Hypophysectomy and Replacement Therapy in Relation to the Growth and Secretory Activity of the Mammary Gland

E. T. Gomez and C. W. Turner

(Publication Authorized May 20, 1937)

COLUMBIA, MISSOURI

TABLE OF CONTENTS

ABSTRACT

This bulletin presents the results of further studies on the endocrine control of the mammary gland. Replacement therapy with ovarian hormones alone and in combination with various purified pituitary hormones, and desiccated whole sheep pituitaries was ineffective in stimulating the growth of glandular parenchyma of the mammary gland of hypophysectomized male and female laboratory mammals. The implants of adult normal male rat pituitaries were likewise ineffective. Twenty to twenty-five daily implants of one adult male rat pituitary obtained from donors which had previously been injected with estrogen daily for 10 to 20 days stimulated complete hyperplasia of the mammary gland parenchyma of normal and castrated-hypophysectomized male and female guinea-pigs.

Hypophysectomy of lactating guinea-pigs and cats at any time during the lactation period was immediately followed by a rapid cessation of milk secretion. Unlike the crude pituitary extracts, the replacement therapy with galactin alone and in combination with thyroxine or thyrotropic hormone beginning immediately after hypophysectomy or after the complete cessation of lactation after the operation, did not prevent the rapid cessation or re-establish lactation. The injection of eschatin or adrenotropic hormone alone was ineffective in stimulating the secretion of milk. The simultaneous injection of galactin, eschatin or the adrenotropic hormone and a glucose solution prevented the cessation of lactation after hypophysectomy in the guinea-pigs, and by continued treatment lactation was maintained for periods comparable to that observed in normal parturient animals. The above factors are indispensable for the initiation and maintenance of lactation. In the light of the results presented, a discussion of the endocrine control of the mammary gland is also included.

ACKNOWLEDGMENT

Hypophysectomy and Replacement Therapy in Relation to the Growth and Secretory Activity of the Mammary Gland

E. T. Gomez* and C. W. Turner

The mammary glands undergo two distinct phases of activity during the animal's successive sexual epochs, namely (1) that of growth and development and (2) secretion of milk by the mammary alveolar epithelium. For over 30 years these changes have been attributed to the action of chemical substances or hormones. At the present time two groups are recognized; those elaborated by (1) the ovaries and (2) by the pituitary gland.

The numerous investigations following the pioneer work of Lane-Claypon and Starling (1906), Grueter (1928) and Stricker and Grueter (1928-1929) have indicated that the growth and development of the mammary apparatus is under the governing influence of the anterior pituitary gland. However, the mechanism by which the internal secretions of these glands exert their action upon the mammary apparatus is not definitely established beyond the fact that the injections of the ovarian hormones (estrogen and progestin) into male and castrate male or female mammals produce growth and proliferation of the lobule-alveolar system, while the administration of pituitary extract following ovarian hormone treatment or under favorable conditions stimulate the secretion of milk.

There is a considerable amount of evidence at the present time indicating that the anterior lobe of the pituitary gland regulates at least in part, a dozen or more of the animal's physiological functions. Thus, the question arising as to whether this gland acts directly or indirectly, through its action upon other endocrine glands, and is at least a contributing factor in mammary development and lactation, cannot be disregarded and obviously requires investigation in hypophysectomized animals.

The more recent and limited studies of the effect of estrogenic hormones on the development of the mammary gland of hypophysectomized laboratory mammals presents conflicting results. Individual studies on rats (Ruinen, 1932; Freud and de Jongh, 1935), rabbits (Asdell and Seidenstein, 1935) and guinea-pigs

(Nelson, 1935) seemed to show that normal mammary development may be obtained in the absence of the animal's pituitary. Other investigators report that in no case were mammary glands able to respond to relatively large dosages of estrogen even following long continued treatment (Reece, Turner and Hill, 1936; Lyons and Pencharz, 1936; Gomez and Turner, 1936 and Gomez et al. 1937).

Though investigators are in perfect accord in their observations on the effect produced by hypophysectomy on lactation, there occurs a discrepancy in the results obtained with replacement experiments. In hypophysectomized dogs (Lyons et al., 1933; Evans, 1934 and Houssay, 1935), cats and ferrets (McPhail, 1935), the induction of lactation has been reported to be stimulated by extracts of anterior pituitary glands containing the lactogenic hormone. On the other hand, the injection of a somewhat purified lactogenic hormone failed to initiate or to maintain lactation in hypophysectomized guinea-pigs (Nelson, 1935; Gomez and Turner, 1936). It is evident, as will be pointed out elsewhere, that this discrepancy lies in all probability upon the hormone content of the various extract preparations used by the different investigators. Further, there is an increasing amount of evidence that the secretion of the endocrine glands controlled by the pituitary gland may be involved in maintaining the functional activity of the mammary glands. The works of Grueter (1931), Graham (1934), Gaunt and Tobin (1936), Nelson and Gaunt (1936), Gomez and Turner (1936-1937) and Gaunt and Nelson (1937) indicate the importance of the thyroid and the adrenal cortex in milk secretion.

The present study is a continuation of the investigations which have been under way in this laboratory for the last six years on the hormonal control mechanism or factors affecting growth and development and secretion of the mammary gland. In turn it is a phase of the general problem of the "physiology and inheritance of milk secretion."

Although the approach in this investigation has been mainly physiological, it was found necessary to determine certain detailed structural changes in the experimental material. As a preliminary to this study, the anatomy and histology of the mammary glands of the common laboratory and domestic mammals have been intensively investigated. (Turner, 1930-1931; Turner and Gomez, 1933-1935).

Specifically, the present study was prompted as a result of the need for determining (1) the influence of the pituitary and other internal secretions or hormones upon the growth and development and secretory activity of the mammary gland and (2) the interrelationships of these hormones in their action on the mammary apparatus.

MATERIALS AND TECHNIQUE

Experimental Animals

Though guinea-pigs have been used most extensively in the work to be reported here, rats, mice, cats, rabbits and ground squirrels have also been used. Sexually immature males and females and adult males or castrate females were used in the study of the experimental development of the mammary gland. In the study of the factors affecting the secretory activity of the mammary gland, animals with either normal or experimentally stimulated mammary glands were used. Observations of either the intact or removed gland, however, have been relied upon for verification of the condition of the mammary glands.

Operative Technique

Hypophysectomy of the rat, mouse, guinea-pig and cat was performed by a slight modification of a parapharyngeal technique described by Selye et al. (1933), Smith (1930), Thomson (1932) and McPhail and Parkes (1933). The rabbit and ground squirrel were hypophysectomized by the transbuccal technique described by Smith and White (1931), White (1933), and Wells and Gomez (1937).

Ovariectomies and laparotomies were performed by either ventral or lateral incision under ether anaesthesia. As feed was kept before our animals at all times and the intestines were always filled, the lateral method was used. Incisions were made on both sides for the inspection or removal of both ovaries. After the removal of the ovary, bleeding was stopped by either ligature or cautery. The muscle layer and skin were separately sutured. Gonadectomy in males was carried on through a mid-ventral inguinal incision.

Ovarian graft and experimental cryptorchidism of male guinea-pigs was necessitated in preparing the animals for the experimental initiation of lactation following hypophysectomy. Partial gonadectomy was performed through a mid-ventral inguinal incision. A piece of ovarian tissue from an immature donor is implanted into

the remaining testis underneath the tunica albuginea and perferably in direct contact with ruptured blood vessels. The tunica muscularis is sectioned to prevent the testis from being pulled by it into the scrotum. The testis bearing the ovarian graft is sutured to the abdominal muscle with *00* cat gut. The skin incision is closed with a silk ligature.

When control glands were desired, the operation was performed either under local or general anaesthesia. A cranial-caudal incision was cut loose from the underlying gland. With reasonable care in the preservation of the blood vessels to the skin and in leaving as much as possible of the subcutaneous connective tissue, necrosis seldom occurred. The glands were then cut free from the underlying muscle and removed. Hemostats were sufficient to prevent bleeding unless the large mammary arteries and veins were cut. The skin was closed with metal clips or sutured with silk thread.

Care of Hypophysectomized Animals

Hypophysectomized animals require great care, presumably because of the general physiological disturbance which follows the operation. In spite of the strictest nutritive care and hygienic regimen, hypophysectomized animals frequently succumb to hypoglycemic coma, especially during the period of low food intake which prevails for some time after hypophysectomy. However, the high post-operative mortality experienced in the earlier experiments was successfully controlled to a great extent by the regular parenteral administration of glucose solution in addition to *ad libitum* feed, beginning shortly after the operation and continuing daily thereafter throughout the period of observation or experimental period. Non-lactating animals were given 20 mgs. of glucose solution for every 100 grams body weight, administered once daily. Lactating animals were given 40 to 50 mgs. of glucose for every 100 grams body weight, since in these animals the development of fatal hypoglycemic coma presents a constant potential danger. The animals were placed in open cages in a warm room, with jars of food and water readily available.

Autopsy

All experimental animals were carefully autopsied at the termination of each experiment. The degree of completeness of hypophysectomy was determined by examination of the sella turcica under the binocular microscope and later verified by serial sections.

Histological Technique

The mammary glands were prepared for histological study either by whole mounts or sections according to the method described by Turner and Gomez (1933). The glands were pinned out flat on thin cork plates immediately upon removal and fixed in Bouin's fluid for 12 to 24 hours or more depending on the thickness of the glands. They were then washed in running water; all muscles and as much connective tissue as possible removed under the dissecting microscope; stained 12 to 24 hours in Mayer's hemalum, cleared in xylol, and mounted in balsam on slides of suitable size. Section of glands for histological study were not trimmed following fixation, but washed, dehydrated, cleared and embedded in paraffin. Sections were cut from 6 to 10 micra in thickness and stained in Delafield's hematoxylin and eosin, or by Mallory's connective tissue stain. The endocrine glands, such as the thyroids, adrenals, and gonads were fixed in Bouin's fixative, sectioned and stained in Delafield's hematoxylin and eosin.

For verification of the completeness of hypophysectomy serial sections were made of the sella turcica. The sellae were fixed in Bouin's fluid, decalcified in 67 per cent acid alcohol, dehydrated, embedded, serially sectioned at 8 to 10 micra in thickness and stained in Mallory's connective tissue stain.

Method of Extract Injections

All injections of the pituitary and other extracts or hormones except for the injection into rabbits for the determination of the gonad-stimulating content have been made subcutaneously. Pregnancy urine (untreated), Antuitrin-S or extracts of desiccated whole pituitary powder have been injected intravenously for the purpose of initiating ovulation. In all instances injections have been made once daily during the period indicated. The injections of the hormones were started in the hypophysectomized animals (unless otherwise stated) immediately after the operation.

Method of Extract Preparation and Source of Extracts

The extract of the adrenal cortex (eschatin), estrogen (theelin-in-oil) and antuitrin-T (thyrotropic hormone) have been kindly and generously supplied by Dr. Oliver Kamm of Parke, Davis and Company. The sodium salt of pure thyroxine was prepared by the British Drug House Ltd., London. Progynon-B was kindly supplied by Dr. E. Schwenk of the Schering Corporation, Bloomfield, New Jersey. The corpus luteum hormone (progestin) was

prepared and assayed in our laboratory according to the method described by Turner and Frank (1932). The lactogenic and adrenotropic hormones were prepared in our laboratory from sheep pituitaries obtained directly from Swift and Company, Kansas City, by a modification of the methods described by Bates and Riddle (1935) and Perla (1936).

Preparation of the Adrenotropic and Lactogenic Hormones

One pound of whole pituitaries of sheep were ground fine through a meat grinder; the tissues dehydrated with acetone, dried, powdered and passed through a 100 mesh copper sieve. A sample extraction is as follows:

Ten grams of whole sheep pituitary powder were extracted with 0.5 per cent glacial acetic acid at ice box temperatures for 48 hours or more and then centrifuged. The insoluble precipitate recovered by centrifugation contained a high concentration of lactogenic hormone, and hence the active lactogenic material was extracted from this precipitate using the method described by Bates and Riddle (1935).

The acid filtrate was titrated with 10 per cent NaOH to pH 7.2-7.8. It was then suspended in a boiling water bath for 20 to 30 minutes and the precipitate which formed while heating was removed by filtration or centrifugation and discarded. The heated alkaline filtrate contained high adrenotropic activity and was ready for use after being neutralized or slightly acidified (pH 6.6-6.8) by the addition of 20 per cent HCl or stored, if desired, in colored bottles at ice box temperature. Since the adrenotropic activity is lost rapidly on standing in either acid or alkaline solution, it was found necessary to recover this material in solid form. This was accomplished by adjusting the alkaline solution after heating to pH 6.6 using 20 per cent HCl. The adrenotropic material was then precipitated by liberal treatment with (reagent) acetone. The active adrenotropic material which settled out of the clear acetone solution in the form of a fine snow-white precipitate was recovered by centrifugation. The yield was very small as only about 150 mgs. of active adrenotropic material were obtained from 5000 mgs. of whole dry pituitary of the sheep.

Assay of the Lactogenic and Adrenotropic Hormones

The lactogenic hormone (galactin) obtained by the method indicated above contained 40 Bird Units per milligram assayed according to Lyons and Page's (1935) intradermal injection technique. Five milligrams of this material (200 B. U.) injected

over a period of two days were required to initiate lactation in guinea-pigs which had been previously prepared for lactation by the injection of estrogen, and 15 mgs. (600 B. U.) administered over a period of six days in pseudo-pregnant rabbits. In amounts ranging from 5 to 10 mgs. the injection of galactin into hypophysectomized guinea-pigs did not prevent the atrophy of the adrenal cortex and the thyroid, and in long continued treatment neither stimulated the growth of the mammary gland nor increased body weight. The intravenous injection of 20 mgs. of galactin into sexually mature female rabbits did not induce ovulation after 18 to 48 hours. These reactions were taken to indicate that the galactin preparation was to a great extent free of adrenotropic, thyrotropic, gonadotropic, mammary gland growth stimulating, and body growth factors.

The daily injection of 0.05 mg. (equivalent to 35 mg. of dry pituitary powder) of adrenotropic preparation was required to prevent the atrophy of the adrenal cortex of hypophysectomized guinea-pigs. Four injections of 0.8 mg. daily were required to restore the normal weight of the adrenal gland of guinea-pigs which had been hypophysectomized 8 days preceding the injection. Five milligrams of this material injected over a period of 4 days produced an average increase of 50 per cent in the weight of the adrenal glands in a group of four guinea-pigs weighing an average of 160 gms. (150-180 gms.) over that of the controls. This preparation was found to be free of growth, gonadotropic, and lactogenic factors when assayed according to the methods indicated above.

PHYSIOLOGICAL AND ANATOMICAL CHANGES AFTER HYPOPHYSECTOMY AND REPLACEMENT THERAPY IN MAMMALS AND OTHER FORMS

Mainly as the result of the earlier experiments on dogs, the pituitary gland had been regarded as essential to life (Cushing, 1912). Recent workers, however, have shown that the removal of the gland itself is not necessarily lethal, although an intensification of the resulting physiological disturbances might lead to a very high mortality. A fair survival rate was reported in the dog (Houssay and Biasotti, 1931; Houssay, 1932), cat (Allan and Wiles, 1932), ferret (Hill and Parkes, 1932), guinea-pig (McPhail and Parkes, 1933; Gomez and Turner, 1936) and ground-squirrel (Wells and Gomez, 1937) after complete hypophysectomy.

Aside from the immediate though transient polydipsia and polyurea, hypophysectomy in laboratory mammals gives an invariable syndrome, the main features of which are an almost complete inhibition in body growth in young animals and a progressive loss of body weight (cachexia) in the adult; an atrophy of the genital system (in the female and immediate cessation of the sexual cycle; an atrophy of the thyroid, parathyroid and the adrenal cortex and general impairment, characterized by a lower. resistance to operative procedures, loss of appetite, weakness and flabbiness which readily distinguish the hypophysectomized from the normal animals. Other organs such as the liver, spleen and kidney also decreased in weight following hypophysectomy.

The physiological and anatomical alterations of the various organs after hypophysectomy of laboratory animals have recently been reviewed by Van Dyke (1936). However, in view of the importance of the influence of the various endocrine glands controlled by the pituitary in relation to the activity of the mammary gland, the anatomical and functional alterations of these glands, particularly those directly or indirectly concerned with the metabolic functions, after hypophysectomy will be considered. The effects produced by hypophysectomy on the mammary gland will be considered in the following section so that they may be considered in the light of the present studies.

