THE PHYSIOLOGY OF THE MAMMARY GLAND.

Thesis for the Degree of M.D.

by

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THE PHYSIOLOGY OF THE MAMMARY GLAND.

In Man the mammary glands are immature at birth and remain so until the time of puberty when the glands in the female begin to grow rapidly and assume their adult proportions, coincident with the onset of the ovarian functions. With each recurring oestral period there is a slight and transitory enlargement of the gland. With the commencement of pregnancy a great development of the gland substance takes place, and becomes more rapid as pregnancy advances. With the termination of pregnancy, growth ceases and secretion begins and continues for many months. These changes are all prevented if the ovaries be removed and these organs must be primarily responsible for the growth of the mammary glands, at any rate prior to the time of conception. After conception the ovaries, uterus, and products of conception, foetus and placenta, must be taken into account as possible sources of a stimulus to be carried to the mamma.

The possible means of conveyance of this stimulus are two, (1) by nerves, (2) by the blood stream. Until within recent years the controlling factors in this series of changes had been investigated almost entirely from/

from the nervous side. It was thought that the stimulus to hypertrophy of the mamma was a reflex one from the uterus and ovaries through the central nervous system, and this view Basch* still holds. This theory was negatived by an experiment of Goltz and Ewald*. They removed the spinal cord of a bitch from the lower dorsal region downwards. Pregnancy followed coitus and terminated with successful parturition. The mammary glands hypertrophied and the puppies were suckled normally. Routh* reports the case of a woman, in whom the spinal cord was shown at a post mortem examination to be completely destroyed at the level of the seventh dorsal segment, and in whom the breasts enlarged during pregnancy, lactation was normal and the quality of the milk was good. Mironow* found that section of the mammary nerves in a pregnant animal did not prevent hypertrophy of the glands, nor did it interfere with their function after parturition.

Ribbert* made a very conclusive experiment on the mamma, showing its independence of nerve impulses. He transplanted the mammary gland of a guinea pig from its normal/

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* Ergebnisse der Physiologie Vol. 2. p.325, 1903.
* Archiv. f.d. ges. Physiol., Bonn, 1896 Bd.LXIII, S. 362
* Trans. Obstet. soc., London 1897.
* Archiv. des Sciences Biol. de St. Petersburg, Vol.III, 1894.
* (Ribbert) "Ueber Transplantation von Ovarium, Hoden und Mamma" Arch. f. Entwick. Mechanik, Vol. VII 1898.

normal situation to the region of the ear. This gland enlarged when pregnancy took place and lactation followed delivery.

Pfister* performed a similar experiment on a rabbit with like results.

It is a well known fact that the secretion of milk can be profoundly affected by emotional states such as grief or fear, which diminish or may altogether stop the secretion.* It was inferred that the secreting cells of the mamma were regulated in their activity by nervous impulses. Eckhard* divided all the nerves supplying the udder in a goat, and found that the secretion was uninfluenced either in quantity or quality. In other experiments he divided the nerves going to one side of the udder and found no difference in the milk obtained from the two sides. Röhrig* found that stimulation of the nerves to the mamma diminished secretion, section increasing it. Mironow* likewise found that stimulation of the nerves caused a reduction in secretion, but he states that section also causes a diminution, which comes on gradually after a few days. He considers that/

* (Pfister) "Ueber die reflektorischen Beziehungen zwischen Mamma und Genitalia Muliebria" Beiträge zur Geb. u. Gynal., Vol. V. 1901.
* Williams "Obstetrics", London 1904.
* Beitr. z. Anat. u. Physiol., Giessen 1855, Bd.I, S12, 1877, Bd. VIII, S. 117.
* Virchow's Archiv. f. Path. Anatomie 1876, Bd. 67, S.119.
* loc. cit.

that these effects may be due to interference with the vaso-motor fibres and alteration of the blood supply of the gland. In his experiments he also found that stimulation of sensory nerves diminished secretion, and that this effect was not obtained when the mammary gland was isolated from the central nervous system.

There is some plain muscular tissue around the lactiferous ducts and in the nipple, and this doubtless functions in the discharge of the milk, that around the ducts compressing them and driving on the milk, while that in the nipple may act by stiffening this structure and so holding open the orifices of the ducts.* The sucking of the young animal is however the chief factor in the discharge of milk. Hellier* describes a "nipple reflex". Firm compression of the nipple and areola for a few seconds produces contraction of the plain muscular tissue and erection of the nipple. He thinks that the pressure of the infant's lips normally excites this reflex, and during lactation causes an ejection of milk.

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* Schäfer "Mechanism of secretion of Milk"
Text Book of Physiol., Vol. I, Edin. 1898.

* Brit. Med. Jour. Nov. 1896.

EFFECT OF INTERNAL SECRETIONS ON THE GROWTH OF THE MAMMARY GLAND.

From the experiments mentioned above it is certain that the blood contains a substance or substances, which act as stimulants to the hypertrophy and subsequent secretory activity of the mammary gland. The growth of the gland at puberty and its temporary enlargment at the oestrous period are due in all probability to an excitant having its source in the ovary. Halban* removed the ovaries from the guinea pig shortly after birth and found that the mammary glands did not develop. If the ovaries were removed from their normal position and grafted into another site, the mamma developed normally. After conception the ovary increases greatly in size, chiefly on account of the formation of the corpus luteum of pregnancy, and the question naturally arises whether the great mammary hypertrophy of that time may not be associated with the increased ovarian activity. The corpus luteum may be considered as a ductless gland producing an internal secretion, whose function is to control the nutrition of the uterus, and to/

* "Ueber den Euifluss der Ovarien auf die Entwickelung des Genitales" Monatschr f. Geburt. u. Gynak., Vol. XII, 1900.

to prepare its mucous membrane for the reception of the ovum.* The growth of the corpus luteum and the mammary gland do not run parallel. The growth of the former is most marked during the first half of pregnancy, and at mid term it attains, in the human female, a length of about an inch. In the second half of pregnancy there is a retrogression, and at full term the corpus luteum is but half an inch in length*. The growth of the mamma is comparatively slow in the first half of pregnancy, the greater part of the growth taking place during the second half. Arcel and Bouin* state, however, that there is, in the rabbit, a parallelism between the growth of the corpus luteum and the development of the mammary gland. Numerous cases have been recorded in Man in which double cophorectomy has been performed during the course of pregnancy, without any interference with mammary hypertrophy or subsequent lactation. The removal of ovaries in pregnant rats has been done with similar results*. Cows which have been castrated during lactation continue to give milk for years, and may in the aggregate yield more milk than cows/

