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Studies on the physiology and bacteriology of the cecum with special reference to the avian subject

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STUDIES ON THE PHYSIOLOGY AND BACTERIOLOGY OF THE CECUM WITH SPECIAL REFERENCE TO THE AVIAN SUBJECT

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INTRODUCTION

The anatomical and physiological significance of the cecum, as related to alimentation in animal biology, has for years been a subject for discussion. Although this organ is, in many respects, comparable anatomically and physiologically to the large intestine, the literature concerning the cecum is meagre. A large and extensive bibliography is available on the intestinal functions in man and animals, yet comparatively little has been published on the bacteriology of the cecal contents or upon the physiology of this structure.

The cecum varies in size and morphology in different animals. The position of it and its intricate connections with the rest of the intestines makes conditions almost impossible for its satisfactory extirpation, an operation quite essential for experiments to furnish data on functional aspects of any organ. However, it has been found from the experiments of this investigation that the domestic fowl offers a satisfactory intact subject for study.

The domestic fowl is well suited for these studies on account of the straight short large intestine and the anatomical position of the ceca, which lie one on each side of the ileocecal junction. The vascular supply to the ceca is distributed from the large superior and inferior mesenteric arteries to the mesentery which joins organs to the surrounding tissues and to the small intestines. The bilateral arrangement of the ceca makes possible observations upon the blood supply which would be impossible with a long and convoluted large intestine.

The two ceca as they are encountered anatomically represent diverticulae varying in length from three to six or seven inches. The total surface area may be estimated as approximately five times that of the large intestines.

The above facts indicated to the author that successful cecectomization could be accomplished.

Since the avian large intestine, in comparison with other subjects, is so short and affords such a limited surface, the ceca may be augmentive structures to it assisting in absorptive functions. Therefore, the author has planned and conducted a series of experiments to obtain information regarding the absorptive activities of these organs and something concerning the bacteriology of their contents. In the procedures have been included a plan and technique for removing the ceca and establishing intact subjects. This has made it possible to study the physiology in some respects before and after cecectomization.

The ceca may, under ordinary conditions of life, harbor bacteria and parasites of sufficient variety to be of interest to the avian pathologist. Biochemically these organs may contain materials in a state of decomposition and putrefaction. Therefore, the question first suggested was: what groups of animal or plant saphrophytes or parasites may under ordinary conditions of healthy living inhabit the ceca; second, what part do such microorganisms play through the decomposition products resulting from their activities; third, how does absorption of these products take place; and, finally, how much do these products influence the intermediate metabolism of the avian subject?

The aim and scope of this investigation is, therefore, to study the domestic fowl before and through cecectomization and subsequently to correlate the physiological and bacteriological studies. Thus, information may be obtained concerning the physiological role of the ceca in alimentation incident to the avian subject. And in general the data may have some application to the biology of animals.

Physiological

In the studies on the physiology of alimentation it is of interest to study some biochemical compounds known to be absorbed in the large and small intestines of man and animal. The fact that sodium chloride is absorbed during alimentation in the large intestine is borne out by many investigations upon human and animal subjects. Thus it has been assumed that the ceca participates in this absorption. The cecal contents are known to be composed of large masses of digested and undigested proteins and protein derivatives. Their presence indicates a possible absorption by the cecal membranes.

Among these protein derivatives or decomposition products are the amino acids, and also the phenols which are derived from such phenyl sources as tyrosin. It is the plan of the author to investigate the levels of these three substances, amino acids, phenols, and sodium chloride, in the whole blood of the experimental subjects before and after cecectomy to ascertain the absorption powers, if any, of these organs and to correlate the findings with any rearrangements in intermediate metabolism incidental to the loss of the ceca.

Bacteriological

The purpose of this phase of these studies has been to examine

the cecal contents, to the end of classifying the significant bacteria present. The results would contribute to knowledge of cecal bacteriology, per se, and furnish information concerning an established flora. This knowledge might be used to correlate the type of organism found with any decomposition in the ceca. Such decomposition might be the source of increased phenols and amino acids and aid in the interpretation of the absorption of these compounds before and after cecectomization.

OPERATIVE TECHNIC

In order to study an organ in the body, it is essential to study it first as to what effect the absence of it will have upon the organism. This can be done either by establishing a fistula or by complete removal. In this investigation a complete cecectomy was performed upon the avian subjects.

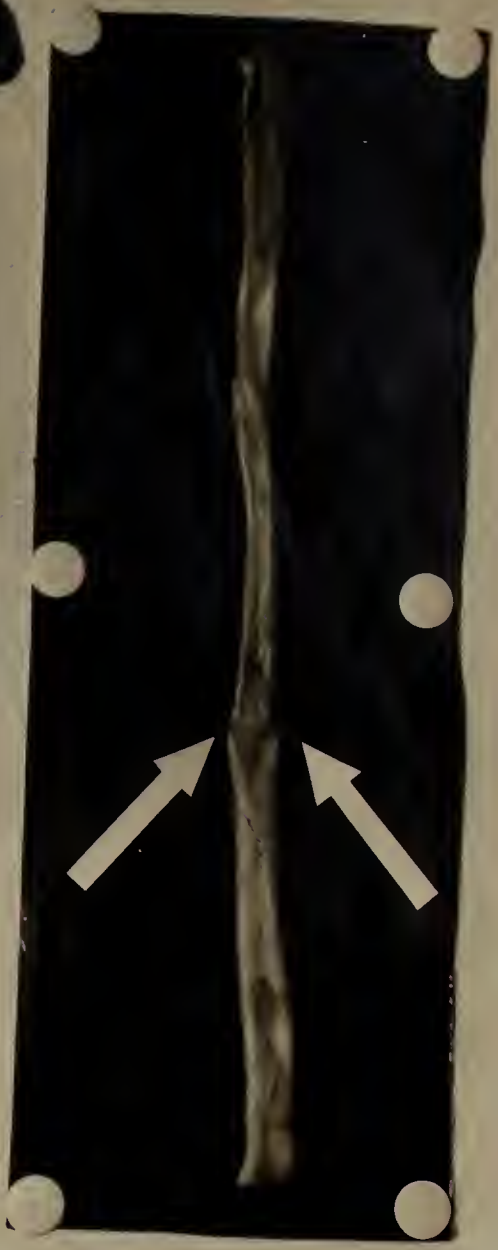
This operation was performed with some degree of success upon avian subjects by Maumus[1]. His technic was, in its essentials, similar to that employed in this investigation. Gage, G. E., has performed the operation and it is recorded in some unpublished work. The author learned the Gage technic through personal communication.

The most important factor in any operation is the method of producing anaesthesia. Several general anaesthetics were tried with unsatisfactory results. Tri-brom-ethanol in amylene hydrate, avertin, was used first. The average dosage for human subjects is 100 mgm. per kilogram body weight. Amounts as high as 500 mgm. per kilogram body weight of the avian subject were used with only slight anaesthesia. This solution was administered per rectum. A 10% solution of urethane was administered per os resulting only in a marked drowsiness. Above this amount the subjects went into complete anaesthesia from which they did not recover in 24 hours.

Having experimented with liquid anaesthetic which took effect through the alimentary tract with no practicable results, it was decided to return to the original technic using ether.

Before ether was applied, the subject was first injected in axilla at the junction of the hind leg with the body with 1 cc. of 1/200 grains atropine sulphate in water. The purpose of this drug

THE LOWER INTESTINAL TRACT AT THE ILEOCECAL JUNCTION BEFORE AND AFTER CECECTOMY.



was to paralyze the nerve endings of the nerves to the involuntary muscles. This drug is known to have inhibitory effect upon the sympathetic and parasympathetic nervous system. The same effect applies to the sensory nerve endings to the visceral organs, so that any excess strain upon the nerve plexes around the operative area would not effect the central nervous system of the subject. The specific nerve trunks and plexuses inervating the ceca and closely related organs are cutaneous nerves and the sympathetic abdominal plexus which leads from the abdomen to the thoracic plexus, thence to the cervical sympathetic trunk, thence to the cervical nerve ganglion. This ganglion communicates anteriorly with the glassopharyngeal and vagus nerves and the sympathetic system.

Following the injection of atropine, complete anaesthesia was established by the use of ether. Since the atropine does not affect the pupillary muscle in this bird, as the iris is controlled by voluntary muscle, it is possible to ascertain as to the point of complete anaesthesia by watching for complete dilation of the pupil. Due to the large alveolar surface of the avian respiratory system, large quantities of ether are required to anaesthetize. It is, therefore, difficult to regulate the amount of ether and thus easy to over anaesthetize.

The Cecectomy.

The subject is firmly secured to the operating table. The injection of the atropine is made and the anaesthesia administered with the ether. When the majority of the body muscles are relaxed, as is evidenced by the ease with which the feathers can be removed, the abdominal region from sternum to cloaca and from one leg to the

other is de-feathered. The abdomen is then washed with 2.5% phenol. When anaesthesia is general the operation is started. Sterile instruments were used throughout and the operator's hands were kept as sterile as possible by the use of phenol. An incision is made with a scalpel starting at a point about 2 cm. below the sternum and about 2 cm. to the right of the linea alba continuing parallel to this line for a distance of from 6 to 7 cm. This incision is best done with one movement. The next incision is started at the same point with entrance into the abdominal cavity. Using the forefinger of the left hand as a spreader and director the incision into the cavity is completed. The forefinger is then inserted into the cavity and by careful exploration the large intestine is located. The point of junction of the ceca is then pulled gently to the surface of the skin. Care must be taken that none of the large blood vessels are broken and that too great pressure is not exerted on the mesenteries, since any excess such as this and pinching of the cecum appears to affect the sympathetic system to a degree. The distal end of one of the ceca is then sought and very carefully disengaged from its mesenteries for its entire length. A piece of catgut is then passed around the proximal portion of the cecum and tied close to the ileocecal junction. The cecum is then excised close to the tied portion with serrated scissors. Precaution must be taken that none of the cecal contents escape into the intestinal cavity because of possible infection. The other cecum is removed in the same manner. The cavity is then dried of blood and any large blood clots are removed.

Interrupted sutures of surgical silk are used to close the incision.

The surface of the incision is finally washed with phenol and dusted with di-thymol iodide.

PHYSIOLOGY

The study of the physiology of an organ in the body of man or animal presupposes certain factors. The first of these is what are the possible functions of the organ, such as absorption through its walls of substances which play a part in the metabolism of the individual. Second, that the substances absorbed are of significance to a degree to make possible their study in the organism.

The fact that the ceca of the domestic fowl contain a similar mucosa to that of the small and large intestines is well established by the work of Looper and Looper[2] and Calhoun[3]. These histologists found villi with mucous cells and glands of Lieberkuhn nearly the whole length of the cecum. Near the distal end there was a tendency for the villi to become less distinct and to show only between the plica circularis which were more prominent here.

In order that absorption may take place there must be an adequate blood supply to the organs in question. The arterial supply and venous outflow from the ceca is described by Kaupp[4].

The ceca receive blood from two sources: first, the branches of the recurrent ilio-celiacus from the anterior mesenteric artery and, second, branches from recurrent ilio-colicus from the recurrent intestinalis artery from the ciliac axis. The venous blood from base of ceca is carried by the vena hemorrhoidalis to the arcus hypogastricus to the vena coccygomesenterica to the vena mesenterica communis, thence to vena portalis dextra. The vena pancreatico-duodenalis passes along both ceca to the vena portalis dextra. It is of importance later to note that all the venous blood goes to the portal vein. This would

indicate that the blood containing products of intermediary metabolism in the ceca goes to the liver.

Phenols and amino acids are known to be produced in the lower intestinal tract of man and animals from products of protein decomposition. Phenols of course are of no metabolic significance from the standpoint of use by the organism. However, this waste product is present to an extent in all animals to make the study of it in the blood of significance. Amino acids on the other hand are the building blocks of proteins in the body and on this ground are of value to study. That sodium chloride is absorbed in the lower intestinal tract makes this substance of interest to study. The red blood cell content and percentage hemoglobin is of interest as indicative of the general state of the blood in the subjects under study.

It is the purpose, then, to study the levels of phenols, amino acids, sodium chloride, red cell counts, and percentage hemoglobin to determine the limits of the amounts of these chemical substances in the blood of the avian subject. Knowing the blood values of these compounds, the ceca are to be removed by surgical operation and studies made upon the blood of the intact animals in terms of the above substances.

Subjects for Study, their Environment and Feed.

The subjects for study were composed of several groups of White Leghorn and Rhode Island Red adult male and female birds. They were normal physiologically insofar as possible to determine. They were kept in a large well lighted enclosure on a floor in the building in which the studies were carried out. No extraneous food could be taken since the floor was of cement covered with wood shavings.

By observation of the feeding trays as to whether or not the feed was cleaned up, it was found that each bird should have 55 grams of the dry mash each day. This metabolic mixture was composed of:

Water	9.28%
Protein	19.14%
Fat	5.55%
Extract matter (carbohydrate or starchy)	51.98%
Fiber	6.35%
Ash	7.7%

1.0% of the latter was salts of chlorine as determined by extraction of the ash with silver nitrate.

On the basis of the analysis of the feed and the amount fed per day to each subject, the approximate amount of each food material received by each is:

Protein	10.56 grams
Fat	3.03 grams
Extract matter (carbohydrate or starch)	28.59 grams
Fiber	3.52 grams
Ash	3.69 grams
Salts of chlorine	0.55 grams

The experimental subjects were maintained on the above amounts of feed and, with the exception of fibrous green food for vitamines occasionally, were not the recipients of any other food. From the above metabolic mixture, the phenols, amino acids and sodium chloride must be derived.

Methods of Obtaining Blood Samples.

Six to eight cc. of blood were drawn by incision from the vein at the base of the elbow (vena humeri profundi) into test tubes containing three drops of a saturated solution of potassium oxalate as an anti-coagulant.

Methods of Blood Examination.

All of the blood examinations were made on the protein free filtrate prepared as described by Folin and Wu[5]. Special care must be employed in laking the red blood cells since the avian erythrocytes carry such a large nuclear structure. 10% sodium tungstate and 2/3 N. sulphuric acid were used to precipitate the proteins. The final filtrate contained one part blood to ten parts water and tungstic acid. The examination of the filtrate for free phenols requires, according to the method used, a one to seven filtrate but satisfactory results were obtained from a one to ten filtrate. From point of convenience all examinations were made on the latter dilution.

Thirty to thirty-five cc. of filtrate are necessary for the determinations done during the examinations of each subject.

Free blood phenols were determined by the method of Theis and Benedict[6]. The examination employs freshly diazotized p-nitro aniline as a basis for colorimetric estimations. The standard is made of pure phenol standardized by the iodine, starch and thiosulphate method. Colorimetric comparisons should be made within a short time as the colors are not stable.

Amino acid nitrogen in the blood filtrate was determined by Folin's[7] method which employs (3 -naphthoquinone as a basis for colorimetric comparison.

The estimation of the amounts of sodium chloride in the blood filtrate were made by the Whitehorn[8] technic. The chlorides are precipitated by silver nitrate in the presence of an excess of nitric acid. The titration for excess silver nitrate is made with standard ammonium or

potassium thiocyanate solution using ferric ammonium sulphate as an indicator.

The red blood cell count was made according to a standard technic in which the important factor was a dilution sufficiently great to assure an even distribution of the large avian erythrocytes. A dilution of one to two hundred was found satisfactory.

The hemoglobin estimation was made by the original Sahli method; Sahli values were converted into percentages using the human male normal of 80%.

Phenols.

The study of phenols in the animal organism can be resolved into two phases: first, production in the intestine; and second, absorption and metabolism in the body.