Effect of Hypophysectomy and Replacement Therapy on the Adrenal

Smith (1930) first reported that hypophysectomy in toads and later in rats, was followed by a rapid regression of the adrenal cortex. If sufficient time was allowed to elapse without replacement treatment the cortex was reduced to a narrow mantle which practically obliterate the cortical zones. The medulla was never affected. Houssay and Samartino (1931) reported similar observations in the dog.

The atrophy of the adrenal cortex is undoubtedly accompanied by adrenal insufficiency, for the hypophysectomized animals showed after several weeks, symptoms characteristic of Addison's disease, which Atwell (1932) has shown is relieved by the administration of adrenal cortical extract.·

Smith (1932) reported that the structure of the atrophic adrenals of rats could be restored to normal or near normal by daily homotransplants of anterior pituitary tissue. Houssay et al.

(1933) reported an average of 34 per cent increase in weights of the adrenals of thyroidectomized, castrated, and hypophysectomized dogs after 7 daily injections of pituitary extracts.

Collip, Anderson, and Thomson (1933, 1935) prepared a pituitary extract that was strong in adrenotropic activity. These authors reported that the injection of this extract into hypophysectomized rats produced an increased adrenal weight of 50 to 300 per cent over the controls removed at the time of the beginning of the injections.

Lyons (1937) and Moon (1937) prepared an extract of the pituitary with high adrenotropic activity. Their extract produced hypertrophy of the adrenal cortices in normal infantile rats or restored to normal or near normal the adrenal cortices of hypophysectomized rats.

The immediate consequence of adrenal insufficiency, either induced by hypophysectomy or adrenalectomy, appears to be a disturbance of the salt and fluid metabolism. Gaunt (1937) is of the opinion that following hypophysectomy there occurred a rapid shifting of the salts and fluid from the tissues and intercell spaces into the blood stream. The resulting polyurea after hypophysectomy and the associated increased salt (NaCl) excretion caused a gradual loss of fluid and consequently a dehydration of the tissues.

Effect of Hypophysectomy and Replacement Therapy on the Thyroid

The changes in the thyroid and the associated alterations in metabolism, directly influenced by this gland, have been reviewed by Van Dyke (1936). The function of the thyroid in the regulation of basal metabolism is now well established. Following hypophysectomy, the thyroid becomes inactive and even atrophic (Aschner, 1912; Houssay, 1916; Smith, 1926, 1930; McPhail, 1935 and others). Collip (1935) reported the thyroid of hypophysectomized rats decreased in weight and the microscopic picture was one of marked involution of the cellular elements.

The atrophy of the thyroid may be prevented or restored to normal, both in structure and functional activity, by the administration of pituitary tissues or extracts in rats (Smith, 1926, 1930) and in dogs (Houssay et al. 1932). Loeb and Basset (1929) and Aron (1929), quite independently of each other, produced hyperplasia of the thyroid of immature guinea-pigs by means of anterior pituitary extracts. The feeding of anterior pituitary gland, even in massive amounts, produced no reparative effects on the thyroid of hypophysectomized rats.

In thyroid deficiency, induced by thyroidectomy or hypophysectomy, the basal metabolic rate of laboratory mammary is markedly reduced. In the hypophysectomized rat, Foster and Smith (1926) reported a decrease in metabolic rate of 35 per cent, Collip (1934) and Anderson and Collip (1934) 23 per cent, and Collip (1935) 74 per cent. Similar observations were reported in hypophysectomized pigeons (Riddle et al. 1933). Hypophysectomy in dogs (Houssay, 1934) produced a decrease in the basal metabolic rate by about 16 per cent. This decrease was apparently due to anatomical and functional hypothyroidism, since thyroidectomy further decreased the basal metabolism, from −12 per cent when hypophysectomized only to −22 per cent when the thyroid gland was removed in a second operation. Thyroidectomy alone decreased the metabolic rate to −24 per cent of normal.

The daily implants of anterior pituitary tissue for a period of approximately three weeks restored the normal basal metabolic rate of hypophysectomized rats (Smith, 1930).

Studies have shown that the thyroid is under the control of the thyrotropic principle of the pituitary. Anderson and Collip (1933) reported that hypophysectomized rats were very sensitive to minute doses of thyrotropic hormone, since the reduced metabolic rate of hypophysectomized rats was brought back to normal with very small amounts (0.01 cc.) of the purified thyrotropic hormone. Collip (1935) reported that the metabolic rate of hypophysectomized rats has frequently been restored to normal within a week with doses twice daily of as little as 0.005 cc. of purified thyrotropic extract.

In addition, the thyrotropic hormone caused an increased excretion of creatin, in both normal and hypophysectomized rats (Pugsley, Anderson, and Collip (1934) and an increase in the excretion of calcium by the intestines (Pugsley and Anderson, 1934). It is evident from the facts noted above that the thyrotropic hormone is an entity having physiological properties that distinguish it from other pituitary hormones.

Effect of Hypophysectomy and Replacement Therapy on the Parathyroid

The effect of hypophysectomy on the parathyroid has been investigated by only a few investigators. Smith (1927) reported an atrophy of the parathyroid gland of rats after hypophysectomy. Houssay and Samartino (1933) reported atrophic changes in the parathyroid in about two-thirds of a group of hypophysectomized

dogs. Collip (1935) reported no consistent degenerative changes even after several months after hypophysectomy of rats and dogs.

Anselmino, Hoffmann, and Herold (1933-34) reported the administration of pituitary extract caused hyperplasia of the chief cells as well as a hypertrophy of the parathyroid of male rats. Hertz and Kranes (1934) reported enlargement and hyperemia of the parathyroid of rabbits after the administration of anterior lobe extract. These authors concluded that these effects were due to the parathyrotropic activity of the pituitary gland.

The experimental data reviewed by Van Dyke (1936) indicate that the parathyrotropic hormone or the secretion of the parathyroid influenced the metabolism of calcium. Teel and Watkins (1929) reported a fall in blood calcium of dogs after treatment with pituitary fractions containing growth hormone. An increased concentration of calcium in the blood of rats (Charles and Hogben, 1932) and dogs (Hoffman and Anselmino, 1934) was reported after the administration of pituitary extracts.

Geesink and Koster (1929) reported subnormal levels in the blood calcium of hypophysectomized dogs. Gerschman (1931) found a normal blood calcium level in hypophysectomized dogs. The contradictory results were probably due to the difference in the ages of the experimental animals employed, since the former authors used very young dogs in which calcium metabolism is always more easily disturbed. Houssay and Mazzocco (1932) reported a drop of 11 per cent of the serum calcium after hypophysectomy of dogs. Perla and Sandberg (1936) found a negative calcium balance during the period of low food intake which prevails after hypophysectomy in rats. Hypophysectomized rats on a low calcium diet showed negative calcium balances which became positive on administration of growth hormone.

After nine daily intraperitoneal injections of 1 cc. of alkaline pituitary extract into immature guinea-pigs, Friedgood and McLean (1937) found a significantly elevated serum calcium level as compared with the untreated controls (11.1 and 12.0 mgms. per cent).

Effect of Hypophysectomy and Replacement Therapy upon other Metabolic Functions

In addition to the metabolic effects mentioned in the above discussion of the effect of hypophysectomy and replacement therapy on the thyroid, adrenal cortex and the parathyroid, there have been studies made on some special relations of the pituitary

to metabolism. These deal largely with the metabolism of carbohydrate, fat, protein, and mineral matter.

Carbohydrate Metabolism.—A factor which is at least responsible for the greater proportion of death following hypophysectomy of laboratory mammals appears to be a disturbance of the carbohydrate metabolism. This phenomenon was first reported by Houssay and Magenta (1925) who noted that hypophysectomized dogs developed marked sensitivity to insulin. In 1930, Biasotti and Houssay extended to mammals an experience with toads to the effect that hypophysectomy lessened the severity of pancreatic diabetes. In 1932, Evans and his co-workers and Houssay reported the production of hyperglycemia and glycosuria in normal animals by the injection of anterior pituitary extracts.

In hypophysectomized dogs, Koster and Geesink (1929), Kobayashi (1931), D'Amour and Keller (1933) and Lucke et al. (1934); in rabbits, Fujimoto (1932), Corkill et al. (1933); birds, Hill and Parkes (1934); and in monkeys, Smith et al. (1936) reported sub-normal sugar values. Similar observations were reported in a single hypophysectomized human female (Elden, 1936). In untreated hypophysectomized animals, hypoglycemia frequently develops to the point of convulsions and death (White, 1933; Gomez and Turner, 1936-1937).

The reduced blood sugar level appears to be partly due to the inability of hypophysectomized animals to absorb glucose. Phillips and Robb (1934) and Bennet (1936) reported that the glucose absorption in hypophysectomized rats was about 35 per cent below normal.

Fischer and Pencharz (1936) reported that hypophysectomized rats continued to oxidize carbohydrate for a longer period of time during fasting and derived a larger proportion of their energy from carbohydrate after glucose feeding than did normal rats. Without the compensatory glycogenic function, hypophysectomized animals evidently derived their carbohydrate supply, to a great extent, from the glycogen reserves in the liver and muscles.

Nakamura (1931) and Corkill et al. (1932) found a rapid diminution of the glycogen in the liver and muscle after hypophysectomy of the rabbit. Russell (1936) reported that during a fasting period of 8 to 18 hours, the amount of carbohydrate in the liver, muscle, and blood was reduced to a greater extent in the hypophysectomized rats than in the normal controls. This may

be due to the greater oxidation of carbohydrates by hypophysectomized animals observed by Fischer and Pencharz.

Houssay et al. (1932) reported that the diminution of the glycogen content in the liver following hypophysectomy in toads could be inhibited by the administration of an alkaline extract of the pituitary gland. Russell and Bennet (1936) reported that the abnormal fall of body carbohydrate suffered by hypophysectomized rats during fasting could be prevented and the muscle glycogen values could be raised to super-normal level for the fasted conditions by the administration of pituitary extracts.

There is some evidence indicating that the adrenal and the islet tissue of the pancreas are concerned with the metabolism of carbohydrate. In hypophysectomized-pancreatectomized cats, the diabetes was strikingly ameliorated by the removal of both the pancreas and the adrenal glands (Long and Lukens 1935). The interpretation of such studies is especially difficult, however, because of the many species used, the varying techniques of operation, the many types of extracts employed, chemical analysis and the acknowledged interrelationship of the many factors other than the pituitary gland involved in general metabolism. The only conclusion now warranted is that, while there is a substance in the anterior pituitary that affects carbohydrate metabolism, the isolation of a separate *carbohydrate metabolism hormone* has not yet been effected.

Fat Metabolism.—The role of the pituitary gland in fat metabolism is generally recognized. Burn and Ling (1928-1929) noted an increased acetone excretion in rats maintained solely on a butter diet following injection of anterior pituitary preparations.

Anselmino and Hoffmann (1931) focused attention upon a special fat metabolism hormone that can be extracted from the anterior lobe. Injected into animals, this hormone elicited an increase in the acetone bodies of the blood (acetone, acetoacetic acid, and beta-hydroxy-butyric-acid). The hormone is discharged into the circulation after the ingestion of a fatty meal and gives rise to the same significant results as are obtained by the injection of pituitary extracts (Funk and Zeffrow, 1932; Boenheim and Heiman, 1932; Magistris, 1933).

In 11 of 16 hypophysectomized dogs, Chaikoff et al. (1936) reported that while the lipid content of the blood obtained was significantly above normal, all the lipid constituents, total fatty acids, phospholipids and free and esterified cholesterol, were present in normal amounts. Further, they reported that the rapid

accumulation of large amount of lipids in the liver after complete hypophysectomy was not prevented by pancreatectomy. Soskin et al. (1935) are of the opinion that fasting dogs are unable to replenish their depleted blood sugar from ingested fat whereas the normal fasting dog can do so. In other words, the demand for the fat metabolism hormone is actually less in hypophysectomized animals than in the normal.

The action of the fat metabolism hormone is an indirect one by way of the endocrine glands. MacKay and Barnes (1936-37) reported that a pituitary extract which had the property of producing ketosis in fasting rats also caused a deposition of fat in the liver. With the removal of the adrenal glands, however, the injection of this extract caused the disappearance of ketosis, prevented the accumulation of and reduced the amount of fat in the liver during fasting.

Best and Campbell (1936) and Fry (1936) independently reported that the ketogenic response to the injection of anterior pituitary extract did not occur in the absence of the adrenal cortical tissue. Further, the ketogenic function exhibited by adrenal cortical tissue was not restored by adrenal cortical extract therapy. The ketogenic action of phlorrhizin or the anterior pituitary extract was not suppressed by the removal of the thyroid gland. These observations appear to indicate the existence of a specific adrenal cortical factor for ketogenic functions.

Protein Metabolism.—There is a considerable amount of evidence indicating that the anterior pituitary plays an important role in protein metabolism. In hypophysectomized dogs, the urinary and fecal nitrogen excretion decreases to the extent of 35 per cent below normal after the operation (Aschner, 1929; Braier, 1932). Non-fasting hypophysectomized dogs eliminated from 9 to 18 per cent of creatin, while under fasting conditions these values decreased 30 to 35 per cent. In the rat, the urinary nitrogen excretion was increased from 21 per cent of the nitrogen intake during the control period to 54 per cent during the following nine weeks after hypophysectomy. Similar observations were reported in partially hypophysectomized rats, though less pronounced, from 26 per cent of the intake to 36 per cent during the first three weeks after the operation (Perla and Sandberg, 1936).

Analysis of the tissues and carcasses of 16 pair-fed normal rats showed a loss of 60 per cent of the fat content in 33 days but all body protein was retained, while the 16-pair-fed hypophysectomized litter mates lost 28 per cent of the initial fat stores

and 19 per cent of the organic nitrogen content. The energy metabolism in both groups, however, was the same but 23 per cent less than the *ad libitum* fed controls. The nitrogen partition of the tissue of 27 hypophysectomized rats showed a higher level of amino acids, urea and total non-protein nitrogen in their liver than in 23 pair-fed controls (Lee and Ayres, 1936).

In phlorrhizinized hypophysectomized dogs, the sugar and nitrogen excretion is markedly less than in thyroidectomized animals given phlorrhizin (Houssay et al. 1933). The former die of hypoglycemic crisis whereas the latter do not. Death was prevented by feeding meat or sugar but not by feeding fat. These observations were interpreted to mean that in the absence of the pituitary gland there is an interference with the conversion of fat to carbohydrate.

Reiss, Schwarz and Fleischmann (1933) reported a marked lowering of the non-protein nitrogen and an increase in urea nitrogen in the blood, especially in dogs, within 4 to 6 hours after the administration of hypophyseal growth hormone. The fall in non-protein nitrogen varied from 10 to 40 per cent while the blood arginine fell 16 to 43 per cent. The rise in urea nitrogen ranged from 21 to 66 per cent. Shaeffer and Lee (1935) found a striking decrease in body protein in hypophysectomized rats which was proportional to the length of time after hypophysectomy.

Mineral Metabolism.—Earlier in the review (p. 15) the disturbance evoked by hypophysectomy on the metabolism of calcium had been indicated. Perla and Sandberg (1936) reported that in hypophysectomized rats the excretion of phosphorus increases progressively in the feces while that of the urinary excretion remains constant irrespective of the amount of phosphorus intake. The retention of phosphorus dropped from 27 per cent of the intake during the control period to 6 per cent after hypophysectomy; however, the phosphorus balance remained positive throughout. The retention of phosphorus returned to normal three weeks after hypophysectomy. Marenzi and Gerschman (1936) found that while the concentration of magnesium in the blood was only slightly decreased following hypophysectomy in dogs, the concentration of potassium was decreased 20 per cent. Further, these authors reported that the injection of bovine pituitary extracts increased the general plasma protein, calcium and magnesium and decreased sodium and chlorine. No changes occurred in plasma potassium.

The metabolism of copper and iron was not disturbed following hypophysectomy of adult rats (Perla and Sandberg, 1936).