* Marshall Physiol. of Reproduction. Chap.IX, London, 1910.
* Marshall loc. cit. Chap. IV.
* "La Developpement de la glande mammaire pendant la gestation est determinè par le corps jaune". C.R. de la soc. de Biol., Vol. LXVII, 1909.
* Marshall and Jolly "Contributions to the Physiology of Mammalian Reproduction". Phil. Trans. B. Vol. CXCVIII, 1906.

cows calving annually in the usual way.* It is thus clearly shown that the ovaries are not essential for mammary hypertrophy during pregnancy nor for lactation.

Miss Lane-Claypon and Starling* carried out a series of experiments with a view to determine the presence and source of the hormone causing mammary growth. They used virgin rabbits, into which they injected, intraperitoneally, extracts of various tissues of pregnant animals of the same species, uterus, ovaries, placenta or foetus. With the first three extracts they obtained negative results, but with foetal extract, they found, after from 15 to 30 injections had been given, that well marked changes had been produced in the mammary glands, which were considerably hypertrophied and showed active proliferation of the duct epithelium, with commencing formation of alveoli at the margins of the gland. Boiled foetal extract was found to be as efficacious as unboiled. Thus there was determined the presence of a specific mammary hormone in the foetal tissues, but the site of formation in the foetus was not ascertained, extracts both of the viscera and of other tissues giving positive results. The production of/

* Marshall "Physiol. of Reproduction" Chap. IX.
* "An experimental inquiry into the factors which determine the growth and activity of the mammary glands." Proc. Roy. Soc., Vol. 77, p.505, 1906.

of the hormone will increase in quantity with the growth of the foetus, hence mammary hypertrophy is greatest as the end of pregnancy approaches. The hormone may also act to some extent on the mammary glands of the foetus, for there is a growth of the glands during the last month of intrauterine life and there may be some secretion for a few days after birth. Foa* has shown that the hormone is not specific for one species of animal, for he obtained similar results by the injection of extract of ox foetus into a virgin rabbit.

Halban* states that the placenta is the active agent in causing mammary growth during pregnancy. Ziedl and Königstein* have confirmed the results of Lane.-Claypon and Starling. They injected extracts of placenta and of foetus into virgin rabbits, and obtained a positive result only with the foetus.

As regards the onset of lactation it has been suggested* that this is the result of the removal of the stimulus, the foetal hormone, which during pregnancy has occasioned the growth of the glands, the hormone having/

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*"Sui fattori che determinano l'accresimento e la funzione della ghiandola mammaria."
Arch. di fisiol. Vol. V. p.520, 1908.
* Arch. f. Gynäk. Bd. 75, S. 353.
* Geitschr. f. exp. Path. u. Therapie Vol. VIII, p.358, 1910.
* Hildebrandt "Hofmeister's Beitrage" Vol. V. p.413, 1904.

having given rise to anabolic changes, and its removal allowing of katabolic changes and the formation of a secretion. D'Errico* has performed experiments to show the presence in the blood of pregnant animals of a substance inhibitory to lactation. He injected into lactating bitches the defibrinated blood of pregnant bitches and found that thereby the secretion of milk was much diminished. In Man there is mammary secretion if the pregnancy be terminated at any time after the third month. In rabbits with a gestation period of 28 - 30 days, removal of the uterus and ovaries at any period after the 15th day causes the production of mammary secretion*. Foa* states that the injection of foetal extract has no inhibitory influence on the secretion of milk.

Against the foetal hormone theory cases have been recorded in which milk was secreted by males, virgin girls and by a woman far past the menopause*. In the Monotremata, which are oviparous, and in which the embryo does not enter into any connection with the uterine wall, there is lactation. It is suggested in this case that the mammary stimulant diffuses through the porous/

* "Sul determinano dell'attivita funzionale delle glandole mammarie". La Pediatria No. 4,1910.
* Lane - Claypon and Starling. loc. cit.
* loc. cit.
* Knott "Abnormal Lactation" Amer. Med. Vol.II, 1907.

porous egg shell and so reaches the uterine mucous membrane where it is absorbed into the circulation.

Lombroso and Bolaffic* have performed experiments on rats by the procedure of parabiosis, suturing rats together in pairs so that a vascular anastomosis was established between the two. They performed numerous experiments of which few were successful. They joined together a virgin female rat and one in an early stage of pregnancy. After three days the pregnant animal showed commencing changes in the mammary glands, whereas after nine days there was no change observed histologically in the glands of the virgin. Two pregnant animals of 20 days difference of impregnation were joined together. When the first was at term and with full mammae, the second showed no change from the ordinary virgin state, and there was no change to be seen in the second when the first began to lactate.

Morpurgo* performed parabiosis of a pair of rats, a male and a pregnant female. During the pregnancy of the female no changes were to be observed in the male mammae.

I have performed the operation of parabiosis of rats/

* "La parabiosi e la questione dei fattori che determinano la funzione mammaria e l'insorgenza del travaglio di parto".
Atti della soc. Ital. di Obstet. e Ginec.
Vol. XV, 1909.

* "Ueber Parabiose künstlich vereinigter Warmblutter"
Munchener Med. Wochenschr. 1908, n.4.

rats several times, but without any successful results.

The case of the pygopagous twins Rosa-Josepha Blazek is a most interesting one in this connection and strongly supports the hormone theory of mammary growth. When these twins were in Edinburgh some months ago, I saw them and made inquiries concerning them. The twins are united by a common sacrum, the iliac bones being separate. There is a common anus, vulva and urinary meatus. There are two uteri and two vaginae. Rosa became pregnant and during the pregnancy there was amenorrhoea in both. In both, the mammary glands hypertrophied and after parturition produced secretion.

EFFECT OF INTERNAL SECRETIONS OF ORGANS UPON THE SECRETION OF MILK.