According to Folin and Denis[9], phenols may be produced in two ways in the intestinal tract. The first of these is through the oxidation of Benzene to phenol and to dioxybenzenes. The second is an entirely different one and results from bacterial decomposition. The important amino acids in this connection are tyrosine and phenylalanine. In the ordinary processes of intestinal metabolism these substances are oxidation and decomposition products of proteins and their derivatives. Phenols should be considered as waste products of decomposition within the intestinal tract. Hydroxy benzene occurs in considerable quantities in the large intestine[10].

Tsudji[11], Rhein[12], Hanke and Koessler[13] state that organisms of the colon group have the power of producing phenols from compounds

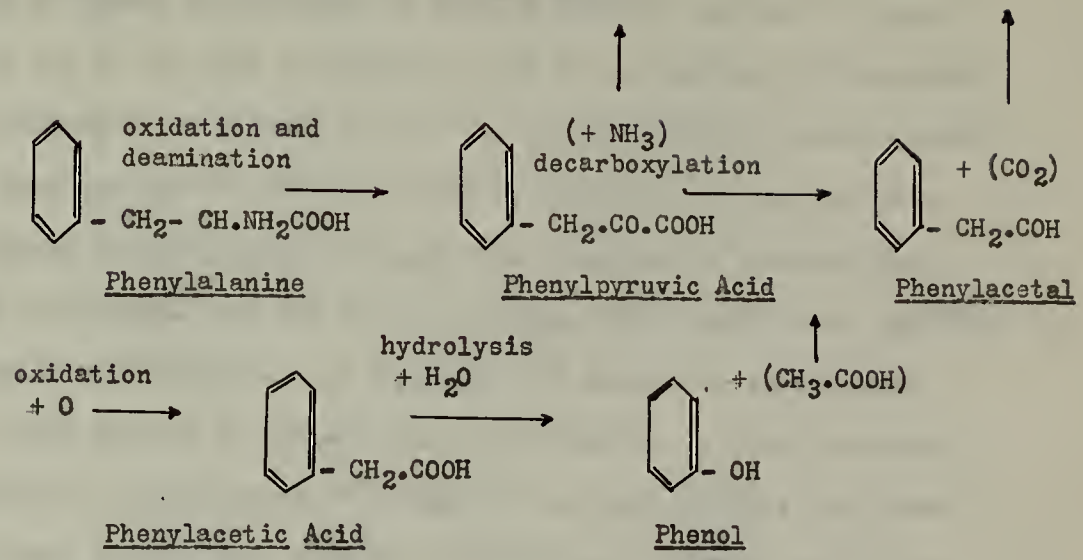
like tyrosine. Certain of the aromatic amino acids are the sources of phenols. When such acids, or proteins that contain them, are fed, phenols increase in the urine[14, 15].

It is not possible to recover quantitatively phenol equivalent to the amount of amino acid compounds administered and the amount recovered after the ingestion of protein is less than after tyrosine[14]. The body must possess some powers to detoxicate the phenols by conjugation with sulphates or glucuronic acid. This is done, however, to a small degree. Folin and Denis[9] give the values ranging from 30% to 90% not conjugated. Rakestraw[16, 17] found 10% to 20% phenol conjugated. Folin and Denis[9] also state that glucuronic acid is the compound which combines to the greatest extent with phenol.

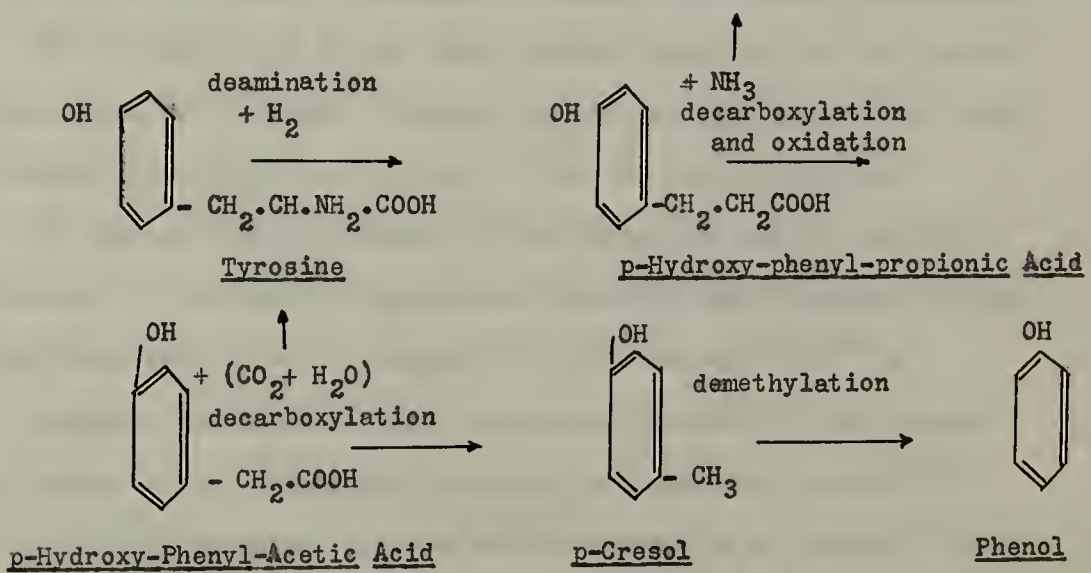
In connection with the conjugation of phenols in the body of the organism, Dubin[14] states that the intestines and liver play the most important part in conjugation. This function is nearly always constant but free phenols may rise upon ingestion of phenolic substances. Fasting also reduces phenols to a low level[14]. This author also suggests that bile plays an important part in conjugation of phenols.

Morse[18] illustrates the conversion of tyrosine and phenylalanine to phenols. These formulae will serve to show transformations involved in the production of phenol compounds.

1. The formation of phenol from phenylalanine.



2. The formation of p-cresol and phenol from tyrosine.



Marenzi[19] in studies of the predominance of any organ in elimination of phenol states that the kidneys regulate the level of blood phenols but do not play an important role in conjugation. He concludes that conjugation of phenols is an extra hepatic function since ligation slows down conjugation but this function is carried on just as well. This worker states that the conjugational function is possessed by divers tissues and that the small intestine plays a part since upon its ablagation the conjugation of injected free phenols is slowed down.

Small amounts of phenols can be detected in the blood of normal individuals. Rakestraw[16, 17] found, in an examination of the blood of a number of patients, values of from 1.9 to 8.0 mgms. per 100 cc. This investigator could detect no conjugated phenols. Other investigators report the presence of conjugated phenols and ethereal sulphates.

The production of phenols must depend, therefore, on two factors: first, oxidation of benzene compounds by tissues and, second, the extent of decomposition activities of bacteria in the large intestine.

The decomposition processes in the intestines and the excretion of phenols are increased by intestinal stasis[14] and diminished by conditions that accelerate the passage of intestinal contents[21].

Complete starvation reduces phenol excretion while high protein diet increases it. It has been suggested by Hanke and Koessler[13] that the acidity or alkalinity may affect production of phenols in the large intestine. High carbohydrate diet may also diminish the production of phenols because organisms like the colon group attack protein derivatives only when carbohydrate is lacking.

Therefore, since free phenols are considered to be the important members of the phenol group from the standpoint of physiological activities, it is the purpose of this part of the investigation to determine, first, the levels of free phenols in the blood.

The results of these levels are recorded in Table 1. Following the establishment of the levels of phenols in the blood, the subjects were cecectomized and the blood of these intact animals was studied as to the effect of removal of the absorption area.

Amino Acids.

Protein metabolism is essentially the metabolism of the amino acids. All present data indicates that these substances are the products into which food proteins are broken during digestion, out of which the body proteins are synthesized, and into which the body proteins are again broken when tissue disintegration occurs. They are also known to serve as sources of essential non-protein substances in the body, such as creatine, purines, etc. Experiments have largely shown that there is rarely important disturbances in the amino acid content of blood. Even in disease the amino acid content of the blood is rarely altered. This indicates that the proper handling of these substances is of such vital importance to the body that capacities of the various bodily mechanisms are provided with a great margin of safety. Therefore, an understanding of them is essential to any conception of the field of nitrogen physiology.

Since it is true that the absorption of amino acids during intermediate metabolism in the body is of great importance, as indicated by

this discussion in relationship to men, and since this absorption is carried out even to the lower part of the intestinal tract, it is conconceivable that the effect of removal of organs like the ceca in the domestic fowl that may contain amino acids would significantly be shown by blood examination. The amino acids also are known to be products of proteolyzing bacteria and from this standpoint, as well as removal of the bacteria and the protein medium, an idea may be obtained as to the significance of the ceca from two angles.

The presence of amino acids in the blood was first conclusively demonstrated by Delaunay[22] who used the formol titration for his estimation and by Van Slyke and Meyer[23].

At the present time most determinations for amino acid nitrogen are made with either the Folin[24] naphthoquinone sulphonic acid colorimetric method or the Van Slyke gasometric method.

Both methods indicate that primary NH_2 nitrogen in the non-protein fraction of normal human blood is between 5 and 8 mgm. per 100 cc. of blood. Digestion of protein food has been shown in many experiments to be capable transiently of raising the amino nitrogen content of dogs, rabbits, etc., several milligrams per 100 cc. of blood[22, 23, 25, 26].

Age, sex and fasting, according to Van Slyke and Meyer[27] and Folin and Berglund[28] appear to have no effect upon the amino acid levels in the blood. However, Edgar[26] gives conclusive data indicating that amino acids in the blood of children do vary from adult levels and that fasting blood gives lower results as to amino acid nitrogen content than do non-fasting. It is considered, however, that the levels will never go below the fasting levels. During a wasting disease[25] it is

possible for an increase to take place in the amino acids of the blood. The presumable explanation for this behavior is that when amino acids are no longer supplied to the blood by food, they are provided by autolysis of body tissues.

Free amino acids appear to be continually originating in the body; first, from digested food proteins and, second, from autolyzed tissue proteins. Intestinal digestion yields products a large proportion of which are free amino acids and during digestion of protein food in man and most animals the amino nitrogen content of the blood increases. The increase affects both the portal blood[29] and the blood of the general circulation. Evidence everywhere indicates conclusively that amino acids are absorbed into the circulation from the intestine during protein digestion[28, 30]. After digestion there is no disappearance of amino nitrogen from the blood. Autolysis and synthesis of tissue proteins, the one utilizing amino acids and the other liberating them, appear both to be continuous processes of which one or the other dominates according to the conditions[24].

Having through these investigations demonstrated that amino acids are the protein digestion products which are absorbed during alimentation, led to many experiments proving that diets containing all their nitrogen in the form of free amino acids suffice for maintenance of animals in nitrogenous equilibrium for considerable periods. From a long series of investigations by L. B. Mendel and his students, the only amino acids which can with certainty at the present time be called indispensible are: histidine, tryptophane and cystine[31].

This is an important problem from the standpoint of nutrition for nature presents proteins that vary in nutritive values which are incapable of supporting nitrogen equilibrium because they completely lack certain amino acids. Casein, as a satisfactory example of a protein, contains or provides the essential amino acid units in nearly ideal proportions.

Complete excision of the liver was shown by Bollman, Mann and Magrath[32] to result in a steady hour by hour increase in blood amino acids. Deaminization and urea formation are not noticeably impaired by removal of less than 90% of the liver. The fact that complete excision of the liver appears necessary in order to prevent approximately normal regulation of amino acid metabolism, indicates that the factor of safety for this function is enormous.

Thyroidectomy was found by Okada and Hayashi[33] to have no effect on the blood amino nitrogen content. Pancreatectomy was found by the above authors[33] to cause a transitory rise of about 1 mgm. per 100 cc. of blood.

According to Bock[34] bird blood contains more amino acid than mammalian blood. There is individual variation, however, and in turkey blood the amino acid nitrogen is about 20 mgm. per 100 cc. Blau[35] in studying Bock's technic finds that some of the proteins were hydrolyzed and thus gave considerably higher results of amino acid nitrogen content of various bloods.

Gyorzy and Zunz[36] and also Bock[34] found that in the blood of the domestic fowls studied the corpuscles contain about two-thirds of the total amount of amino acid nitrogen. Turkey blood analyzed by Constantino[37] showed 3 mgms. of amino acid nitrogen per 100 grams of serum and 34 mgms. in 100 grams corpuscles.

Becker and Hermann[38] report that animals having nucleated cells show a corresponding amino acid nitrogen content of blood to human patients with leukemia. The inference is that the amino acid nitrogen content of the blood is contained mostly in the nucleated red or white cells.

In connection with this is a report by Loeper, et. al.,[29] that more amino acids are found in blood clots than in the serum expressed.

The production of amino acids from protein by bacterial catabolism in the large intestine will be discussed in the section on the bacteriology of this investigation.

It is the purpose of this study of blood amino acids to establish the quantitative amounts of this substance in the blood of avian subjects. The cecectomized subjects will then be studied in the same manner to detect any effects upon the absorption of amino acids. Since amino acids are products of enzymatic action by tissues and bacteria, the removal of an absorptive area of the ceca may remove a source of these compounds. The preliminary results on subjects prior to cecectomy are recorded in Table 2.

Chlorides.

Because the reaction of the tissues is to the alkaline side of the isoelectric point of proteins, the inorganic chlorides presumably exist entirely in the form of salts of the alkali metals, a conclusion supported by the potentiometer measurements of Neuhaussen[39] on blood plasma and by results of Van Slyke, Wu and McLean[40]. The base in the cells being chiefly composed of potassium and that in the extracellular fluids of sodium, it follows that potassium chloride predominates in the cells and sodium chloride in the plasma, lymph and serous fluids. These facts indicate that cell boundaries are in general pervious to the chloride ion. The distribution of this ion between cells and plasma is unequal. Van Slyke, Wu and McLean[40] found that the chief causes of the inequalities of Cl^- and HCO_3^- distribution are due to the fact that in the cells an important part of potassium is in the form of the hemoglobin salt leaving only a balance to combine with Cl^- and HCO_3^-.

During the entrance and exit of CO_2 in the respiratory cycle, there is a rearrangement in the distribution of the diffusible anions Cl^- and HCO_3^-. According to Donnan's Law[41] the distribution of these anions should be equal when the system is at equilibrium, so that

$$HCO_3^-{}_{cells} : HCO_3^-{}_{plasma} = Cl^-{}_{cells} : Cl^-{}_{plasma}$$

Through the absorption of CO_2 by the blood from the tissues, the formation of additional HCO_3^- in the cells unbalances this equilibrium. To restore the normal condition HCO_3^- diffuses out of the cells

into the plasma forming $NaHCO_3$ and releases Cl^- from NaCl which diffuses into the cells until the cell to plasma ratios of the active concentrations of Cl^- and HCO_3^- anions are restored to equality. As a result of these reactions bicarbonate of both cells and plasma is increased. The cells gain some chloride from the plasma and the Cl^- and HCO_3^- concentrations in the two phases become more nearly equal. Furthermore, the transfer of HCO_3^- from cells to plasma, by increasing the $NaHCO_3$ of the latter, serves to diminish the effects of the acid HCO_3^- by maintaining the proper pH of the blood.

Increase in sodium bicarbonate decreases the hydrogen ion concentration according to the familiar Henderson equation: $H^+ = K^1 \frac{H_2CO_3}{NaHCO_3}$ The effect of increase in H_2CO_3 in raising the H^+ is therefore in part neutralized by the immediate increase in $NaHCO_3$. The bicarbonate increases in the plasma are due indirectly to the chlorides since the HCO_3^- could not have been transferred from cells to plasma without an equivalent exchange of Cl^-. Thus the chlorides play an important part in the interchange of buffer effects between the richly buffered cells and the poorly buffered plasma. There is increasing evidence that the chlorides also assist in a similar interchange between the tissue cells and intercellular fluids of the body. This interchange stabilizes the acid-base balance of the fluids.

