Date of 2nd service: **-**

Date of collection: **-**

At time of collection Dam was alive ( ), dead ( ).

Embryos collected hour(s) after death.

Litter received from: **Edinburgh Surgery**

Remarks:

Number of embryo:	**D 73**	Date of processing:	**January 13, 1962**
Size before fixation:	**-**	Block:	**Paraffin**
Size after fixation:	**180 mm. C.R.L.**	Stain:	**H. & E.**
Age:	**-**	Plane of section:	**Sagittal**
Position on uterus:	**-**	Thickness of section:	**10 microns**
Size of loculus:	**-**	Series made:	**D 73 (1 - 16)**
Fixed in:	**10% Formalin**		
Stored in:	**10% Formalin**		

From litter:		**21**
Number of embryos in litter:		**7**
DAM	Breed:	**-**
	Age:	**-**
	Weight:	**-**
SIRE	Breed:	**-**
	Age:	**-**
	Weight:	**-**
Number of services:		**-**
Date of 1st service:		**-**
Date of 2nd service:		**-**
Date of collection:		**-**

At time of collection Dam was alive ( ), dead ( ).

Embryos collected hour(s) after death.

Litter received from: **Edinburgh Surgery**

Remarks: **For a picture see that of litter mate D 72 (Fig.IV.160.)**

Number of embryo:	**DD 5 - DD 9**	Date of processing:	**February, 1962**
Size before fixation:	**-**	Block:	**-**
Size after fixation:	**178 - 185 mm. C.R.L.**	Stain:	**-**
Age:	**-**	Plane of section:	**-**
Position on uterus:	**-**	Thickness of section:	**-**
Size of loculus:	**-**	Series made:	**Used for dissectio**
Fixed in:	**10% Formalin**		
Stored in:	**10% Formalin**		

From litter:		**21**
Number of embryos in litter:		**7**
DAM	Breed:	**-**
	Age:	**-**
	Weight:	**-**
SIRE	Breed:	**-**
	Age:	**-**
	Weight:	**-**
Number of services:		**-**
Date of 1st service:		**-**
Date of 2nd service:		**-**
Date of collection:		**-**

At time of collection Dam was alive ( ), dead ( ).

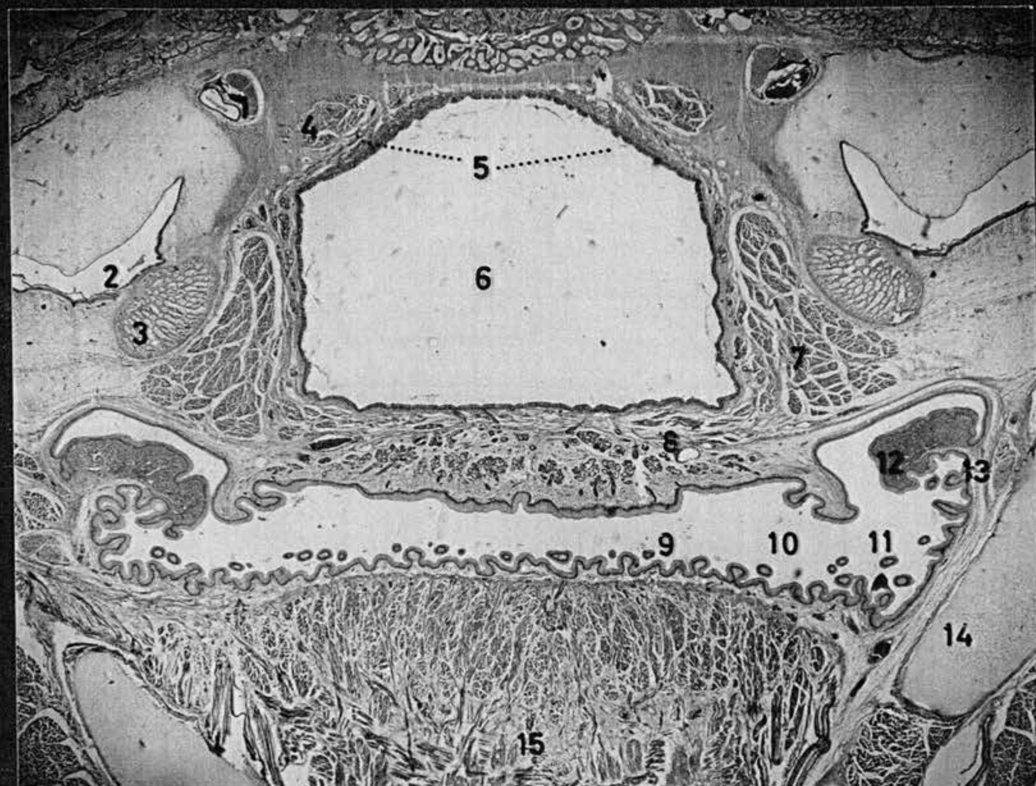
Embryos collected hour(s) after death.

Litter received from: **Edinburgh Surgery**

Remarks: **For pictures see that of litter mate D 72 (Fig.IV.160.)**

Fig. IV.161. Transverse section of pharynx, term fetus D 72 (183 mm. C.R.L.), (X 15).

1 Internal carotid artery and carotid nerve; 2 Tubotympanic recess; 3 Tympanic ring; 4 M. longus capitis; 5 Pharyngeal tonsil; 6 Nasopharynx; 7 M. pterygopharyngeus; 8 M. palatopharyngeus; 9 Oropharynx; 10 Plica semilunaris; 11 Tonsillar sinus; 12 Palatine tonsil; 13 Glossal branch of glossopharyngeal nerve; 14 Epihyoid; 15 Root of tongue.



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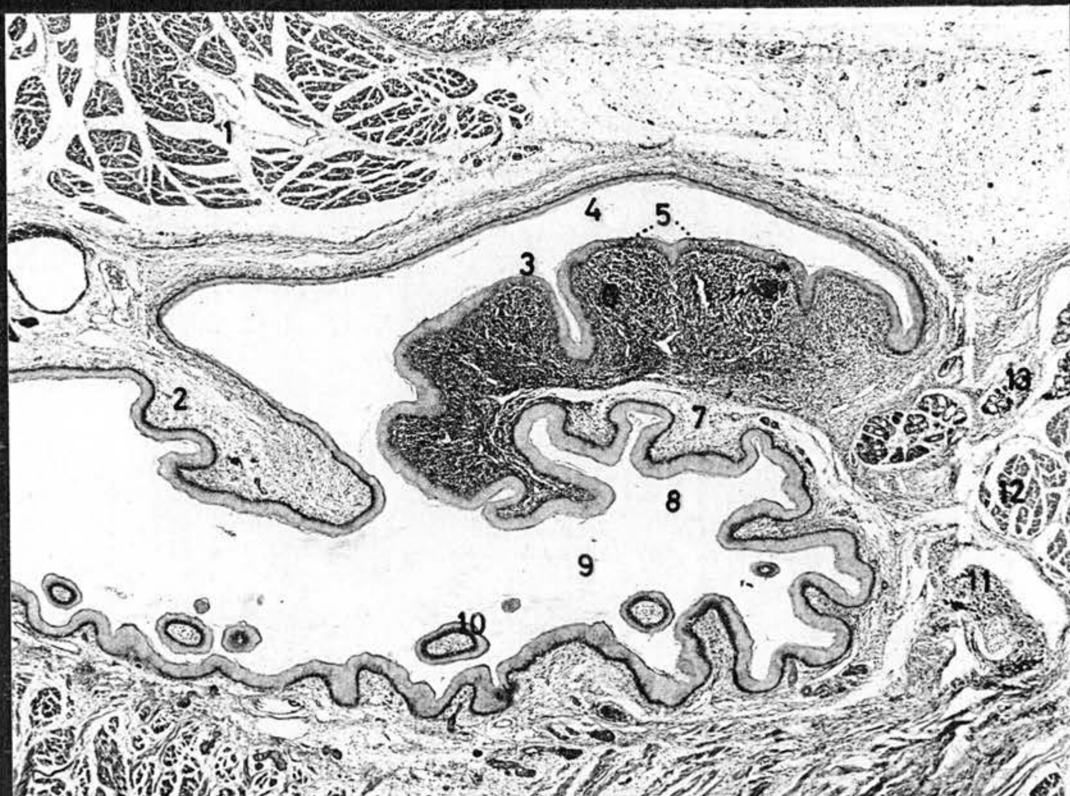
The Shape of the Pharynx

The pharynx has increased in size but has retained in general the morphology previously observed. Its longitudinal measurement is about 13 mm. and its width, taken in the naso-pharynx in front of the pharyngeal openings of the auditory tubes, is between 3 and 4 mm. The morphology of the pharynx at term is illustrated by three dissections and one histological preparation (Figs. IV.164.; IV.165.; IV.166.; IV.161.). The epiglottis was again found in a forward-bent position in all specimens (Fig. IV.165./7). The nasopharynx, when seen in cross section has taken on an even more rectangular shape when compared with that seen in the 120 mm. fetus (Fig. IV.161./6).

Minor but interesting changes have occurred in the palatine tonsils (12) which figure prominently on the lateral walls of the oropharynx (9). They lie well protected in the tonsillar sinuses (11) and are overhung medially by the Plicae semilunares (10), which are two connective tissue folds springing from the ventral surface of the soft palate, and on the medial sides of which small secondary folds have developed. The palatine tonsil is a shelf-like projection from the lateral wall of the tonsillar sinus about 1.5 mm. wide and between 3 and 4 mm. long. In the fully grown animal the tonsils are described as being four to six times as long as they are wide (Lentz and Lee, 1947). Thus it would appear that the tonsils at term are rather wide in relation to their length, and that from now on more longitudinal enlargement can be expected.