It is generally held that the onset of lactation is determined by the removal at parturition of an inhibitory hormone produced by the foetus. The question of the existence of hormones, which act as stimulants to the milk flow thus set up, has next to be investigated. Bouchaccurt* states that there was an increased production of milk in women fed on sheep's placenta. Basch*/

* Compt. rend. Soc. de Biol. Feb. 22nd, 1902.
* Monatschr. f. Kinderheilk Bd. VIII, S. 513, 1909.

Basch injected placental extract into a dog and established a flow of milk in an animal a month after its lactation period had terminated. Lederer and Pribram* made direct observations on the action of extract of placenta on the milk flow. They inserted fine catheters into the milk ducts of the goat and gave intravenous injections of placental extract of animals of the same species. By this method they found, (1) that the intravenous injection of a fresh extract of placenta caused, in from three to seven minutes, a great increase in the rate of milk secretion, (2) that the extract gradually weakens on standing for a time, and altogether loses its power when warmed to 65°C., (3) that the placenta has a specific action in producing this stimulant, ovarian extracts giving doubtful and liver extracts definite negative results, (4) that large doses of the fresh extract not only caused increased milk flow but also intravascular coagulation and death of the animal.

Fraenkel* ascribes to the corpus luteum an influence in stimulating lactation. Ferroni* found in a rabbit/

* Pflügers Archiv. f. Phys. Vol. 134 p. 153, 1910.
* Arch. f. Gynäk. Bd. 68, S. 438.
* "Le attuali questione sulla secrezione mammaria". L'annali d'ost. e ginee. XXXI April, 1909.

rabbit, which had failed to lactate after previous pregnancies, that the injection of an extract of corpus luteum after a parturition resulted in a plentiful secretion of milk.

In a pamphlet by Ott on "Internal Secretions" there is inserted at page 123 a typewritten slip dated October 28th, 1910, and to the effect that "Infundibulin is a rapid and powerful galactagogue". A month later Ott and Scott* read a paper on the "Action of infundibulin on the mammary secretion." They experimented on a goat in the early lactation period. They inserted cannulae into the nipples, applied external pressure to the glands, and used the suction of a water aspirator to draw off the milk. They found that when infundibulin (the active principle of the posterior lobe of the pituitary body) was injected into a subcutaneous vein, there was a rapid and great increase in the production of milk. The milk was caught in a graduated flask and measured periodically. In one experiment they found an increase from 4 to 405 drops per five minutes, immediately following the injection of/

* Proc. Soc. for Exp. Biol. and Med.
New York, Dec. 21st. 1910, p. 48.

of infundibulin. This great effect rapidly passed off. The dose was repeated 30 minutes later with a resulting increase from 4 to 75 drops per five minutes. The commencement of the increased flow was about one minute after injection and the maximum was reached at four minutes, after which there was a rapid fall to normal.

At the same time these authors read a communication on the "Galactagogue action of the thymus gland and the corpus luteum"*. They found a four fold increase of milk flow in five minutes with intravenous injection of corpus luteum, thymus gland or pineal body. Extract of ovary minus corpus luteum had no effect.

In my series of experiments the animals used were cats, at varying stages of lactation. The animals were anaesthetised with chloroform and then kept under with subcutaneous injections of chloral hydrate. A trachea tube was inserted and connected to an artificial respiration pump. The blood pressure in the carotid was recorded by means of a mercurial kymograph. A vein tube was inserted into the external jugular vein for the purpose of injection of the extracts. Various methods/

* loc. cit. p. 49.

methods were tried for the determination of the amount of milk flow. In the cat there are from three to five pairs of mammary glands, the posterior pair being usually the most actively functioning. In the experiments one pair of glands, or from 20 - 30% of the whole mammary tissue was used for the estimation. The first means tried was that of inserting fine glass cannulae into the nipples, the tips of which had been cut off. These cannulae were led off to an electro-magnetic drop recorder. With this method it was found that the milk did not flow at all freely into the cannulae and it was given up. Another method was that of inserting a large hollow needle into the gland so that its point lay just beneath the nipple. This method was found to answer fairly well in cases where the mammae were well developed and the milk secretion free, but it was not satisfactory in small mammae with little secretion.

The most satisfactory method and the one chiefly used was an exudation method. In this a transverse incision was made into a pair of mammae just behind the nipple, the incision passing nearly through the mammary tissue. The main ducts were then cut across as they converged towards the nipple. There was as a rule

free/

free bleeding at first but this was easily stopped by pressure forceps and the oozing soon ceased. There was also a free exudation of milk from the cut glandular surface, but within ten or fifteen minutes this became reduced to an occasional drop. In the incision was then laid a strand of absorbent wick, the end being carried over the side of the animal so that the drops from the wick might fall on the electromagnetic recorder and be registered by it on the smoked paper of a revolving drum. This method had the disadvantage that a certain amount of secretion was lost by evaporation from the exposed raw surface and from the wick. It had, however, the advantage that it eliminated any action of the plain muscular tissue of the milk ducts and the nipple in producing milk flow. During the period of lactation the alveoli secrete milk not only whilst the gland is being drawn by the process of sucking or milking, but also in the intervals of such processes, and the milk then produced accumulates in the alveoli and ducts.* It is possible in the first method that part of the milk flow caused by certain injections might be due to stimulation of the muscular tissue, contraction of which would drive out the milk which had been/

* Schäfer Text book of Phys. Edin. 1898. Vol. I, p.663.

been accumulating in the reservoirs of the milk ducts in the ordinary ccurse of continual secretion. This possible scurce of fallacy is obviated by the exudation method.

METHOD OF PREPARATION OF SUBSTANCE USED FOR INJECTION.

In most cases fresh organs were taken, chopped up and ground thoroughly in a mortar with sand and then Ringer's solution was added and well mixed with the paste. The whole was then boiled, so that the proteids were coagulated, and filtered, the filtrate usually being sterilised by again boiling in a flask. The solutions were in most cases made of such a strength that 5 ccs was equal to ·5 gram. of the fresh organ. In other cases the solutions were of greater strength, and in others again e.g. pineal and pituitary, the solution was made up independent of weight, but in proportion to the number of glands contained.