The normal human body contains between 560 and 630 mgms. of chlorides per 100 cc. of blood. If a 0.9% NaCl solution can be used as isotonic comparable with serum, it would indicate that the chlorides contribute about two-thirds of the osmotic pressure of the blood. The other third is made up of HCO_3^-, protein, SO_4, PO_4, and unknown ions.

Chlorides, therefore, make up the most abundant source of electrolytes for maintaining the osmotic balance of the body.

The electrolyte balance in the body fluid is a function of the kidneys. These organs are influenced by the chloride concentration in the removal of water from the blood. This important function regulates blood composition first and volume second. For example, water drunk by the dehydrated subject will not be retained by the kidney unless salt is taken with it.

Chlorides in relation to gastric acidity are of interest. Shortly after the intake of food the bicarbonate of the plasma of a normal individual rises and at the same time the acidity of the urine diminishes. It has long been assumed that the "alkaline tide" was a reflection of the acidity of the stomach in secreting hydrochloric acid. This has been supported by Hubbard[42] and others[43]. The curve of urinary acidity tends to parallel that of chlorine. Prolonged administration of a salt-poor diet results in the development of gastric acidity from which it may be inferred that a certain supply of chlorine is essential for the production of the normal acid secretion of the stomach. If the pyloris is obstructed[44], secretion of chloride in the gastric juice may continue after plasma chlorine has been reduced to one-half the normal concentration. If reabsorption of chlorides from the intestines is prevented by the production of a gastric fistula, pyloric obstruction, or persistent vomiting, the chloride of the plasma becoms depleted.

Loss of chloride in gastric secretions may continue even after serum Cl^- has fallen to an extremely low level. On the other hand, urinary excretion of chlorides becomes minimal or ceases entirely when

serum chlorine falls appreciably below normal concentration. The urine at first becomes alkaline because the excretion of bicarbonate excess in serum and body fluids prevents the alkalinity from becoming intolerably great. A point may be reached, apparently, where the organism will not permit further reduction of the volume and osmotic pressure of its fluids in behalf of acid-base equilibrium. Under these circumstances the administration of sodium chloride may result in the excretion of an alkaline urine[45, 46, 47].

The effects on plasma electrolytes of obstruction of the alimentary canal below the pyloris does not differ greatly from those of pyloric obstruction[46, 47].

In all such conditions dehydration and base deficiency develop. The lower in the intestine the point of obstruction lies, the less evident are likely to be chloride reduction and bicarbonate excess. This is due to the fact that the secretions of intestines are alkaline[48]. It is conceivable that an acid condition that might exist in some parts of the intestines in some animals may effect the absorption of chlorides. Fistulae of the gastro-intestinal tract give rise to much more varied disturbances of the plasma electrolyte because of the differences in the inorganic composition of the various alimentary secretions[49]. With pancreatic fistulae or fistulae lower down in the small intestines, bicarbonate deficit and chloride excess are encountered because pancreatic juice is alkaline. All such fistulae lead to dehydration and loss of sodium chloride and bicarbonate. The proportions in which these ions are lost vary with the site of the fistula[49].

From this discussion the physiological significance of sodium chloride in the maintenance of the acid-base balance of the body fluids is evidenced. The effect upon absorption of chlorides from the intestines by removal of absorptive areas is well established(44, 45, 46, 47).

Therefore, by cecectomy, a removal of a possible absorption area may effect the levels of sodium chloride in the blood. Table 3 expresses the results obtained by blood examinations for sodium chloride prior to the studies of the cecectomized subjects.

Erythrocyte Count and Hemoglobin Content.

Since the red blood cells and more especially their hemoglobin content play an important part in the maintenance of the alkali reserve and buffering action of the blood as was described in the discussion of blood chlorides, a study of the blood content of the former substances would be of value. That is, a reduction in the red blood cells and hemoglobin percentage due to subtle physiological effects following the operation might influence the chloride balance and acid-base equilibria in the blood. Since the erythrocytes in the aves are nucleated, a reduction in their number might also influence the amino acid content of the blood(38).

These determinations are of value also by indicating the general state of the blood as well as contributing to scientific information on the blood of the avian subject. In this connection, Kaupp(4) records the avian erythrocyte count to be 3,000,000 to 4,000,000 per c.m.m. of blood. Cook and Dearstyne(50) report red cell counts averaging 2,000,000 to 3,500,000 per c.m.m. and hemoglobin 57.1% to 100%.

TABLE 1.

Blood determinations for free phenols on subjects prior to cecectomy; figures in mgm. per 100 cc. of blood.

Subject No.	Day of Examination				
	1	6	11	15	Avg.
2	6.0	5.5	6.2		4.4
5	3.2	3.4	3.9		3.6
8	3.1	3.4	4.1		3.5
17	7.8	4.7	9.2		7.2
18	7.3	6.2	8.9		7.4
19	8.7	6.8	8.5		8.0
19W	7.5	6.4	6.9		6.5
24	5.7	5.3	3.9		5.0
36	8.5	6.0	10.7		8.4
39	7.1	8.9	6.5		7.5
42	4.8	5.4	6.3		5.5
43	4.9	6.1	5.2		5.4
44	5.1	2.9	5.2		4.4
45	6.2	5.6	7.7		6.5
47	9.1	9.2	3.9		7.4
33	3.5	3.1	5.7	3.1	3.8
66	4.3	3.5	4.7	4.7	4.3
77	5.9	4.8	5.0	4.2	4.9
88	6.9	7.7	6.4		7.0
7839	7.5	4.4	4.7	4.7	5.3
7831	5.2	4.8	7.6	4.8	5.6
9005	6.0	5.0	6.1		5.7
16788	5.5	4.1	5.4		5.0

TABLE 2.

Blood determinations for amino acid nitrogen on subjects prior to cecectomy; figures in mgm. per 100 cc. of blood.

Subject No.	Day of Examination				
	1	6	11	15	Avg.
5	7.6	9.3	9.3		8.7
8	7.6	8.3	11.6		9.2
66	7.6	9.6	10.3	9.2	9.2
77	9.2	8.3	11.4	9.5	9.6
88	9.2	8.1	11.8	8.9	9.5
7831	8.0	8.8	10.8		9.2
7839	7.8	8.5	11.6	10.4	9.6
9005	9.9	9.6	11.6		10.3
16788	7.6	9.6	10.9	10.5	9.7

TABLE 3.

Blood determinations for sodium chloride on subjects prior to cecectomy; figures in mgm. per 100 cc. of blood.

Subject No.	Day of Examination			
	1	6	11	Avg.
2	280	396	495	390
5	379	379	445	401
8	445	437	437	439
17	320	396		338
18	390	520	462	457
19	380	495		392
19W	300	396		348
24	290	462	198	316
33	445	478	503	475
36	300	264		282
39	440	429	512	460
42	320	363		341
43	310	297	528	378
44	300	240	328	289
45	310	462	561	444
47	360	363		362
66	428	289	413	376
77	429	445	432	435
88	363	371	429	387
7831	458	462	454	458
7839	421	449	494	454
9005	445	504	487	478
16788	503	498	486	495

TABLE 4.

Blood counts for erythrocytes on subjects prior to cecectomy; figures in number of cells per c.m.m. blood.

Subject No.	Day of Examination			
	1	6	11	Avg.
2	3,400,000	2,800,000		3,100,000
5	3,200,000	2,400,000	3,200,000	2,900,000
8	2,400,000	2,400,000		2,400,000
17	3,200,000	2,800,000		3,000,000
18	3,200,000	2,800,000		3,000,000
19	3,200,000			3,200,000
19W	3,200,000	2,800,000		3,000,000
24	2,000,000	2,800,000		2,400,000
36	3,200,000	3,200,000		3,200,000
39	2,800,000	2,800,000		2,800,000
42	3,200,000	2,800,000		3,000,000
43	2,800,000	2,800,000		2,800,000
44	3,200,000	3,200,000		3,200,000
45	3,200,000	3,600,000		3,400,000
47	3,200,000	2,800,000		3,000,000
33	3,200,000	2,400,000	2,400,000	2,600,000
66	2,400,000	2,400,000	2,400,000	2,400,000
77	3,200,000	2,400,000	2,400,000	2,600,000
88	2,400,000	2,400,000	2,400,000	2,400,000
7839	2,400,000	3,200,000	2,400,000	2,600,000
7831	3,200,000	2,400,000	2,400,000	2,600,000
9005	2,400,000	2,400,000	2,400,000	2,400,000
16788	3,200,000	2,400,000	3,200,000	2,900,000

TABLE 5.

Hemoglobin percentages on subjects prior to cecectomy; figures in percent hemoglobin.

Subject No.	Day of Examination			
	1	6	11	Avg.
2	56.3	51.3	68.8	58.8
5	62.5	62.5	60.0	61.6
8	62.5	66.3	67.5	65.4
17	83.8	56.2	60.0	66.6
18	56.3	50.0	75.0	60.4
19		56.3	57.5	56.9
19W		60.0		60.0
24	47.5	56.3	57.5	53.4
36	50.0	47.5		48.7
39	67.5	57.5	72.5	65.8
42	63.7	62.5	62.5	62.9
43	53.8	56.3	63.8	57.9
44	56.3	50.0	58.8	55.0
45	52.5	63.0	62.5	59.3
47	67.5	63.8		65.6
33	60.0	50.0	62.5	57.5
66	66.2	68.8	67.5	67.5
77	68.7	74.2	73.5	72.1
88	71.2	66.2	68.7	68.7
7831	67.5	71.5	68.7	65.9
7839	75.0	71.2	72.5	72.9
9005	70.0	65.0	65.0	66.6
16788	65.0	62.5	65.0	64.2

Forkner[51] established averages on eleven birds as 3,267,000 per c.m.m. and hemoglobin as 62.9%.

The red blood cell count and hemoglobin content of the blood of several subjects prior to cecectomy will establish to some extent the blood picture in the domestic fowl. These results are recorded in tables 4 and 5. The effect of cecectomy upon the amounts of these blood constituents will be shown in connection with the studies of the intact subjects.

Discussion of Preliminary Experimental Data and Introduction to Studies on Intact Subjects.

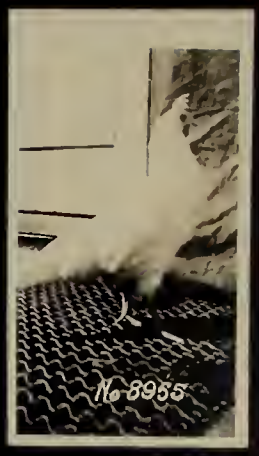
The quantitative estimations of phenols, amino acids, sodium chloride, hemoglobin and erythrocytes in a number of normal birds prior to operative procedures are reported in Tables 1, 2, 3, 4, and 5.

This data will strengthen the information obtained in the studies of the birds used as intact subjects by establishing the limits of the substances under investigation in the types of domestic fowls used in these experiments. The average quantitative limits of these chemical and physiological substances are: phenols 3.5 to 8.4 mgm. per 100 cc. of blood; amino acid nitrogen 8.7 to 10.3 mgm. per 100 cc. of blood; sodium chloride 282 to 475 mgm. per 100 cc. of blood; erythrocytes 2,400,000 to 3,400,000 per c.m.m. and hemoglobin 48.7 to 72.9%. Thus, if the amounts of these physiological compounds in the blood examination of the intact subjects preliminary to cecectomy are within the above ranges, it will be expected that a reduction in the quantitative

estimates of these compounds in the blood of the subjects following the operation will be indicative of the part the ceca play in intermediate metabolism of the avian subject.

The discussion of each of the case studies provides only the average values of phenols, amino acids, sodium chloride, erythrocyte count and hemoglobin percentage obtained in the investigation. Reference to the accompanying table to each case study will give the individual amounts of each blood examination. Charts I, II, and III present in graphic form the trend of the results obtained in these studies.

CECECTOMIZED SUBJECTS USED FOR CASE STUDIES IN THIS INVESTIGATION



Case Studies of Cecectomized Subjects Used in this Investigation.

Case I.

Subject #6. Rhode Island Red female adult, weighing 1,800 gms. before operation. Normal in so far as possible to determine. The blood examinations made on February 20, 25, 28 and March 3, 1934, previous to the operation, showed, per 100 cc. blood: Phenols 5.0 mgm.; Amino acids nitrogen 9.1 mgm.; NaCl 429 mgm.; Red Blood Cell Count 2,580,000 per c.m.m. and Hemoglobin 63.3%. The operation was performed March 10, 1934 with the use of atropine sulphate and ether as anaesthetics. The subject recovered from the anaesthesia within a short time after the operation and sought food. Subject continued to eat well and within a few days laid eggs. Blood examinations were made on the eighth and ninth days following the operation and the results were, per 100 cc. of blood: Phenols 2.4 mgm.; Amino acid nitrogen 7.4 mgm.; NaCl 193 mgm.; Red Blood Cell Count 1,720,000 per c.m.m. and Hemoglobin 54%. The above averaged results indicated a reduction, per 100 cc. of blood of: Phenols 2.7 mgm.; Amino acid nitrogen 1.7 mgm.; Red Blood Cells 860,000 per c.m.m. and Hemoglobin 9.3%. The subject was bled to death from the carotid artery on March 20, 1934 having lost considerable weight prior to this. The autopsy showed heart normal, tympany of small intestine above ileo-cecal junction, few adhesions, points of cecal excision healed, slight decomposition of intestinal contents above cecal junction, and the gall bladder was enlarged.
The results of each blood examination are reported in Table 6.

Table 6.

Blood studies before and after cecectomy. Phenols, amino acids and sodium chloride expressed in mgm./100 cc. of blood.

Mgm./100 cc.	2/20	2/25	2/28	3/3	Avg.
Phenol	4.7	4.8	6.0	4.5	5.0
Amino Acid N	8.4	9.3	11.1	9.7	9.1
NaCl	406	404	479		429
R.B.C./cmm.	2,960,000	2,320,000	2,480,000		2,580,000
Hgb. %	67.5	60.0	62.5		63.3

Mgm./100 cc.	3/19	3/20	Avg.	Change
Phenol	2.2	2.4	2.3	- 2.7
Amino Acid N	7.5	7.4	7.4	- 1.7
NaCl	260	125	193	- 236
R.B.C./cmm.	1,360,000	2,000,000	1,720,000	- 860,000
Hgb. %	58.5	48.3	54.0	- 9.3

Case II.

Subject #7. Rhode Island Red female adult weighing 1,830 gms. before the operation. Normal in so far as possible to determine. The blood examinations on February 20, 25 and 28, 1934, previous to the operation showed the following averages, per 100 cc. of blood: Phenols 5.4 mgm.; Amino acids N 9.4 mgm.; NaCl 456 mgm.; Red Blood Cell Count 2,660,000 per c.m.m. and Hemoglobin 64.5%. The operation was performed March 7, 1934 with the use of atropine sulphate and ether as anaesthetics. The subject recovered from the anaesthesia within a short time after the operation and sought food. It continued to eat well and within a few days laid eggs. Blood examinations were made on the tenth and eleventh days following the operation and the average results per 100 cc. of blood were: Phenol 1.5 mgm.; amino acid N 5.2 mgm.; NaCl 366 mgm.; Red Blood Cell Count 1,840,000 per c.m.m. and Hemoglobin 57.0%. The above results indicated an average reduction per 100 cc. blood of: Phenol 3.9 mgm.; Amino acid N 4.2 mgm.; NaCl 90 mgm.; Red Blood Cells 820,000 c.m.m. and Hemoglobin 7%. The subject was bled to death March 18, 1934. The autopsy showed heart normal, lungs normal, proventriculus and gizzard normal; sutures of operation healed, slight tympany above ileocecal junction, large intestine empty, broken egg shell in cloaca, oviduct ruptured but not extensively, and no infection in the abdominal cavity. The results of each examination of the blood are reported in Table 7.