Fig. IV.162. Transverse section of tonsillar sinus, term fetus D 72 (183 mm. C.R.L.), (X 43).

1 M. pterygopharyngeus; 2 Plica semilunaris; 3 Temporary tonsillar crypt; 4 Supratonsillar fossa; 5 Areas of epithelial destruction; 6 Organisation of lymphoid tissue in thick lymphoid zone of tonsillar shelf; 7 Connective tissue zone (hilus) of tonsillar shelf; 8 Ventral longitudinal groove of tonsil; 9 Tonsillar sinus; 10 Filiform papilla of root of tongue in section; 11 Lingual ganglion on glossal branch of glossopharyngeal nerve; 12 M. styloglossus; 13 Part of sublingual salivary gland.



<u>Fig. IV.162</u>. Transverse section of tonsillar sinus, term fetus D 72 (183 mm. C.R.L.), (X 43).

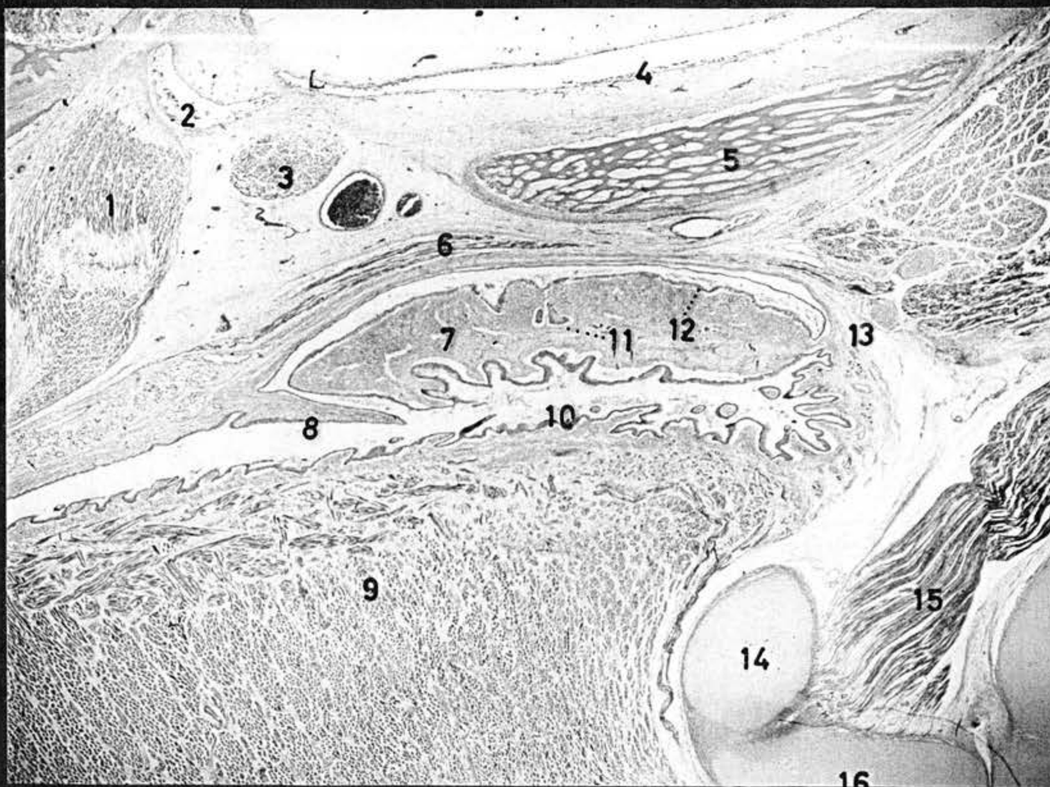
1 M. pterygopharyngeus; 2 Plica semilunaris; 3 Temporary tonsillar crypt; 4 Supratonsillar fossa; 5 Areas of epithelial destruction; 6 Organisation of lymphoid tissue in thick lymphoid zone of tonsillar shelf; 7 Connective tissue zone (hilus) of tonsillar shelf; 8 Ventral longitudinal groove of tonsil; 9 Tonsillar sinus; 10 Filiform papilla of root of tongue in section; 11 Lingual ganglion on glossal branch of glossopharyngeal nerve; 12 M. styloglossus; 13 Part of sublingual salivary gland.

The medial edge of the shelf is turned ventrad, producing a convex dorsal and a concave ventral surface when seen in cross section (Fig. IV.162.). The ventral concavity is thrown into short longitudinal folds and can be regarded as representing what was described in the adult by Lentz and Lee (1947) as the tonsil's "ventral longitudinal groove" (8). The convex dorsal surface of the tonsillar shelf has developed six to seven slit-like epithelial invaginations, distributed evenly over the surface of the organ (3). The slits are orientated longitudinally and are of varying lengths, the average lying between 0.5 and 1.0 mm. The invaginations have the appearance of tonsillar crypts. The German term for (tonsillar) crypts is "Bälge" (bellows), which would imply that crypts are slit-like and flat, rather than finger-like invaginations. On the basis of this it should be permissible to apply the term crypts to the structures under description, although the palatine tonsils of the adult are without them (Lentz and Lee, 1947; Nickel, Schummer, Seiferle, 1960). Obviously, these crypts must disappear again. Whether they are straightened out by the general growth of the organ, or they degenerate by epithelial apposition as was seen in numerous occasions in earlier embryos, or they are invaded by lymphocytes and thus masked, can only be determined by further investigation. At term however, these crypts exist and impart a cobble-stone appearance to the dorsal surface of the tonsil (Fig. IV.164./4). Three small epithelial folds extend from the dorsal surface of the tonsil to the laterodorsal wall of the tonsillar sinus, being directed somewhat caudally and lying parallel to each other. They are equally spaced and together with the palatoglossal arch at the anterior pole of the organ and the palatoepiglottic fold at the posterior pole they divide the sulcus between tonsil and dorsolateral wall of the sinus into four uniform cells, which are lined by epithelium and are easily distinguished from the tonsillar crypts. It seems that these folds disappear as the animal matures, because reference to them cannot be found in the current literature dealing with the anatomy of the region.

The interior of the tonsillar shelf can be divided into two layers (Fig. IV.162.). A thin ventral layer of connective tissue (7) and a much thicker dorsal layer of lymphoid tissue (6). The ventral layer acts as a hilus to the organ and extends its entire length, being continuous laterally with the connective tissue of the lateral pharyngeal wall. It is rich in lymph channels, blood vessels and nerves and from it they have easy access to the overlying lymphoid layer. The base of the human palatine tonsil is much broader and the layer of connective tissue at this base is referred to as Tonsillar capsule (Gray, 1958). The connective tissue layer described here is probably homologous to the tonsillar capsule of the human, but its position in the dog at term is such that "capsule" does not really describe it. Indeed, it requires imagination to understand how it can do so in the human. The lingual ganglion of the glossopharyngeal nerve (11)is situated lateral to where the connective tissue shelf blends with the

Fig. IV.163. Sagittal section of palatine tonsil, term fetus D 73 (180 mm. C.R.L.), (X 18).

1 M. tensor veli palatini; 2 Auditory tube; 3 M. levator veli palatini; 4 Tympanic cavity; 5 Tympanic ring; 6 M. pterygopharyngeus; 7 Palatine tonsil; 8 Plica semilunaris; 9 Root of tongue; 10 Tonsillar sinus; 11 Temporary tonsillar crypt; 12 Supratonsillar fossa; 13 Glossal branch of glossopharyngeal nerve; 14 Epihyoid; 15 M. hyopharyngeus; 16 Keratohyoid.



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1 M. tensor veli palatini; 2 Auditory tube; 3 M. levator veli palatini; 4 Tympanic cavity; 5 Tympanic ring; 6 M. pterygopharyngeus; 7 Palatine tonsil; 8 Plica semilunaris; 9 Root of tongue; 10 Tonsillar sinus; 11 Temporary tonsillar crypt; 12 Supratonsillar fossa; 13 Glossal branch of glossopharyngeal nerve; 14 Epihyoid; 15 M. hyopharyngeus; 16 Keratohyoid.

lateral pharyngeal wall. It is related to the epihyoid laterally. The heavy layer of lymphoid tissue is composed of a connective tissue reticulum in the meshes of which lie masses of young lymphocytes, obscuring most of the structures that are present. Round cell invasion of this area had first been observed in the previous stage (120 mm. C.R.L.). At term this area looks denser and in some places follicle-like organisation seems to be taking place, especially in the slightly protruding areas between the tonsillar crypts (6). What looks like a process of destruction goes on within the surface epithelium overlying such protruding areas (5). It appears as though the basal layer of the stratified squamous epithelium is losing its cytoplasm and being left with a layer of what one might call naked nuclei. Many of the latter have become elongated, giving the epithelia of these regions the appearance of a comb, whose body, or base, is formed by the undisturbed surface layer of epithelium, while the comb's teeth are represented by the naked long nuclei of the basal layer, the whole pointing, teeth first, towards the relatively undisturbed mass of lymphocytes. A large number of the flat surface-cells have been shed and are found in the tonsillar sinus (Fig. IV.163./10, 12). Only the occasional lymphocyte has migrated into the tonsillar epithelium, suggesting that the epithelium undergoes changes such as those described as preparation for the actual invasion. Lymphocytes have also appeared below the epithelium of the tonsillar sinus immediately above the tonsillar shelves. This is only the case however in the anterior portion of the sinus. The term