In the case of pituitary and pineal glands, the glands were dried before being extracted. Alcchclic extracts were used in some instances e.g. pituitary and/

and corpora lutea. These were made by grinding the substance with sand and absolute alcohol, filtering and evaporating the filtrate to dryness, the residue after evaporation being dissolved in Ringer's solution and boiled. The organs were taken from lactating or non-lactating animals of the same species, but in some instances the organs of other species were used.

DESCRIPTION OF AN EXPERIMENT.

A freely lactating cat, weight 3 kilos., was anaesthetised with chloroform and then ·75 gram. chloral hydrate 1% solution was injected subcutaneously, followed 30 minutes later by ·5 gram more chloral, after which scarcely any chloroform was used. Artificial respiration was used, and blood pressure taken from the carotids. A vein tube was inserted into external jugular. An incision was made across a pair of mammae and the secretion led off by a wick to the drop recorder. A large hollow needle was inserted into the substance of one of the hindmost pair of mammae so that the point lay just beneath the nipple, and the drops from/

from it fell on to a second drop recorder. After about half an hour, when only an occasional drop was falling from the wick, the injections were begun. In all cases the dose of the substance was contained in solution in 5 ccs. of Ringer's fluid, and unless otherwise specified the extract of the organ had been made with the same fluid and subsequently boiled. The injections used were as follows:-

(1) Extract of one thymus gland of a lactating cat. No effect produced.

(2) Extract of 1 gram of the involuting uterus of a lactating cat. Fifteen seconds after the commencement of the injection, milk began to flow from the needle and the flow was so rapid for 30 seconds that the drops could not be separately recorded by the dropper, at least from 40 - 50 drops falling, after which the flow suddenly ceased.

At about the time of the conclusion of this flow drops began to fall very quickly from the wick. (Part of the delay is to be accounted for by the time taken for the fluid to pass along the wick). The flow continued rapid for 5 minutes after which drops fell at regular/

regular intervals for fully half an hour, at first at 20-second interval and then at 60-second, fully 100 drops falling during the whole period.

The effect was unaccompanied by any change in the blood pressure. Greater fulness of the mammary vessels was observed during the active secretion, and slight oozing of blood from the cut surface. This latter had no appreciable effect upon the dropping as the amount was not more than sufficient to tinge the milk.

(3) Extract of one ovary of a lactating cat.

The flow after the last injection was still continuing regularly when this injection was given, and no change was produced.

(4) Extract of muscle (·5 gram) of a lactating cat.

No change produced on the dropping.

(5) Serum of a lactating cat 2·5 ccs.

No effect produced.

(6) Extract of involuting uterus (1 gram) repeated.

Forty five minutes had elapsed since the previous injection. Twenty seconds after commencement of injection there was a flow from the needle and 20 drops fell within 15 seconds, after which there was a sudden cessation. Thirty seconds from the time of injection dropping began from/

from the wick, at first so rapidly that separate marks could not be produced, then gradually more slowly. The effect lasted for fully 15 minutes and at least 70 drops fell. There was no effect on blood pressure.

An interval of 30 minutes was then taken in order that the gland might have a chance to recover itself after so great a secretion. When the injections were recommenced drops were falling only at long intervals.

(7) Cow's milk 5 ccs injected.

No effect produced.

(8) Lactose 5 ccs of a 20% solution.

No effect produced.

Another interval of 30 minutes was now taken.

(9) Extract of pancreas (1 gram) of lactating cat.

No effect.

(10) Alcoholic extract of the anterior lobe of the pituitary gland Ox ·25 gram dry gland.

No effect.

(11) Ringer extract of the anterior lobe of the pituitary body Ox ·25 gram dry gland.

A few drops of milk fell. (This was probably accounted for/

for by some contamination with or imperfect separation of the posterior lobe, as other preparations of anterior lobe in experiments gave no effect on the milk flow.) There was however no rise of blood pressure with this effect.

(12) Alcoholic extract of the posterior lobe of the pituitary body Ox (3 glands).

No effect.

(13) Ringer extract of the posterior lobe of the pituitary body Ox (3 glands).

An immediate and tremendous effect was produced upon the mammary secretion.

After a preliminary fall, the blood pressure rose considerably and the rise was sustained for several minutes.

Ten seconds after the commencement of the injection milk began to flow from the needle and this flow continued for 1½ minutes during which time 57 drops fell. The flow began during the first depression of the blood pressure. The secretion from the cut surface of the two mammae appeared nearly at the same time and ran down the wick in an almost continuous stream for some little time, so that for 20 seconds there was no separate/

separate marking of the drops. After this there was gradual slowing till at the end of 8 minutes, the drops were falling at intervals of about 40 seconds. During the period of action certainly over 200 drops fell. The flow was well maintained after the blood pressure had returned to its former height.

(14) At the end of 8 minutes the dose of pituitary extract was repeated. There was a moderate fall in blood pressure but no effect on the milk flow. Thirty minutes interval was again taken.

(15) Alcoholic extract of Corpus Luteum of Sheep .

2 Corpora Lutea.

No effect.

(16) Ringer extract of Corpus Luteum of Sheep.

2 Corpora Lutea.

No effect.

(17) Extract of Thyroid Gland of a lactating cat.

Half a gland.

No effect.

Fifteen minutes interval taken.

(18) Extract of Brain of Lactating cat 1 gram.

No effect.

(19) Extract of Pituitary of non-lactating dog.

One gland.

No effect.

(20) Extract of Pituitary of lactating cat.

One Gland.

No effect.

(21) Extract of Pineal gland of sheep.

One Gland.

No effect.

Fifteen minutes interval.

(22) Extract of Corpus luteum of sheep.

2 Corpora lutea.

There was no effect on the blood pressure.

After an interval of more than a minute, milk began to be secreted from the cut surface of the glands and a steady flow continued for an hour. At first the drops came at intervals of 5 seconds, then at 10 seconds, after 30 minutes at 20 seconds, and at the end of the hour at 40 seconds.

(23) At the end of 45 minutes an extract of one suprarenal gland of a lactating cat was injected. There was a characteristic rise in blood pressure, but no change was produced in the regular dropping of milk set up by the corpus luteum..

This terminated the experiment which had extended over a period of seven hours. This time was largely taken/

taken up by the resting intervals which were given after a galactagogue action had been obtained, in order that the mammary gland might have a chance of recovery after the activity which it had shown.