Table 7.

Blood studies before and after cecectomy. Phenols, amino acids and sodium chloride expressed in mgm./100 cc. of blood.

Mgm./100 cc.	2/20	2/25	2/28	3/3	Avg.
Phenol	6.0	6.5	4.5	4.7	5.4
Amino Acid N	9.5	8.9	9.8		9.4
NaCl	445	429	494		456
R.B.C./cmm.	2,800,000	2,640,000	2,560,000		2,660,000
Hgb. %	65.0	63.8	65.0		64.5

Mgm./100 cc.	3/17	3/18	Avg.	Change
Phenol	1.6	1.5	1.5	- 3.9
Amino Acid N	5.0	5.3	5.2	- 4.2
NaCl	420	380	366	- 90.0
R.B.C./cmm.	1,840,000		1,840,000	- 820,000
Hgb. %	57.0		57.0	- 7.0

Case III.

Subject #22. Rhode Island Red female adult, weighing 2,380 gms. following operation. Normal in all respects. The blood examinations on November 9, 16, and 24, 1933, preceding the operation, showed the following averages per 100 cc. of blood: Phenols 6.7 mgms.; Amino acid N 5.8 mgm.; NaCl 318 mgm.; Red Blood Cells 2,960,000 per c.m.m.; Hemoglobin 73%. The operation was performed February 7, 1934 with the use of atropine sulphate and ether as anaesthetics. The subject recovered from the anaesthesia within a short time after the operation and sought food. It continued to eat well and within a few days laid eggs. Blood examinations on March 21, 26, 28 and 30 showed the following averages per 100 cc. blood: Phenol 3.2 mgm.; Amino acid N 8.3 mgm.; NaCl 355 mgm.; Red Blood Cells 2,530,000 c.m.m. and Hemoglobin 60%. The above results indicated the following average variance per 100 cc. blood. Phenol reduced 3.5 mgm.; Amino acid N gained 2.5 mgm.; NaCl gained 27 mgm.; Red Blood Cells reduced 430,000 per c.m.m. and Hemoglobin reduced 13%. This subject is still alive, May 18, 1934, laying eggs every other day and is in good condition from external manifestations. Results of individual blood examinations are reported in Table 8.

Table 8.

Blood studies before and after cecectomy. Phenols, amino acids and sodium chloride expressed in mgm./100 cc. of blood.

Mgm./100 cc.	11/9	11/16	11/24	Avg.
Phenol	7.1	6.3		6.7
Amino Acid N	6.7	5.8	5.0	5.8
NaCl	240	396		318
R.B.C./cmm.	3,040,000	2,880,000		2,960,000
Hgb. %	80.0	75.0	62.5	73.0

Mgm./100 cc.	3/21	3/26	3/28	3/30	Avg.	Change
Phenol	3.6	3.5	2.9	2.8	3.2	- 3.5
Amino Acid N	7.2	10.6	9.3	8.4	8.3	+ 2.5
NaCl	350	355	380	370	355	+ 37.0
R.B.C./cmm.	2,920,000	2,560,000	2,320,000		2,530,000	- 430,000
Hgb. %	60.0	60.0	60.0		60.0	- 13.0

Case IV.

Subject #40. Rhode Island Red female adult weighing 2,240 gms. following the operation. Normal in all respects. The blood examinations preceding the operation on November 9 and 16, 1933 showed the following averages per 100 cc. of blood: Phenol 6.1 mgms.; Amino Acid N 5.9 mgms.; NaCl 348 mgms.; Red Blood Cells 3,100,000 per c.m.m.; Hemoglobin 53.0%. The operation was performed February 7, 1934 with the use of atropine sulphate and ether as anaesthetics. The subject recovered from the anaesthesia after a short time and sought food. It continued to eat well and within a few days laid eggs. Blood examinations on March 26, 28, and 30, 1934 showed the following averages per 100 cc. of blood: Phenol 2.6 mgms.; Amino acid N 9.3 mgms.; NaCl 362 mgms.; Red Blood Cells 1,760,000 per c.m.m.; Hemoglobin 55%. The above figures indicate the following average variances per 100 cc. of blood: Phenol reduced 3.4 mgms.; Amino acid N increased 3.3 mgms.; NaCl increased 14.0 mgms.; Red Blood Cells reduced 1,340,000 per c.m.m.; Hemoglobin reduced 2%. The subject is still alive, May 18, 1934, laying eggs every other day and is in good condition as far as possible to determine. Results of individual blood examinations are reported in Table 9.

Table 9.

Blood studies before and after cecectomy. Phenols, amino acids and sodium chloride expressed in mgm./100 cc. of blood.

Mgm./100 cc.	11/9	11/16	Avg.
Phenol	5.9	6.3	6.1
Amino Acid N	5.9	6.0	5.9
NaCl	300	396	348
R.B.C./cmm.	3,120,000	3,080,000	3,100,000
Hgb. %	55.0	50.0	53.0

Mgm./100 cc.	3/26	3/28	3/30	Avg.	Change
Phenol	3.2	2.1	2.0	2.6	- 3.4
Amino Acid N	10.6	8.9	8.3	9.2	+ 3.3
NaCl	375	350	360	362	+ 14.0
R.B.C./cmm.	1,920,000	1,600,000	1,760,000	1,760,000	- 1,340,000
Hgb. %	56.0	56.0	54.0	55.0	+ 2.0

Case V.

Subject #2930. White Leghorn female, weighing 2,120 gms. preceding the operation. Normal in all respects. The blood examinations on February 20, 25 and 28, 1934 showed these averages per 100 cc. of blood: Phenol 4.3 mgms.; Amino acid N 8.3 mgms.; NaCl 484 mgms.; Red Blood Cells 2,560,000 per c.m.m.; Hemoglobin 64.8%. The operation was performed March 10, 1934 with the use of atropine sulphate and ether as anaesthetics. The subject recovered from the anaesthesia within a short time after the operation and sought food. It continued to eat well and within a few days laid eggs. A blood examination was made on March 22, 1934 and the results per 100 cc. of blood were: Phenol 1.8 mgms.; Amino acid N 6.3 mgms.; NaCl 355 mgms.; Red Blood Cells 2,400,000 per c.m.m.; Hemoglobin 56.0%. The above figures indicated an average reduction per 100 cc. of blood: Phenol 2.5 mgms.; Amino acid N 2.0 mgms.; NaCl 129 mgms.; Red Blood Cells 160,000 per c.m.m.; Hemoglobin 8.8%. The subject was bled to death March 22, 1934. The autopsy showed abdominal cavity full of gas with general distention. Sclerotic tissue about incision with many connective tissue adhesions in the abdominal cavity. Large intestine distended to twice normal size. There was a general intestinal stasis due to the blockage of the cloacal opening by coagulated egg. Results of individual blood examinations are reported in Table 10.

Table 10.

Blood studies before and after cecectomy. Phenols, amino acids and sodium chloride expressed in mgm./100 cc. of blood.

Mgm./100 cc.	2/20	2/25	2/28	Avg.	3/22	Avg.	Change
Phenol	4.7	4.0	4.1	4.3	1.8	1.8	- 2.5
Amino Acid N	7.5	8.5	8.3	8.3	6.3	6.3	- 2.0
NaCl	470	495	478	484	355	355	- 129
R.B.C./cmm.	2,930,000	2,320,000	2,560,000	2,560,000	2,400,000	2,400,000	- 160,000
Hgb. %	62.5	66.8	65.0	64.8	56.0	56.0	- 8.8

Case VI.

Subject #3245. White Leghorn female adult weighing 1,820 gms. before the operation. Normal in all respects. The blood examinations on February 20, 25 and 28, preceding the operation, showed these averages per 100 cc. of blood: Phenol 6.4 mgms.; Amino Acid N 9.3 mgms.; NaCl 462 mgms.; Red Blood Cells 2,480,000 per c.m.m.; Hemoglobin 60.8%. The operation was performed on March 11, 1934 using atropine sulphate and ether as anaesthetics. The subject recovered from the anaesthesia a short time after the operation and sought food. It continued to eat well and within a few days laid eggs. Blood examinations were made on March 17, 1934 and March 18, 1934 and the average results per 100 cc. of blood were: Phenol 1.7 mgms.; Amino acid N 5.0 mgms.; NaCl 431 mgms.; Red Blood Cells 2,000,000 per c.m.m.; Hemoglobin 57.0%. These results indicate a reduction per 100 cc. of blood of: Phenol 4.7 mgms.; Amino acid N 4.3 mgms.; NaCl 31 mgms.; Red Blood Cells 480,000 per c.m.m.; Hemoglobin 3.8%. The bird died March 18, 1934 probably from loss of blood and ruptured oviduct. The heart and lungs were normal. Autopsy showed tympany of small intestine, no inflammation, oviduct ruptured and slight adhesions. The results of each blood examination are reported in Table 11.

Table 11.

Blood studies before and after cecectomy. Phenols, amino acids and sodium chloride expressed in mgm./100 cc. of blood.

Mgm./100 cc.	2/20	2/25	2/28	Avg.
Phenol	7.2	5.9	6.3	6.4
Amino acid N	9.2	9.1	9.5	9.3
NaCl	470	463	454	462
R.B.C./cmm.	2,720,000	2,240,000		2,480,000
Hgb. %	66.2	50.0	66.2	60.8

Mgm./100 cc.	3/17	3/18	Avg.	Change
Phenol	1.3	2.2	1.7	- 4.7
Amino Acid N	5.7	4.4	5.0	- 4.3
NaCl	400	460	430	- 31
R.B.C./cmm.	2,000,000		2,000,000	- 480,000
Hgb. %	57.0		57.0	- 3.8

Case VII.

Subject #8955. White Leghorn female adult, weighing 1,630 gms. before operation. Normal in all respects. The blood examination previous to the operation on February 20, 25 and 28, 1934 showed these averages per 100 cc. of blood: Phenol 7.3 mgms.; Amino acid N 9.2 mgms.; NaCl 428 mgms.; Red Blood Cells 2,890,000 per c.m.m.; Hemoglobin 61.0%. The operation was performed March 7, 1934 using atropine sulphate and ether as anaesthetics. The subject recovered from the anaesthesia a short time after the operation and sought food. It continued to eat well and in a few days laid eggs. Blood examinations were made on March 26, 28 and 30, 1934 and the average results per 100 cc. of blood were: Phenol 3.3 mgms.; Amino acid N 9.9 mgms.; NaCl 352 mgms.; Red Blood Cells 2,930,000 per c.m.m.; Hemoglobin 72.0%. These results indicated the following reduction or increase per 100 cc. of blood: Phenol reduced 4.0 mgms.; Amino acid N raised 0.7 mgm.; NaCl reduced 76 mgms.; Red Blood Cells increased 40,000 per c.m.m.; Hemoglobin increased 11.0%. The subject was bled to death April 2, 1934 for a cell volume per cent determination. The results from this showed 26.5% of erythrocytes and leucocytes. The autopsy showed that the excision had healed perfectly and that the abdominal cavity held no adhesions. The results of each blood examination are reported in Table 12.

Table 12.

Blood studies before and after cecectomy. Phenols, amino acids and sodium chloride expressed in mgm./100 cc. of blood.

Mgm./100 cc.	2/20	2/25	2/28	3/3	Avg.
Phenol	7.5	6.8	7.7		7.3
Amino Acid N	7.4	9.3	11.4	8.9	9.2
NaCl	437	412	434		428
R.B.C./cmm.	2,640,000	3,280,000	2,740,000		2,890,000
Hgb. %	60.0	60.0	62.5		61.0

Mgm./100 cc.	3/26	3/28	3/30	Avg.	Change
Phenol	4.2	3.3	2.6	3.3	- 4.0
Amino Acid N	10.4	10.0	8.1	9.9	+ 0.7
NaCl	355	345	355	352	- 76
R.B.C./cmm.	3,410,000	3,080,000	3,320,000	2,930,000	- 40,000
Hgb. %	55.0	80.0	80.0	72.0	+ 11.0

Case VIII.

Subject #8967. White Leghorn female adult, weighing 1,710 gms. following the operation. Normal in all respects. The blood examinations on November 9, 16 and 24, 1933 showed these averages per 100 cc. of blood: Phenol 6.4 mgms.; Amino acid N 5.5 mgms.; NaCl 390 mgms.; Red Blood Cells 2,600,000 per c.m.m. and Hemoglobin 50.8%. The operation was performed January 20, 1934 using atropine sulphate and ether as anaesthetics. The subject recovered from anaesthesia within a short time and sought food. It continued to eat well and within a few days laid eggs. The blood examinations following the operation on March 21, 26, 28 and 30 showed these average results per 100 cc. of blood: Phenol 3.3 mgms.; Amino acid N 8.7 mgms.; NaCl 389 mgms.; Red Blood Cells 1,840,000 per c.m.m.; Hemoglobin 52.0%. These results showed an average increase or reduction per 100 cc. of blood: Phenol reduced 3.1 mgms.; Amino acid N increased 3.2 mgms.; NaCl reduced 18 mgms.; Red Blood Cells reduced 760,000 per c.m.m.; Hemoglobin increased 1.2%. The subject is still alive, May 18, 1934, normal in all respects and laying eggs daily. The results of each blood examination are recorded in Table 13.

Table 13.

Blood studies before and after cecectomy. Phenols, amino acids and sodium chloride expressed in mgm./100 cc. of blood.

Mgm./100 cc.	11/9	11/16	11/24	Avg.
Phenol	9.1	6.8	3.4	6.4
Amino Acid N	6.5	5.3	4.9	5.5
NaCl	280	396	415	390
R.B.C./cmm.	2,800,000	2,400,000		2,600,000
Hgb. %	50.0	48.7	53.8	50.8

Mgm./100 cc.	3/21	3/26	3/28	3/30	Avg.	Change
Phenol	3.1	3.0	3.4	3.4	3.3	- 3.1
Amino Acid N	8.3	8.6	8.8	9.3	8.7	+ 3.2
NaCl	385	360	385	360	372	- 18
R.B.C./cmm.	2,320,000	1,600,000	1,600,000		1,840,000	- 76,000
Hgb. %	60.0	52.0	45.0		52.0	+ 1.2

Case IX.

Subject #16670. White Leghorn female adult, weighing 2,230 gms. before the operation. Normal in all respects. The blood examinations on February 20, 26 and 28, 1934, previous to the operation, showed these averages per 100 cc. blood: Phenol 3.9 mgms.; Amino acid N 9.7 mgms.; NaCl 481 mgms.; Red Blood Cells 2,560,000 per c.m.m.; Hemoglobin 71.2%. The operation was performed March 7, 1934 using atropine sulphate and ether as anaesthetics. The subject recovered from the anaesthesia within a short time and sought food. It continued to eat well and in a few days laid eggs. Blood examinations were made on March 26, 28 and 30 and the results were: Phenol 2.1 mgms.; Amino acid N 9.9 mgms.; NaCl 345 mgms.; Red Blood Cells 2,240,000 per c.m.m.; Hemoglobin 49.0%. These results indicated a reduction of: Phenol 1.7 mgms.; Amino acid N increased 0.2 mgm.; NaCl reduced 136 mgms.; Red Blood Cells reduced 320,000; Hemoglobin reduced 22.2%. The subject was bled to death March 30, 1934. The autopsy showed normal healing of incision, no inflammation, adhesion throughout mesenteric area and kidneys, liver and heart normal. The gall bladder was enlarged. The oviduct was burst near the cloaca and there was a large loss of blood. The results of each blood examination are recorded in Table 14.

Table 14.

Blood studies before and after cecectomy. Phenols, amino acids and sodium chloride expressed in mgm./100 cc. of blood.