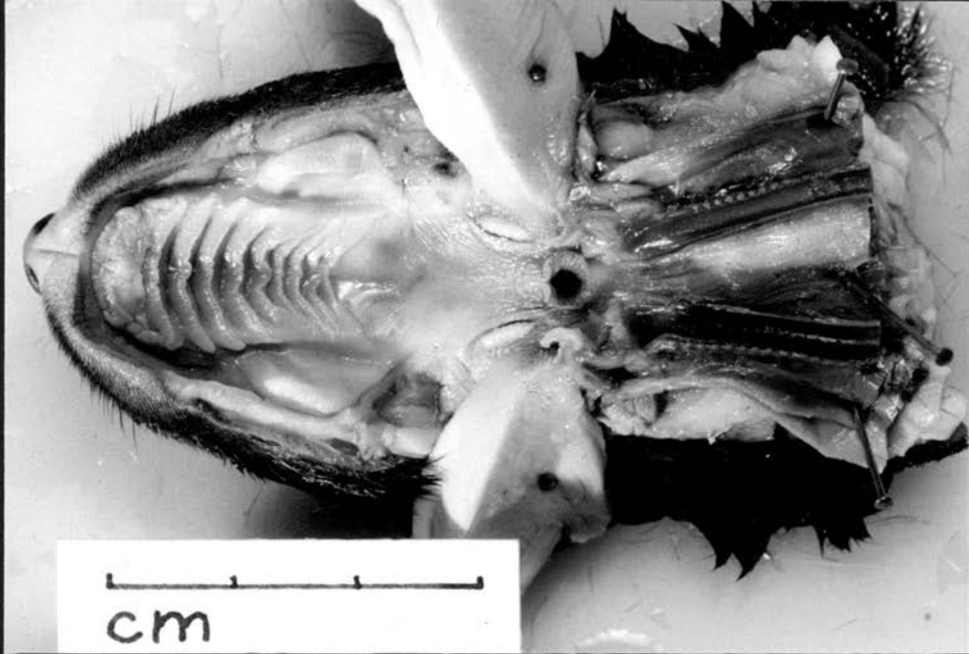
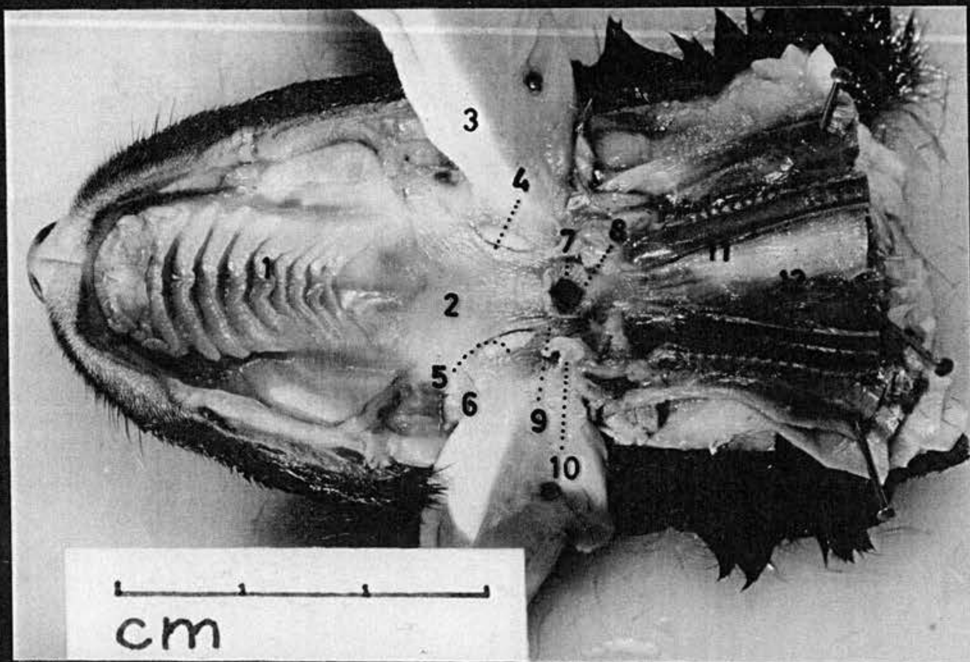


Fig. IV.164. Canine pharynx opened ventrally, term fetus DD 8 (178 mm. C.R.L.).

1 Hard palate; 2 Soft palate; 3 Tongue split in the median plane and turned laterally; 4 Palatine tonsil; 5 Plica semilunaris; 6 Glossopalatine arch; 7 Pharyngeal isthmus; 8 Palatopharyngeal fold; 9 Palatine arch; 10 Epiglottis; 11 Trachea; 12 Esophagus.



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Supratonsillar fossa, by which the space above the tonsil is commonly described, would therefore be inappropriate, because this cavity is not above the tonsil but is between two portions of the tonsil, so that Intratonsillar fossa would be more applicable here. In the middle and posterior portions of the elongated tonsillar sinus round cells are not encountered in the wall above the tonsil and, therefore, the name Supra-tonsillar fossa, as applied to the portion of the sinus above the tonsil, would be correct.

The Arcus veli palatini at term still retains some of the median indentation observed since the closure of the palate and has a rather swollen appearance (Fig. IV.164./9). It is continued caudally by two folds. The palatopharyngeal fold is the more dorsal of the two and extends along the lateral wall of the pharyngeal chamber, diminishing slowly in height until it fades out before reaching the midline (Fig. IV.166./12). It forms, together with the Arcus veli palatini, the anatomical boundary between the nasopharynx and the oropharynx, forming at the same time part of the pharyngeal isthmus (Foramen intrapharyngicum) through which the two chambers communicate (Figs. IV.164./7; IV.165./9). A second but less prominent fold lies ventral to the first and runs from the Arcus veli palatini to the vicinity of the epiglottis (palatoepiglottic fold) (Fig. IV.166./11). It is obscured by a covering of filiform papillae which extend backwards from the root of the tongue. This fold is described as being present in the adult

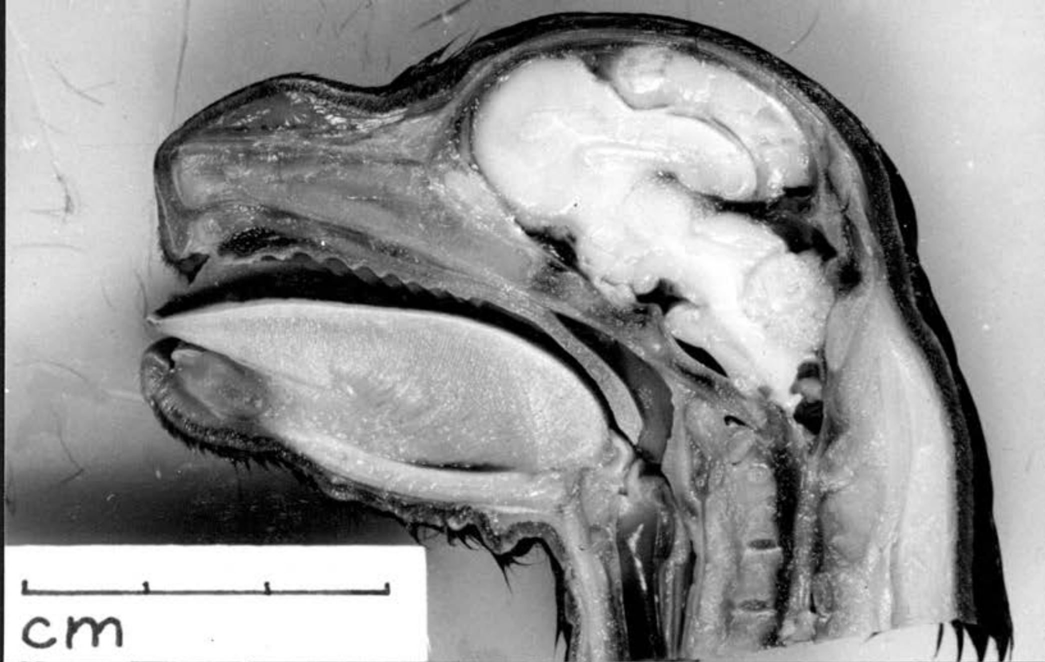
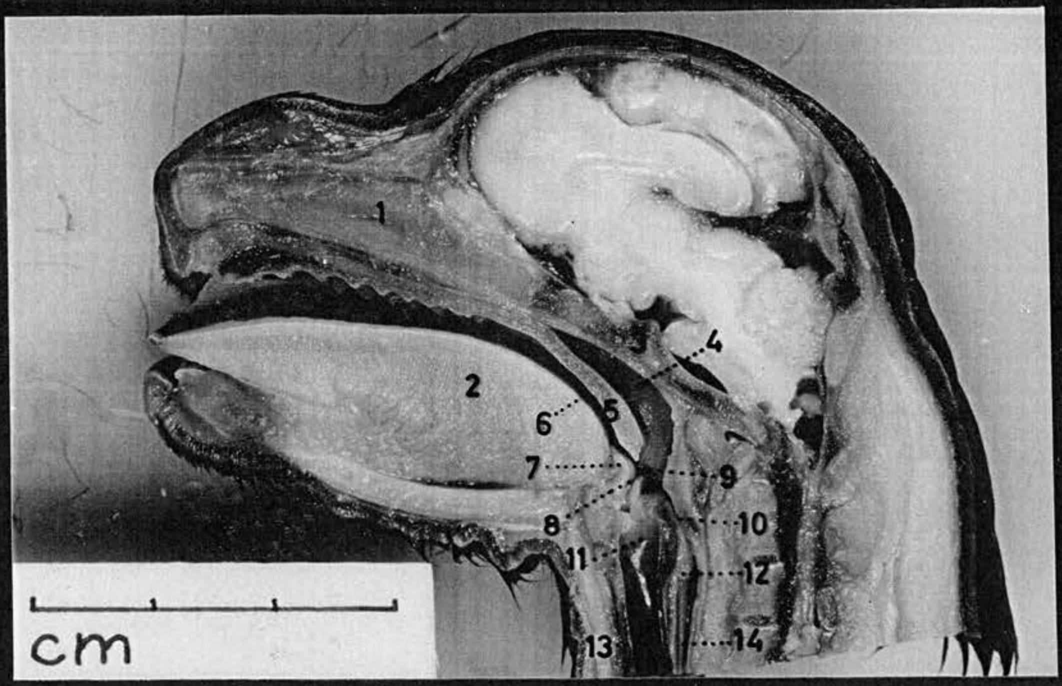


Fig. IV.165. Median section of head, term fetus DD 6 (185 mm. C.R.L.).