RESULTS.

By such experiments the action upon the mammary gland of the extracts of many organs was investigated. An extract was deemed to be a galactagogue when its injection was followed, in several experiments, by a well marked increase in the rate of milk production. An extract was considered to be inactive as regards the mammary gland when it had been used in several experiments and, when after its administration in an early stage of the experiment without result, the administration of one of the active substances produced a free milk flow.

Five organs were found to contain a constituent possessing the power of stimulating the mammary gland to increased secretion.

1. The Pituitary Body.
2. The Corpus Luteum.
3. The Pineal Body.
4. The Mammary Gland.
5. The Involuting Uterus.

The/

The results with the first three were confirmatory of those obtained by Ott and Scott.

1. The Pituitary Body. (Figs. 1 - 8).

An extract of this body is the most powerful of galactagogues. In these experiments the pituitary of the ox was chiefly used. The anterior and posterior lobes were carefully separated and independently investigated. The mammary stimulant is contained in the posterior lobe, extracts of the anterior lobe being entirely inactive in this respect. The active substance is not destroyed by drying, even though the dried glands be kept for several years. It is easily extracted by a weak saline solution. It is insoluble in alcohol, but is unaffected by treatment with it, saline solutions of the gland substance, previously treated with alcohol, being quite active. Repeated boiling of the saline extract does not in any way impair its activity. With intravenous injection the action is very prompt, the effect being produced in from 20 to 40 seconds after the commencement of the injection. The flow of milk rises to its maximum almost immediately, and then gradually diminishes, ceasing after from 10 to 15 minutes.

*The/

*The following is a description of the results obtained by the injection of a Ringer extract of the posterior lobe of the pituitary, the dose used in each instance being three glands in 5 ccs. of Ringer's solution. The animal used was a freely lactating cat. The first injection produced the usual blood pressure effect, a short-lasting preliminary fall followed by a moderate rise, sustained for several minutes. The galactagogue action was immediate and great. In less than 20 seconds from the commencement of the injection, milk began to run freely from all parts of the cut surfaces of the two mammae used. A maximum was reached in a few seconds and the flow was very abundant, a steady stream down the wick, so that for 60 seconds the individual drops could not be recorded. Then the flow slackened and gradually diminished and at the end of 5 minutes, there was an interval of 30 seconds between the drops. The amount of milk produced by the two mammae during the action was 15 ccs. The flow was independent of the increase in blood pressure, commencing during the preliminary fall, and continuing after the raised pressure had again returned to normal.

A/

* Figs. 1,2,3.

A resting interval of 20 minutes was given to the gland and the dose of pituitary was repeated. There followed the usual fall of blood pressure produced by a second dose of pituitary extract. The galactagogue action began 50 seconds after the injection was made. The amount of milk was small in comparison with that following the first dose, but the flow was a free one. A maximum was reached after 15 seconds and the action continued for five minutes, about 80 drops falling.

Another interval of 20 minutes was taken and a third dose was given. After 70 seconds milk began to flow, a maximum was reached in 40 seconds, the action lasted for five minutes and about 40 drops fell.

In other experiments the pituitary of the cat was used, and an attempt was made to compare the galactagogue power of extracts of the gland in lactating and in non-lactating animals, but no definite result was obtained to show greater activity of the gland in the lactating animal. This, however, may be presumed. It has been noted that there is an hypertrophy of the pituitary body during pregnancy*. I have examined the/

* Comte "Contribution á l'etude de l'hypophyse", 1898.

the pituitaries of cats and have compared the weights in lactating and non-lactating females, with the result that I have found a constant increase in size during the lactation period. In six non-lactating cats the average weight of the gland was 31 milligrammes , highest 33, lowest 29. In six lactating cats the average weight was 35 milligrammes, highest 40, lowest 32. There was thus in the lactating animal an increase in weight of more than 10%.

Oliver and Schäfer* determined the presence in the pituitary body of a substance with the property of acting on the heart and blood vessels. Howell* showed this substance to be confined to the posterior or infundibular part of the body. Schäfer and Vincent* found that there are present in the posterior lobe two substances which affect the blood pressure in opposite directions, a pressor and a depressor body, the former giving a marked rise of blood pressure when a first dose of the extract is given, the latter producing a fall when a second dose is given within a short time of the first. The depressor substance is soluble/

* "On the physiological action of extracts of the pituitary body and certain other glandular organs". Journ. of Physiol. Vol. XVIII p. 277, 1895.
* The physiological effects of extracts of the Hypophysis Cerebri and Infundibular body." Amer. Journ. of Exper. Med. Vol. III p. 245, 1898.
* "The physiological effects of extracts of pituitary body." Journ. of Physiol. Vol. XXV No. 1. 1899.

soluble in alcohol, the pressor substance insoluble, and the two can thus be separated. A diuretic action of the posterior lobe of the pituitary body was found by Magnus and Schäfer*, and Schäfer and Herring*. The increased flow of urine is accompanied by increase in kidney volume. This effect is independent of the general vascular action, being prolonged after the rise of blood pressure due to a first dose has passed off, and being produced when there is given a second dose, causing fall in blood pressure. The action is a direct one, a stimulation of the renal epithelium to increased activity. Other glands were observed, pancreas, salivary and sweat glands, but in them there was no increased secretion. To the above mentioned properties of infundibular extract must now be added that of a powerful galactagogue. This action resembles the diuretic action in being independent of the general vascular effects and being produced by successive doses. The active substance is probably a direct stimulant of the secreting epithelium. It is present in the pituitary body not only of lactating females, but also of non-lactating females and of males. It is not specific to any/

* "Action of pituitary extracts on the kidney". Proc. physiol. soc. 1901.
* "Action of pituitary extracts on the kidney." Phil. Trans. Roy. Soc. B. Vol. CXCIX p.1, 1906.

any one species of mammals, nor is it even confined to the mammals, but is present also in the pituitary bodies of birds. Herring* has shown that extracts of the posterior lobe of the pituitary body of the fowl produce, on injection into mammals, effects on the blood pressure, kidney volume, and urine flow very similar to those produced by extracts of mammalian pituitary. I also have used the pituitary of the fowl, making saline extracts. Injection produced a very decided galactagogue action (Figs. 5 -6). In one experiment a freely lactating cat was taken, the milk flow from two mammae being recorded. An intravenous injection of a Ringer extract of fowl's pituitary was given, equivalent to eight glands. A slight rise in blood pressure was produced. Within 20 seconds a great flow of milk began from all parts of the cut glandular surface, pouring down in a continuous stream for 30 seconds and then gradually slowing, the effect passing off in four minutes. The amount of milk secreted in this time was 10 ccs. Other experiments confirmed this action. This result is a particularly interesting one, showing the presence in an oviparous animal of a substance capable of stimulating mammary secretion.