EFFECT OF HYPOPHYSECTOMY AND REPLACEMENT THERAPY ON THE MAMMARY GLAND

The normal development and secretory activity of the mammary gland of the experimental animals used in this study, with the exception of the ground-squirrels, have been reported (Turner and Gomez, 1933-1935; Turner and de Moss, 1934). Reference to the more important literature concerning the normal development and functional activity of the mammary gland may be found in the review by Turner (1932) and more recently by Nelson (1936). In view of the importance of its bearings upon the experiments to be described later, a brief review of the literature concerned with the hormones stimulating the growth of the mammary and the initiation ànd maintenance of lactation will be made.

Growth of the Duct System

Effect of Estrogen.—The influence of estrogen upon the stimulation of mammary gland growth has been extensively investigated in several species of laboratory mammals, including the rabbit (Parkes, 1930; Turner and Frank, 1930; Turner et al. 1932), rat (Laquer et al. 1927; Turner and Schultze, 1931; Turner et al. 1932), mouse (Allen et al. 1924; Turner et al. 1932; Bradbury, 1932; Turner and Gomez, 1934; Gardner, 1935; Gardner et al. 1935; Burrows, 1936, Bonser, 1936), cat (Turner and de Moss, 1934), dog (Turner and Gomez, 1934) and guinea-pig (Champy and Keller, 1927; Haterius, 1928; Loeb and Kountz, 1928; Dingemanse et al., 1930; Nelson and Smelser, 1933; Smelser, 1933, Turner and Gomez, 1934 and a host of other investigators).

Estrogen induced growth of the duct system but did not induce development of the alveoli, except in the guinea-pig, and to a limited extent in the rat and mouse. Recent studies have shown that the extended administrations of certain estrogen preparations, such as folliculin benzoate (Gardner, 1935; Gardner et al. 1935), estriol, estradiol, equilin (Burrows, 1936) and estrone (Bonser, 1936) stimulated a varying degree of lobule-alveolar proliferation of the mammary gland parenchyma of male mice.

Effect of Estrogen on Mammary Duct Growth in Hypophysectomized Animals.—There is a growing interest in the studies on the effect of ovarian hormones upon the growth and development of the mammary gland of hypophysectomized animals. The limited

studies which have been undertaken in the past few years present only limited and contradictory results. Ruinen (1932) reported that the injection of 50 mouse units of crystalline estrogen twice daily for 14 days after the operation produced mammary development comparable to the injected animals with their pituitary intact. Freud and de Jongh (1935) treated a series of young castrate female rats the first two weeks with 100 I. U.* of estrogen daily. The animals were then hypophysectomized and during the following week were treated with estrogen and progestin. In these animals the authors reported an extension of the lobular system of the mammary gland with side branches along the ducts. Nelson (1935) reported mammary development in four hypophysectomized guinea-pigs which received 40 rat units of estrogen daily comparable to that obtained in non-hypophysectomized animals receiving similar treatment. In the hypophysectomized rats, the mammary response to estrogen injections was erratic (Nelson, 1936).

In contrast to the above observations are the works of Lyons and Pencharz (1936) in the guinea-pig, Reece et al. (1936), in the rat and Gomez et al. (1937) in male mice. Lyons and Pencharz observed nipple development in four hypophysectomized male guinea-pigs equal to that obtained in six normal male animals under similar treatment after two months injection of progynon-B, yet there was only limited growth of ducts in the hypophysectomized animals in comparison to functional alveolar development found in the injected intact controls. These observations were confirmed by Gomez and Turner (1936) in male and female guinea-pigs using various estrogens, theelin (aqueous and in oil) and the benzoate of dihydrotheelin (progynon-B and oestroform-B) in amounts ranging from 25 to 100 I. U. daily for a period of 20 to 65 days. Reece et al. obtained no evidence of mammary duct growth in five female and four male immature hypophysectomized rats which received progynon-B treatment in amounts ranging from 25 to 500 I. U. daily for 18 to 35 days. Nelson and Tobin (1936) and more recently, Nelson (1937) reported similar observations in rats.

In partially hypophysectomized animals, the presence of even microscopic fragments of the anterior pituitary gland was effective in causing the administered estrogen to produce growth of the duct system of the mammary glands (Gomez et al. 1937).

*International Units.

Table 1.—Summary of Results on the Effect of Estrogens on the Mammary Gland of Completely and Partially Hypophysectomized Laboratory Mammals.

Animal	Sex	No. days of injection	Estrogen		Observations	
			Kind	Daily dose I.U.	Nipples	Mammary Gland
			Guinea-pig—Complete			
G35-12	F	40	T.A.	100	Well developed	No duct growth
G35-1	F	32	P.B.	100	Well developed	No duct growth
G35-3	F	65	P.B.	150	Well developed	No duct growth
G35-4	M	25	P.B.	1000	Well developed	No duct growth
G36-12	F	20	T.O.	50	Well developed	No duct growth
G364	M	30	T.O.	50	Well developed	No duct growth
G362	F	30	T.O.	50	Well developed	No duct growth
G363	M	25	T.O.	25	Well developed	No duct growth
G365	M	20	O.B.	50	Well developed	No duct growth
			Guinea-pig—Partial			
G35-14	M	25	P.B.	100	Well developed	Complete hyperplasia
G35-12	F	35	T.A.	100	Well developed	Complete hyperplasia
G35-13	M	30	T.A.	100	Well developed	Complete hyperplasia
G35-15	M	30	T.A.	100	Well developed	Complete hyperplasia
			Mouse—Complete			
HM-2	M	30	P.B.	50		No duct growth
HM-7	M	20	T.O.	50		No duct growth
HM-8	M	20	T.O.	50		No duct growth
HM-13	M	20	O.B.	50		No duct growth
HM-14	M	25	O.B.	50		No duct growth
			Mouse—Partial			
HM-1*	M	20	P.B.	50		Slight duct growth
HM-5*	M	20	P.B.	50		Slight duct growth
HM-3	M	60	P.B.	50		Good duct growth
HM-6	M	20	P.B.	50		Good duct growth
HM-9	M	20	T.O.	50		Extensive duct growth
HM-10	M	20	T.A.	50		Extensive duct growth
HM-11	M	20	T.A.	50		Extensive dict growth
HM-15	M	25	O.B.	50		Extensive duct growth
			Rat—Complete			
HR-01	M	30	T.A.	100		No duct growth
HR-02	CF	25	T.A.	100		No duct growth
HR-03	M	45	T.O.	100		No duct growth
HR-04	M	35	T.O.	100		No duct growth
HR-05	M	25	T.O.	100		No duct growth
HR-06	M	25	T.O.	100		No duct growth
			Rat—Partial			
HR-10	M	20	T.O.	100		Good duct growth
HR-12	M	25	T.O.	100		Good duct growth
Control	M	20	T.O.	100		Good duct growth
			Rabbit—Complete			
Rb-1	F	60	T.O.	100	Slightly developed	No duct growth
Rb-3	F	50	T.O.	100	Slightly developed	No duct growth
			Rabbit—Partial			
Rb-2	F	50	T.O.	100	Slightly developed	Extensive duct growth
			Cat—Complete			
CH-5	M	30	T.O.	500	No growth	No duct growth
CH-6	M	40	T.O.	500	No growth	No duct growth
CH-1	M	100	T.O.	500	Slightly developed	No duct growth
CH-2	F	270	T.O.	500	Slightly developed	No duct growth
			Cat—Partial			
CH-3	F	90	T.O.	500	Slightly developed	Good duct growth
			Ground Squirrel—Complete			
SH-1	A.F.	40	T.O.	50	Slightly developed	No duct growth
SH-4	F	80	T.O.	50	Slightly developed	No duct growth
SH-5	F	40	T.O.	50	Slightly developed	No duct growth
SH-10	F	35	T.O.	50	Slightly developed	No duct growth
SH-12	F	25	T.O.	50	Slightly developed	No duct growth
SH-13	F	30	T.O.	50	Slightly developed	No duct growth
			Ground Squirrel—Partial			
SH-2	F	25	T.O.	50	Well developed	Good duct growth
SH-3	F	20	T.O.	50	Well developed	Good duct growth
Control	F	25	T.O.	50	Well developed	Good duct growth

*Only microscopic fragments of pituitary tissue were left in the sella turcica.

T.A. = Aqueous theelin.
T.O. = Theelin in oil.
P.B. = Progynon-B.
O.B. = Oestroform-B

C.F. = Castrated female.
A.F. = Adult female.

It was probable that the lack of agreement in the findings noted above may be due to the variability in the animals and in the ovarian hormone preparations employed, the dosages and period of injections of the ovarian hormones or the degree of the completeness of the removal of the anterior pituitary gland, may be accountable for these differences. In order to determine the factor or factors responsible for the conflicting observations noted above, studies were conducted on the effects of various estrogens alone and in combination with progestin upon the mammary gland of hypophysectomized young male and female laboratory mammals.

Experiment I. Studies with estrogen.*—Thirteen hypophysectomized male and female guinea-pigs were used. The animals were given various estrogen preparations in amounts ranging from 25 to 1000 I. U. beginning immediately after hypophysectomy and continuing daily thereafter for periods of 20 to 65 days (Table 1). Nine of these animals, which at autopsy and upon microscopic examination of the sectioned sellae revealed complete hypophysectomy, showed no evidence of growth of the mammary gland tissue, although in all instances the nipples were well developed. The mammary glands of the four remaining animals showed development of the nipples and mammary glands comparable to that of the intact animals which received estrogen for 20 days. Tiny fragments were found in the sella turcica upon microscopic examination of the sections.

In view of the inability of the estrogen to stimulate mammary gland growth in the guinea-pig, the experiments were extended to other species of laboratory and small domestic mammals in order to determine whether the effect obtained in the guinea-pig represents only a species difference or they were generally applicable.

Hypophysectomy which was carried on by the parapharyngeal technique was well tolerated by the albino mouse and the postoperative recovery was similar to that of the guinea-pig or rat. However, regardless of the time of the beginning of the injection of estrogen after hypophysectomy, a great percentage of the experimental animals succumbed to the treatment before an adequate injection period was determined. In this study, only the animals surviving the estrogen treatment for periods of 20 days or more were included in the report.

*A considerable number of animals which succumbed to the treatment too soon to allow for the necessary observation or produce comparable effect in normal animals were omitted from this report.

Of some fifty hypophysectomized male mice used, 13 survived the treatment of 50 I. U. of estrogen daily for 20 to 60 days. Five of these animals were completely hypophysectomized, while the rest revealed fragments of varying size, ranging from macroscopic to microscopic fragments. The mammary glands of the completely hypophysectomized animals revealed only a rudimentary duct system comparable to the mammary glands of the adult untreated controls. In partially hypophysectomized animals, the mammary glands showed varying degrees of duct growth.

Five completely hypophysectomized immature male and castrate female rats which received 100 I. U. of estrogen (theelin-in-oil) daily for 25 to 45 days, revealed no growth of the duct system. Two animals with pituitary fragments left in the sella turcica showed an appreciable extension of the duct system with side branches comparable to the duct growth of intact treated controls.

Six young male rats were injected with 20 rat units of theelin (in oil) daily for 20 days. After 20 days the animals were hypophysectomized. A gland removed at the beginning of the theelin injection and at the time of hypophysectomy served as controls. The mammary glands taken after 20 days of estrogen injections showed an extension of the duct system with side branches and prominent end-buds distinctly differentiated from the compact glandular tissue present at the time of the initiation of injections (Figs. 5 and 6). Immediately after hypophysectomy the animals were injected with the same amount of estrogen and continued daily for 20 days. One of the animals died on the second day after the operation, and two others six days later. The mammary glands of these animals showed mammary structures similar to those present in the gland at the time of hypophysectomy. The three remaining animals, which were able to tolerate the post-operative treatment, were sacrificed after 20 days. Two of these animals were completely hypophysectomized. The mammary glands of these animals still showed the extension of the duct system but the prominent end-buds observed at the time of hypophysectomy were no longer present and the ducts had undergone regression. The mammary glands of the partially hypophysectomized rat, on the other hand, showed further extension and arborization of the duct system.

Three immature female rabbits were hypophysectomized. One week after the operation, each of these animals was given
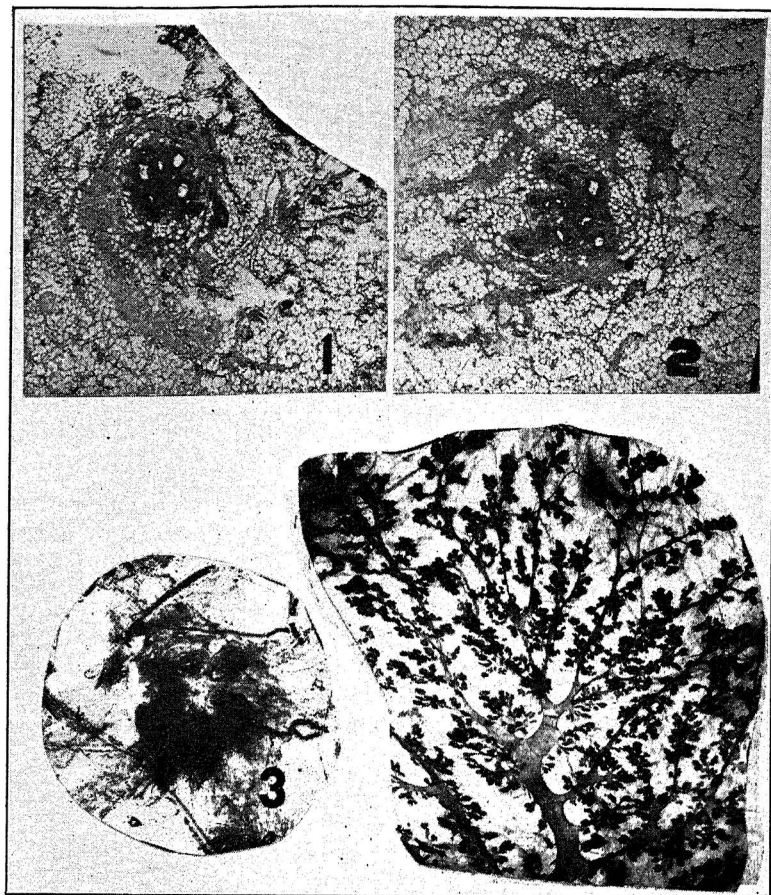
Fig. 1.—A microphotograph of a cross section at the base of the nipple of a completely hypophysectomized female ground squirrel which received 50 I. U. of estrogen daily for 25 days beginning immediately after the operation. The growth of the duct system was not stimulated. Enlarged approximately 4 times.

Fig. 2.—A microphotograph of a cross section at the base of the nipple of a partially hypophysectomized female ground squirrel which received 50 I. U. of estrogen daily for 20 days. Note the extension of the duct system in comparison with that shown in Fig. 1. Enlarged approximately 4 times.

Fig. 3.—A photograph of a whole mount of the gland of a completely hypophysectomized female rabbit which received 100 I. U. of estrogen daily for 60 days. The glandular parenchyma consisted only of a rudimentary duct system equal to that observed in immature male or female untreated controls. Enlarged approximately 4 times.

Fig. 4.—A photograph of a section of the whole mount of a mammary gland of a partially hypophysectomized female rabbit which received 100 I. U. of estrogen daily for 20 days. Enlarged approximately 4 times.

100 I. U. of estrogen (theelin-in-oil) daily for 50 to 60 days. A gland was removed for control at the time of the beginning of the injections, a second after 20 days, and a third at autopsy. Two of these animals were completely hypophysectomized, while in a

third pituitary fragments to the extent of approximately 5 per cent (0.0015 gms.) by weight were left in the sella turcica. The mammary glands taken at different intervals during the treatment in two completely hypophysectomized animals revealed no evidence of duct growth, while the glands of the third animal taken after 20 days showed extensive growth of the duct system (Figs. 3 and 4).

Three immature male and two female cats were hypophysectomized. Beginning immediately after hypophysectomy, each of the animals was given 500 I. U. of estrogen (theelin-in-oil) and continued daily thereafter. Two of the males succumbed after 30 and 40 days, respectively. The nipples of these animals were slightly developed. The mammary glands, however, revealed only a rudimentary duct system extending slightly beyond the base of the nipples. The third male and one of the females were sacrificed, the male after 100 and the female after 270 days. Both of these animals showed marked development of the nipples but no growth of the duct system. Hypophysectomy of all of the above mentioned animals was complete. The remaining female was sacrificed after 90 days. The nipples and mammary gland of this animal showed development equal to that of the intact control which received similar treatment. This animal was only partially hypophysectomized.