1 Nasal septum; 2 Tongue; 3 Base of skull; 4 Nasopharynx; 5 Soft palate; 6 Isthmus faucium; 7 Epiglottis; 8 Arytenoid cartilage; 9 Pharyngeal isthmus; 10 Vestibulum esophagi; 11 Larynx; 12 Limen pharyngoesophagicum; 13 Trachea; 14 Esophagus.



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1 Nasal septum; 2 Tongue; 3 Base of skull; 4 Nasopharynx; 5 Soft palate; 6 Isthmus faucium; 7 Epiglottis; 8 Arytenoid cartilage; 9 Pharyngeal isthmus; 10 Vestibulum esophagi; 11 Larynx; 12 Limen pharyngoesophagicum; 13 Trachea; 14 Esophagus.

by Bradley (1959) and Ellenberger and Baum (1891) but not by Nickel, Schummer and Seiferle (1960).

The Structure of the Pharyngeal Wall

The epithelial lining of the pharyngeal chamber consists, as it does in the adult animal, of two types of epithelium. The nasopharynx is lined with immature pseudostratified columnar epithelium, still retaining its previously observed basal cuboidal layer. The latter consists sometimes of a single, and sometimes of a double row of small polyhedral cells which contain very darkly staining compact nuclei. This is followed by a layer of pseudostratified columnar cells. Primordial goblet cells are present in this layer and appear like non-staining oval spaces with centrally placed round nuclei. The previously observed red-staining band overlying the latter layer has become transformed into a brush border reminiscent of those seen in adult preparations. The epithelial lining of the oropharynx is of the stratified squamous type but lacking, with the exception of the part covering the tongue, an underlying papillary body. The cells in the surface layer of this epithelium are very flat and many of them have been shed into the pharyngeal lumen. These are all nucleated. The line of transition from the nasopharyngeal (respiratory) epithelium to the oropharyngeal (upper digestive) does not coincide with the anatomical boundary between the two cavities, which is formed by the Arcus veli palatini in front and the Arcus palato-

<u>Fig. IV.166</u>. Canine pharynx opened dorsally, term fetus DD 5 (180 mm. C.R.L.).

1 Tongue; 2 Median sulcus; 3 Circumvallate papillae; 4 Glosso-palatine arch; 5 Soft palate split in the median plane and turned laterally; 6 Root of tongue; 7 Palatine tonsil; 8 Plica semi-lunaris; 9 Epiglottis; 10 Arytenoid cartilage; 11 Palato-epiglottic fold; 12 Palatopharyngeal fold; 13 Vestibulum esophagi; 14 Limen pharyngoesophagicum; 15 Esophagus.

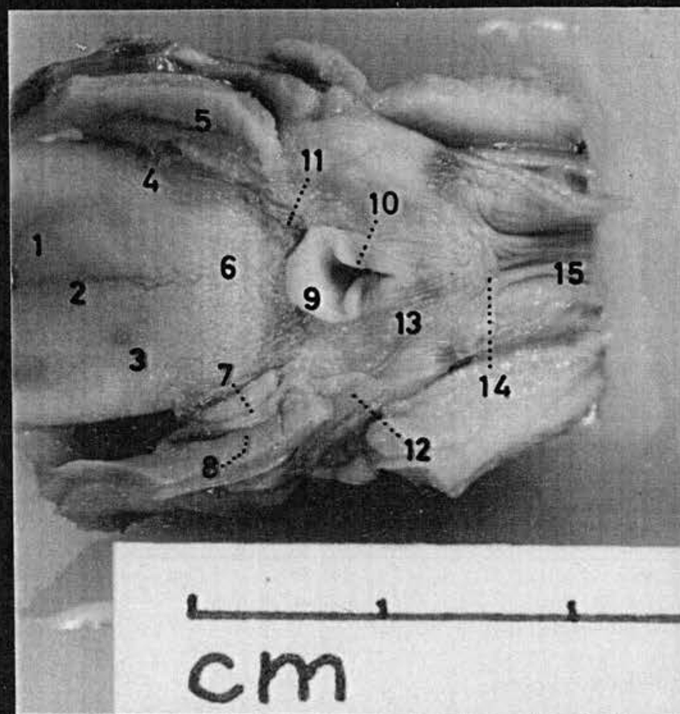

Fig. IV.166. Canine pharynx opened dorsally, term fetus DD 5 (180 mm. C.R.L.).

1 Tongue; 2 Median sulcus; 3 Circumvallate papillae; 4 Glossopalatine arch; 5 Soft palate split in the median plane and turned laterally; 6 Root of tongue; 7 Palatine tonsil; 8 Plica semilunaris; 9 Epiglottis; 10 Arytenoid cartilage; 11 Palatoepiglottic fold; 12 Palatopharyngeal fold; 13 Vestibulum esophagi; 14 Limen pharyngoesophagicum; 15 Esophagus.

pharyngei at either side encircling the Foramen intrapharyngicum, since narrow areas of nasopharynx are lined by stratified squamous epithelium. In the full-term fetus a band of nasopharyngeal lining about 1 mm. wide and situated immediately above the Foramen intrapharyngicum is of the stratified squamous type. Therefore, the entire surface of the palatopharyngeal folds and the free fold of the soft palate are lined by stratified squamous epithelium, which compares with the findings for the adult of Jaenicke (1908) who gives the line of transition as being between 3 and 6 mm. from the free edge of the soft palate, but not with Grundmann's (1894) who states that stratified squamous epithelium extends to about the middle of the third fourth of the length of the soft palate. The presence of cutaneous epithelium on the nasal surface of the soft palate suggests that this surface is exposed to friction in the mature animal; and indeed during nasal breathing, the soft palate is wedged below the epiglottis (Fig. II.2.). The epithelium of the nasopharyngeal roof and the roof of the Vestibulum esophagi is thrown into fine longitudinal folds (Fig. IV.166./13), which come abruptly to an end at the Limen pharyngoesophagicum, a ring-like epithelial swelling at the beginning of the esophagus (14). Posterior to this the esophageal epithelium forms more conspicuous longitudinal folds (15).

The filiform papillae over the root of the tongue are well developed at term (6) (Fig. IV.162./10). Light staining spindle-shaped cell aggregations reminiscent of taste buds have appeared in the epithelium on either side of the moat and along the flat surface of the circumvallate papillae.

The pharyngeal glands have continued to grow and form well circumscribed clusters a short distance below their respective epithelia. They are more numerous and better developed in the oropharynx than in the nasopharynx and this can be seen to best advantage in a section of the soft palate, where the lower cushion of glands is about twice as thick as that under the respiratory surface (Fig. IV.161.). The walls of the Vestibulum esophagi are very rich in glands. A rather peculiar distribution of glands is observed in the root of the tongue. Clusters of glands congregate around the circumvallate papillae and empty with their ducts into the moats of these structures. Two more glandular aggregations are in the dorsolateral edge of the tongue about opposite the anterior pair of vallate papillae. Their ducts converge radially and open into a slight depression on the tongue at that point.

Of the two tonsillar elements present in the pharyngeal wall at term the palatine tonsils have been described in the preceding section. The primordium of the other overlies the roof of the nasopharynx in the vicinity of the fornix (5). It consists of a cushion of free round cells stretching across the roof. Formerly, it will be recalled, two such aggregations were found, one on either side of the midline. More cells are present however at term, and the two original aggregations have united in the midline and have formed a single organ. The epithelium covering them has not been invaded by round cells, nor is it in any way disintegrating. Follicle formation has not begun in the primordium. Lymphoid aggregations other than the two mentioned are not present at this stage of development.

Regarding the musculature of the region only a few minor changes for the palatine group can be reported. It seems that the heavy glandular cushion that has developed in the lower stratum of the soft palate has displaced the muscular elements dorsally (Fig. IV.161.). Furthermore, the rapid proliferation of the glands and their invasion of the muscles has obliterated the fairly clear picture of the muscular pattern in the soft palate, that was evident in the previous few developmental stages. Careful study however reveals that the distribution of muscle has remained the same. The palatinus, sometimes referred to as M. uvulae (Bradley, 1959), does not reach far enough rostrally to be attached to the horizontal portion of the palatine bones. The aponeurosis of the tensor (palatine aponeurosis) however is now firmly attached to this bone. A narrow space has appeared between the fibres of the tensor and the ventral aspect of the pterygoid hamulus. This is probably the forerunner of the bursa present there in the more mature animal. Bony structures to provide origin for both tensor and levator have not yet formed.