An/

* "A contribution to the comparative physiology of the pituitary body."
Quart. Journ. of Exp. Phys. Vol. I, No. 3, 1908.

An analysis made of the milk obtained after the injection of pituitary extract resulted as follows:-

Fat = 9.4%

Sugar = 5.26%

This was a milk very rich in fat for an analysis of normal cats' milk shows.

Fat = 3.33%

Sugar = 4.9%

In a second specimen of cats' milk obtained after injection of pituitrine (P.D.) the percentages were:-

Fat = 18·40

Sugar = 3·00

THE CORPUS LUTEUM.

In the experiments the corpora lutea of sheep were used, and were obtained from the ovaries of non-lactating animals. They were carefully separated from the ovarian tissue, and extracts were made from them, as in the case of the pituitary body, with alcohol and with Ringer's solution, the extracts subsequently being boiled. The dose usually employed for intravenous injection was the extract of two corpora lutea in 5 ccs of Ringer's solution.

The/

The following is an account of the result obtained in one of the experiments. (Figs. 10,11,31,32). The animal used, a cat, had but a moderate amount of mammary development and only a small drop of milk could be squeezed from the nipple at the beginning of the experiment. Two mammae were incised and led off with a wick. No drops of milk had fallen for fully fifteen minutes when a dose of two corpora lutea was injected into the external jugular vein. The blood pressure showed a slight and temporary fall, following the injection. After 80 seconds milk began to flow freely and 25 drops fell in four minutes, after which there were occasional drops, at intervals of about a minute, for ten minutes longer. Thirty minutes interval was allowed. The secretion quite ceased and the dose of corpus luteum was repeated. After 90 seconds the milk flow began and the effect was practically equal to that on the former occasion, 20 drops falling in three minutes, and occasional drops afterwards. An hour's interval was allowed and again the dose was repeated. The effect this time was greater than before, 30 drops falling within the space of two minutes and the latent interval this time being but one minute.

Thirty/

Thirty minutes interval was given and then a fourth dose injected, with greater effect than on any of the previous occasions. After one minute the flow began and 40 drops fell in four minutes.

The corpus luteum is thus shown to have, in addition to its regulating power over the growth of the uterus and its preparation for reception of the ovum, a distinct influence on the mammary gland, by the production of an internal secretion which acts as a stimulant to the secretion of milk. This stimulant is readily soluble in weak saline solution, it is insoluble in alcohol, but is not destroyed by treatment with it. Nor is its activity impaired by repeated boiling, nor by standing for some weeks in solution.

During lactation the corpora lutea are large and visible to the naked eye. Microscopically they resemble the corpora lutea of pregnancy, and are much larger than those developed in the absence of pregnancy or lactation. In rats they have been examined in animals at periods ranging from 36 hours to 23 days after parturition, and no diminution in size has been observed in that time.* The active substance, however, is/

* Watson "On the state of the ovaries during lactation with special reference to the luteal tissue." Journ. of Phys. Vol. XXXIV, June 1906.

is present not only in the corpora lutea of lactating animals, but also in those obtained at any other time. Intravenous injection of corpus luteum has been tried also on non-lactating cats. In one case there was a well marked flow of serous fluid following an injection, but this was not repeated in subsequent experiments.

I have made some experiments with the subcutaneous injection of extract of corpus luteum. Six virgin female rats were taken, three being used for the injection of corpus luteum, and three being used as controls, the latter being injected with Ringer's solution. The injections were continued over a period of twelve days, boiled extract of one corpus luteum of the sheep in 2½ ccs. Ringer's solution being given to each rat daily. The rats were killed on the day after the last injection, and the mammary tissue was examined. No change could be detected either naked eye or microscopically between those injected with corpus luteum and the controls.

THE PINEAL BODY.

The pineal bodies of the sheep were used. They were dried, extracted with Ringer's solution and the extract boiled. Intravenous injection causes a small but undoubted effect on mammary secretion. One gland extracted by 5 ccs. of Ringer's solution was the dose used. In one experiment (Fig. 13) in which no drops of milk had been produced for fifteen minutes, an oozing from the cut surface was observed within 30 seconds of the injection and three drops fell at intervals of 40 seconds, after which no other drops fell for fifteen minutes.

In another experiment (Fig. 12) oozing from the gland appeared within 30 seconds of the injection of a dose, and seven drops were produced and fell at gradually increasing intervals, the whole effect occupying ten minutes. During fifteen minutes prior to this injection but one drop had fallen, and for fifteen minutes subsequent to the passing of the effect no drop fell.

This galactagogue action is a surprising result, for hitherto no active function of the pineal body was known, and it was considered to be a functionless vestigial structure.*

* Dixon and Halliburton "The pineal body." Quart. Journ. Exper. Phys. Vol. II, No. 3. 1909.

THE INVOLUTING UTERUS.

An extract of the involuting uterus was found to possess a strong galactagogue action. The uterus of a cat within a week after parturition was extracted with Ringer's solution, the extract then being boiled and filtered.

The following experiment (Fig.14) illustrates its action. A freely lactating cat was taken and two mammae led off by a wick. The extract of one gram of the involuting uterus in 5 ccs of Ringer's solution was injected intravenously. Forty seconds after the commencement of injection, drops began to fall quickly and a rapid flow continued for five minutes, after which drops continued to fall regularly for half an hour, fully 100 drops being recorded during that period. The effect was unaccompanied by any change in blood pressure. Greater fulness of the mammary vessels and a slight oozing of blood from the cut surface were observed. Several other extracts were then injected without effect, and the extract of involuting uterus was then repeated, again with a most marked effect, though not so great as the first, 70 drops falling/

falling during the fifteen minutes that the action extended over.