Six female ground squirrels were hypophysectomized. One week after the operation the animals were injected with 50 to 100 I. U. of estrogen daily for periods of 25 to 60 days. The mammary glands of these animals revealed no mammary gland growth although hyperplasia of the nipples and the vagina were observed during the treatment. The duct system of the mammary glands was limited to a few millimeters in extent around the base of the nipples. A portion of the pituitary stalk and tiny fragments but no granular cells were found in the sectioned sellae of these animals.

In addition two other females, which at autopsy revealed large pituitary fragments in the sella turcica, showed an extended growth of the duct system comparable to that of the normal injected controls after 20 to 24 days of injection (Fig. 2).

Summary.—The duct or lobule-alveolar complexes of the mammary glands of completely hypophysectomized guinea-pigs (9 cases), rats (6 cases), mice (5 cases), ground squirrels (6 cases), rabbits (2 case) and cats (4 cases) have not been observed to

respond to long continued administration of large dosages of estrogen. It would appear that the inability of estrogen to stimulate the growth of the mammary gland parenchyma in the absence of the pituitary gland is probably not a species difference but applies generally.

Growth of the Lobule-Alveolar System

Effect of Progestin.—In view of the rapid hyperplasia of the gland which occurs during pregnancy or pseudo-pregnancy, the existence of a functional relationship between the active corpora lutea and the growth of the mammary gland has been assumed by early investigators, including Ancel and Bouin (1911), Hammond and Marshall (1914), O'Donoghue (1912), and Loeb and Hesselberg (1917). In other words, it was thought that the corpus luteum was the source of a hormone which stimulated the growth of the lobule-alveolar system.

The injections of aqueous (Loeb and Hesselberg, 1917) or lipoid (Bencan and Keller, 1927) extracts of bovine corpora lutea produced no noticeable influence on the growth of the mammary gland of the guinea-pig. Corner (1930) and Turner and Frank (1930-1932) failed to stimulate lobule-alveolar proliferation with progestin in rabbits.

Turner and Schultze (1931) obtained no lobule-alveolar proliferation in rats with progestin. Selye et al. (1936) reported that the daily injection of 4 mg. of synthetic progesterone were without effect upon the mammary gland of female rats.

In the guinea-pig, Nelson and Pfiffner (1931) obtained marked hypertrophy of the glands and nipples following the injection of progestin believed to be free of estrogen. Turner and Gomez (1934) reported that the injection of progestin alone with or without preliminary treatment with estrogen was ineffective in stimulating the growth of the mammary gland beyond that observed with estrogen alone. Nelson (1936) stated that the postive results obtained by them in their earlier experiments with progestin (Nelson and Pfiffner) in comparison with the above observations were probably due to the presence of estrogen in their extracts.

Effect of Estrogen and Progestin.—Because of the fact that estrogen has been shown to be secreted continuously during pregnancy, Turner and Frank (1930-1932) assumed that estrogen prob-

ably was essential for the action of progestin. On the basis of this assumption, these authors injected progestin and estrogen simultaneously into castrated male rabbits immediately following the preliminary treatment with estrogen. They reported that the mammary glands of the animals so treated underwent development comparable to that observed during pseudo-pregnancy or the first-half of pregnancy. These observations were confirmed in the rat (Turner and Schultze, 1931; Freud and de Jongh, 1935), cat (Turner and de Moss, 1934), mouse (Turner and Gomez, 1934) and rabbit (Anselmino et al. 1935; and MacDonald, 1936). Selye et al. (1936) reported negative results with rats.

Effect of Progestin and Estrogen on the Mammary Gland of Hypophysectomized Animals.—Asdell and Seidenstein (1935) ovariectomized a series of rabbits and then kept them for two months to exhaust their supply of ovarian hormone. The animals were then hypophysectomized and the injections begun two months later. The rabbits were injected daily with 25 rat units of progynon-B and 0.5 cc. of progestin (equal to about 4 rabbits units). The animals were injected daily for 15 days and then sacrificed. These investigators stated that the degree of development of the mammary glands was about the same in hypophysectomized animals as in those in which the hypophysis was not disturbed or was incompletely removed. Freud and de Jongh (1935) treated a series of young castrated female rats the first two weeks with 100 I. U. of menformon per day. The animals were then hypophysectomized and during a following week they were treated with menformon and progestin. These investigators reported an extension of the alveolar system with branching along the ducts. These authors concluded that the hypophysis is not involved in the changes of the mammary gland produced by estrogen and progestin.

Experiment II. Studies with Estrogen and Progestin.—Two young male guinea-pigs were given 100 I. U. of estrogen daily. One of these animals was hypophysectomized after 7 days and the other after 22 days. In both instances, 100 I. U. of estrogen and 0.66 of a rabbit unit of progestin were injected simultaneously daily beginning immediately after the operation, and continuing daily thereafter for 20 and 12 days, respectively. The nipples of both of these animals were developed equal to those of the intact animals receiving similar treatment. The mammary gland of the former animal showed no extension of the duct system beyond

the extent attained during the 7 day pre-operative estrogen treatment. The extensive lobule-alveolar growth produced during the 20 day pre-operative treatment with estrogen in the latter animal had undergone involution during the post-operative estrogen and progestin treatment for 12 days.

Four immature female guinea-pigs were injected with 100 I. U. of estrogen and 0.33 of a rabbit unit of progestin simultaneously daily for 20 to 30 days beginning immediately after hypophysectomy. Two of these animals which were autopsied after 25 and 30 days, showed well developed nipples but no evidence of growth of the duct system. Hypophysectomy was complete in these animals. The remaining two animals autopsied after 20 days showed good nipple development and extensive hyperplasia of the glandular parenchyma. Large pituitary fragments were found in the sella turcicae of these animals.

Five young male rats were given 100 I. U. of estrogen (theelin-in-oil) daily for 20 days. They were then hypophysectomized and immediately injected with 100 I. U. of estrogen and 0.2 to 0.33 of a rabbit unit of progestin simultaneously daily for 20 days. A control gland was removed at the time of the beginning of the estrogen injections and at hypophysectomy. The mammary glands obtained at the time of hypophysectomy showed a slender duct system with side branches and prominent end-buds extending out of the peripheral region of the dense mesh of original glandular tissue (Figs. 5 and 6).

Four of these animals, which at autopsy after 20 days were found to be completely hypophysectomized, showed no further extension of the duct system beyond that attained during the pre-operative estrogen treatment (Fig.7). The prominent end-buds observed in the duct system as a whole showed marked indications of involution.

Complete lobule-alveolar proliferation of the gland complex occurred in the fifth animal (Fig. 8). This animal was only partially hypophysectomized.

Summary.—Like estrogen, the administration of progestin or progestin and estrogen with or without preliminary treatment with estrogen was incapable of stimulating the growth of the mammary gland in completely hypophysectomized male or female rats or guinea-pigs.
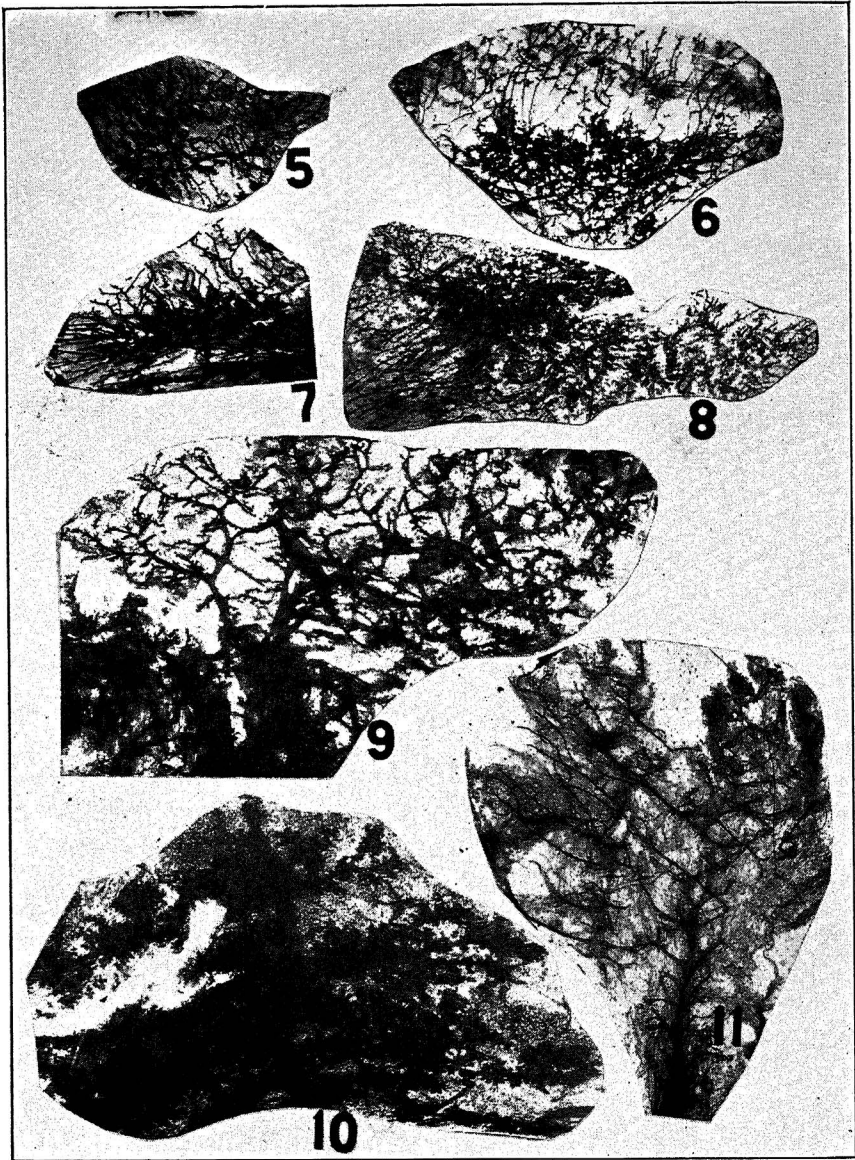
Fig. 5.—A photograph of a whole mount of a mammary gland of a young male rat taken at the time of the initiation of the injection of 100 I. U. of estrogen. Enlarged approximately 2 times.

Fig. 6.—A photograph of a mammary gland of the same animal (Fig. 5) taken at the time of hypophysectomy after 20 daily injections of estrogen. Note the extension of the duct system from the compact mass of original glandular complex. Enlarged approximately 2 times.

Fig. 7.—A photograph of a portion of a whole mount of the mammary gland from the same animal (Figs. 5 and 6) after 20 stimultaneous daily injections of 0.66 of a rabbit unit d progestin and 100 I. U. of estrogen. No further extension of the ducts or proliferation of the lobule-alveolar system occurred. Enlarged approximately 2 times.

Fig. 8.—A photograph of a whole mount of a mammary gland of a partially hypophysectomized male rat which received 0.33 of a rabbit unit of progestin and 100 I. U. of estrogen daily for 20 days beginning immediately after the operation. Further arborization of ducts and proliferation of the lobule-alveolar system occurred. The animal received 20 daily injections of estrogen preceding the operation. Enlarged approximately 2 times.

Fig. 9.—A photograph of a whole mount of a mammary gland of a completely hypophysectomized-castrate male guinea-pig which received 10 daily implants of rat pituitaries. The donor rats were previously treated with estrogen daily for 10 days. Enlarged approximately 4 times.

Fig. 10.—A photograph of a portion of the mammary gland of a completely hypophysectomized male guinea-pig after 20 daily implants of rat pituitaries obtained from donors which had previously been injected with estrogen daily for 10 to 20 days. Enlarged approximately 4 times.

Fig. 11.—A photograph of the mammary gland of a completely hypophysectomized male guinea-pig which received 30 daily implants of normal adult male rat pituitaries. Note the condition of the duct system of this gland as compared to that shown in Fig. 9. Enlarged approximately 4 times.

Influence of the Other Endocrine Gland in Mammary Development

In view of the consistent failure of the mammary glands of hypophysectomized animals to respond to ovarian hormone injections (p. 12), a working hypothesis was developed which took into account a possible reciprocal relationship between the ovaries and the pituitary in the control of mammary gland growth and development. In short, it was suggested that during sexual or reproductive activity, i. e., during recurring estrum and pregnancy, either the ovarian secretions, estrogen or progestin required the supplementing action of the pituitary hormone(s) or the secretion of some of the endocrine glands controlled by it, particularly the thyroid and the adrenals, in stimulating the growth of the ducts and lobule-alveolar system or (2) that estrogen and progestin stimulated the elaboration of a specific hormone(s) by the pituitary gland which in turn exerted a direct action upon the mammary gland.

In order to test the merit of the first hypothesis, hypophysectomized male and female laboratory mammals were injected with thyroxine, eschatin, the pituitary thyrotropic (antuitrin-T) and adrenotropic hormones and desiccated whole pituitaries of sheep, alone and in combination with estrogen to determine their effect upon the mammary gland.

Influence of the Thyroid in Mammary Development

Little is known at the present time of the influence of the thyroid gland in mammary development. Dragstedt et al. (1924) and Dragstedt (1927) reported that the complete absence of the thyroid and parathyroid glands in dogs did not prevent the hypertrophy of the mammary gland during pregnancy. If the development of tetany is prevented during gestation, normal litters may be delivered and reared solely on the mother's milk. Chandler (1927-1932) reported that thyroidectomized rats passed through estrum and became pregnant with about the same regularity as the controls.

While the above observation seemed to indicate that the thyroid glands are not essential in mammary development, other studies in rats have shown that the growth of the mammary gland was precociously developed in hyperthyroid animals, induced by desiccated thyroid feeding. Weichert and Boyd (1933) reported growth of the mammary gland of pseudo-pregnant rats induced by desiccated thyroid feeding. However, the typical lobule-alveolar

proliferation observed in pseudo-pregnant animals induced by sterile copulation was not observed in experimentally induced hyperthyroid rats. In their later studies, Weichert and Boyd (1934) observed precocious development of the mammary gland complex of thyroid fed pregnant rats beginning on the 5th day of pregnancy as compared to the mammary glands of the normal pregnant controls. Cohen (1934) reported changes in the mammary gland of male rats fed with desiccated thyroid gland.

Experiment III. Studies with Thyroxine.—In order to determine whether the thyroid glands exert a direct influence or only supplement the effect of ovarian hormones in stimulating mammary gland growth, the sodium salt of pure thyroxine was tried alone and in combination with estrogen, in hypophysectomized rats and guinea-pigs.

Three young male rats were injected with 0.05 to 0.1 mg. of thyroxine daily for 20 to 25 days beginning immediately after hypophysectomy. The mammary glands of these animals showed only a rudimentary duct system equal to that observed in the hypophysectomized control after 25 days. Tiny fragments and portion of the pituitary stalk but no granular cells were found in the sellae of these animals.

The mammary glands of a completely hypophysectomized young male and female guinea-pig which received 0.05 and 0.01 mg. of thyroxine daily showed no development of the nipples and mammary gland parenchyma after 20 days. One incompletely hypophysectomized male guinea-pig which received 0.1 mg. of thyroxine daily for 20 days showed very slight development of the mammary gland after 20 days injection.

Experiment IV. Studies with Thyroxine and Estrogen.—The mammary glands of three completely hypophysectomized young male guinea-pigs which received 0.05 to 0.1 mg. of thyroxine and 50 I. U. of estrogen each daily, showed only a rudimentary duct system equal to that observed in hypophysectomized controls after 20 days.

One completely hypophysectomized-castrated female guinea-pig which received 0.05 mg. of thyroxine and 50 I. U. of estrogen daily showed marked development of the nipples after 20 days. The extent of the mammary gland parenchyma, however, was limited to a short or rudimentary duct system equal to that observed in hypophysectomized or untreated normal controls.

Summary.—The administration of the sodium salt of pure thyroxine alone or in combination with estrogen was ineffective in stimulating duct growth of completely hypophysectomized young male or female guinea-pigs and rats at the dosage level and length of injection indicated.

Influence of the Adrenal Cortex in Mammary Development

The influence of the adrenal cortex in mammary development is indicated by the fact that in adrenal insufficiency, the reproductive function of the animal is held in abeyance (Wyman, 1928-1929; Loeb and Stahl, 1935). Grollman (1936) stated that when rats are maintained in a state of chronic adrenal insufficiency and when no growth has occurred for several months, spontaneous estrus may still occur at intervals of several months, as compared to 4 to 7 days for normal rats. The animals conceive and may rear a normal litter. About half of the animals die during either pregnancy or lactation. The surviving animals showed no evidence of failure of mammary function and the young are normally nourished.