The Relationships of the Pharynx

The pharyngeal hypophysis was observed in only one of the sectioned specimens. It consists of several epithelial islands, each surrounding a small elongated lumen. The principal epithelial make-up is of the stratified squamous type, but small areas of respiratory epithelium were encountered also. At its point of attachment to the pharyngeal roof the pharyngeal epithelium has differentiated into a stratified squamous type which lies like a small island embedded in the generally pseudostratified lining of this cavity ("Pharyngeal epithelial plate"; Kingsbury, 1942). The craniopharyngeal canal through the base of the skull can be followed in serial sections and appears as a connective tissue strand passing through the ossification centre below the Sella turcica. Several remnants of the previous stalk, thinner walled than those found below the base, were encountered along the canal. There was also a small epithelial island (parahypophysis) above the base of the skull, but it had no connection with the overlying Hypophysis cerebri.

Lateral to the pharynx hair follicles associated with clusters of sebaceous glands have appeared in the ventral wall of the external acoustic meatus just below the almost horizontally placed tympanic membrane. The medial extremity of the latter is separated from the nasopharynx by only the width of the osseous tympanic ring and the pterygopharyngeus muscle, in all a little more than 1 mm. Ventrally, the basihyoid is just beginning to ossify.

V. REGIONAL REVIEWS

The Pharyngeal Tube

The transformation of the early pharynx becomes clear only when considered as a part of the continuous growth process of the branchial region as a whole. From its earliest appearance the pharynx lies between the brain and the heart and without doubt is influenced by the changes in growth these two organs perform. Notable amongst these changes are (a) the tremendous growth of the neural tube and its subsequent folding, by which is produced an anterior and a posterior flexure in the underlying pharynx, (b) the precocious enlargement of the heart, which hinders the ventral expansion of the pharynx, and (c) the descent of the heart, by which process the aortic arches are withdrawn from the posterior branchial region, thereby bringing about, in the wake of this apparent ventral movement, a migration of the appendicular pharyngeal elements away from the pharyngeal tube. Furthermore, the extensive lateral expansion of the first and second visceral arches, and concurrent subsidence, in effect, of the third and fourth arches, together with the precocious development of the ventral respiratory elements, which, despite their being postbranchial in origin, invade the posterior pharynx from below against the more lateral caudally flowing structures, also produce their individual influences on the pharyngeal shape as a whole.

The embryonic pharynx is, without reservation, the most interesting region in the scope of morphological developmental studies, and consequently it is small wonder that a wealth of literature is available on this subject. The bulk of previous work is confined to the study of the branchial derivatives, few investigators (Sudler, 1902, and Kingsbury, 1915) having followed the transformations of the pharyngeal tube itself. Regrettably, they stop short once the fourth pouches have become isolated from the pharynx proper. A short phase in later pharyngeal development is covered by Peter (1924) in his comprehensive review on the development of the secondary palate. In the main, the observations of these workers, those of the first two being based on human embryos, and those of the last covering a number of mammalian species, but excluding the dog, correspond with those described herein for that animal, although naturally as morphogenesis progresses the adult anatomical differences become increasingly more marked.

In the dog the pharynx first appears as a dorsoventrally flattened semilunar pouch (Fig. IV.10.) which, in the 3 - 4 mm. embryo (represented by Fig. IV.7.), encroaches upon the space between the neural tube and the heart. The visceral arches have not as yet developed, therefore the lateral borders of the foregut evince no corresponding pouch formation. Thereafter, without increase in width, the foregut rapidly elongates until in the 5 - 6 mm. (!) stage (represented by Fig. IV.16.) its shape is reminiscent of that of a cat's tongue (Fig. IV.20.). At this time its lateral margins become indented by the expanding first and second visceral arches, between which the first two pharyngeal pouches are formed. The planes of these

pouches lie almost parallel to the long axis of the foregut (5, 9). Concurrently, the overlying neural tube begins its rapid enlargement and initial folding and, its anterior tip being held in place by the ectodermal envelope, causes the cephalic flexure to appear on the pharynx, which, due to the rupture of the oral membrane, seems to have elongated ventrally, rendering the flexure much more pronounced. At this time also the formation of the placenta necessitates the precocious development of the heart which presses against the pharynx from below, as if moulding it against the undersurface of the neural tube. The heart maintains this position and concomitant influence on the pharynx until such time as the head grows forwards, bringing about, in effect, the heart's descent.
More visceral arches have developed in the 4 - 6 mm. stage (represented by Fig. IV.24.), each embracing the pharynx laterally and below; between them the full complement of four pharyngeal pouches has developed, the last of which is as yet immature (Fig. IV.34.). Lung buds have made their appearance on the anterior surface of what will later be the esophagus. This pharyngeal concept, typical because of its dorsoventral compression and the presence of flexures and pouches, is retained throughout the following 6 - 8 and 7 - 9 mm. stages (represented by Figs. IV.39. and IV.51. respectively), the two flexures becoming even more pronounced with the appearance of Seessel's pocket on the cephalic, and the median pharyngeal recess on the cervical flexure. The latter outgrowths are temporary, and are the result of tension built up in the

anterior and posterior mesenchyme by the lengthening of the overlying hindbrain (Fig. IV.56.). Other than this, the pharyngeal pouches, with the exception of the first, begin to elongate ventrad, a development which gives the impression of their being suspended from the lateral border of the pharynx (Figs. IV.43.). The rapid lateral expansion of the first two visceral arches and the apparent subsidence of those remaining, becomes evident in the 8 - 10 mm. stage (represented by Fig. IV.64.). This process is evidenced on the pharyngeal tube when the first and second pouch area is drawn laterally, while that of the third and fourth pouches on the vertical portion of the pharynx, remains at the same distance from the midline as before (Fig. IV.69.). Meanwhile, the ventral migration of the aortic arches draws the third and fourth complexes away from the pharyngeal tube, while the respiratory elements, being separated now from the digestive, push upwards and invade the ventral pharyngeal region in the median plane. These same processes also cause the lateral edges of the posterior pharynx to be turned ventrally, thereby producing in the 9 - 12 mm. stage (represented by Fig. IV.80.) the inverted-trough appearance of this area which is retained in the Vestibulum esophagi until maturity (Fig. IV.81.). At the same stage, pouch II becomes reabsorbed into the lateral wall of the pharynx, pouch III breaks away, while pouch IV still retains its attachment by means of its pharyngobranchial duct.

It is, however, the 15 mm. stage of development which can be considered the most significant of all the developmental phases under study in this work, since it is then that the pharyngeal tube first becomes moulded into a configuration resembling that of the adult structure. Nevertheless, the tubotympanic recesses still remain relatively large in comparison with the chamber, and without a soft palate the pharynx looks as yet immature (Fig. IV.97.). The moulding influence of the heart has ceased some time previously, but it seems that the tongue has temporarily taken its place, since the hitherto flattened pharyngeal tube becomes moulded over it in such a manner that the pharynx appears as an inverted trough. At 22 mm., the pharyngeal chamber is invaded from in front by the caudal extremities of the lateral palatine processes. These take the form of two shelf-like structures projecting from the lateral pharyngeal walls immediately above similar, but much shorter, tonsillar swellings which have recently been pushed from their origin on the pharyngeal floor to the lateral wall of the chamber (Fig. IV.113.). Later, in the 29 mm. stage, the palatine shelves close, thereby dividing the pharyngeal chamber into an upper respiratory and a lower digestive channel (Fig. IV.122.). Thereafter, from the 40 mm. stage onwards, the pharyngeal chamber can be considered as having attained mature adult configuration.

The First and Second Pharyngeal Pouches

For a long time it was considered that the first pharyngeal pouch was transformed into the auditory tube and tympanic cavity, while the second pouch was thought to give rise to the palatine tonsil. These concepts, although not accurate, seemed at the time reasonable, and indeed today some parts of them are handed on to students in modern embryological texts (Zietzschmann and Kroelling, 1955; Patten, 1958). Doubts raised by several investigators in the late 19th century remained unnoticed; and especially when Hammar (1902, 1903), almost eighty years after the concept was first established, in a classical comparative study restated the original ideas, it was considered that there was little doubt the true fate of the first and second pouches had been explained. Frazer, in three analytical studies (1910, 1914 and 1922) was the first whose challenge seemed to change embryological thinking with regard to middle ear formation. He proposed and proved, for the human at any rate, that not only pouch I but also the dorsal portion of pouch II took part in the formation of the auditory tube and tympanic cavity and proposed the name "Tubotympanic recess" for this dorsolateral outgrowth of the embryonic pharynx. The present work is not designed to clarify this point farther, although what little has been learned of the first and second pouch transformations during this description of the entire pharyngeal region, tends to support Frazer's findings.

Similarly, the concept that pouch II is responsible for the formation of the palatine tonsil, or at least remains as the tonsillar sinus is difficult to dispel, despite the early efforts of Kingsbury (1915) and later those of Kingsbury and

Rogers (1927), Levin (1930) and Ramsay (1935) who by observation and deduction proved that such was not the case. Very recently, Hamilton and Hinsch (1957), depositing carbon particles in the second pouches of chick embryos, found that these pouches leave no derivatives. Embryologists today are generally agreed, although some current texts do not bear out the fact, that the dorsal portion of the second pouch contributes to the tubo-tympanic recess, while its ventral portion becomes resorbed by the pharyngeal wall due to mesenchymal pressure especially of the third visceral arch. The most that can be said is that the tonsillar sinus develops at the former site of the pouch, but probably anterior to it as the present study would suggest.