In another experiment (Fig. 15) a cat was used which had kittened prematurely and in which the amount of milk was very small. In this case an injection was followed by the falling of 20 drops in ten minutes, a considerable effect, for no drop had fallen for fifteen minutes previously.

Here again a substance is produced in an organ, passed into the blood stream and carried to the mammary gland, where it acts as a stimulant to the secretory cells to pour out their secretion. It was not determined for how long after parturition this substance is produced in the uterus, but it was found to be absent from the uterus of pregnant and non-lactating animals.

MAMMARY GLAND.

An extract of the mammary gland of one lactating animal, when injected into another lactating animal, produces a marked increase in the rate of mammary secretion. The mammary glands of a lactating cat were used in these experiments, the gland substance being/

being extracted with Ringer's solution, boiled and filtered.

The following (Fig. 16) is an experiment illustrative of the effect produced by this extract. A cat was used, anaesthetised with chloroform and chloral. Two mammae were incised and led off by a wick to a dropper. Various tissue extracts were tried with negative results. Then an injection of corpus luteum was given and was followed by a fall of seven drops of milk. Thirty minutes interval was allowed and then there was injected the boiled extract of one gram of lactating mammary gland in 5 ccs. of Ringer's solution. This injection was followed by a slight fall of blood pressure. After an interval of 40 seconds a rapid dropping of milk began, fully 50 drops falling in four minutes. The effect soon ceased. Ten minutes later the dose was repeated and again a milk flow was obtained, this time 10 drops in three minutes.

In another experiment (Fig. 18) there was an even greater effect, 60 drops of milk being recorded within two minutes of the injection of the extract of one gram of mammary tissue.

An alcoholic extract of mammary gland failed to give/

give any galactagogue effect. Saline extract of mammary gland of a non-lactating animal was also without action. Milk, lactose and the salts of milk in concentration all gave negative results.

There is thus shown to be present in the mammary gland of a lactating animal a substance possessing the power of stimulating the secretory cells of the gland itself to increased activity. This substance is not passed out into the external secretion, but is of the nature of an internal secretion.

Ott and Scott* state that an extract of mammary gland contains a substance which has the power of increasing the extent of uterine contractions.

THE INHIBITORY INFLUENCE OF CERTAIN EXTRACTS UPON THE MAMMARY SECRETION.

Several extracts were investigated with a view to determine if they had any influence in diminishing or altogether inhibiting milk flow. It was more difficult to be sure of the results in these cases. The procedure adopted was as follows. It was found by experiment that the repeated injection of extract of corpus luteum gave practically equal results if a resting interval of thirty minutes were allowed between the/

* Journ. of Exp. Med. No. 11, 1909.

the injections. A similar result was produced by pituitrine, Parke Davis & Co's preparation of the posterior lobe of the pituitary body, and by Burrough & Wellcome's Infundibular extract, but in these cases the first result was always greater than those following, which were about equal. In the experiments to test inhibition a dose of one of the above mentioned substances was given and the result recorded. Half an hour later a dose of the extract to be tested was given, followed in a few minutes by a repetition of the galactagogue substance, and the effect again recorded. Another interval of thirty minutes was allowed and a third dose of the galactagogue was given. If the first and the third doses produced a well marked effect, and the second dose produced little or none, it was held to be shown that the extract injected immediately before the second dose had an inhibitory effect on the secretion. By this method I have found both placental extract and foetal extract to be possessed of this inhibitory power. This is in strong confirmation of the theory that hormones produced by the uterine contents prevent lactation during pregnancy, and that on their removal at parturition, milk secretion is allowed to take place.

The/

The following (Figs. 22 - 25) is the description of an experiment in which foetal extract was used. The foetus was that of a cat well advanced in pregnancy. A saline extract was made, boiled, and filtered. A freely lactating cat was taken, two mammae being led off to a dropper. A dose of 1 cc. of pituitrine was given. There was a marked rise in blood pressure and in 30 seconds milk began to run down in a continucus stream for nearly three minutes, 13 ccs. of milk being obtained. After thirty minutes interval a second injection of pituitrine was given and again a free flow resulted after 50 seconds, continued for three minutes, and 120 drops fell. Thirty minutes interval was taken and then an extract of 2 grams of foetal cat in 5 ccs Ringer was injected. One minute later 1 cc. of pituitrine was given. In 40 seconds a slight ooze of milk was observed and one drop fell. The dose of pituitrine was repeated in 90 seconds and then a somewhat freer secretion was produced, seven drops falling in a space of four minutes. Thirty minutes interval was taken and, as the supply of pituitrine was used up, 1 cc. of infundibular extract was injected. In 90 seconds drops began to fall and fell rapidly/

rapidly for four minutes, over 60 drops falling.

This experiment seems clearly to show the inhibitory effect of foetal extract, for a dose of pituitary extract given early and late in the experiment produced a free flow, whereas a double dose given between these, but immediately preceded by a dose of foetal extract, produced scarcely any effect at all. The inhibitory, like the stimulating effect of extracts, is evidently of not very long duration and had passed off before the fourth dose of pituitary extract was given.

In another experiment (Figs.26-27) 1 cc. of pituitrine was injected with a resulting flow of 80 ~~drops~~ drops. Thirty minutes interval was taken and then an extract of 2 grams of foetal cat was injected, followed in 90 seconds by 1 cc. pituitrine which produced seven drops in four minutes. Another dose of pituitrine was given at the end of that time. An oozing of milk was observed but no drop fell. In this experiment the animal unfortunately died soon after the last mentioned injection.

My results with the injection of placental extract were exactly the reverse of those of Lederer and Pribram who considered it to have a galactagogue effect.

I/

I have found it tc have an inhibitory effect. In one experiment (Fig. 21) where doses of corpus luteum had at half hourly intervals produced practically equal effects, a fifth dose, preceded by an injection of boiled extract of two grams of human placenta and one of unboiled extract of four grams of the same, produced only a slight exudation from the cut surface, insufficient to cause a drop to fall. In another experiment (Figs.20 & 28) in which a dose of pituitrine had resulted in a flow of 30 drops and then a half hour interval had been taken, unboiled extract of two grams of cats' placenta was injected, and followed in two minutes by a dose of pituitrine, the effect of which was the production of a slight ooze, and the fall of a single drop. Another dose of pituitrine was given two minutes afterwards and 12 drops of milk were produced in four minutes.