Mgm./100 cc.	2/20	2/25	2/28	3/3	Avg.
Phenol	3.6	3.2	5.0		3.9
Amino Acid N	7.6	10.4	11.4	9.6	9.7
NaCl	463	512	486	462	481
R.B.C./cmm.	2,400,000	2,560,000	2,160,000		2,560,000
Hgb. %	67.5	78.7	67.5		71.2

Mgm./100 cc.	3/26	3/28	3/30	Avg.	Change
Phenol	2.1			2.1	- 1.8
Amino Acid N	9.9			9.9	+ 0.2
NaCl	345			345	- 136
R.B.C./cmm.	2,080,000	2,400,000		2,240,000	- 320,000
Hgb. %	50.0	42.0	55.0	49.0	- 22.2

Case X.

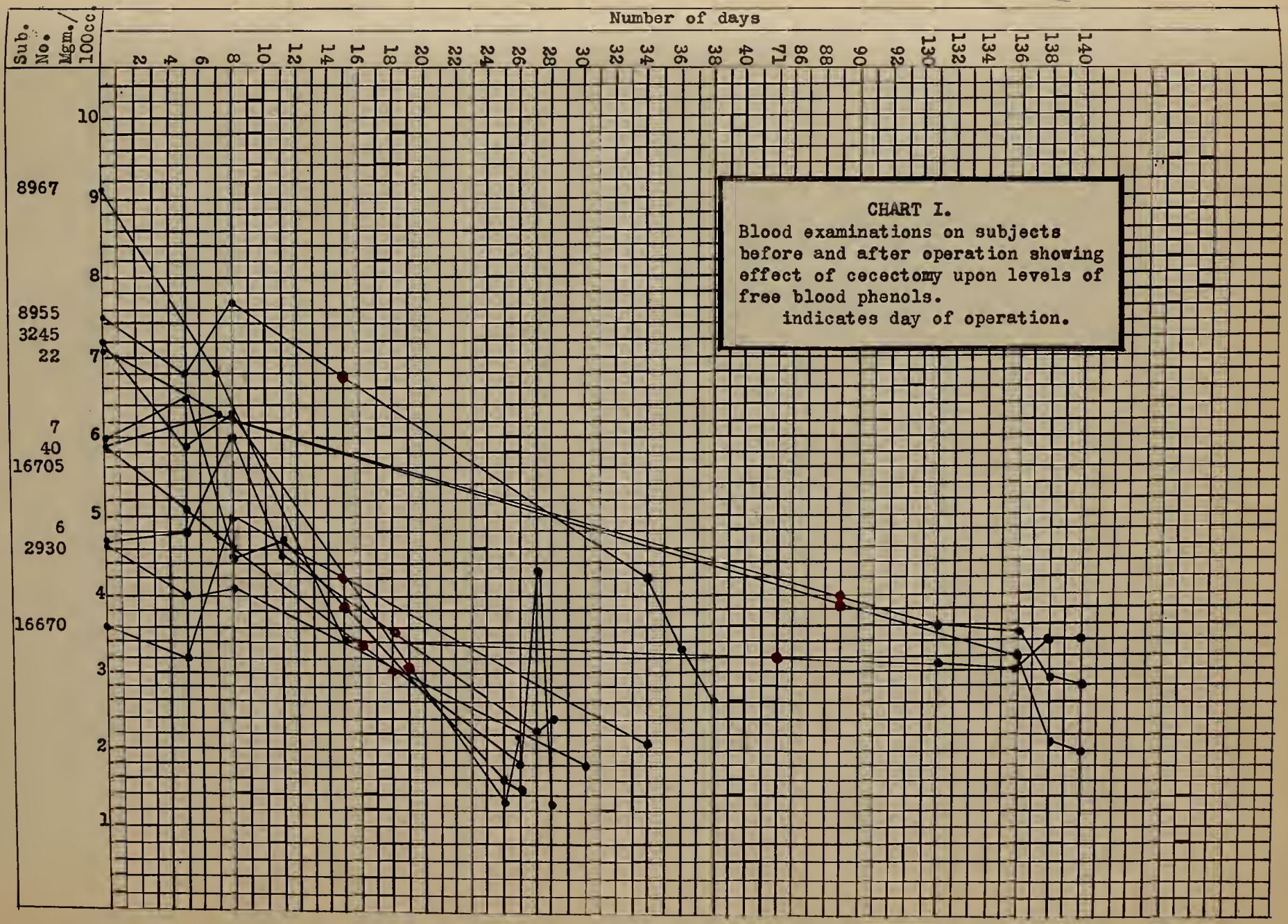
Subject #16705. White Leghorn female adult, weighing 1,480 gms. preceding operation. Normal in all respects. The blood examinations on February 20, 25, and 28 and March 3, 1934 showed these averages per 100 cc. of blood: Phenol 5.2 mgms.; Amino acid N 9.6 mgms.; NaCl 440 mgms.; Red Blood Cells 2,640,000 per c.m.m. and Hemoglobin 66.3%. The operation was performed March 8, 1934 using atropine sulphate and ether as anaesthetics. The subject recovered from the anaesthesia within a short time and sought food. It continued to eat well and within a few days laid eggs. Blood examinations were made on March 18, 19, and 20 and the averages per 100 cc. of blood were: Phenol 2.5 mgms.; Amino acid N 6.3 mgms.; NaCl 325 mgms.; Red Blood Cells 1,680,000 per c.m.m.; Hemoglobin 48.0%. The results indicated a reduction per 100 cc. of blood of: Phenol 3.7 mgms.; Amino acid N 2.8 mgms.; NaCl 87 mgms.; Red Blood Cells 960,000 per c.m.m.; Hemoglobin 18.3%. The subject was bled to death from the carotid artery March 20, 1934. The autopsy showed heart, liver, gizzard and intestines normal. Tympani of intestines below gizzard to cecal openings and the oviduct was ruptured. There was no inflammation or clots in the abdomen. The results of individual blood examinations are reported in Table 15.

Table 15.

Blood studies before and after cecectomy. Phenols, amino acids and sodium chloride expressed in mgm./100 cc. of blood.

Mgm./100 cc.	2/20	2/25	2/28	3/3	Avg.
Phenol	5.9	5.1	4.6		5.2
Amino Acid N	7.7	8.6	10.7	9.5	9.1
NaCl	458	425	330	437	412
R.B.C./cmm.	2,720,000	2,800,000	2,400,000		2,640,000
Hgb. %	65.2	68.8	65.0		66.3

Mgm./100 cc.	3/18	3/19	3/20	Avg.	Change
Phenol	1.8	4.3	1.3	2.5	- 3.7
Amino Acid N	5.3	8.9	4.9	6.3	- 2.8
NaCl	340	345	300	325	- 87
R.B.C./cmm.	1,600,000	1,600,000	1,840,000	1,680,000	- 960,000
Hgb. %	46.6	42.0	46.0	48.0	- 18.3


Sub. No.
Mgm./100cc
Number of days
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 71 86 88 90 92 130 132 134 136 138 140
10
9
8
7
6
5
4
3
2
1
8967
8955
3245
22
7
40
16705
6
2930
16670
CHART I.
Blood examinations on subjects before and after operation showing effect of cecectomy upon levels of free blood phenols.
indicates day of operation.

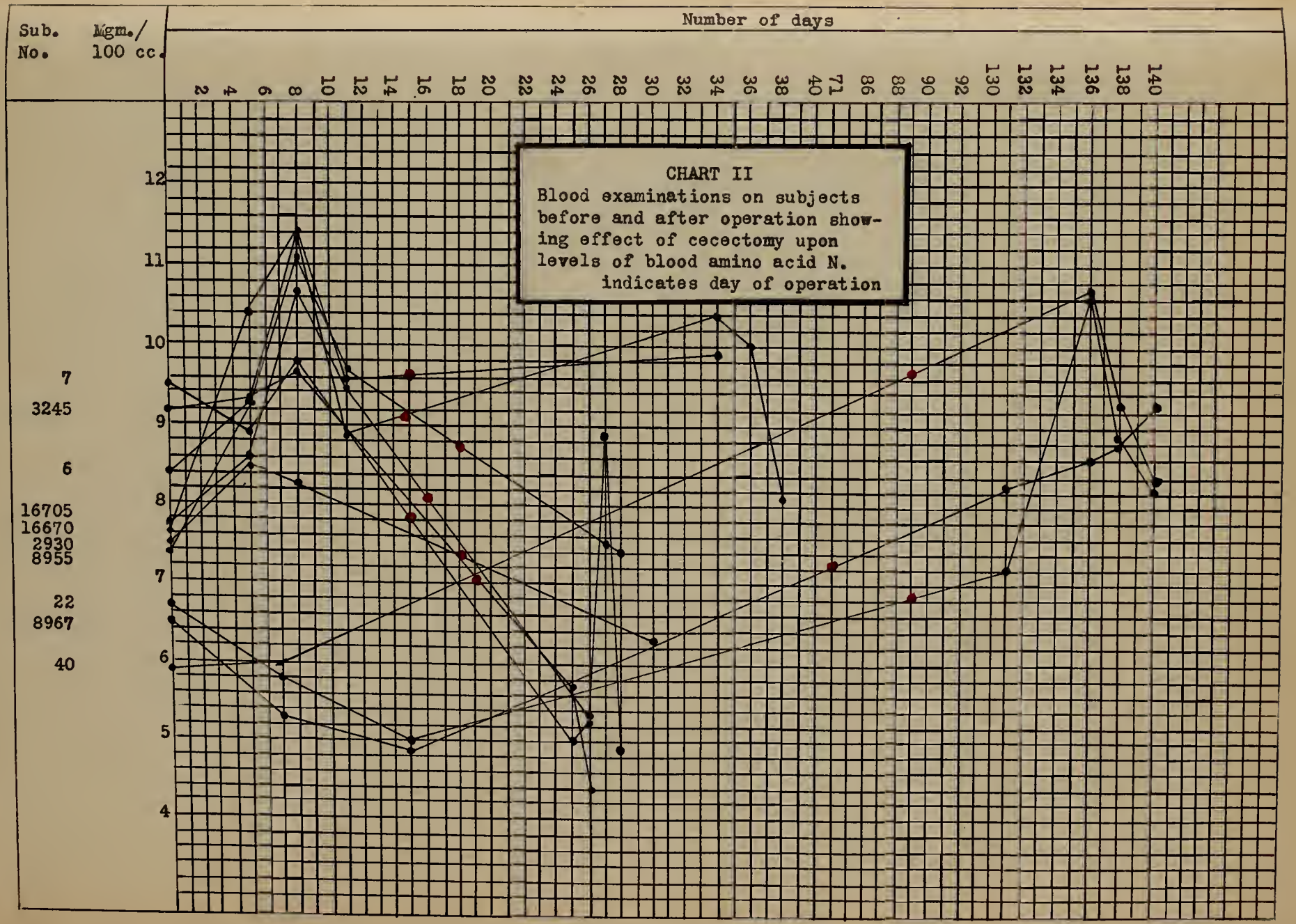
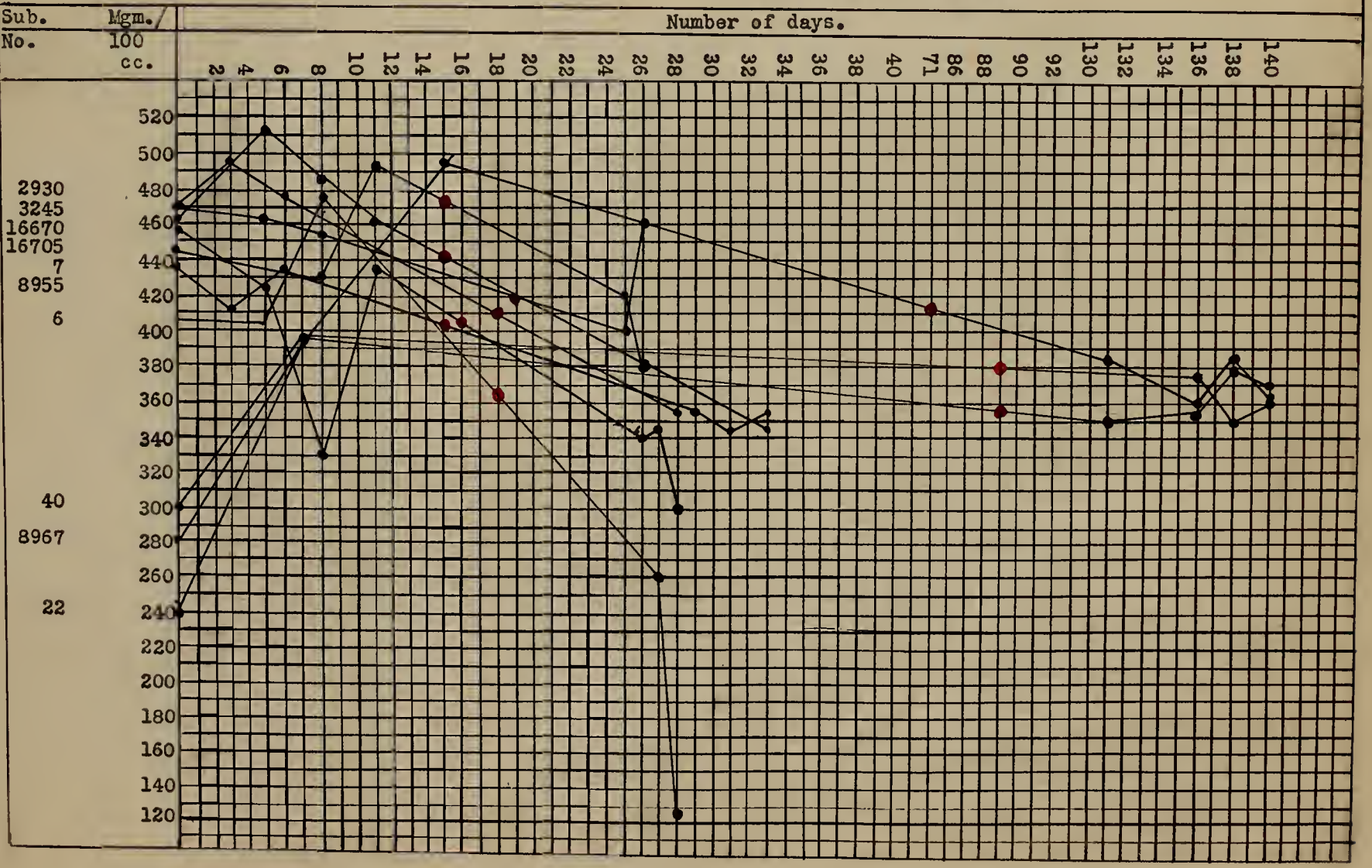

Sub. No.
Mgm./ 100 cc.
Number of days
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 71 86 88 90 92 130 132 134 136 138 140
12
11
10
9
8
7
6
5
4
7
3245
6
16705
16670
2930
8955
22
8967
40
CHART II
Blood examinations on subjects before and after operation showing effect of cecectomy upon levels of blood amino acid N.
indicates day of operation

CHART III. Blood examinations on subjects before and after operation showing effect of cecectomy upon levels of blood sodium chloride. indicates day of operation.



Discussion of experimental work on the physiology of the ceca of the avian subjects.

The experimental work on the subjects preliminary to the studies on the intact animals indicate the levels or limits of the chemical and physiological substances determined in the blood. These values are only of importance to the end of studying the limits of these substances in the blood, whereas the immediate application of these studies to the physiology of the ceca has been made by means of the cecectomized subjects. In making comparisons in these studies, most of the material must be drawn from work on human subjects.

Phenols.

Rakestraw[16, 17], Folin and Denis[9] have established phenol limits in human subjects of 1.9 to 8.0 mgms. per 100 cc. of blood. The limits obtained from 74 determinations on 23 subjects used in the preliminary part of this investigation are 3.1 to 10.7 mgms. per 100 cc. of blood as recorded in Table 1.