Britton and Kline (1935-1936) reported that adrenalectomized female rats seldom become pregnant, and cases in which pregnancy occurs are apparently possible because of enlarged accessory cortico-adrenal tissue. Even the removal of one adrenal in female rats frequently upsets the normal course of pregnancy and often results in sterility. Further, they report that pregnant animals which have been adrenalectomized fail to go through the normal process of parturition; abortion is commonly observed; and lactation does not follow. Male or female in chronic adrenal insufficiency show impotence with atrophy of the reproductive system similar to that observed in hypophysectomized animals. Repair of the reproductive system and a return of sexual activity occurs in these animals after the administration of adrenal cortical hormone. Adrenal cortical extract therapy gives adequate protection to adrenalectomized pregnant rats and allows normal reproduction, parturition and lactation to take place.

In addition, the clinical observations on the association of gynecomastia and lactation with adrenal-cortical tumor in male patients (Bittorf, 1919; Zum Busch, 1929; Holl, 1930 and more recently Lisser, 1936) further support the concept of a relationship between the adrenal and reproductive organs. The gonads of these

patients were atrophied and the interstitial tissue completely degenerated. These observations, however, seemed to indicate a direct stimulation of the mammary gland by the adrenal cortical secretion.

In order to determine the relation of the adrenal cortex and the mammary gland, studies were made on the effect of the injections of the adrenal cortical extract (eschatin) alone and in combination with estrogen on the mammary gland of hypophysectomized immature male and female guinea-pigs.

Experiment V. Studies with Eschatin.—Four completely hypophysectomized young male and female guinea-pigs which received 0.2 to 1.0 dog units of eschatin each daily beginning immediately after the operation showed no nipple or duct development after 40 to 50 days of injection. One completely hypophysectomized male guinea-pig which received 2.0 dog units of eschatin daily succumbed after 10 days. The nipples and mammary gland parenchyma showed no evidence of stimulation.

One partially hypophysectomized and one intact young male guinea-pig showed no nipple and duct development after 25 days of daily injections with 0.2 and 1.5 dog units of eschatin respectively.

Experiment VI. Studies with Eschatin and Estrogen.—Two completely hypophysectomized young male guinea-pigs which received 0.2 dog units of eschatin and 100 I. U. of estrogen simultaneously each daily showed marked development of the nipples but no duct growth after 20 days. The duct systems of these animals were similar to those observed in the untreated normal or hypophysectomized controls.

One partially hypophysectomized guinea-pig showed extensive lobule-alveolar proliferation after 20 daily injections of 0.2 dog units of eschatin and 100 I. U. of estrogen. The effect was attributed to the estrogen.

Summary.—The administration of eschatin alone and in combination with estrogen at the dosage level and length of injection indicated was incapable of stimulating mammary gland growth of completely hypophysectomized male or female guinea-pigs.

Influence of the Pituitary Gland in Mammary Development

Effect of Pituitary Extracts.—The numerous studies on the effect of pituitary extracts or implants in mammary development

have yielded contradictory results. Parkes (1929) reported that the injection of pituitary extracts daily for a period equal to that of normal pregnancy in rabbits produced mammary gland growth and slight milk secretion. After an extended period of pituitary extract injections, Corner (1930) stated that the gland of mature ovariectomized rabbits underwent development comparable to the condition observed during pregnancy. Using similar extracts, Asdell (1931) reported mammary development in mature ovariectomized rabbits but not in immature males or females. Lyons and Catchpole (1933) reported mammary gland growth with anterior pituitary extracts containing lactogenic hormone. Lyons (1936) reported that alveolar proliferation occurred within a day following the injection of lactogenic hormone, so that large alveoli and small formed lobules such as those seen in normal lactation are formed after one week of injections.

In contrast are the observations of Turner and Schultze (1931), Turner and Gardner (1931), Riddle et al. (1932) Gardner and Turner (1933), Nelson (1934) and Sardi (1935). These authors reported that mammary response comparable to that induced by estrogen or estrogen and progestin did not occur following the injections of pituitary extracts rich in lactogenic hormone.

Effect of Pituitary Implants.—Turner and Schultze (1931) reported that implants of whole pituitaries of castrate rats for short periods of time at least seemed to be incapable of producing significant changes in the mammary glands of castrated female rats. Weichert, Boyd, and Cohen (1934) obtained no mammary gland growth in rats which received implants of rat pituitaries for an extended period of time.

Effect of Pituitary Extracts and Estrogen on the Mammary Gland of Hypophysectomized Animals.—Selye and Collip (1936) reported no mammary development in hypophysectomized rats which received injections of a pituitary extract and estrogen. Nelson and Tobin (1936) reported that the injection of purified lactogenic hormone and estrogen into hypophysectomized rats was no more effective in stimulating mammary gland growth than estrone alone. However, when crude pituitary extract was used in place of the lactogenic hormone, a well developed mammary gland was obtained.

Although quite contradictory, the foregoing observations on the influence of pituitary extracts on the mammary gland of

normal and hypophysectomized animals suggest that the pituitary gland in some way plays a role in mammary development. In this series of experiments the object was to determine the relation of the various known pituitary principles alone and in conjunction with estrogen in stimulating the growth and development of the mammary gland of hypophysectomized male and female guinea-pigs.

Experiment VII. Studies with Thyrotropic Hormone (Antuitrin-T) and Estrogen.—The nipples and mammary gland parenchyma of one hypophysectomized male guinea-pig which received 1.0 guinea-pig unit of antuitrin-T daily, beginning immediately after the operation, showed no development after 30 daily injections. One other completely hypophysectomized young female guinea-pig which received 1.0 guinea-pig unit of antuitrin-T and 100 I. U. of estrogen daily, showed marked development of the nipples after 20 daily injections. However, the duct system of the gland of these animals was very rudimentary, indicating that it was not stimulated by the treatment.

Experiment VIII. Studies with Adrenotropic Hormone and Estrogen.—The mammary ducts and nipples of two completely hypophysectomized male and one female guinea-pig were not developed after 30 days of daily injections with 0.05 mg. each of the adrenotropic hormone.

Three completely hypophysectomized young male guinea-pigs which received 0.1 mg. of adrenotropic hormone and 100 I. U. of estrogen simultaneously each day, showed marked growth of the nipples but not of the duct system after 25 days of injection. The duct system of these animals was similar in extent to that observed in untreated intact or hypophysectomized controls.

Two partially hypophysectomized young males which received 100 I. U. of estrogen and 0.1 mg. of adrenotropic hormone each simultaneously daily, showed well developed nipples and extensive lobule-alveolar proliferation of the glandular parenchyma after 20 daily injections.

Experiment IX. Studies with Galactin and Estrogen.—Two completely hypophysectomized female and one male guinea-pig which received 100 I. U. of estrogen (theelin-in-oil) and 10, 30, and 40 mgs. of galactin daily all showed marked development of the nipples after 25 days. The mammary glands of these animals at autopsy revealed only duct systems equal to the uninjected hypo-

physectomized control. One partially hypophysectomized guinea-pig receiving similar treatment showed marked development of the nipples and extensive proliferation of the lobule-alveolar complex in the mammary gland after 25 days of injection.

It would appear from the above described experiments that the purified thyrotropic, adrenotropic and lactogenic hormones do not represent the factors which in conjunction with estrogen stimulate mammary gland growth. In view of the fact that the other known pituitary principles are not available in pure or isolated form, an experiment was conducted in order to determine grossly whether the whole pituitaries of sheep contained the factor or factors which supplement the estrogen in its action on the mammary gland.

Experiment X. Studies with Desiccated Pituitary Powder.— The mammary gland parenchyma and nipples of two completely hypophysectomized immature female guinea-pigs which received 5 mgs. of desiccated whole pituitary powder of sheep daily revealed no evidence of stimulation of duct growth after 25 daily injections. One other completely hypophysectomized guinea-pig which received 10 mgs. of desiccated whole pituitary powder daily succumbed after 10 days. The nipples and mammary gland parenchyma of these animals showed no evidence of growth stimulation at autopsy.

The above observations were taken to indicate that the whole pituitaries of sheep did not contain the principle(s) concerned in mammary development. It seemed desirable, therefore, to turn to the second part of the working hypothesis, namely, that the ovarian hormones estrogen and progestin, stimulate the production of an as yet unrecognized hormone(s) of the pituitary which in turn causes the growth of the mammary gland. According to this hypothesis the pituitaries of animals during estrum, pregnancy, or those which have been receiving estrogen treatment might be expected to contain a high concentration of this hormone(s), and the administration of such pituitaries to castrated or hypophysectomized animals would stimulate the growth and development of the mammary gland complex. Experiments were therefore conducted having as their objective the test of this hypothesis.

Experiment XI. Studies with Pituitary Implants.—Three completely hypophysectomized young male and one female guinea-pig were each given an implant daily of one rat pituitary. The

donor rats had previously received 10 to 20 daily injections of 100 I. U. of estrogen. The mammary glands of these animals showed extensive proliferation of the lobule-alveolar system equal to that observed in intact animals which received estrogen injections for 20 days (Fig. 10)

Two completely hypophysectomized-castrated young male guinea-pigs were each given a daily implant of one rat pituitary which had received 100 international units of estrogen daily for 10 days. One of the animals succumbed after 10 days. The mammary gland parenchyma of this animal showed extensive growth and arborization of the duct system equal to that observed in an intact animal which received 10 daily injections of estrogen (Fig. 9). The nipples, however, showed no evidence of growth stimulation.

The mammary glands of two castrated young male guinea-pigs which received 20 daily implants of one-half of a rat pituitary daily showed extensive hyperplasia of the gland parenchyma. The donor rats received daily injection of 100 I. U. of estrogen for 10 days.

One completely hypophysectomized and one intact castrated immature male control, which received daily implants of a normal adult rat pituitary beginning immediately after the operation, showed no evidence of duct growth after 30 days (Fig. 11). Likewise, the duct system and the nipples of one normal male guinea-pig which received one rat pituitary daily showed no evidence of stimulation after 20 days of injection.

Summary.—The administration of desiccated whole sheep pituitaries or the purified extracts of the thyrotropic, adrenotropic, or lactogenic hormones, either individually or in combination with estrogen, were incapable of stimulating the growth of the mammary gland of completely hypophysectomized animals. Without estrogen, the above mentioned pituitary principles were ineffective in stimulating mammary gland growth of partially hypophysectomized or normal intact guinea-pigs.

The daily implants of the pituitary glands of rats which received injections of estrogen daily for 10 to 20 days produced extensive hyperplasia of the mammary gland of completely hypophysectomized or completely hypophysectomized-castrated immature male and female guinea-pigs. The pituitaries of adult normal rats were ineffective in stimulating mammary gland growth of either hypophysectomized or normal male or female guinea-pigs.

These observations were taken to indicate that estrogen stimulates the production of a "mammary gland growth stimulating hormone" by the pituitary gland of rats.

LACTATION

It is generally recognized that during the latter part of pregnancy, the cells of the lobules gradually assume functional activity. At the approach of parturition the secretion present in the gland takes on the characteristics of colostrum. Following parturition there is a burst of secretory activity. The studies of Grueter (1928), Stricker and Grueter (1928-1929) and Corner (1930) may be considered to be the first to have shown that the secretory activity observed was due to a lactogenic hormone produced by the pituitary gland. The numerous investigations which have followed their announcements have confirmed and extended the work to several species of mammals.

Under favorable conditions the crude pituitary extracts or the somewhat purified lactogenic hormone have been shown to stimulate lactation in the rabbit (Turner and Gardner, 1931; Gardner and Turner, 1933; Lyons and Catchpole, 1933; Donahue, 1934 and Gardner et al., 1935), the guinea-pig (Nelson and Pfiffner, 1931; Gardner and Turner, 1933; Lyons and Catchpole, 1933; Nelson and Smelser, 1933 and Turner and Gomez, 1934), the cat (Turner and deMoss, 1934), the dog (Lyons et al., 1933; Gardner and Turner, 1933; Evans, 1934; Asimoff, et al. 1934; Anselmino and Hoffmann, 1934 and Houssay, 1935), the goat (Asdell, (1931-1932), the cow (Stricker and Grueter, 1929) and the monkey (Riddle et al., 1933 and Allen et al. 1935). Schultze and Turner (1933), Gardner and Turner (1933), and Riddle et al. (1933) reported either slight or negative results in rats and mice.

The physiological state or condition of the gland parenchyma at which time they are responsive to the stimulating action of the lactogenic hormone had some particular significance in the light of the experiments to be described later, since it was necessary to secure or prepare the mammary gland of the animals for lactation before hypophysectomy was performed and hormone replacement therapy initiated. There has been considerable disagreement concerning the developmental condition of the mammary glands when they are responsible to the lactogenic hormone. Catchpole and Lyons (1933) were of the opinion that young virgin female rabbits possessed mature mammary glands and there-

fore were responsive to the lactogenic hormone. Gardner and Turner (1933) disagreed with them on this point, since completely grown glands showing both ducts and lobule-alveolar proliferation had never been observed in virgin female rabbits. These authors further reported that pseudo-pregnant rabbits with complete hyperplasia of the gland parenchyma were most responsive to the extracts of the pituitary glands. The susceptibility of the mammary gland of the pseudo-pregnant rabbit to pituitary extracts has been used by the latter authors in the assay of lactogenic hormone.

The mammary glands of laboratory mammals which have been receiving estrogen treatment daily for some time will respond readily to the lactogenic hormone. Gardner, Gomez, and Turner (1935) have shown that the mammary gland ducts of ovariectomized immature male or female rabbits developed under the influence of estrogen will lactate provided the lactogenic hormone is injected within 72 hours after the last injection of estrogen. Likewise, the mammary duct system persisting after previous ovariectomy of multiparous females, will lactate when injected with lactogenic hormone beginning immediately after the cessation of estrogen treatment.

In similar manner, the male or female guinea-pigs which received estrogen injections daily for some time (20 days or more) will lactate within 36 to 72 hours following the cessation of estrogen and the initiation of lactogenic hormone.

In partially castrated and experimentally cryptorchid males carrying functional ovarian grafts for 6 to 8 weeks, the injection of lactogenic hormone was invariably followed by the initiation of lactation in 2 to 3 days (Nelson, 1933; Turner and Gomez, 1934).

During the declining phase of lactation the administration of pituitary extract rich in lactogenic hormone was usually followed by a transient rise in milk yield. In the cow, Evans (1934), Asimoff et al. (1934) and Asimoff (1935) and in the sheep and goat, Kabak and Kisilstein (1934) and Kabak and Margulis (1935) reported an increased milk yield ranging from 25 to 30 per cent over the pre-injection level following the administration of pituitary extracts. Asimoff et al. (1934) reported that a single injection of 10 to 15 cc. of pituitary extract had no effect on the milk yield of cows. Margulis et al. (1935) observed no change in the milk yield of cows which received daily injections of 20 to 40 cc. of an alkaline extract of the pituitary glands.

The foregoing observations add to the evidence that the pituitary gland is functional in the initiation and maintenance of the secretory activity of the mammary gland. It is not clear, however, whether the lactation process, once initiated, can be carried on without further mediation or stimulation of galactin to maintain lactation and whether the persistency of secretion during the declining phase of lactation curve is regulated by this hormone. In order to answer these important questions and further establish the function of the lactogenic hormone in relation to the initiation and maintenance of lactation, it became necessary to remove the pituitary, observe the deficiency which follows, and attempt to repair that deficiency by the administration of extracts of the pituitary gland.

Effect of Hypophysectomy on the Initiation and Maintenance of Lactation

Allan and Wiles (1932) first reported that cats hypophysectomized late in pregnancy delivered normally but never suckled their young. Pencharz and Lyons (1934) reported that hypophysectomy of guinea-pigs on the 40th or 41st day of pregnancy did not prevent the delivery of viable young at term. However, the animals secreted only a small amount of milk which persisted for only 12 to 18 hours *post-partum.*

Selye et al. (1933) reported that hypophysectomy of lactating rats and mice at any stage during the lactation period promptly led to a rapid cessation of milk secretion. Similar observations were reported in ferrets and cats (McPhail, 1935), rats (Reece, Turner and Hill, personal communication), goats (Hill et al., 1935 and Gomez and Turner, 1936) and guinea-pigs (Gomez and Turner, 1936 and Macchiaurulo, 1936) after hypophysectomy.