These two experiments show a definite inhibitory influence of some substance produced in the placenta. In no experiment did extract of placenta, either boiled, or fresh and unboiled, show the slightest indication of being possessed of any galactagogue effect.

The blood serum of a pregnant cat was also tested for/

for the presence in it of inhibitory substances. It was difficult to say whether it had any such effect, but if it had, it was much less than that possessed by placenta or foetus. In an experiment on a poorly lactating cat (Fig. 28) 10 ccs. of blood serum of a pregnant cat was injected intravenously. A minute and a half later 1 cc. of pituitrine was given and almost immediately there was a free flow, 27 drops falling in 80 seconds, a considerable quantity in view of the poor mammary development. It is not of course likely that there would be present in the circulation any great quantity of the inhibitory substance at one time, but that it would be constantly passed in small amount from its sources of manufacture, the foetus and placenta, into the blood stream.

NEGATIVE RESULTS.

Extracts of a great number of organs were tried and found to be without any effect upon the secretion of milk. The following is a list of those used, the organs being taken from a lactating animal unless otherwise specified.

1. Thyroid Gland.
2. Thymus Gland.
3. Suprarenal Body.
4. Spleen.
5. Ovary minus corpus luteum.
6. Uterus of a non-lactating animal.
7. Testis.
8. Liver.
9. Pancreas.
10. Duodenum.
11. Kidney.
12. Muscle.
13. Serum.
14. Brain.

With extracts of thymus, Ott and Scott obtained a galactagogue action. This observation I have been quite unable to confirm. In many experiments I have given/

given injections of thymus both of lactating and non-lactating animals, but in none of them was there produced the slightest effect upon milk secretion, though the administration shortly afterwards of pituitary extract caused free secretion (Fig. 22).

In the case of the suprarenal an attempt was made to prepare an extract of the cortical part of the gland free from the medullary substance. This was not found practicable and the interrenal body of the skate, which is the homologue of the mammalian cortical substance, was used instead, but was found to be without any influence on the mammary gland. In one experiment (Fig. 30) where a steady dropping of milk had been set up by the injection of corpus luteum, an extract of one suprarenal body of the cat was given. This caused the characteristic blood pressure rise but was without influence on the milk flow.

INVESTIGATION OF THE INFLUENCE OF NERVES ON THE MAMMARY GLAND.

The mammary gland in the cat is supplied by branches from the intercostal nerves. These carry vaso-motor nerves to the blood vessels of the gland, but it has never been demonstrated that there are definite secretory fibres running to the epithelium of the alveoli.

Various experiments were performed with the object of ascertaining whether the nervous system has a controlling influence over the secretory activity of the gland. Stimulation of the nerves to the gland with weak or strong interrupted shocks had no effect, either in producing a milk flow or in inhibiting one which had been set up by the injection of a galactagogue. Section of the nerves was also done without any effect upon the normal secretory activity or upon the action of galactagogue extracts. Stimulation of the skin of the areola and nipple, either mechanically or electrically also gave entirely negative results. No effect on mammary secretion was obtained on stimulation of sensory nerves.

From these experiments it appears that the

mammary gland is but little, if at all, under the direct influence of the nervous system. This view is strengthened by the results which were obtained on the injection of atropine and which are detailed in the next paragraph.

INVESTIGATION OF THE ACTION OF DRUGS UPON THE MAMMARY SECRETION.

There are no drugs which are known to have a definite galactagogue action. Hammarbacher* found pilocarpine to be without effect upon mammary secretion. He injected a cow with .25 gram daily without causing any increase in milk production. This finding I have confirmed. Pilocarpine nitrate (.003 gram) was injected intravenously in a lactating cat. Within two minutes there was a copious flow of saliva, and the pads became moistened with sweat, but no drop of milk was secreted. Several other drugs which act as stimulants to other glands were also used, eserine, nicotine and caffeine, but without any effect.

* Arch. f.d. ges. Physiol. Bonn, 1884 Bd. XXXIII, S. 228.

THE ACTION OF ATROPINE.

Cushny* states that atropine diminishes the secretion of mammary gland as it does that of other glands, by paralysing the secretory nerve endings in the gland. Hammarbacher* found that atropine diminished the amount but increased the concentration of the milk.

In my experiments atropine has shown no paralysing effect whatever upon mammary secretion. In an experiment (Figs. 31 & 32) on a lactating cat, doses of extract of two corpora lutea were twice given at half hourly intervals, producing a flow of 25 and 20 drops respectively. Two further doses were given at similar intervals, preceded by .00005 and .0001 gram respectively of atropine sulphate, and produced 30 and 40 drops of milk. There was no prolongation of the latent interval before secretion began. Other experiments (Fig. 33) in which pituitrine was used confirmed this result.

This absence of action of atropine on the mammary gland is in correlation with the absence of effects on nerve stimulation or section.

* Text Book of Pharmacology, 1906, pp. 280, 281.
* loc. cit.

CONCLUSIONS.

1. That the Mammary gland is, as regards its secretory activity, little if at all under the influence of the nervous system.

2. That the stimuli to activity, and to inhibition of the action of the gland, reach it by means of the blood stream.

3. That five different organs of the body produce hormones possessing the power of stimulating the mammary gland to activity. These organs are (a) pituitary body, (b) corpus luteum, (c) pineal body, (d) involuting uterus, (e) the mammary gland itself.

4. That the pituitary body is the most powerful organ in this respect, that the active galactagogue substance is formed in the posterior lobe of the gland, and that it is not specific to mammals but is present also in the pituitary glands of birds.

5. That the injection of pituitary extract causes the production of a milk exceedingly rich in fat.

6. That hormones inhibitory of mammary secretion are produced by the foetus and the placenta.

7. That none of those drugs which cause marked activity of other glandular organs have any effect on the mammary gland.

8. That atropine, as tested by this method, has no paralysing effect on the secretory activity of the mammary epithelium.

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