Only pouches I and II are present in the 5 - 6 mm. (!) stage (represented by Fig. IV.16.). Both are dorsoventrally flattened lateral extensions of the foregut, the first pouch being slightly larger than the second. Their planes lie more or less parallel to the long axis of the foregut, and both have formed branchial membranes along their sharp lateral margins (Fig. IV.20.). Due to rupture of the oral membrane and subsequent ventral elongation of the foregut, and due to the fact that the visceral arches arrange themselves more transversely than before, the first two pouches rotate clockwise through an angle of about 60 degrees, until in the 4 - 6 mm. stage (represented by Fig. IV.24.) their planes stand at right angles to the long axis of the flexed pharyngeal tube (Fig. IV. 34.). The pouches can then be described as being flattened from before back, both at this stage having developed dorsal and ventral wings, of which the latter, being the more medial, impart to the pouches, according to Kingsbury (1915) a "thyropetal" inclination. Subsequent growth in the 6 - 8 mm. stage (represented by Fig. IV.39.) with concurrent shiftings and enlargement of the first three visceral arches, push the first pouch upwards, giving it the appearance of possessing a large dorsal and a small ventral wing (Fig. IV.43.). In contrast, although by the same influences, pouch II is forced ventrally, whereupon its ventral wing becomes quite pendulous, while that on the dorsal aspect diminishes. Both pouches maintain long and narrow contact with the ectoderm of their respective branchial clefts, part of the second having ruptured to form a short temporary gill slit (9). In the following 7 - 9 mm. stage (represented by Fig. IV.51.) there is a gradual anticlockwise rotation of the plane of pouch I until it becomes arranged almost parallel to the horizontal portion of the pharynx, simultaneously losing contact with first cleft ectoderm. Pouch II flattens further and elongates in a dorso-ventral direction, its branchial membrane still partially ruptured (Fig. IV.55.). Increased lateral expansion of the horizontal portion of the pharynx in the 8 - 10 mm. stage (represented by Fig. IV.64.) completes the transformation of the first pouch into a dorsoventrally compressed extension of the flat pharyngeal tube (Fig. IV.69.). It is continuous caudally with the dorsal (anterior) portion of pouch II, the two combining to form the tubotympanic recess. Pouch I has just broken contact with the ectoderm, and the second pouch ectodermal contact is drawn out into a cellular strand ending at the cervical sinus (5). The rapid expansion of the third visceral arch that now follows is making itself felt in the 15 mm. stage by beginning to press, from behind, on to the recess, thereby gradually diminishing the proximal portion of the recess and giving it a dorsocaudal direction (Fig. IV.97.). Undercut now by the caudally extended lateral edge of the oral cavity (5), the recess appears almost as if protruding from the pharyngeal roof. The influences of the third visceral arch continue during the following developmental stages and combine with the effects of the expansion of the otic cyst above and the growth of the malleus below the distal portion of the recess in shaping a tubotympanic outgrowth as illustrated in Fig. IV.127. During the 40 mm. stage the tympanic ring forms below the distal portion of the recess by intramembranous ossification. It can be followed until full term, by which time its interior harbours the tympanic membrane (Fig. IV.161.).

The Third Pharyngeal Pouch

The transformations of the third pharyngeal pouch in the dog are typically mammalian and conform in general with those described by Godwin (1937 a), and for other species by Zuckerkandl (1903), Badertscher (1915), de Winiwarter (1933) and Weller (1933). The pouch appears as an anteroposteriorly flattened outgrowth from the pharyngeal tube in the 4 - 6 mm.

stage (represented by Fig. IV.24.) a little anterior to the cervical flexure, and forms a long contact with overlying cleft III ectoderm, as well as a ventral wing which is capped by a true ventral diverticulum (Fig. IV.34.). Growth processes in the 6 - 8 mm. stage (represented by Fig. IV.39.) move it ventrally and posteriorly in relation to the cervical flexure, and it can be described as having become pendulous, while its contact with ectoderm is diminishing (Fig. IV.43.). A circumscribed area of taller cells on its anterior surface marks the anlage of parathyroid III(Fig. IV.48./4). The latter increases in size and becomes raised in the following 7 - 9 mm. stage (represented by Fig. IV.51.), at which time also the anlage of thymus III appears as a rounded medial protuberance (Fig. IV.57.). The complex is then being drawn from the pharyngeal tube in the 8 - 10 mm. stage (represented by Fig. IV.64.) and a branchiopharyngeal duct remains the only connection (Fig. IV.69./10). Parathyroid III is raised well above the complex, while thymus III (9) has continued to elongate anteromedially and appears now as a long finger-like outgrowth. Posteriorly, the complex has made contact with the cervical vesicle (8). In the 9 - 12 mm. stage (represented by Fig. IV.80.) the complex breaks away and is independent of the pharynx from now on (Figs. IV.84.; IV.86.). By being caught in the ventral flow of material, the complex is drawn out and finally separates into thymus and parathyroid III. The former is found at the entrance into the thorax (Fig. IV.99./26), and the latter is applied laterally to the thyroid gland at the commencement of the trachea in the 15 mm. embryo (Fig. IV.105./20).

The Fourth Pharyngeal Pouch

The fourth pharyngeal pouch, due to its relation to the ultimobranchial body is without doubt the most extensively studied portion of the embryonic pharynx, while the possible presence of a thymus IV has added appreciably to the bulk of the literature on this subject. A study of any of the major investigations such as those carried out by Badertscher (1918), Rogers (1927), Watzka (1933), Godwin (1937 b, 1940), Norris (1937), van Dyke (1945) and Klapper (1946) will lead to comprehensive lists of references which need not therefore be repeated here. The fourth pouch was only studied in this work until such time as it breaks away from the pharyngeal tube. No significant developmental differences were encountered between the dog and the other species examined by previous workers.

The canine fourth pouch is first seen in the 4 - 6 mm. stage (represented by Fig. IV.24.) as a small round outgrowth at the level of the cervical flexure (Fig. IV.30.). Its interior is in communication with the pharyngeal lumen in common with the third pouch (Fig. IV.29.). Ectodermal contact is not yet established. A ventral saccular outgrowth appears on the pouch in the 6 - 8 mm. stage (represented by Fig. IV.39.) and grows towards the pericardium (Fig. IV.43./13).

This is the anlage of the ultimobranchial body. The pouch itself has developed short dorsal and ventral wings, the former making brief contact with overlying ectoderm. The complex becomes pendulous and drawn out in the following 7 - 9 mm. stage (represented by Fig. IV.51.). Its bulk is comprised of the ultimobranchial body (Fig. IV.57./6), while the remnant of the previous pouch can still be made out by a small thyropetally orientated ventral diverticulum (5). The dorsal diverticulum has been carried down and away from the pharyngeal attachment and is but a small tubercle on the dorsal aspect. The complex becomes thicker walled and club-shaped in the 8 - 10 mm. stage (represented by Fig. IV.64.), dorsal and ventral diverticula having been lost. Parathyroid IV has appeared on the anterolateral aspect of the complex (Fig. IV.72./7, 8). Further growth away from the pharynx draws out a pharyngobranchial duct IV in the 9 - 12 mm. stage (represented by Fig. IV.80.), parathyroid IV being now raised above the level of the complex (Fig. IV.81.). At 15 mm., connection with the pharynx is broken and the complex is found embedded within the thyroid (Fig. IV.104./15, 16).

The Thyroid

Interest in the development of the mammalian thyroid involves one immediately in the problem, as yet unsolved, of whether the gland grows from only one primordium or is the product of three separate anlagen. Born (1883) was one of the first to postulate the existence of a median and two lateral thyroid primordia, the former comprising a ventral outgrowth from the pharyngeal floor at the level of the first pouches, and the latter being considered as secondary pouches developing from the posterior aspects of the fourth pouches. Soon afterwards, the lateral anlagen of the thyroid became the subject of extensive comparative studies, and the names "Ultimobranchial, Postbranchial or Telobranchial bodies" (the first is now in general use) replaced Born's initial term "Lateral thyroids". It may be that the noncommittal nature of the new term has guaranteed its perpetuation. It is well known that the ultimobranchial bodies after their detachment from the pharynx are incorporated within the laterally expanding median thyroid primordium. Their fate after inclusion is still unknown however. Two schools of thought on this aspect are in existence. The first, supported by Stewart (1918) studying the cat, and more recently by Kingsbury (1939) who worked on human embryos, and by Klapper (1946) who worked on the guinea pig, is that the ultimobranchial bodies degenerate within the thyroid tissue. The second, and the more favoured, line of thought was first proposed by Rogers (1927) who, working on white rats, thought that ultimobranchial tissue proliferated into thyroid tissue by a process of "homeogenetic induction". Such views were shared by Godwin (1937 b) who worked on the dog, Politzer (1936) and Norris (1937) both studying thyroid development in the human embryo, although the latter favoured a return to the initial view of a "Lateral thyroid". Others who studied the early development of the median thyroid anlage were Kallius (1903) and Waterman and Gorbman (1956) on the rabbit, Rabl (1923) on the guinea pig, Moody (1910) on the pig, Weller (1933), Sagalitzer (1941), Politzer and Stockinger (1954), Politzer (1955) and Orts-Llorca and Genis Galves (1958) on the human, Godwin (1936) on the dog, and Sugiyama (1941) on the rat and mouse.