It would appear from the above observations that the pituitary is necessary for both the initiation and maintenance of milk secretion. Lyons et al. (1933), Evans (1934) and Houssay (1935) reported success in initiating lactation in hypophysectomized dogs with alkaline extracts of the pituitary glands. McPhail (1935) reported similar results in hypophysectomized cats and ferrets. Gomez and Turner (1936) and Nelson and Gaunt (1936) were able to initiate lactation in hypophysectomized guinea-pigs with mammary glands previously prepared for lactation by the injection of estrogen or by ovarian grafts, with crude extracts of the pituitaries of sheep.

Using a somewhat purified lactogenic hormone, galactin, Gomez and Turner (1936) failed to initiate, reinitiate or prevent the rapid cessation of lactation in hypophysectomized goats, cats and guinea-pigs. Nelson and Gaunt (1936) reported similar observations in hypophysectomized guinea-pigs after the injection of the purified lactogenic hormone. These results were interpreted as due to a loss of some of the essential hormones during the process of purification, which in conjunction with galactin stimullated the secretion of milk. Assuming that the pituitary supplied the necessary stimuli, the question as to which of the pituitary hormones were concerned and the mechanism regulating these stimuli arose. There is an increasing amount of evidence that these hormones can act indirectly through the other endocrine glands, particularly the thyroid and the adrenals. The influence of these glands in lactation will be considered in a later section so that they may be considered individually in the light of the present studies.

Recent studies indicated that the pituitary secretes a hormone which influences carbohydrate metabolism. The physiological influence of this hormone on the organism as a whole has been indicated by the effect of hypophysectomy upon the level of glucose in the blood. This hormone requires first consideration in studies which involve the use of hypophysectomized experimental animals, since much is dependent upon the level of glucose in the blood for the maintenance of their well being. Nelson (1936) and Gomez and Turner (1936) reported that the development of spontaneous fatal hypoglycemic coma which frequently occurs among hypophysectomized animals was successfully controlled to a great extent by the daily parenteral administration of glucose solution. Gomez and Turner (1936) are of the opinion that carbohydrate metabolism plays an important indirect role in milk secretion, since it regulates the level of glucose in the blood which is one of the important percursors of milk.

In the experiments to be described, it should be recognized, therefore, that the regular administration of glucose after hypophysectomy of experimental mammals prevented the development of hypoglycemic coma and further maintained the level of blood glucose. The first of the series of experiments was undertaken to determine whether glucose would supplement the action of galactin upon the lobule-alveolar epithelium and ultimately initiate milk secretion.

Experiment XII. Studies with Galactin.—Five completely hypophysectomized, experimentally induced cryptorchid males carrying functional ovarian-testicular grafts for 6 weeks, which received 10 mgs. of galactin daily, showed no evidence of lactation after 10 days. In two completely hypophysectomized guinea-pigs as above, lactation which would have been established within 3 days after the removal of the testis grafts, failed to appear within 10 days following hypophysectomy and removal of the ovarian-testicular grafts.

Two partially hypophysectomized guinea-pigs which received 10 mgs. of galactin each daily were in lactation after 2 and 3 days, respectively. Two control guinea-pigs which received 5 mgs. of galactin daily and one with the ovarian testicular graft removed were in heavy lactation after three days.

These observations indicate that galactin alone will not initiate lactation in hypophysectomized guinea-pigs.

Effect of postponed galactin treatment on lactation.—Seven lactating guinea-pigs were hypophysectomized during the first week after delivery. Lactation at this time was well established and would normally continue for 2 to 3 weeks. All of these animals showed a rapid decline in milk secretion the first day after the operation, being completely dry 2 to 3 days later. Five of these animals, which received from 20 to 50 mgs. of glucose each daily, beginning immediately after complete cessation of lactation, and the remaining two with 200 mgs. of glucose alone, all showed no evidence of milk secretion after 10 days. The mammary glands at autopsy and later verified by microscopic examination, revealed in all instances marked involution of the glandular parenchyma.

Six partially hypophysectomized lactating guinea-pigs showed a decline in milk secretion during the first few days (2 to 3 days) following the operation, but soon returned to a level which was sufficient to raise their young without other feed. The mammary glands at autopsy after 15 days showed abundant milk secretion.

Effect of immediate galactin treatment.—Three completely hypophysectomized lactating guinea-pigs which received 30 to 40 mgs. of galactin and 200 mgs. of glucose each daily, beginning immediately after hypophysectomy, ceased lactating completely after 2 to 4 days. One partially hypophysectomized lactating guinea-pig which received 20 mgs. of galactin and glucose solution daily was in heavy lactation after 10 days.

One completely hypophysectomized cat (with 4 young), which received 100 mgs. of galactin and 5 cc. of saturated aqueous glucose solution daily, was completely dry after 7 days. All of her young died after 7 days, evidently due to a lack of nourishment. Histological examination of the mammary gland obtained at autopsy revealed collapsed alveoli indicating the occurrence of an involutionary process of the glandular complex following the cessation of lactation. One partially hypophysectomized cat (with 5 young) which received similar treatment as above, was in lactation after 10 days, and continued in lactation without galactin for 40 days.

One completely hypophysectomized rabbit which received 20 mgs. of galactin and glucose solution, beginning immediately after the operation, failed to lactate during an injection period of 6 days.

The failure of galactin and glucose to stimulate or support lactation in hypophysectomized guinea-pigs (15 cases), rabbits (1 case) and cats (1 case) was not taken to indicate the inadequacy of these therapies in stimulating the secretory activity of the lobule-alveolar epithelium, but was due rather to a general physiological disturbance in hypophysectomized animals which invariably reduced to a low level the available precursors of milk in the blood. Since the regulation of the precursors of milk is under direct control of the endocrine glands maintained directly or indirectly by the pituitary, the need for the study of the influence of these glands or their secretions on lactation was indicated.

Influence of the Thyroid Gland in Lactation

It was well established that the thyroid glands became inactive and atrophic following hypophysectomy (p. 13). This atrophy is prevented and the normal structures and functions restored by pituitary implants or by the administration of extracts containing the thyrotropic hormone. The relation of the thyroid to milk secretion is not definitely established beyond the fact that thyroidectomy caused a decline in milk yield while thyroid feeding or the parenteral administration of thyroxine during the declining phase of lactation increases milk production. It is thus apparent that the thyroid glands are in some way concerned in the mechanism of milk secretion.

Dragstedt et al. (1924) reported that thyroid-parathyroidectomized dogs conceive and pass through normal pregnancy and lactation after parturition, provided that the tetany which frequently developed during pregnancy was controlled. Nelson and

Tobin (1936) reported that rats thyroid-parathyroidectomized on the 13th to 15th days of pregnancy bore normal litters and in all instances raised their young.

Graham (1934) reported that the feeding of desiccated thyroid gland during the declining phase of milk production after thyroidectomy or in normal lactating cows caused a rapid increase of milk and fat production after which the gradual decline continued. Excessive thyroid feeding of thyroidectomized cows caused a rapid decline of milk production. Further, he stated that thyroid feeding during the 4th to 6th weeks after parturition, the time at which milk production normally increases had no effect on milk production.

Jack and Bechdel (1935) stated that the injection of thyroxine, so as to increase the basal metabolic rate of cows by 10 per cent, produced an increase in milk production of about 11.2 per cent, while thyroxine injections in dosages which raised the basal metabolic rate 30 per cent, increased milk production by only 6.5 per cent. The injection of thyroxine was effective only during the declining phase of lactation.

Folley and White (1936) reported an average increase of 28 per cent in the milk production of 4 Shorthorn dairy cows by the injection of 10 mgs. of thyroxine daily for 15 days. During the four days following the cessation of thyroxine injection, milk production declined to approximately 12 per cent below the base line. They noted the decline in milk yield occurred in the morning milking.

In order to determine the role of the thyroid in milk secretion, studies were undertaken concerning the effect of thyroxine on lactation in hypophysectomized guinea-pigs. The experimental animals were given glucose injections along with the hormones, except during the period when the animals were receiving crude pituitary extract injections.

Experiment XIII. Studies with Thyroxine and Galactin.—In the study on the effect of thyroxine in relation to the initiation and maintenance of lactation in the guinea-pig, the experimental animals were injected with 10 mgs. of desiccated whole sheep pituitaries daily for 2 days in order to prevent the cessation of lactation attributable to the shock of the operation.

Two completely hypophysectomized lactating guinea-pigs which received 0.05 and 0.1 mg. of thyroxine daily, beginning immediately after the operation, were dry after 2 days.

The rapid cessation of lactation which followed complete hypophysectomy was not prevented in two normal lactating females and one experimentally induced lactating male by the injection of 5 and 10 mgs. of galactin and 0.025 and 0.05 mg. of thyroxine daily beginning on the third day after complete removal of the pituitary gland, the animals being completely dry in 2 to 3 days. Three completely hypophysectomized normal lactating guinea-pigs showed no evidence of lactation after three daily injections of 10 mgs. of galactin and 0.05 mgs. of thyroxine. Lactation was re-initiated in these animals following three daily injections of 10 mgs. of galactin, 0.2 to 0.4 dog units of eschatin and 200 mgs. of glucose each beginning immediately after complete cessation of lactation during the thyroxine and galactin injections.

Three operated guinea-pigs in lactation after 6 daily injections of thyroxine and galactin were found only partially hypophysectomized at autopsy.

The inability of thyroxine (2 cases) or thyroxine and galactin (5 cases) to stimulate lactation in hypophysectomized guinea-pigs was taken to indicate that thyroxine is not a limiting factor in the rapid cessation of lactation which normally followed the complete removal of the pituitary gland. It was not interpreted as indicating the lack of importance of the thyroid in lactation.

Influence of the Adrenal Cortex in Lactation

The observations which have been reported on the relationship of the adrenal glands to lactation appear in many cases very limited and indefinite. As indicated elsewhere, the reproductive function of animals in adrenal insufficiency was held in abeyance. Adrenalectomy of lactating rats was immediately followed by a rapid cessation of lactation. Carr (1931) reported that the Swingle-Pfiffner's extract does not sustain lactation after the removal of the adrenals. Britton and Silvette (1931) reported similar results with adrenal cortical extracts in adrenalectomized rats. Hartman et al. (1933) reported that their adrenal cortical extract "cortin" was not able to maintain lactation in adrenalectomized rats. Another preparation from the adrenal cortex "cortilactin", however, was said to be effective.

Gaunt and Tobin (1936) reported that the life sustaining dosage of the adrenal cortical hormone was insufficient to maintain lactation in adrenalectomized rats. However, the administration of twice the life sustaining dosage supported lactation and normal litters were raised by the mother. The oral administration of salt solution in the drinking water reduced the requirement of the

hormone. Grollman (1936) stated that spontaneous .estrum and reproduction may still occur in rats maintained in a state of chronic adrenal insufficiency. In such animals there was no evidence of failure of mammary function and the young were normally nourished. Britton and Kline (1935) reported that female rats adrenalectomized during pregnancy failed to go through the normal process of parturition; abortion was commonly observed; and lactation did not follow.

They further reported that extracts of the adrenal cortex gave adequate protection to the adrenalectomized rats and allowed normal reproduction and lactation. Levenstein (1936) reported a deficiency of the secretory activity of the mammary gland of adrenalectomized rats as indicated by their inability to nourish their young.

In adrenalectomized dogs maintained in a state of normal health by the injection of cortin, Swingle and Pfiffner (1932) reported that pregnancy and lactation were not impaired. Gaunt and Parkins (1933) reported that adrenalectomized dogs kept alive by the injection of highly purified Swingle-Pfiffner's extract reproduced normally. They did not report, however. on the condition of the mammary gland.

Hypophysectomy of lactating animals elicit effects similar to those observed in adrenalectomy. Unlike the adrenalectomized animals, however, the administration of the adrenal cortical extract, eschatin, into hypophysectomized guinea-pigs, failed to initiate or prevent the rapid cessation of lactation after the operation (Gomez and Turner, 1936). Nelson and Gaunt (1936) reported that the administration of purified lactogenic hormone and Swingle-Pfiffner's adrenal cortical extract initiated lactation in 12 hypophysectomized guinea-pigs with mammary glands previously prepared for lactation by the injection of estrogen. In a preliminary report, Gomez and Turner (1936) confirmed these observations. These authors further reported that galactin, eschatin and glucose prevented the rapid cessation of lactation in hypophysectomized lactating guinea-pigs and by continued treatment, lactation was maintained for periods corresponding to the normal duration of lactation in these animals. Since the observations were very limited, additional experiments were conducted in order to secure additional evidence on the role of the adrenal cortex in lactation and also to determine whether the above therapies would be

effective in stimulating or maintaining lactation in other species of laboratory mammals, such as the rabbit and cat.

Experiment XIV. Studies with Galactin and Eschatin.—Initiation of Lactation—Six completely hypophysectomized guinea-pigs, consisting of three males and three females which were previously prepared for lactation by the injection of estrogen (theelin) daily for 20 days, were in lactation after three days following the daily injection of 0.2 to 0.4 dog units of eschatin, 5 mgs. of galactin and 200 mgs. of glucose beginning immediately after hypophysectomy. In two other animals which received similar treatment but found to be only partially hypophysectomized at autopsy, lactation was established after three injections of galactin and eschatin. One completely hypophysectomized guinea-pig which received 5 mgs. of galactin and another 0.2 dog units of eschatin daily beginning immediately after hypophysectomy as controls, failed to lactate after 7 days. One completely hypophysectomized rabbit which received simultaneous injections of 25 mgs. of galactin and 2 dog units of eschatin in conjunction with glucose, showed a lactation response of plus three (3+) after 4 days.

Maintenance of lactation.—A total of ten lactating guinea-pigs completely hypophysectomized 2 to 3 days after parturition were maintained in lactation for periods ranging from 5 to 20 days by the simultaneous injection of 0.2 to 0.4 dog units of eschatin, 3 to 5 mgs. of galactin and 200 mgs. of glucose. The presence of milk secretion in these instances was determined by manual manipulation of the teats of the animals before the young, which had been kept away for several hours, were allowed to nurse. A gradual decline of milk secretion between the 10th and 20th days was observed as indicated by the size attained by the glands between milkings at 4 to 6 hour intervals. Histological examination of the mammary gland obtained at autopsy 10 to 20 days after hypophysectomy and treatment showed a varying degree of involution of the glandular complex (Fig. 13 and 14).

Two completely hypophysectomized lactating guinea-pigs which received 10 mgs. of galactin and 200 mgs. of glucose immediately after hypophysectomy, ceased lactating after three days. Lactation was re-initiated in these animals within 3 days by the injection of 5 mgs. of galactin and 0.2 dog units of eschatin, beginning immediately after the complete cessation of lactation, and by continued treatment lactation was maintained for 10 days.
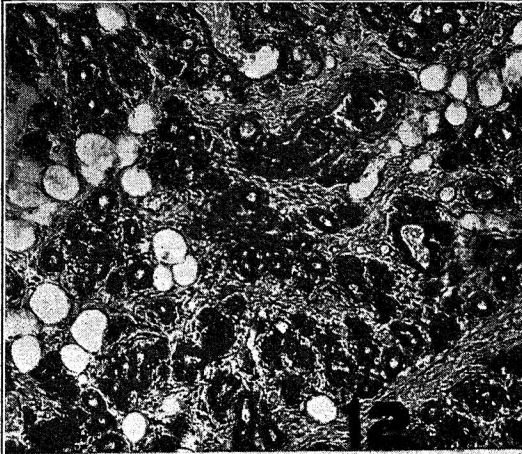
Fig. 12.—Microphotograph of a sectioned mammary gland of a completely hypophysectomized guinea - p i g which received 20 daily implants of rat pituitaries. The donor rats received estrogen injections for 20 days. Enlarged approximately 52 times.
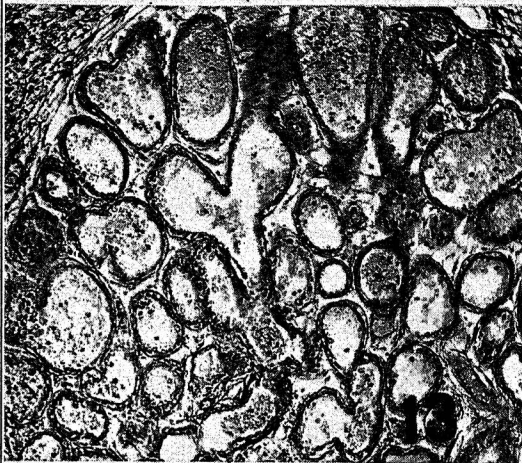
Fig. 13.—Microphotograph of a sectioned mammary gland of a completely hypophysectomized guinea-pig taken 10 days after the operation and simultaneous treatment with 10 mgs. of galactin, 0.5 cc. of adrenotropic hormone and a glucose solution. Enlarged approximately 52 times.
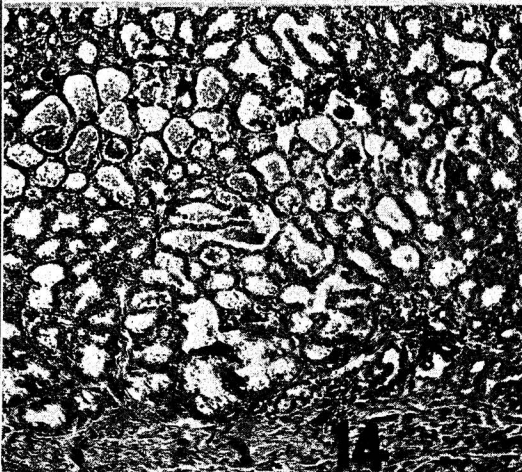
Fig. 14.—Microphotograph of a sectioned mammary gland of a completely hypophysectomized lactating guinea-pig taken 20 days after the operation and treatment with galactin, adrenotropic hormone and a glucose solution. The gland parenchyma showed evidence of involution although the secretion of milk was maintained. Enlarged approximately 52 times.