The intact subjects before cecectomy showed levels of from 3.2 to 9.1 mgms. per 100 cc. of blood. These values are well within the normal limits established in the preliminary work. Following cecectomy, the blood analyses of all subjects reveal a marked reduction in free phenol content. The limits observed on these studies were 1.3 to 4.3 mgms. per 100 cc. of blood. In the individual cases the average reductions were 1.7 to 4.7 mgms. per 100 cc. of blood. This data is presented in Tables 6 to 15 and in Chart I. These results indicate that

the ceca play some part in the intermediate metabolism of phenol. That this function is disturbed over a long period of time is evidenced by subjects #22, #40 and #8967, which were bled for post operative blood examination from 45 to 60 days following cecectomy. A possible explanation for this condition is offered by Folin and Denis[21] who claim that blood phenols are diminished by conditions accelerating the passage of intestinal contents. Removal of blind sacs as the ceca, into which intestinal contents are evidently pushed, may be similar to acceleration of the passage of intestinal contents. This operation, removing as it does a possible absorption area of the body, may be a comparable condition to a mild form of starvation. If this contention is plausible, Dubin's[14] statement that starvation reduces phenol excretion by the kidney in man may offer an explanation for the results obtained in this investigation.

Amino Acids.

The 32 blood analyses on 9 subjects studied preliminary to the investigation as to the physiological value of the ceca disclose normal limits of 7.6 to 11.8 mgms. of amino acid nitrogen per 100 cc. of blood as recorded in Table 2. Van Slyke and Meyer[23] and Folin[24] state the amino acid nitrogen content of human blood is 5 to 8 mgms. per 100 cc. Bock[34] reports that the amino acid nitrogen content of turkey blood is approximately 20 mgms. per 100 cc. Even though Bock's determinations have been criticized by Blau[35], the facts are evident from this investigation that the amino acid content of avian blood is higher than human.

The blood amino acid nitrogen determination of the case subjects prior to the operation show limits of 5.0 to 11.4 mgms. per 100 cc. of blood. After cecectomy the blood analyses of the intact subjects disclosed limits of 4.4 to 10.6 mgms. per 100 cc. of blood. (Tables 6 to 15 and Chart II). In subjects #6, #7, #2930, #3245, and #16705, a reduction of amino acid content of the blood was apparent. The averaged results indicated these reductions to be between 1.7 and 4.3 mgms. per 100 cc. of blood. Subjects #22, #40, #8955, #8967 and #16670, on the other hand, show an increase in amino acid nitrogen.

Most investigators(25, 26, 27, 28) agree that the amino acids of the blood vary little unless a general wasting of the body is in progress. It is believed(22, 23, 25, 26) that protein ingestion may transiently raise the amino acid level of the blood a few milligrams per 100 cc. Edgar(26) contends that fasting blood gives lower blood amino acid nitrogen values than does blood under normal conditions. On this basis, the removal of the ceca may, by removing such an absorption area, be comparable for a time to a lowering of the fasting levels of amino acids in the avian blood. This may possibly explain the reduction of amino acid nitrogen content in the blood of some of the intact subjects. As on subjects #22, #40 and #8967, post operative blood examinations were made 45 to 60 days following the cecectomy, it is probably that a readjustment of the absorption of the intestines took place. The increase or maintenance of the normal amino acid content of the blood in subjects #8955 and #16670 is difficult of interpretation, although it is indicative of a transient effect upon these levels by cecectomy

since these birds underwent the same time period from operation to post operative blood examinations as did #6, #7, #2930, #3245 and #16705. It is possible that the readjustment of the former subjects to a normal condition was more rapid than in the latter.

Chlorine as NaCl in avian blood.

The 63 blood examinations for chlorides on the 23 subjects preliminary to the investigation on the intact subjects yielded results which, when studied, showed that the limits of NaCl were 198 to 528 mgms. per 100 cc. of blood. These values are recorded in Table 3.

The subjects studied prior to the operation revealed NaCl limits from 240 to 495 mgms. per 100 cc. of blood. The NaCl limits following the operation upon these subjects were between 125 and 460 mgms. NaCl per 100 cc. of blood. The averaged results, both before and after cecectomy in these intact subjects, presented figures showing a reduction in blood NaCl from 3 to 236 mgms. per 100 cc. of blood. These conditions obtained in all instances except in subjects #22 and #40, which were examined for blood NaCl 30 to 60 days following the cecectomy. The results of these studies are shown in Tables 6 to 15 and Chart III.

Although for a time at least the subjects were maintained in bodily function at a lower level of Cl^- concentration of the blood, they continued in normal activity as was evidenced by a continual appetite and laying of eggs. Therefore, it obtains that the acid-base equilibrium in the body was not upset to a great extent by the lower Cl^- concentration of the blood, for chloride makes up two thirds of the plasma ions.

The normal consumption of CO_2 in the body forming H_2CO_3 is necessarily neutralized by $NaHCO_3$ immediately to prevent H^+ ion increase. The bicarbonate increases in the plasma is due indirectly to the chloride, because the HCO_3^- could not have been transferred from cells to plasma if the plasma had no Cl^- anions to exchange for them. The chlorides by making such an exchange possibly play an important part in the interchange of buffering effects between richly buffered cells and poorly buffered plasma. Thus, any marked derangement in the chloride balance of the body would seriously effect the health of the bird.

Furthermore, as the subjects maintained their general state of health, such bodily functions as that of the kidney must have played a part in maintaining body balance of electrolytes within narrow limits.

The removal of the ceca may be compared to an intestinal obstruction in the sense that removal of these organs obstructs the passage of intestinal contents into them. The obstruction of the passage of the NaCl into this absorption area, for a time at least, will lower the amount of NaCl absorbed by the body. Thus the acid-base balance in the body is maintained at a lower level as is shown by general healthy state of all subjects. Gamble and McIver[48] state that the lower the intestinal obstruction, the less evident is the chloride decrease and bicarbonate excess in the blood. This is due, they maintain, to the alkaline nature of the secretions the lower down one goes in the intestinal tract. On the contary, in avian subjects studied in this investigation, the cecal contents were definitely acid, pH 6.1, as is shown in Table 17. Therefore, it is probable that in the avian subject the contention of Gamble and McIver[48] does not hold.

It is to be expected, then, that due to the acid condition of the intestinal contents in the region of the ceca, NaCl absorption is effected by removal of these organs.

The fact that subjects #22, #40 and #8967 attained approximately pre-operative normal levels within 45 to 60 days indicates a readjustment of the absorption of NaCl by the alimentary tract.

Erythrocytes and Hemoglobin.

The limits of erythrocytes in the avian blood, as established by the work done by Kaupp(4), Cook and Dearstyne(50) and Forkner(51), are from 2,000,000 to 4,000,000 per c.m.m. of blood, and hemoglobin from 57% to 100%. The results obtained on the preliminary part of this investigation of 55 examinations of 23 subjects are 2,400,000 to 3,400,000 red blood cells per c.m.m. and hemoglobin 47.5% to 75%. The results are within the limits described by the previous authors.

The blood counts and hemoglobin percentages obtained on the subjects preceding cecectomy are 2,160,000 to 3,280,000 erythrocytes per c.m.m. and hemoglobin 50% to 80%. Following cecectomy there was slight variance from operative levels in the erythrocyte count (Tables 6 to 15). Hemoglobin or respiratory pigment remained quite constant while the cell content dropped to some extent. This indicates the maintenance of the physiological chemical balance of the hemoglobin in the blood.

Since the reduction in amounts of hemoglobin and erythrocytes was varied and not complementary, it is possible that mechanically the operation may have interfered with hematopoiesis in some instances.

BACTERIOLOGY

Bacteriological investigation of intestinal contents requires methods of procedure which will bring out within limits the predominant microbial forms. The methods selected will be described later. These methods established, the project resolved itself into two phases. First, the identification of species of bacteria representative of those found in the ceca of subjects kept under conditions of housing and feeding established for the experiment. The question then is, what can be learned, under the standardized conditions of this experiment, concerning the bacterial flora of the ceca of avian subjects fed on a standard fat, carbohydrate, and protein metabolic mixture, living in an environment which would be maintained for the time of the complete investigation. Second, the differential bacteriology completed, it was proposed to study the predominance and distribution of the different forms. The prominent types of bacteria are to be studied for their biochemical activities, insofar as these activities might, through the metabolic end products, produce such substances as phenols and amino acids which have been discussed in connection with the physiological studies. Thus it is desirable to identify the production of phenols and amino acids with the powers of certain species to produce these compounds by their properties to deaminize, decarboxylyze, proteolyze and peptolyze the more complex food substances found in the cecal substrate.

Other species of microorganisms identified which did not possess to a significant degree the enzymatic powers stated above, would furnish information and be significant concerning the cecal contents as a medium of bacterial propagation.

It was hoped that any data obtained would be a contribution to the bacteriology of the ceca and incidently substantiate the findings of other workers in the field of the bacteriology of the large intestine and its appendices.

Subjects for study.

The domestic fowl of an adult age was used for these experiments. The subjects were varied as to breed, sex, and place of origin, and were all from Pullorum tested flocks. They were subjected without exception to the same conditions of running ground, feed and freedom of movement.

Procedure for preparing cecal contents for study.

The surgery involved in the cecectomy has been described. The ceca were removed aseptically and placed immediately into sterile Petri dishes for subsequent examination.

The organs were opened with the aid of sterile forceps and scalpel by slitting the wall longitudinally for about 2 to 3 cm. Using the tip of a scalpel a small amount of the cecal contents were removed and placed in two small covered glass dishes, the first containing 20 cc. of sterile saline and the second 20 cc. of distilled water. The contents were then thoroughly emulsified with the aid of a sterile wire loop.

At this point in the examination of the cecal contents, an attempt was made to correlate putrefaction as defined by Rettger[52] with malodors associated with the production of indol, skatol and mercaptan. The observations reported show that in some of the cecal contents studied as to

the above conditions that there is very definitely a lack of putrefactive odors. Subjects #7, #2930, #3245, #8955, #16670 and #16705 gave ammoniacal sweet odors while in subject #6 only a very slight acid odor was observed.

An electrometric pH determination was made on the distilled water emulsion of the contents. The apparatus used was the standard Leeds and Northrup model using quinhydrone and a calomel electrode.

Methods of bacteriological study of the cecal contents.

Smears were made of the above emulsion and stained according to Hucker's modification of the Gram staining technic. The percentages of Gram positive and negative forms were recorded.

A loopful of the suspension was inoculated into a tube of cooked egg meat medium. The medium was heated at 80° C. for 10 minutes to kill the aerobic non-spore formers. The oxygen tension was then reduced by the use of the Wright plug technic. The tube was then incubated at 37° C. for 48 hours. This technic was used as a presumptive test for proteolysis by anaerobic spore formers.

Varied culture methods and procedures as described below were carried out under both anaerobic and aerobic conditions as established by this experiment at 37° C. for 48 hours. Reduced oxygen tension for plates under this condition was obtained by placing 25 gms. pyrogallic acid and 250 cc. of 40% NaOH in the bottom of a 2500 cc. Scheibler desiccator. Blood agar plates were streaked with a loopful of the cecal contents suspension to furnish a substratum for propagation of Gram positive and hemolytic forms. Endo's agar plates were poured after

inoculation with a loop of the suspension for preliminary differentiation within the coli-salmonella-typhi-alkaligenes groups. Plain nutrient agar plates were poured with the same amount of inoculum for propagation of any other forms such as the higher bacteria which would not grow well on the other media.

Following the incubation period all different types of colonies were transferred on to plain nutrient agar slants for cultural characteristics and morphological studies. These transfers after a growth period were again transferred into Krumwiede's triple sugar medium. This medium was used as it gave a good picture of the fermentative powers of the organisms. For further differentiation specific acid and gas production was determined by the use of double barreled fermentation tubes containing broth and Andrade's indicator in which dextrose, lactose and sucrose were used singly.

Differentiation within the colon-aerogenes group was carried out with a few modifications of modern technics. Clark and Lubs phosphate broth was used for the methyl red and Voges-Proskauer reactions, the latter being carried out by O'Meara's modification. Escherichia coli was differentiated from Escherichia communior on the basis of reaction to sucrose. Escherichia coli was differentiated from Escherichia acidilactici on basis of the salicin reaction.

Thus the aerobic microorganisms were identified as to species on their morphological, staining, cultural and fermentative properties.

The identification of spore forming anaerobes was carried out under three main procedures: first, a presumptive test in Dorset's egg meat medium; second, isolation of organism through plating technics;

third, purification following in part the methods devised by Hall.

In the presumptive test, the inoculated tubes were studied as to possible putrefaction by describing odors, color of the medium, and proteolytic activities. Odors such as H_2S, mercaptan and ammonia were used as indices. By the term 'color' is meant a marked change in the color of the medium, such as a deep reddening. Proteolytic activities were noticeable by the zone of digested egg-meat and liquefied state of the top of the medium.

Isolations and part of purification of the anaerobes were made by the use of Roux tubes in which was placed dextrose broth. After inoculation the constriction was blocked with a sterile rubber stopper to effect anaerobiosis. Five drops of saturated gentian violet were placed in the medium above the plug to prevent any aerobic contamination. The inoculum used was from the surface liquid of the presumptive egg-meat tubes which showed any degree of positiveness according to the criteria described above. Also the positive presumptive tubes were examined by Gram stained smears for the presence of large anaerobic type spore formers.

If any of the Roux tubes showed growth either by cloudiness or production of acid or gas and if the smears of the original inoculum showed presence of spore forms, inoculations were made by a single wire loop to plating agar enriched with dextrose and plated. These plates were incubated under reduced oxygen tension as previously described. From these plates colonies typical of known obligatory anaerobes were transferred to the Roux tubes containing dextrose broth and again purified. Examination of morphological characteristics such as to size

and spore position were made by microscopic examination of smears. If the Roux tubes showed the same reaction as to acid and gas and only the spore former subcultured was found in a stained smear, the organisms were considered isolated. The classification of the anaerobic organism into species was made by the type of odor, proteolytic powers, fermentative powers, colony formation, morphological characteristics and motility.

Identification of the higher forms of bacteria was made only through cultural characteristics on agar slants and morphology in Gram stained smears. In this manner, the yeasts, molds, actinomyces and chlamydo-bacteriales were identified. The biochemical characteristics of the above types were not considered of enough value to use in identification.

Environment of subjects under study.

Since the birds were brought from different localities, it may be assumed that because of previous environmental conditions that the intestinal flora may be correspondingly varied. It was realized that a set of conditions of diet must be established before a comparable bacterial picture might be obtained. Consequently none of the ceca of the subjects were removed until the subjects had been under the established conditions at least three weeks during which time a comparable flora in all subjects was expected.

Discussion of methods employed.

The work of MacNeal, Latzer, and Kerr(53) has established a technic for quantitatively determining the relative numbers of Gram positive and

Gram negative organisms in the feces of healthy men. This method is modified from that of Klein and Eberle. The technic is similar to the cover glass method of making a blood smear. MacNeal, et. al.[53], have determined approximately the numbers of bacteria per unit weight of feces. They state that Gram negative forms are as high as 70%, the other 30% being made up of organisms staining positive or indifferently to Gram's method. Strasburger[54] using a gravimetric method determined more accurately the per cent by weight of bacteria in feces but arrived at nearly the same results as MacNeal and his coworkers.

The determination of the Gram positive or negative organisms in excreta, however, leaves one still in question as to the specific types and species of organisms found in the large intestine. The investigations in differential bacteriology as applied to large intestinal contents have proceeded along two avenues. The first of these is concerned purely with examination of feces for isolation of transmissible disease organisms involved in the intestinal tract; and the second to the end of studying the bacteriology per se and insofar as it affects the host by the biochemical actions of the microorganisms. This investigation deals only with the second consideration.