The development of the canine thyroid begins with the appearance of a slightly concave thyroid plate composed of tall laterally compressed columnar cells in the 5 - 6 mm. (!), 18 - 20 somite embryo (represented by Fig. IV.16.). The plate is roughly circular in outline and has a diameter of about 130 microns (Fig. IV.21./10). After widening to become an oval of over 200 microns greatest diameter in the 4 - 6 mm. embryo (represented by Fig. IV.24.), the plate throws out several finger-like projections from its mesenchymal surface, which come in contact with the underlying aortic sac (Fig. IV.31.). These finger-like epithelial extensions elongate from the plate in a caudoventral direction in the 6 - 8 mm. embryo (represented by Fig. IV.39.), their tips keeping close to the descending aortic sac, while the original plate starts to contract and decrease in size. Several stages of this process are illustrated in Figs. IV.44.; IV.46.; IV.49. The continuing downward growth of the thyroid draws the pharyngeal epithelium ventrally to form a transitory

Foramen cecum and a small lingual duct, while the upper portion of the primordium thins out into a short thyroglossal stalk (Fig. IV.46./5, 4). In the 7 - 9 mm. embryo (represented by Fig. IV.51.) the primordium breaks away from the pharyngeal floor by the rupture of the thyroglossal stalk where it was continuous with pharyngeal epithelium, leaving no trace on the floor except for a shallow depression which in succeeding stages however returns to the general level of the floor (Fig. IV.56.). In one specimen of this group the break occurred farther ventrally along the thyroglossal stalk leaving, in association with the pharynx, a small amount of thyroid tissue, which doubtless would suffer a similar fate to the "lingual accessory thyroid", found by Tehver (1940) to be present in a cat. It is of interest to note that Tehver observed the accessory thyroid tissue to be anterior to the level of the circumvallate papillae. The persisting Foramen cecum in Man is found posterior to this level. This could well indicate that in carnivores the location of the median thyroid primordium with relation to the development of the tongue is more anterior than that in the human.

After detachment from the pharyngeal floor the primordium is found as a cluster of epithelial cords and plates enveloping the anterior aspect of the aortic sac and it has now reached a width of some 500 microns (Fig. IV.54./21). Norris (1918), primarily working on human embryos, stated that he found small vacuoles in the thyroid primordium of the dog - one of several species he examined for its comparative value - after it had lost contact with the pharyngeal floor. Such lumina were not observed in the course of this study. Godwin (1936) also could not find them. Further thyroid development has received little special attention in this study. However, the primordium can be followed in 8 - 10 mm. (Fig. IV.75./12) and 9 - 12 mm. embryos (Fig. IV.90./15) as it descends with the aortic sac in front of the developing larynx. In the latter group of embryos, the distance between the laterally expanding thyroid and the ultimobranchial bodies was 120 microns, only a step away from their integration. The primordium has reached its adult position in front of the primitive trachea in the 15 mm. embryo. It is now bi-lobed, the lobes being connected by a fairly thick glandular isthmus. The ultimobranchial body is contained within the young gland, while parathyroid III and IV are associated with it laterally (Fig. IV.105./19, 20). At the same stage and regularly throughout the remaining stages of this study, accessory thyroid tissue was found medially placed in the vicinity of the basihyoid, mostly at its ventrocaudal aspect. This suggests that small portions of the short thyroglossal stalk are caught up in the developing basihyoid at the 15 mm. stage, although the gland in its descent does not draw out a long thyroglossal duct in the dog as it does in the human. Gilmore, Venzke and Foust (1940) stated that they observed follicle formation in the "25 day old canine fetus". The "25 day old canine fetus" is, in fact, an embryo barely reaching a length of 10 - 12 mm.: thyroid follicle formation was found much later than 10 - 12 mm. in this study.

Rathke's Pocket and the Pharyngeal Hypophysis

Rathke's pocket, although in the strictest sense not a part of the embryonic pharynx, has been included in the general description of this study until such time as it is cut off by the developing basilar plate. Its position in the young embryo immediately anterior to the oral membrane renders it a welcome landmark by which the anterior limits of the pharynx may be recognised throughout most of intra-uterine life. It appears in the 4 - 6 mm. embryo (represented by Fig. IV.24.) as a shallow, crescent-shaped evagination, arranged in a transverse manner immediately anterior to the dorsal remnant of the recently ruptured oral membrane (Figs. IV.33./1; IV.30./1). In the 6 - 8 mm. embryo (represented by Fig. IV.39.), the pocket has pushed upwards somewhat further and has assumed a short quadrilateral outline (Fig. IV.43./1). Further growth transforms it in the 7 - 9 mm. embryo (represented by Fig. IV. 51.) into a somewhat dorsoventrally flattened pouch situated just below the cephalic flexure of the pharynx (Fig. IV.55./1). In front it is closely applied to the diencephalon from which an as yet shallow evagination protrudes above the dorsal extremity of the pouch, indicating the future site of the infundibulum (Fig. IV.56./3, 11). During the following 8 - 10 mm. stage (represented by Fig. IV.64.) the pouch continues to elongate in a dorsal direction, eventually appearing as a tube of some 100 microns in diameter and somewhat quadrilateral in cross section, from the dorsal extremity of which have grown two lateral wings (Fig.IV.69./1).

In further development the tubular portion thins until at the 9 - 12 mm. stage (represented by Fig. IV.80.) its diameter has diminished to about 50 microns, while the wings have expanded more and become dorsoventrally flattened (Figs. IV.83./1; IV.88./3). In the 15 mm. embryo development of the cartilaginous basilar plate has severed the stalk, isolating the bulk of the pouch above the basilar cartilage, and has thus initiated its second phase of development which is spent quite independently of the pharynx. However, a short portion of the connecting stalk remains associated with the pharyngeal roof as the Pharyngeal Hypophysis (Fig. IV.99./3, 5).

According to Boyd (1956), who has given the most recent account of the developmental anatomy of the pharyngeal hypophysis in Man, Froriep in 1882 and Killian in 1888 were the first to have mentioned, in passing, the persistence of the aforementioned ectodermal vestige. It was Erdheim (1904) however, who recognised it as a constant feature in the human embryo and named it "Rachendachhypophyse". Kingsbury and Roemer (1940), describing the morphogenesis of the canine hypophysis, referred to Tourneux as being the first to have described the structure in the dog in 1912, although Haberfeld in 1909 and Pende in 1910, as quoted by Boyd, could not detect it in this species. It would seem from the work of Kingsbury (1942) that Man and Dog are the only species in which a pharyngeal hypophysis occurs with constancy.

In pursuance of the present work a pharyngeal hypophysis was observed in all specimens until term. It first appeared in the 15 mm. embryo as an epithelial process, comprising of an upper tubular and lower globular portion, and retaining its connection with the pharyngeal roof (Fig. IV.101./3). Thence the process became irregularly tubular until the first signs of what appeared to be cell degeneration were noted in the 40 mm. embryo. Thereafter, the original epithelial tube gradually broke up, appearing at term as a string of small cystic islands, their cells having differentiated to, in the main, stratified squamous epithelium, although small areas of ciliated columnar could also be observed on occasions. The craniopharyngeal canal at term was represented by a connective tissue strand which traversed the anterior ossification centre of the basilar plate. Epithelial islands along the course of the canal, similar to those in the pharyngeal hypophysis, but with thinner walls, were noted only in a few specimens. A para-hypophysis was present in one.

The Pharyngeal and Palatine Muscles

The appropriate literature has many references to the anatomy and morphology of both pharyngeal and palatine muscles found in the adult mammal. Their developmental course however, as far as the writer can ascertain, has been rather neglected. Indeed, it would appear that Edgeworth, already noted for his treatise on the cranial muscles of vertebrates (1935), stands alone in the field of pharyngeal and palatine morphogenesis with his observations on mammals, especially Sus (1916), and Manis and Tatusia (1923). The latter study, whilst containing a wealth of observations on the cranial muscles as a whole, unfortunately falls short, in that little detailed reference is made to those groups of muscles of interest here. Similarly, only sparse reference is made by Frazer (1953) as to the branchial origin of the muscles of the soft palate in the human. Innervation studies in the present work however, confirm that the findings of the previous author are also applicable to the dog.

<u>8 - 10 mm. stage (represented by Fig. IV.64.)</u>. The primordium of the pharyngeal musculature is represented by a cord-like condensation of mesenchyme which runs the dorsal length of the sharp lateral border of the posterior pharynx (Fig. IV.72./14).

<u>9 - 12 mm. stage (represented by Fig. IV.80.)</u>. The primordium is now denser and, underlying the lateral edge of the pharynx, is associated anteriorly with the glossal branch of the glossopharyngeal nerve (Fig. IV.86./12, 9). Thence, the cell cord gains the dorsal pharyngeal aspect by traversing a temporary groove between the former sites of the second and third pharyngeal pouches (Fig. IV.87./6, 7, 8). Thereafter, it passes caudally, dorsal to the lateral pharyngeal edge, being associated with the mesenchymal density which surrounds the anterior laryngeal nerve (Fig. IV.86./16), until it becomes confluent with the primordium of the esophageal muscle (20).

15 mm. stage (represented by Fig. IV.94.). The cell cord has flattened and with that of the other side forms a muscular cap, which overlies the cervical flexure (Fig. IV.103.). It is notched anteriorly for the Fornix pharyngis which later protrudes dorsally at this point. The cap finds attachment on adjacent skeletal structures by means of three muscular extensions on either side (1, 3, 5). Such are recognisable as the anlagen of the stylopharyngeus, hyopharyngeus and thyropharyngeus muscles. The fibres within the muscle cap are arranged in horizontal fashion in the anterior portion, while posteriorly they assume an almost vertical course, necessitating a crossing of fibres in the vicinity of the cervical flexure. Beyond this, the cap presents no more than a pre-muscle mass, too homogeneous to permit the differentiation of individual muscles. In contrast, the small primordium of the Tensor veli palatini is now visible in the root of the lateral palatine process, its fibres running transversely towards the free border. The small nerve associated with the primordium can be traced to its origin from the fifth cranial nerve.

22 mm. stage. Superior to the stylopharyngeal extension of the muscular cap the pterygopharyngeus muscle anlage has appeared, growing anteriorly towards the root of the palatine process. A pre-muscle mass also noted between the cricoid lamina and the esophagus suggests the laying down of the cricoesophageal muscle.