Three completely hypophysectomized lactating guinea- pigs were kept in lactation throughout an observation period of 10 days by the simultaneous injection of 10 mgs. of galactin and 5 to 10 mgs. of desiccated guinea-pigs adrenal gland and glucose beginning immediately after the operation.

One completely hypophysectomized cat (with 3 young) was kept in lactation for 30 days by the simultaneous daily injection of 25 mgs. of galactin and 5 dog units of eschatin. The animal developed coma three times during the period of observations, but was successfully resuscitated on the first two occasions by a gradual intracardial administration of 5 cc. of saturated glucose solution. Sufficient milk was produced as indicated by the maintenance of the health of the young, throughout the period of observations. The simultaneous injection of galactin, eschatin or the desiccated guinea-pig adrenal gland and a glucose solution was capable of initiating or maintaining lactation in completely hypophysectomized guinea-pigs (22 cases), rabbits (1 case) and cats (1 case).

On the basis of the above observations it was assumed that the reason for the failure of galactin and glucose in initiating or preventing the rapid cessation of lactation after hypophysectomy of laboratory mammals was obviously due to the insufficient secretion of the adrenal cortical hormone caused by the withdrawal of the adrenotropic hormone coincident with the complete removal of the pituitary gland. If the above assumption is correct, the injection of galactin and glucose should stimulate or support the continuance of lactation provided the normal function of the adrenal cortex is maintained by the injection of the adrenotropic hormone beginning immediately after hypophysectomy. Studies were therefore undertaken in which the objective was to determine the effect of the adrenotropic hormone on the initiation and maintenance of lactation.

Experiment XV. Studies with Galactin and the Adrenotropic Hormone. Initiation of Lactation.—Lactation was initiated in two completely hypophysectomized male guinea-pigs carrying functional ovarian grafts for 6 to 8 weeks by the simultaneous daily injection of 20 mgs. of galactin and 0.1 cc. and 0.2 cc. of adrenotropic hormone for three days beginning immediately after the operation.

Five completely hypophysectomized, involuted, multiparous guinea-pigs previously prepared for lactation by the injection of

estrogen daily for 20 days, were in lactation 3 to 5 days after the initiation of simultaneous daily injections of 0.1 to 0.2 cc. of adrenotropic hormone, 10 to 20 mgs. of galactin and 200 mgs. of glucose, beginning immediately after hypophysectomy.

One completely hypophysectomized control receiving 10 mgs. of galactin and 200 mgs. of glucose daily and another receiving 0.2 cc. of adrenotropic hormone and glucose daily failed to lactate after 6 days. One non-hypophysectomized control which received 10 mgs. of galactin was in lactation after two days while another which received 0.2 cc. of adrenotropic hormone failed to lactate after 6 days. Two partially hypophysectomized guinea-pigs were in heavy lactation after three days following the initiation of the simultaneous daily injections of 0.1 cc. of adrenotropic hormone and 10 mgs. of galactin.

Of a total of 4 completely hypophysectomized rabbits which received simultaneous injections of 10 to 20 mgs. of galactin and 0.5 to 1.0 cc. of adrenotropic hormone each daily, two showed a lactation response of plus 2 and 3 after 4 days; and a third plus 2 after 6 days. The fourth animal, which succumbed on the third day showed no lactation response. One intact control which received 15 mgs. of galactin daily beginning at the end of pseudo-pregnancy showed a lacation response of plus 4, while a second animal which received 1.5 cc. of adrenotropic hormone showed no lactation response after 6 days.

Maintenance of lactation.—A total of eight guinea-pigs, completely hypophysectomized 2 to 7 days after parturition, were maintained in lactation for periods ranging from 8 to 15 days by the simultaneous injections of 10 to 20 mgs. of galactin and 0.1 to 0.2 cc. of adrenotropic hormone and glucose, beginning immediately after hypophysectomy.

Two completely hypophysectomized lactating guinea-pigs which received 10 mgs. of galactin and 0.5 cc. and 0.25 cc. of adrenotropic hormone succumbed after 3 and 5 days. These animals were still in heavy lactation at the time of death. One completely hypophysectomized lactating guinea-pig which received 0.5 cc. of adrenotropic hormone and another which received 10 mgs. of galactin as controls ceased lactating after three days. Milk secretion was re-initiated in these animals by the simultaneous injection of 0.2 cc. of adrenotropic hormone, 10 mgs. of galactin and 200 mgs. of glucose beginning immediately after the cessation of milk secretion.

The simultaneous daily injection of galactin, adrenotropic hormone and glucose solution was capable of initiating lactation in hypophysectomized guinea-pigs (7 cases) and rabbits (4 cases). Lactation was also maintained in hypophysectomized guinea-pigs (10 cases) by the above treatment.

Summary.—The injection of the somewhat purified lactogenic hormone, galactin and glucose solution immediately after hypophysectomy or after the complete cessation of lactation in hypophysectomized lactating laboratory mammals was incapable of preventing the rapid cessation or of re-initiating lactation. The injection of galactin, however, in conjunction with eschatin or the adrenotropic hormone and glucose solution under favorable conditions initiated lactation in hypophysectomized animals. In hypophysectomized lactating animals, these therapies were capable of sustaining lactation comparable to that which occurred in normal animals. The injection of thyroxine after hypophysectomy in laboratory mammals alone or in conjunction with galactin and glucose did not initiate or prevent the cessation of lactation.

ENDOCRINE CONTROL OF THE MAMMARY GLAND

Marked progress in our knowledge of the endocrine control of the mammary gland has been made since hypophysectomized animals have been employed in experimental work. Before 1936, it was believed that the growth of the duct system of the mammary gland was directly stimulated by the estrogenic hormone. Further, the extensive arborization of ducts and the subsequent proliferation of the lobule-alveolar system which occurs during the first-half of pregnancy or during pseudo-pregnancy were stimulated directly by the estrogenic hormone and the secretion of the corpus luteum, progestin. Recent studies have shown, however, that though the normal growth and development of the mammary gland proceeds only in the presence of a functional ovary, the action of the ovarian hormone is believed to be indirect by way of the pituitary gland.
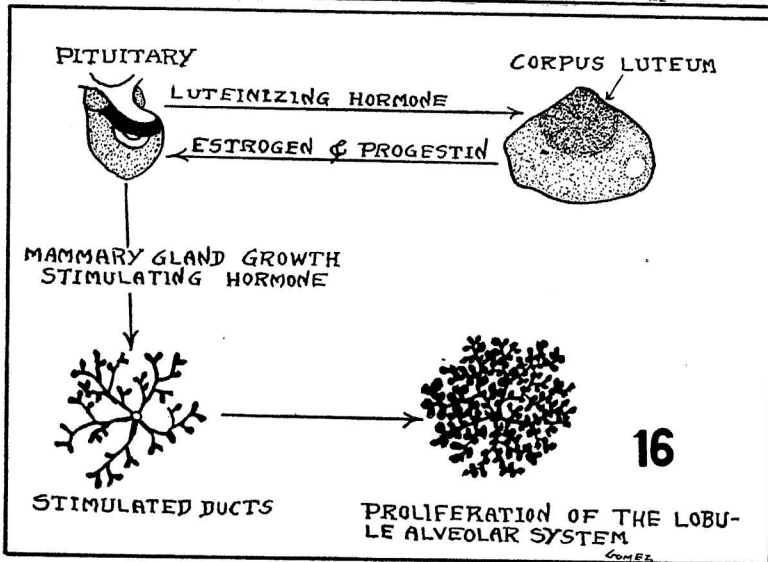
It is now well established that at the approach of sexual maturity, in the female, the pituitary produces a hormone, the follicle stimulating hormone, which causes the growth of the ovaries and the development and maturation of the Graafian follicles. The follicles in turn elaborate a hormone, estrogen, which in most species stimulates only the growth of the duct system. In addition the estrogens exert an influence on the pituitary, suppressing the production of the follicle stimulating hormone and permitting the production of the luteinizing hormone which

induces ovulation and the formation and maintenance, during pregnancy or pseudo-pregnancy, of functional corpora lutea. The estrogen and progestin secretions of the ovary were assumed to exert a direct influence on the mammary gland parenchyma and to stimulate the development of the lobule-alveolar system.

From the evidence presented in the studies on the experimental development of the mammary gland of normal or castrate male and female laboratory mammals by the injection of estrogen and progestin, the direct ovary-mammary gland relationship discussed above appeared attractive. However, this interpretation of the mode of action of the ovarian hormones upon the growth of the mammary gland parenchyma was clearly inadequate when hypophysectomized animals were employed. The adminstration of the estrogen or estrogen and progestin was ineffective in stimulating the growth of the duct system of completely hypophysectomized animals. Likewise, the administration of the purified pituitary hormones or desiccated sheep pituitaries alone or in combination with the ovarian hormones was ineffective. From these observations it appeared that estrogen might act upon the pituitary, stimulating the secretion of a principle either not present or present only in small amounts in the pituitaries of animals unstimulated by estrogen. The daily implants of pituitaries, from donors which had previously been treated with estrogen, into hypophysectomized-castrate animals were found effective in stimulating the growth and development of the mammary gland complex.

The above observations were taken to indicate that estrogen (Fig. 15) and probably also estrogen and progestin (Fig. 16) elaborated by the ovaries stimulated the production of specific hormones by the pituitary gland which in turn exerted a direct action upon the mammary gland thus stimulating the growth of ducts and proliferation of the lobule-alveolar system.

During the second half of pregnancy or in animals with pseudo-pregnancy extending for periods corresponding to the duration of normal gestation, the mammary gland gradually hypertrophied as a result of the initiation of secretory activity and the gradual accumulation of secretion. The initiation of secretory activity has been attributed to the influence of the lactogenic hormone of the pituitary, although until recently the mechanism of its function in the initiation and the part it plays in the maintenance of lactation has been only vaguely understood.

Schematic Diagram Showing the Pituitary-Ovary-Mammary Gland Interrelationship in Mammary Development

Fig. 15.—The estrogenic hormone secretion of the ovary stimulated the production of a specific hormone by the pituitary gland which exerted a direct action upon the mammary gland and stimulated the growth of the duct system.

Fig. 16.—The ovarian hormones, estrogen and progestin, probably stimulated the production of a specific hormone by the pituitary which caused further arborization of ducts and subsequent proliferation of the lobule-alveolar system.
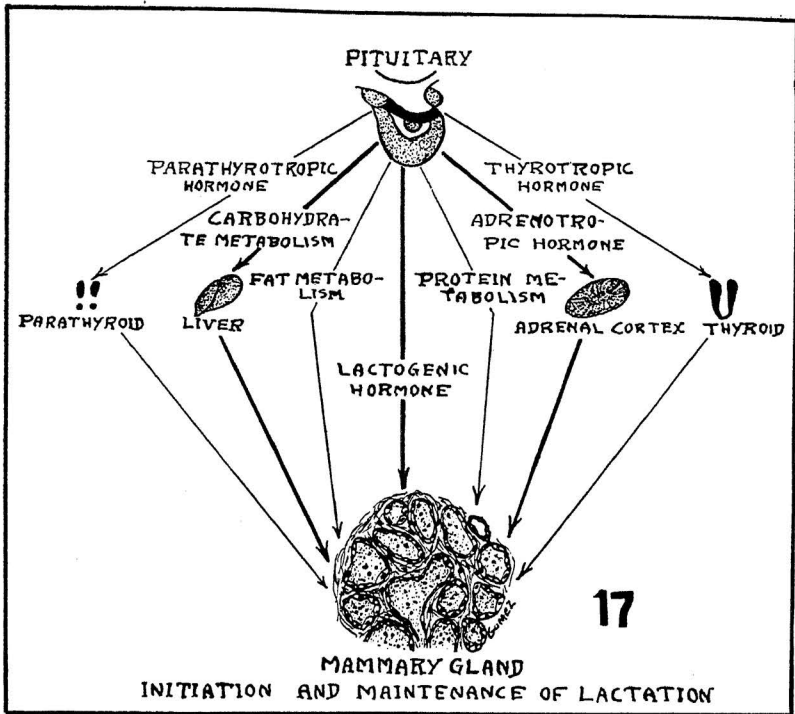
Fg. 17.—Schematic diagram showing the endocrine interrelatonship in the control of normal lactation. The bold lines indicate the pituitary principles indispensable for the initiation and maintenance of lactation. The other principles, indicated by light lines are probably concerned in the metabolism of the precursors of milk and undoubtedly play a role in the secretion of milk.

The present study has established, that in addition to the influence of the lactogenic hormone which is constantly needed during the period of active lactation, the simultaneous though indirect influence of the carbohydrate metabolism and adrenotropic hormones is indispensable for the initiation and maintenance of lactation. Thus a deficiency or withdawal of any one of these hormones or the organs stimulated by them invariably impair the secretory activity of the lobule epithelium and consequently the secretion of milk. The endocrine control mechanism is shown in Fig. 17.

The lactogenic hormone exerts a direct influence upon the lobule epithelium thereby initiating the secretory function, and by continued stimulation maintains the activity of the secretory cells provided the precursors of milk are ample. It is evident

that the lactogenic hormone is concerned primarily in the process of the secretion of milk.

Preliminary work has already shown that it is frequently possible to increase the secretion of milk by the injection of the lactogenic hormone, thus supplementing the action of the normal secretion by the pituitary. These observations were taken to indicate that the amount of secretion of this hormone by dairy cows was one of the important reasons for the variation of milk production.

Since the pioneer work of Kaufman and Magne (1906) the importance of blood glucose in the secretion of milk has been appreciated. Recent studies have shown that the level of glucose in the blood was influenced by the carbohydrate metabolism hormone of the pituitary. In cases of the deficiency, such as that produced by hypophysectomy, the animal's ability to absorb glucose or convert glucose from protein is markedly reduced. The decreased ability of lactating animals to regulate the blood sugar after hypophysectomy and the continued utilization of blood glucose by the mammary gland for milk synthesis caused a rapid depletion of glucose in the glood and ultimately resulted in the development of a fatal hypoglycemic coma. The blood glucose is not only the precursor of the lactose of the milk but undoubtedly is the source of the energy for the metabolism of the gland.

In the absence of a purified carbohydrate metabolism hormone which would regulate the blood sugar of our experimental animals, it was found possible by the frequent parenteral administration of a glucose solution to control this condition. It should be emphasized, however, that the regulation of carbohydrate metabolism by the pituitary was believed to play an important role in permitting the secretion of large quantities of milk by some dairy cows.