All of the methods of isolation of microorganisms from intestinal contents are dependent upon certain fundamental factors. The first of these is the establishment of a proper medium or media for bringing out all types to be found. The second factor is the viability of the bacteria present in the contents , which is all important since some organisms might be crowded out by others through products of metabolism or merely over growth.

In the light of the above factors, the author is of the opinion that fixed rules for specific methods of isolation of intestinal bacteria cannot be set forth, since each experiment in this field involves a unique set of conditions and thus dictates its own methods of approach. The historical literature concerning methods of isolation of intestinal bacteria is voluminous and varied. However, those methods which are most applicable to the problem at hand will be discussed.

MacNeal, Latzer and Kerr[55] state that only a small percentage of bacteria present in fecal contents can be determined. They advocate the use of agar and gelatin plates enriched with simple sugars for both anaerobic and aerobic forms. Also, the use of sugar broths for determination of relative presence or absence of gas producers is advocated. Kendall[56] advocates the inoculation of intestinal contents into sugar broth for enrichment before plating. This is a good plan if the weaker organisms are not over grown. Gage[57] working on the fecal and intestinal contents of the domestic fowl of various ages used agar and gelatin plates under both aerobic and anaerobic conditions as well as egg-meat medium under reduced O_2 tension for anaerobes. Litmus milk and differential sugars were used for classification of types found. Torrey[58] suggests the use of Endo's medium for inhibition of Gram positive forms and enriched media for various types as acetic acid media for aciduric forms. He used blood liver agar for Gram positive forms and blood liver glucose agar under anaerobic conditions for spore forming anaerobes.

Krumwiede and Kohn[59] describe a triple sugar modification of Russell's double sugar media for preliminary differentiation as to the

fermentative powers of microorganisms found in the intestinal tract. Webster[60] in examination of intestinal flora of mice used dextrose fermentation tubes, litmus milk and gelatin for aerobic organisms. For anaerobes he uses kidney ascitic fluid, potato, cooked meat and glucose blood agar plates.

Torrey[61] makes these suggestions for the analysis of fecal flora: for aerobes, brom-cresol agar plates and glucose blood agar plates under reduced O_2 tention for anaerobes. This author also uses cooked meat in tubes as a presumptive test for spore forming anaerobes and putrefactive forms.

Menes and Rochlin[62] use agar plates enriched with dextrose, lactose, or blood as well as Endo's and Conradi-Drigalski medium. For anaerobes in fluid media they use a vaseline seal. For reducing O_2 tension for plating methods, these authors use a desiccator with pyrogallic acid and potassium hydroxide.

From the technics described above, it can be readily seen that a specific series of best methods is impossible to take. However, the author has endeavored to make use of those methods that appear most advisable under the conditions of this experiment. In handling as large a number of birds in as short a time as the conditions of the project required, it was impossible by the means at hand to exhaustively study the bacterial flora of all birds. It was, however, not necessary to do this as the project requires only the representative type species found.

Discussion concerning effect of diet on changing intestinal flora.

In the early part of this bacteriological discussion, it was stated that by subjecting the birds to a standardized set of conditions immediately upon arrival at the place of the experiments, the constant environmental and feeding conditions would be expected in the course of time to change the cecal flora from a relatively varied one in each set of birds to a comparatively constant one in all birds.

The topic of changing the intestinal flora has been under much discussion for many years. From the work by Metchnikov on changing the intestinal flora by feeding lactic acid and later the use of the lactic acid organisms, many authors, such as Rettger and his coworkers and Kendall and his confreres, have tried the effect first of feeding large amounts of certain bacteria and second the effect of changing of the diet on large intestinal flora. Kendall and Herter[63] tried the use of Bacillus bulgaricus feeding on animals with the results that feeding did not change the bacterial flora in the region of the cecum. These two workers[64] then tried the effect of diet upon alteration of the intestinal flora. The conclusions drawn from these experiments were that a protein diet was conducive to a proteolytic flora and carbohydrate diet to one of acidogenic type. At this time began the real study of changing the flora. By this is meant the biology and biochemistry of the bacteria in relation to diet and changes in intestinal flora. Kendall[65] assumed that the sparing action of carbohydrates for proteins could be used to rid the intestine of putrefactive organisms by change of diet and introduction of lactic acid bacteria.

By this statement is meant that bacteria that attack carbohydrates are more rapid in growth than the proteolytic forms, so if an easy source of energy is supplied to the acidogenic types the protein will not be attacked. Rettger and Hull[66] working on rats found that the intestinal flora was influenced by diet. Rats fed white bread and green vegetables showed a similar flora to man. The feeding of milk and lactose made Bacillus bifidus the prominent organism. A bread, vegetable, milk and lactose diet caused the predominant types to be Bacillus bifidus and Bacillus acidophilus. They concluded that the feeding of bacteria even in large numbers had little effect unless the diet was changed. These authors[67] later showed that lactose feeding had a more rapid effect than milk on changing the flora from a proteolytic to aciduric type. It remained however for Torrey[58] to set forth the factors controlling the intestinal flora. His four factors are as follows: first, chemical character of diet as to the availability of decomposition products; second, completeness of digestion and absorption of food in the small intestine; third, rapidity and degree to which food residues are eliminated; fourth, symbiosis and antagonism between groups. Torrey[58] discusses many types of food as to their effects upon changing the flora of dogs. One of these foods in particular is of significance to this problem. He found a high protein vegetable diet suppressed the putrefactive types and stimulated the fermentative organisms. Cannon and Dragstedt[68] confirmed the work of Hull and Rettger[67] that diet had a marked effect upon changing the bacterial flora in the intestines of rats. Cannon and McNease[69] in

TABLE 16.

Percentage Gram + or Gram - forms determined by microscopic examinations of stained smears from cecal content suspension.

Subject No.	Gram +		Gram -	
	Number	%	Number	%
2	57	66.3	29	33.7
6	30	31.6	65	68.4
7	48	36.6	83	63.4
18	191	52.4	210	47.6
22	183	40.2	272	59.8
40	152	47.2	170	52.8
42	79	40.7	115	59.3
43	285	47.7	312	52.3
44	64	38.3	103	61.7
45	157	65.6	103	34.4
47	69	53.0	59	47.0
2930	66	28.3	167	71.7
3245	114	37.0	194	63.0
8955	58	29.8	137	70.2
16770	39	29.7	123	75.3
16705	110	34.8	206	65.2
Total		674.2		925.8
Avg.		42.1		57.9

TABLE 17.

Hydrogen Ion Concentration of cecal contents expressed in pH Values

Subject No.	Cecum	
	1	2
6	5.8	5.8
7	6.0	6.0
2930	6.2	6.2
3245	6.2	6.2
8955	6.2	6.2
16670	6.2	6.2
16705	6.2	6.2
Avg.	6.1	6.1

TABLE XVIII. OCCURRENCE OF DIFFERENT ORGANISMS IN CECA OF INDIVIDUAL SUBJECTS.

	Subject Number																							Percentage.
	1	2	6	7	18	19	19W	22	36	39	40	42	43	45	47	2930	3245	8955	8967	16670	16705			
N. catarrhalis	1				1	1									1	1	1	1	1				8	
G. tetragena	1				2	1			2	2					3	1		1					13	
StreptococcusX				1												1				1			3	
St. albus	4			1	1			2		5			3		12				1	1			30	
St. citreus																	1						1	17.5
M. cereus				1	2		1		1	4						1				1	1		12	
M. luteus															1								1	
S. lutea						1	1											1					3	
S. aurantiaca			1														1						2	
E. coli	5	15	3	7	3	12	11	7	11	6	7	7	3	4	3	8	8	9	10	12	7		158	
E. acidi lact.				1												6	8		2	7	2		26	
E. communior	1		7	6	4		1	3			5	1		8		7		11	1	6	6		67	61.4
A. aerogenes																				2	2		4	
Alk. fecalis																				1			1	0.2
B. subtilis										1				1	3								5	
B. cereus										1			2	1		1	3	1					9	
B. tumescens		1				2	2		1	6	1			1	4			1			1		20	
B. simplex										1													1	
B. fluorescens													1										1	16.4
B. megatherium																				1			1	
B. ruminatus	2	1							2	1			1	2	3				1				13	
B. mesentericus					1	2				2			2						1		1		9	
B. fusiformis					1		2	1	1			1	2		1								9	
Cl. sporogenes										1													1	0.2
Act. asteroides						2			3										1				6	
Lep. ochracea															1		1						2	
D. ferruginea				1				1												1			3	
Sp. dichotoma										1										1			2	4.3
T. rosea	1									1													2	
P. glaucus					1		1			1					2								5	
																							418	
	1	2	6	7	18	19	19W	22	36	39	40	42	43	45	47	2930	3245	8955	8967	16670	16705			

TABLE XVIIIA. COMPARISON OF PERCENTAGES OF GRAM POSITIVE OR NEGATIVE FORMS BY:

Differentiation	Stained Smear
% Gram + 38.6	42.1
% Gram - 61.4	57.9

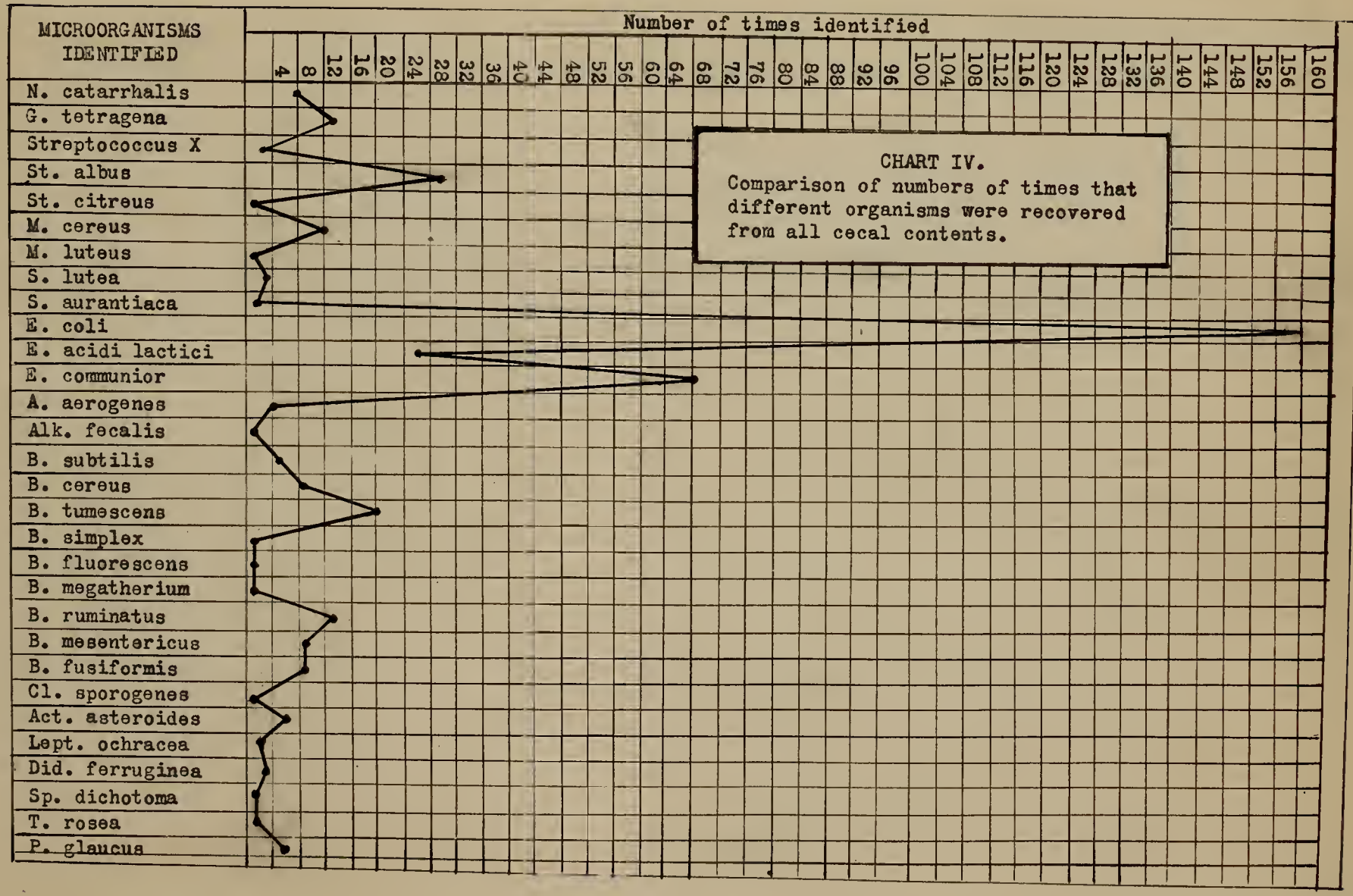
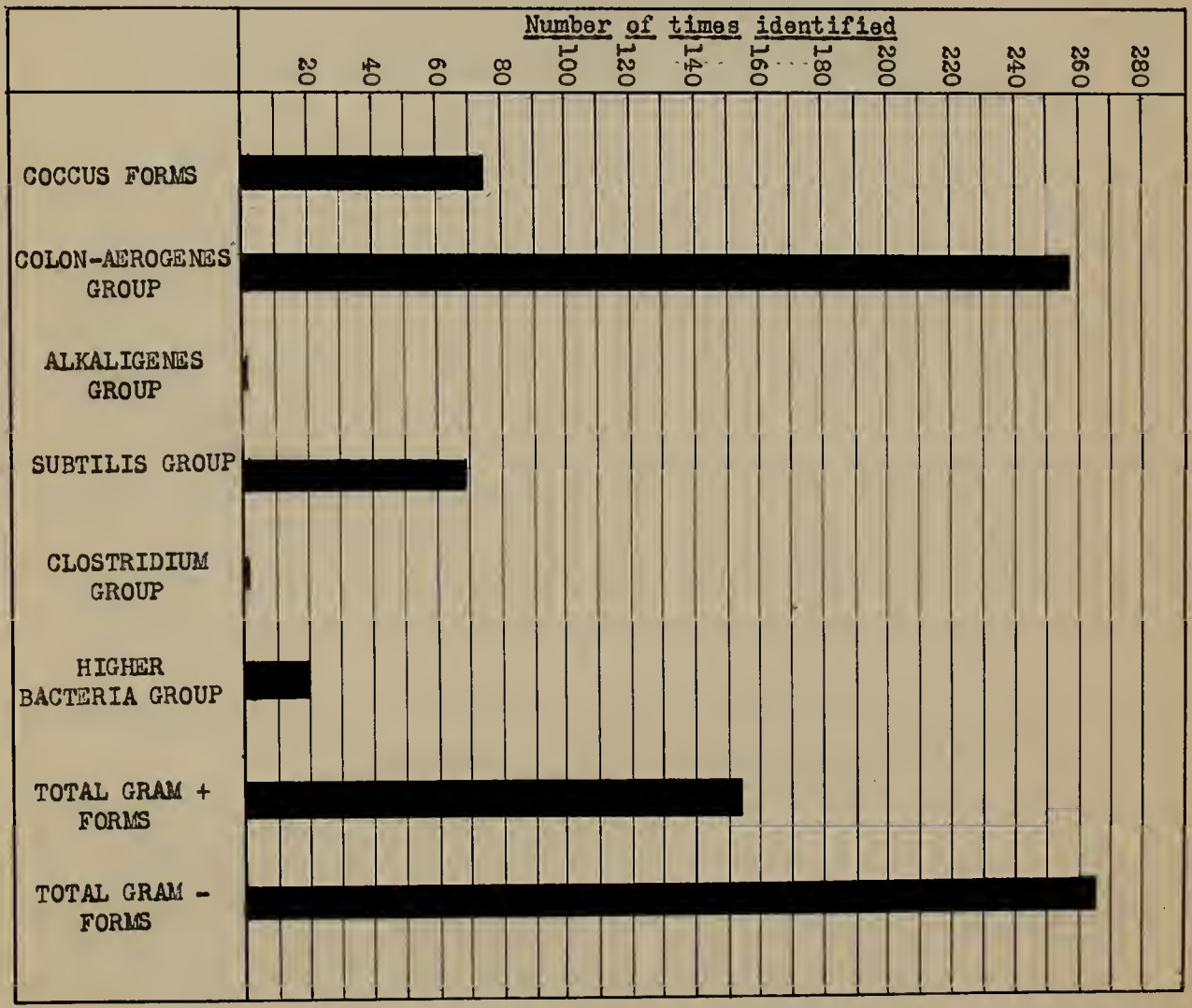


CHART IV.