29 mm. stage. The pterygopharyngeal extension has elongated further, but is still short of the recently formed

pterygoid hamulus. The palatopharyngeal primordium has appeared ventral to the above muscle, while the tensor, lying lateral to the pterygoid bone, has extended several fibres medially around the hamulus. The anlage of the Levator veli palatini can be noted lying transversely within the lateral palatine process. The cricopharyngeus can also be recognised as a small posterior section of the large thyropharyngeus primordium.

40 mm. stage. Separate muscles can now be distinguished within the pharyngeal muscular cap, although their arrangement is as yet indefinite (Fig. IV.132.). The palatinus muscle is present as a faint primordium, while the palatopharyngeus has pushed through to the soft palate inside the recently established palatopharyngeal folds.

50 mm. stage. The pterygopharyngeus has made contact with the pterygoid hamulus (Fig. IV.140./6, 4), and the levator fanned out on approaching the midline. The tensor, having wound medially around the hamulus, is now continued by a non-muscular sheet (palatine aponeurosis). The crico-pharyngeus muscle embraces the esophagus from below, actually becoming contiguous with the internal layer of the esophageal muscle.

70 mm. stage. The Raphe pharyngis is developing from before backwards, it being noted first in the superficial and then in the deeper layers of the pharyngeal muscle coat. The muscular arrangement is illustrated in Fig. IV.148.

90 mm. stage. Immaturity of the palatine glands in this stage enables the muscular arrangement in the soft palate to be observed to best advantage (Figs. IV.154.; IV.155.). The levator passes between the pterygopharyngeus and the palato-pharyngeus muscles, the latter two being wrongly shown by Miller (1952) to form one belly, such finding attachment on the hamulus. However, the description of the adult muscular arrangement in the soft palate by Ellenberger and Baum (1891) and Dyce (1957) is corroborated by this work. The posterior fibres of the stylopharyngeus can be followed under cover of the posterior constrictors until lost in the internal muscular coat of the esophagus. They do not insert in the pharyngeal raphe.

Vessels and Nerves

As far as the writer can ascertain the development of the vessels and nerves supplying the pharynx has not been studied in the dog. Although continued reference has been made to them in the sections on pharyngeal relationships, no claim can be made to have given a comprehensive account of the subject. Nonetheless, the information collected is useful, if only for the benefit of helping to fix any given stage of development.

The first two aortic arches are present and well formed in the 5 - 6 mm. (!) stage (represented by Fig. IV.16.), while the third arch is just beginning to develop. The latter is complete in the succeeding 4 - 6 mm. stage (represented by Fig. IV.24.). At 6 - 8 mm. (represented by Fig. IV.39.) the first aortic arch is already disappearing, while the fourth and sixth arches have developed, so that four aortic arches embrace the pharyngeal tube at this time. The nerves associated with the embryonic pharynx are those contained within the mesenchymal visceral arches. They are, from before backwards: the mandibular, facial, glossopharyngeal and anterior laryngeal, all of them only short stumps, barely reaching the level of the pharynx. The tympanic nerve has also appeared as a short stump on the petrosal ganglion. A model (Figs. IV.54.; IV.55.) illustrates the vessels and nerves in the 7 - 9 mm. stage (represented by Fig. IV.51.). The second aortic arch has now disappeared and its ventral end remains below the pharyngeal tube as the source of the external carotid artery. The section of dorsal aorta between the third and fourth aortic arches (Ductus caroticus) is beginning to diminish in diameter in preparation for the formation of the internal carotid arteries from the dorsal portion of third aortic arch and the dorsal aorta. Of the nerves at this stage only the facial and the glossopharyngeal have developed branches, the former giving off the Chorda tympani, which does not as yet make contact with the mandibular nerve, and the latter its glossal branch. The posterior laryngeal nerve (recurrent) is present in anlage, passing medially behind the sixth aortic arch. A graphic reconstruction (Fig. IV.86.) elucidates schematically the courses and branchings of the nerves encountered in the 9 - 12 mm.

stage. The Chorda tympani has joined the mandibular, the greater superficial petrosal nerve has started to grow from the geniculate ganglion and the recurrent nerve has been drawn ventrad by the receding pulmonary arch. The third, fourth and sixth aortic arches are present. The fourth and sixth are appreciably larger on the left side. The section of dorsal aorta between the third and fourth arches is but a cell cord and at the point of rupture.

At 15 mm. the remaining aortic arches have been withdrawn from the pharyngeal region and can now be located at the caudal end of the recently formed neck of the embryo; this results in the drawing out of the common carotid artery, which divides into external and internal carotids inside the groove formed between the nodose and the anterior cervical ganglia. The external carotid artery runs forwards along the lateral face of the pharynx at about the level of the tonsillar swellings. It gives off in rapid succession (a) a small pharyngeal branch which enters the pre-muscle mass of the pharyngeal musculature, (b) the occipital artery, accompanying the internal carotid in its dorsal course, and (c) a fairly large laryngeal branch which enters the larynx through the thyroid fissure anterior to the anterior laryngeal nerve. Before the external carotid artery reaches the level of Reichert's cartilage it sends the lingual artery medially towards the tongue, then passes outwards between the cartilage and the digastricus muscle, thus losing its relationship with the pharynx. The internal carotid artery continues dorsally inside the groove between the nodose and the anterior cervical ganglia, curves anteriorly at the level of the pharyngeal roof and, accompanied by the carotid nerve, forms close dorsal relations with the pharynx until it disappears through the carotid foramina of the basilar plate. The otic ganglion has appeared on the mandibular nerve at the level of the tubotympanic recess. The three end branches of this nerve (lingual, mandibular alveolar and mylohyoid) are well formed (Fig. IV.105./4, 5, 6). The facial nerve, except for its Chorda tympani branch, has been carried laterad by the rapid expansion of the first and second visceral arches and is no longer related to the pharynx. The glossal branch of the glossopharyngeal nerve has formed a small spherical lingual ganglion below the root of the tongue (Fig. IV.108./14) and distributes itself below the epithelium of that area. The pharyngeal branch receives a twig from the nodose ganglion, and branches from this union (pharyngeal plexus) go to the muscular cap. Some fibres innervate the muscle, while others penetrate it to gain the subepithelial layers of the posterior pharynx. By the 29 mm. stage the adult arterial and nervous pattern as related to the pharynx has been attained.

The Tonsillar Elements of the Pharynx

Two of the components making up the pharyngeal tonsillar ring of the adult are present at birth. They are the paired palatine tonsils in the Isthmus faucium and the unpaired pharyngeal tonsil lying in the roof of the nasopharynx. As has been mentioned in the chapter on the first and second pharyngeal pouches, the palatine tonsils cannot be regarded as being of second pouch origin, although they develop very close to the previous site of the pouch. Furthermore, the incorrect assumption that the tonsillar sinus could be considered a perpetuation of the second pouch cavity and so act as necessary primordium to the tonsil itself, which, therefore, was even thought by proponents of branchiomerism to be a homologue of thymus II, led earlier investigators to believe that the median pharyngeal recess or pharyngeal bursa, as its continuation is known in the human, is an essential anlage for the development of the pharyngeal tonsil (Schwabach, 1887). A study of animals such as the pig, cat and ox where no pharyngeal bursa appears at once indicates that the pharyngeal tonsil is quite independent of a bursa (Kingsbury, 1932). Indeed, in the present study the pharyngeal tonsil appears a long time after the temporary pharyngeal recess has disappeared. Therefore, no portion of the embryonic pouch-bearing pharynx can be considered as being predetermined to give rise to any component of the pharyngeal tonsillar ring. Comprehensive descriptions of the development of the palatine tonsils were given by Kingsbury and Rogers (1927), Levin (1930) and Ramsay (1935), and of the pharyngeal tonsil by Kingsbury (1932) and Snook (1934).

In the dog the palatine tonsils make their appearance in the 15 mm. stage as small dorsal protuberances on the pharyngeal floor on either side of the root of the tongue (Figs. IV.106.; IV.108.). From this position they are moved laterally by an epithelial fold (alveolingual fold, Ramsay) and come to lie on the lateral pharyngeal wall in the 22 mm. stage (Fig. IV.113./21). Meanwhile, the lateral palatine swellings have appeared on the pharyngeal walls and by their closure help create the tonsillar sinus (Fig. IV.125./8, 11). Since their appearance the tonsillar swellings have elongated and appear like shelves on the lateral pharyngeal walls. Short folds (Plicae semilunares) grow ventrally from the undersurface of the soft palate to cover the tonsils medially in the 40 mm. stage (Fig. IV.131./13). From then onwards, the tonsillar complex, apart from general enlargement, seems to lie quiescent until shortly before full term, when in the 120 mm. stage lymphocytes appear in the dorsal surface of the tonsillar shelves (Fig. IV.159.). At term the lymphoid zone takes up most of the tonsillar substance, leaving only a narrow ventral connective tissue hilus. The epithelium covering the lymphoid zone has invaginated at several places, forming what could be called temporary tonsillar crypts (Fig. IV.162./3). Lymphocytic invasion of the overlying epithelium, and follicle formation within the tonsils are just beginning at birth.

The development of the pharyngeal tonsil can first be recognised in the 120 mm. fetus by the appearance of large numbers of lymphocytes in the subepithelial tissues of the nasopharyngeal roof. Two patches of such cells are present, one on either side of the midline (Fig. IV.159./2). At full term these patches have enlarged and met, forming a single unpaired organ. The overlying epithelium is unaffected by the presence of these lymphocytic cushions and similarly neither lymphocytic invasion nor follicle formation has begun.

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