Comparison of numbers of times that different organisms were recovered from all cecal contents.

FIG. I. Number of times that organisms were recovered from all ceca, arranged by groups. Comparison of total Gram + and Gram - forms recovered from all ceca.



studies on the pH of various parte of the intestine found in opposition to Rettger and Cheplin[70] that the pH played an important part in influencing the intestinal flora of white rate. Cannon and McNease[69] showed that a pH of 7.0 was characteristic of gas producing proteolytic types whereas an increasing acidity is characterized by diminution of proteolytic types and replacement by aciduric types. The cecum in rate was found to be the most acid place in the colon and the changee in the bacterial flora took place here to a large extent. Hudson and Parr[71] bear out the work of Cannon and McNease[69] and in opposition to Rettger and Cheplin[70] state that the pH of the cecal contents give a better indication of the pH of intestinal contents than does the pH of the fecal contents.

From the above literature, the author believes he is juetified in his original contention that by establishing a standardized set of living conditions and feeding it would be possible to effect a change in the varied flora of the birds of different groups to a comparative flora in all birde subjected to the estabiiehed experimental conditions.

Diecussion concerning the bacteriological findings.

Through the aid of the literature on this eubject, it will be attempted to ehow the results obtained by other inveetigators in the field of intestinal bacteriology as it applied to animale and, in placee, to the domestic fowl.

In this connection we come upon the work of Kern[72]. This worker identified 21 species of microorganisms in the intestinal contents of

24 birds. Among these species were found a large number of spore formers which liquefied gelatin. Escherichia coli was found to predominate. Rahner[73] in making examinations of the intestinal flora of the domestic fowl found Escherichia coli, Gram positive cocci, molds, Bacillus megatherium and lactic acid bacilli. He believed Escherichia coli to be the common inhabitant crowding out others whose presence and metabolic activities depend upon the food consumed by the host. King[74] found the flora of the intestinal mucosa of birds was constant to a certain extent. The mucosa of the cecum and colon were inhabited by a greater number of species than any other portion of the intestinal canal. Escherichia coli was found to be the predominating organisms in the ceca and colon. The observations of Gage[57] on the flora of intestinal and fecal contents show that Escherichia coli and coli type organisms predominate throughout; the other organisms were found to vary and to be large in number of types. He also found 60% of organisms did not stain by Gram's method and that under ordinary conditions 40% of the organisms were Gram negative. This worker finds in the ceca of chicks and birds up to two years of age chiefly forms of subtilis, colon-aerogenes, Staphylococcus albus and citreus groups, as well as a few of the higher bacteria. He found spore forming anaerobes to be very rare and suggests that this be due to the vegetative nature of the diet. Torrey[58] in feeding experiments upon dogs discovered that the intestinal flora varied. He found that whenever the substrate was fermentative, Bacillus acidophilus, Bacillus bifidus, enterococci and Bacillus welchii were present. On a putrefactive substrate, Clostridium sporogenes, Bacillus maligni oedematis (Koch), Clostridium putrificus, Bacillus mesentericus, Bacillus proteus, Bacillus pyocyaneous,

Bacillus fluorescens and staphylococci were noted. Escherichia coli was present under either condition. Menes and Rochlin[62] in studying the flora of the intestinal tract of the domestic fowl observed only a few species of bacteria which were constant in all parts of the lower alimentary tract. Emmel[75] in examining the flora of the feces of the domestic fowl encountered Escherichia coli and Escherichia communior as constant inhabitants. This group was estimated to take up 60% of the types isolated. The spore forming anaerobes identified were Bacillus bifidus, Clostridium sporogenes, and Bacteriodes liquefaciens.

Discussion concerning the experimental work in connection with the bacteriological investigation.

The results of the Gram stained smears of the cecal contents of 16 of the avian subjects are set forth in Table 16. The percentages obtained, Gram positive forms 42.1% and Gram negative forms 57.9%, are within range of the estimations made by Emmel[75] and Gage[57].

Although it is impossible to determine with accuracy the exact number of organisms of different species, an approximation of the relative numbers of each group of organisms is possible to obtain as is seen in Table 18. The predominance of organisms of the coli group is evident. The percentages of each group of organisms are also given in this table. The coli forms are estimated as 61.4%, the cocci as 17.5%, the subtilis as 16.4%, and the higher bacterial forms including yeasts and molds as 4.3%. It is noteworthy that only one sporulating anaerobe was present, Clostridium sporogenes. As the diet contained vegetable

material in its entirety, it is expected that a putrefactive flora would not be in evidence[58]. Gage[57] also found very few sporulating anaerobes in his studies. The uniform established conditions of this experiment would pre-suppose this condition as well since the diet and running grounds were a constant factor. The pH of the intestinal contents is known to have a marked effect upon the character of the bacterial flora. Cannon and McNease[69] and Hudson and Parr[71] observed that a pH above 7.0 effected a putrefactive flora, whereas one more toward the acid range was indicative of a fermentative flora. The pH of some of the cecal contents studied in this connection reveal an average pH of 6.1 (Table 17). Thus according to the above authors, this latter pH would be indicative of a non-putrefactive bacterial flora which was found to be the case in this investigation.

In Chart IV is expressed the relationship between the numbers of times the organisms were identified in all cecal contents. The predominance of Escherichia coli is to be noted. This observation is in agreement with the work of Rahner[73], Kern[72], King[74], Gage[57], Menes and Rochlin[62] and Emmel[75].

Figure 1 records in comparative ordinates the numbers of times the various groups of organisms were identified in the cecal contents. The second part of this figure represents a comparison of the total Gram positive and Gram negative forms identified.

Discussion of the metabolic end products of organisms identified in the cecal contents.

The experimental work has accorded definite data concerning the groups and species of organisms found in the cecal contents of the avian subject. It is the purpose now to study the microorganisms from the standpoint of their biochemistry and metabolic end products. This is to be done with special regard for the powers of the bacteria to metabolize proteins and protein derivatives to the end of producing amino acids and phenol. Through this material it is expected that some correlation may be established between the presence of the organisms with the special properties previously assigned and the change following cecectomy in the blood levels of amino acids and phenols.

The production of amino acids from protein is an established fact. It is known as well that phenol may be produced from such compounds as tyrosine and phenylalanine. The production of these two compounds by enzymatic action of bacteria is considered possible by many workers. The literature concerning the specific proteolytic powers of all but a few organisms is meagre. Although most of the data concerning these powers is in terms of gelatin liquefaction, the author does not attempt to suggest that all organisms possessing gelatinase are proteolytic for all proteins. It will be necessary to assume that the organisms identified that do possess a proteolytic enzyme decompose to a greater or lesser degree some of the proteins present in the cecal contents for the production of amino acids.

According to Ford(76), Bergey(77), or Kendall(78), the following microorganisms identified in this investigation have proteolytic powers

for some proteins and protein derivates: Staphylococcus citreus, Staphylococcus albus, Sarcina lutea, Sarcina aurantiaca, Alkaligenes fecalis, Bacillus subtilis, Bacillus cereus, Bacillus tumescens, Bacillus simplex, Bacillus fluorescens, Bacillus megatherium, Bacillus ruminatus, Bacillus mesentericus, Bacillus fusiformis, Clostridium sporogenes, and Sphaerotilus dichotomus. According to the same authorities, these organisms possess peptolytic powers, i.e., Escherichia coli, Escherichia acidi-lactici, Escherichia communior. The remainder of the bacteria found in the cecal contents have neither proteolytic or peptolytic powers according to any information available and are to be considered as purely environmental forms.

The discussion concerning the production of phenols from such compounds as tyrosine by the decarboxylizing and deaminizing powers of certain bacteria is taken up fully in the section pertaining to that subject. It is sufficient to note here that but few organisms, namely, Staphylococcus citreus, Staphylococcus albus, Escherichia coli, Escherichia acidi-lactici, Escherichia communior, are accorded the above described powers(13, 76).

Discussion of the bacteriological studies.

The object of the identification of microbial forms in terms of predominance in the cecal content was to establish the significance of their occurrence with their metabolic activities.

The experimental evidence in favor of a non-putrefactive flora is strong since no malodorous contents were observed and only one

anaerobe was identified. The evidence obtained through Gram stained smears correlated within a few per cent with relative percentages of groups of organisms identified (Table 18A). This data is indicative of a predominantly colon group flora. The organisms of the remaining groups are chiefly Gram positive aerobes of the cocci and subtilis forms. These two groups of organisms are not putrefactive or proteolytic to the extent of the sporulating anaerobes. Therefore, any change in the amounts of amino acids or phenols in the blood of the intact subjects following cecectomy will have to be correlated with the metabolic powers of the above groups of organisms. It is possible to expect some degree of change in the blood content of the compounds studied since they are produced to some degree by the organisms found in the cecal contents. Therefore, a removal of the ceca, its contents, and bacteria present would also remove a possible source of amino acids and phenols.

SUMMARY

The physiological significance of the ceca in living subjects has been studied through the aid of the domestic fowl. It has been the purpose to study the levels of phenols, amino acids, and sodium chloride in order to obtain evidence to support any conclusions that might be reached concerning the part played by the ceca in alimentation.

Röseler[79] indicates that the ceca are organs for absorption of liquids and amines, and the place of digestion and bacterial decomposition of crude fiber. These studies were carried out by means of chemical analyses of the feed and the fecal excreta, the absorption being in terms of difference between the two analyses. The author believes that since the exact function of the organs is not known the question as to whether or not absorption takes place in the ceca can only be answered by way of blood studies through cecectomy. An analysis of the excreta chemically is too empirical and inexact a method for proper interpretation of function.

If there is absorption through the cecal membranes, evidence supporting this will be in the form of reduction of amounts of phenol, amino acids, and chlorides studied in the blood following cecectomy.

The levels of phenol were established between 3 and 10 mgms. per 100 cc. of blood. The trend toward reduction in amounts of blood phenol following cecectomy is represented in Chart I. The amino acid nitrogen levels were from 7 to 12 mgms. per 100 cc. of blood. There was some

reduction in the blood amino acids following cecectomy (Chart II). Blood sodium chloride levels were established between 198 and 528 mgms. per 100 cc. Following cecectomy a reduction of blood content of sodium chloride took place to the extent of from 3 to 236 mgms. These results are recorded in Chart III in which the trend is represented.

The erythrocyte count in the avian subjects in this investigation was between 2,400,000 and 3,400,000 per c.m.m. and the hemoglobin content was from 45 to 70% (Tables 4 and 5). That cecectomy influenced hematopoiesis to some extent is shown in Tables 6 to 14. The operation did not markedly influence the hemoglobin content of the blood. This observation does not indicate any great re-arrangement in the physiological chemical structure and maintenance of the hemoglobin level in the blood (Tables 6 to 14).

All evidence obtained in this investigation points to an effect upon the levels of phenols, amino acids and sodium chloride by cecectomy. The most of this data on intact subjects was obtained within 12 to 18 days following the operation.

As to whether the removal of the ceca has a transient or permanent effect upon absorption in the body is shown to some extent on subjects #22, #40, and #8967. These subjects were bled for post-operative blood examination 45 to 60 days following operation and with the exception of free phenol content of the blood exhibit approximately the same levels of amino acid nitrogen and sodium chloride as preceding the operation. There is not enough evidence from this investigation to say that cecectomy has a transient effect or not upon intermediate metabolism in the avian subject. An answer to that problem could only

be obtained by study of the subjects over a much longer period.

Since normal body function such as feeding, oestrus, and ovulation were not interfered with, the inference is that cecectomy has not influenced cellular activity in these general directions. Therefore, these case studies show that the removal of the absorbing surface afforded by the ceca has played its part in influencing the quantitative levels, for some time, of the blood phenols, amino acids and sodium chloride. This unquestionably demonstrates the absorptive powers of the cecal membranes to support the thesis that these organs physiologically play an important part in alimentation.

The bacteriological studies reveal a correlation between the percentage of Gram positive and Gram negative forms observed in smears and the organisms recovered from the cecal contents (Table 18A and Table 16). These results bear out the work of Gage[57], King[74], Emmel[75] and others[72, 73] in that Gram negative forms in the avian intestinal contents are approximately 60% and Gram positive forms 40%.

The microbiological examination of the cecal contents revealed organisms of coccus types, as the genera Neisseria, Gaffkya, Streptococcus, Staphylococcus, Micrococcus, and Sarcina; rod forms, as genera Escherichia, Aerobacter, Alkaligenes, Bacillus and Clostridium; higher bacterial groups as Actinomyces, Leptothrix, Didymohelix, Sphaerotilus, and Torula; and molds as the Penicillium.

The phenol producing organisms such as the colon-aerogenes and staphylococcus forms found in the ceca are present in the greatest

percentages of all organisms identified (Table 18, Chart IV, and Figure I). The organisms of the coccus and subtilis groups which can break down protein and its derivatives for the production of amino acids are present second only in numbers to the colon-aerogenes types.

One sporulating anaerobe was found out of all the microbial types identified. This fact indicates a non-putrefactive flora and accounts for the sweet odor (page 47) and low pH (Table 17) of the cecal contents.

Upon removal of the ceca are also removed all of the bacterial types which are responsible for the production of phenol and amino acid. It is concluded, then, that the phenol and amino acid bacteria do have a function in part at least in producing these compounds to be absorbed through the cecal membranes.

Therefore, since these organs have a similar histology to, absorb similar products as, and possess a similar bacterial flora to the large intestine in the avian subject, the ceca apparently are supplementary absorptive areas to the large intestine and support the intermediate metabolism of this relatively short part of the alimentary tract.

CONCLUSIONS

Under the conditions of this investigation and according to the methods employed, the author makes the following conclusions:

1. Removal of the ceca in the domestic fowl decreases the absorption area for phenols even over a period of 45 to 60 days; of amino acids over a period of 12 or more days; and of sodium chloride over a period of 12 or more days.

2. Re-establishment of normal levels of blood amino acids and sodium chloride occurs after a period of 45 to 60 days.

3. Hematopoiesis is interfered with to some degree by the cecectomy, although hemoglobin content is only slightly disturbed.

4. The removal of the ceca reduces the absorptive surface of the alimentary tract but in no way interferes with other important physiological functions.

5. Thirty species of bacteria were identified; approximately 40% of the forms were Gram positive and 60% Gram negative.

6. Sporulating anaerobes indicating putrefaction of protein compounds were markedly absent in the cecal contents.

7. Of the thirty species of microorganisms, 17.5% cocci, 61.4% colon-aerogenes, 0.2% alkaligenes, 16.4% subtilis group, 0.2% clostridia, and 4.3% of higher bacteria, yeasts and molds, were found.

8. Of the species found, 61.4% were known phenol producers and 34% were known producers of amino acids.

9. The bacteriological work supports that of others that the flora of the avian intestinal tract under normal conditions is not strongly putrefactive in nature.

10. The data obtained in this investigation points to the conclusion that the ceca are supplementary areas to the large intestine and play a similar part in absorptive function.

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