The importance of the adrenal cortex in lactation was first indicated by the fact that adrenalectomy of lactating laboratory mammals was immediately followed by a complete cessation of lactation. The lactation of such animals was restored by the adequate administration of adrenal cortical extracts. In adrenal insufficiency induced either by adrenalectomy or hypophysectomy, there occurred a disturbance of the sale and fluid metabolism. The withdrawal of the adrenotropic hormone caused a rapid shifting of the salts and fluids from the tissue and intercell spaces into the blood stream. The resulting polyurea after hypophysectomy and

the associated increased excretion of salt, i. e. sodium chloride, caused a gradual withdrawal of fluid and consequently a dehydration of the tissue. As a result the passage of the precursors of milk from the blood stream was prevented, thereby depressing the secretory function of the lobule cells.

The fact that the amount of adrenal cortical hormone required for lactation was found to be much greater than the amount required to sustain life in adrenalectomized animals indicates that the secretion of the adrenal cortex might influence the rate of secretion.

Other pituitary hormones directly or indirectly concerned with metabolic functions while apparently not indispensable for lactation, nevertheless, are important for the maintenance of the maximum activity of the secretory cells and the secretion of milk. The function of the thyroid gland in milk secretion is particularly interesting, since the yield of milk is diminished by thyroidectomy and is increased by desiccated thyroid feeding or the administration of thyroxine. Further, the basal metabolic rate and the pulse rate of the animals is markedly reduced in thyroid insufficiency. Since the blood is the medium of the transport of the precursors of milk to the mammary gland, the reduced rate of blood flow through the mammary gland may account for the reduced milk secretion in thyroid deficiency. The increased milk production in hyperthyroid animals may be due to the stimulation of the secretory cells by thyroxine and the increased amount of blood flowing through the mammary gland.

It is probable that the exceptionally high milk producing capacity of some dairy cows may be due, at least in part, to the hyperactivity of the thyroid gland during the period of active lactation.

The importance of calcium in the synthesis of milk is well known. Recent studies have shown that the pituitary stimulates the parathyroid to the secretion of the parathyroid hormone, parathormone, which in turn regulates calcium metabolism.

The significance of the pituitary fat metabolism hormone in relation to lactation has not yet been investigated. As large quantities of fat are required in milk secretion, it is probable that it has an important place in maintaining maximum milk production.

Recent studies have shown that associated with the so-called growth hormone, the pituitary secretes a principle which has been

shown to affect protein metabolism. It would appear that this principle may play a role in the mobilization of protein for the synthesis of protein in the milk.

In general it may be stated, that although the lactogenic, the carbohydrate metabolism and the adrenotropic hormones are indispensable for the initiation and maintenance of lactation, the direct or indirect supplementing effect of the other pituitary hormones or the secretions of the endocrine glands controlled by them are believed to have great significance in normal lactation (Fig. 17.)

It will be appreciated that a great deal of work is yet to be done before the endocrine interrelationships in the control of the mammary gland will be thoroughly elucidated. Though our present knowledge of the mechanism of the endocrine control is gravely incomplete, the present study has established for the first time some of the obscure points in the endocrine physiology of the mammary gland.

SUMMARY AND CONCLUSIONS

1. The administration of the ovarian hormones, estrogen and progestin, which produces complete hyperplasia of the mammary gland complex of normal or castrated laboratory mammals, was ineffective in stimulating the growth of the mammary gland of completely hypophysectomized guinea-pigs, rats, mice, rabbits, cats and ground squirrels.

2. The administration of purified thyrotropic, adrenotropic and lactogenic hormones and desiccated whole sheep pituitaries, alone and in combination with estrogen or estrogen and progestin failed to stimulate the growth of the duct system of the mammary gland of completely hypophysectomized rats and guinea-pigs. These observations were interpreted as indicating that the growth of the mammary gland parenchyma is not due to the synergistic action of pituitary and ovarian hormones.

3. The daily implants for 20 to 25 days of rat pituitaries obtained from donors which had previously been injected with estrogen daily for 10 to 20 days, stimulated the growth of the ducts and the subsequent proliferation of the lobule-alveolar system of the mammary gland of normal or castrated hypophysectomized male and female guinea-pigs comparable to that observed in normal animals which received estrogen treatment. These observations were interpreted as indicating that the growth of the mammary gland parenchyma of the guinea-pig is under the direct influence

of a specific hormone(s) of the pituitary gland. The production of this hormone(s) by the pituitary occurs only after its stimulation by the ovarian hormones.

4. Hypophysectomy of lactating guinea-pigs and cats at any time during the lactation period was immediately followed by a rapid cessation of milk secretion, the animals being dry in two to three days.

5. Replacement therapy with crude pituitary extracts beginning immediately after hypophysectomy of lactating guinea-pigs prevented the rapid cessation of lactation or re-established it when administered immediately after complete cessation of lactation.

6. Replacement therapy with purified lactogenic hormone, galactin, and glucose solution, beginning immediately after the complete cessation of lactation after hypophysectomy, failed to re-establish lactation. Likewise, this therapy was ineffective in preventing the rapid cessation of lactation in guinea-pigs even when the injection was begun immediately after the operation.

7. The simultaneous injection of thyroxine or the thyrotropic hormone, galactin and a glucose solution failed to initiate or support the continuance of lactation after the hypophysectomy of guinea-pigs.

8. The simultaneous injection of galactin, glucose solution and the adrenotropic hormone or the extract of the adrenal cortex was capable of initiating lactation in hypophysectomized guinea-pigs and rabbits. The above mentioned therapy was likewise effective in supporting the continuance of lactation in hypophysectomized lactating guinea-pigs. The lack of any one of the above factors in replacement therapy invariably held the secretory activity of the lobule-alveolar epithelium in abeyance. The above mentioned factors responsible for the initiation of lactation were also responsible for the maintenance of milk secretion during normal lactation.

BIBLIOGRAPHY

Allan, E., and Wiles, P. 1932 *The Role of the Pituitary Gland in Pregnancy and Parturition. I. Hypophysectomy.* J. Physiol. Vol., 74, p. 23.

Allen, E., Francis, B. F., Robertson, L. L., Colgate, C. F., Johnson, C. G., Doisy, E. A., Kountz, W. B., and Gibson, H. F. 1924 *The Hormone of the Ovarian Follicle; its Localization and Action in Test Animals, and Additional Points Bearing upon the Internal Secretion of the Ovary.* Am. J. Anat. Vol., 34, p. 133.

Allen, E., Gardner, W. U., and Diddle, A. W. 1935 *Experiments with Theelin and Galactin on Growth and Function of the Mammary Gland of the Monkey.* Endocrinology, Vol. 19, p. 305.

Ancel, P., and Bouin, P. 1911 *Recherches sur les Fonctions du Corps Jaune Gestatiff. II. Sur le Determinisme Developement de la Glande Mammaire au Cours de la Gestation.* J. de Physiol. et de Path. Gen., T. 13, p. 31.

Anderson, E. M., and Collip, J. B. 1933 *Thyrotropic Hormone of Anterior Pituitary.* Proc. Soc. Exper. Biol. and Med., Vol., 30, p. 680.

Anderson, E. M., and Collip, J. B. 1934 *Studies on the Physiology of the Thyrotropic Hormone of the Anterior Pituitary.* J. Physiol. Vol., 82, p. 11.

Anselmino, K. J., and Hoffmann, F. 1931 *Nachweis der Antidiuretischen Komponente des Hypophysenhinterlappenhormons und Einer Blutdrucksteigernden Substanz im Blute beim Nephrophatie und Eklampsie der Schwangeren.* Klin. Woschr., Bd. 10, S. 1438.

Anselmino, K. J., and Hoffmann, F. 1934 *Studien Zur Physiologie der Milchbildung. I .Das Laktationshemmung* des Hypophysenvordrelappens. Zentrbl. f. Gynäk., Bd. 58, S. 2770.

Anselmino, K. J., Herold, L.. and Hoffman, F. 1935 *Studien zur Physiologie der Milchbildung; Experimenteller Aufbau der Brustdruse und Erzeugung von Lakation bein Männlichen Tieren.* Zentrabl. f. Gynäk., Bd. 59, S. 963.

Anselmino, K. J., Hoffmann, F.. and Herold, L. 1934 *Ueber die Parathyreotrope Wirkung von Hypophysenvorderlappenextrakten.* Klin. Woschr., Bd. 113, S. 45.

Anselmino, K. J., Hoffmann, F., and Herold, L. 1934 *Ueber das Corticotropes Hormon des Hypophysenvorderlappens.* Klin Woschr., Bd. 10, S. 209.

Aron, M. 1929 *Action de la Prehypophyse sur la Thyroide chez le Cobaye.* Compt. rend. Soc. de Biol. T. 102, p. 682.

Aschner, B. 1912 *Ueber die Funktion der Hypophyse.* Arch. f. de ges. Physiol., Bd., 146, S. 1.

Aschner, B. 1929 *Physiologie der Hypophyse, in Hirsch, Handbuch der Inneren Sekretion.* Leipzig. Curt Kabitzsch. Bd. 2, S. 277.

Asdell, S. A. 1931 *Recent Development in the Field of Sex Hormones.* Cornell Vet. Vol. 21, p. 147.

Asdell, S. A. 1932 *The Effect of the Injection of Hypophyseal Extract in Advanced Lactation.* Am. J. Physiol., Vol. 100, p. 137.

Asdell, S. A., and Seidenstein, H. R. 1935 *Theelin and Progestin Injections on Uterus and Mammary Glands of Ovariectomized and Hypophysectomized Rabbits.* Proc. Soc. Exper. Biol. and Med., Vol. 32, p. 931.

Asimoff, J. 1935 *The Influence of Anterior Pituitary Hormones upon Lactation in Cows.* Prob. Zoot. and Exper. Endocrin., Vol. 2, p. 261.

Asimoff, J., Skargiskaya, M., and Ptchelina, V. 1934 *Effect of Alkaline Extracts of Anterior Lobe of the Pituitary Gland on Lactation in Cows*. Prob. Zoot. and Exper. Endocrin., Vol. 1. p. 118.

Atwell, W. J. 1932 *Effects of Administration of Cortical Adrenal Extract to the Hypophysectomized Rat*. Proc. Soc. Exper. Biol. and Med., Vol. 29, p. 639.

Bates, R. W., and Riddle, O. 1935 *The Preparation of Prolactin*. Jour. Pharamacol. and Therap., Vol. 55, p. 365.

Bencan, C. D., and Keller, T. 1927 *Reproduction des phenomenes gravidiques par injections d'hormones ovarienne*. Compt. rend. Soc. de Biol., T. 97, p. 427.

Bennet, L. L. 1936 *Glucose Absorption and Glycogen Formation in the Hypophysectomized Rat*. Proc. Soc. Exper. Biol. and Med., Vol. 34, p. 277.

Best, H., and Campbell, J. 1936 *Anterior Pituitary Extract and Liver Fat*. J. Physiol., Vol. 86, p. 190.

Biasotti, A., and Houssay, B. A. 1932 *Phlorrhizin Diabetes in Fasting or Fed Hypophysectomized Dogs*. J. Physiol., Vol. 77, p. 81.

Bittorf, A. 1919 *Nebennierentumor und Geschlechstdrüsen Ausfall beim Manne*. Klin. Woschr., Bd. 56, S. 776.

Boenheim, F., and Heimann, F. 1932 *Das Fettsoffwechselregulieren Hormon des Hypophysenvorderlappens im Inkretan*. Ztschr. f. d. ges. Exper. Med., Bd. 83, S. 637.

Bonser, G. M. 1936 *The Effect of Oestrone Administration on the Mammary Gland of Male Mice of two Strains Differing Greatly in the Susceptibility to Spontaneous Mammary Carcinoma*. Jour. Path. and Bact., Vol. 42, p. 169.

Bradbury, J. T. 1932 *Study of the Factors influencing Mammary Development and Secretion in the Mouse*. Proc. Soc. Exper. Biol. and Med., Vol. 30. p. 212.

Braier, B. 1932 *Metabolismo Nitrogenado en la Accion Especifica Dinamica de los Perros Hypofisoprivos*. Rev. Soc. Argent. de Biol., T. 7, p. 254.

Braier, B. 1932 *Metabolisme Nitrogenado de los Perros Hypofisoprivos en el Ayuno*. Rev. Soc. Argent. de. Biol., T. 7, p. 140.

Britton, S. W., and Kline, R. F. 1936 *Relation of the Adrenal Cortex to Reproduction and Lactation*. Am. J. Physiol. Vol. 115, p .627.

Britton, S. W., and Silvette, H. 1931 *Some Effect of Cortico-Adrenal Extract and other Substances on Adrenalectomized Animals*. Am. J. Physiol., Vol. 99, p. 15.

Burn, J. H., and Ling, H. W. 1928 *Effect of Insulin on Acetonuria*. J. Physiol., Vol. 65, p. 191.

Burn, J. H., and Ling, H. W. 1929 *Effect of Pituitary Extract and Adrenalin on Ketonuria and Liver Glycogen*. Quart. J. Pharamacol., Vol. 2, p. 1

Burn, J. H., and Ling, H. W. 1930 *Ketonuria in Rats on a Fat diet (a) after Injection of Pituitary (anterior lobe) Extract (b) during Pregnancy*. J. Physiol., Vol. 69, p. 189.

Burrows, H. 1936 *A Comparison of the Changes Induced by Some Pure Estrogenic Compounds in the Mammae and Testes of Mice*. J. Path. and Bact., Vol. 42, p. 161.

Carr, J. L. 1931 *Effect of Swingle's Extract upon Lactation in Adrenalectomized White Rat*. Proc. Soc. Exper. Biol. and Med., Vol. 29, p. 131.

Catchpole, H. R., and Lyons, W. R. 1933 *The Lactation Hormone of the Hypophysis*. Anat. Rec., Vol. 55, p. 49.

Chaikoff, I. L., Reichert, F. L., Larson, P. S. and Mathes, M. E. 1935 *Effect of Hypophysectomy and Cerebral Manipulation in Dog upon Responses of Blood Sugar and Inorganic Phosphorus to Insulin.* Am. J. Physiol., Vol. 112, p. 493.

Champy, C., and Keller, T. 1927 *Development Uterin et Mammaire per Injection d'Hormone Ovarienne.* Compt. rend. Soc. de Biol., T. 97, p. 427.

Chandler, S. B. 1927 *The Relation of Parathyroid to Pregnancy, Lactation and the Growth of Offspring in Albino Rats.* Anat. Rec., Vol. 35, p. 7.

Chandler, S. B. 1932 *The Relation of Parathyroid in Estrus, Pregnancy and Lactation in the Albino Rat.* Anat. Rec., Vol. 53, p. 105.

Cohen, R. S. 1935 *Effect of Experimentally Produced Hyperthyroidism upon the Reproductive and Associated Organs of the Male Rat.* Am. J. Anat., Vol. 53, p. 143.

Collip, J. B. 1933 *The Adrenotropic Hormone of the Anterior Pituitary Lobe.* The Lancet, Vol. 1, p. 347.

Collip, J. B. 1934 *Some Recent Advances in the Physiology of the Anterior Pituitary.* J. Mount Sinai Hosp., Vol. 1, p. 28.

Collip, J. B. 1935 *Diabetogenic, Thyreotropic, Adrenotropic and Parathyrotropic Factor of the Pituitary.* J. A. M. A.. Vol. 104, p. 827.

Collip, J. B., Selye, H., and Thomson, D. L. 1933 *Further Observations on the Effect of Hypophysectomy on Lactation.* Proc. Soc. Exper. Biol. and Med., Vol. 30, p. 915.

Collip, J. B., Selye, H., and Thomson, D. L. 1933 *Beitrage zur Kenntnis der Physiologie des Gehirnanhanges.* Virchow's Arch. Path, Anat., Bd. 290, S. 23.

Corkill, A. B., Marks, H. P., and White, W. E. 1933 *Relation of the Pituitary Gland to the Action of Insulin and Adrenaline.* J. Physiol., Vol. 80, p. 193.

Corner, G. W. 1930 *The Hormonal Control of Lactation. I. Non-Effect of of the Corpus Luteum. II. Positive Action of Extracts of the Hypophysis.* Am. J. Physiol., Vol. 95, p. 43.

Cushing, H. 1912 *The Pituitary Body and its Disorders.* J. B. Lippincot Co., Phila.

D'Amour, M. C., and Keller, A. D. 1933 *Blood Sugar Studies Following Hypophysectomy and Experimental Lesions of the Hypothalamus.* Proc. Soc. Exper. Biol. and Med., Vol. 30, p. 1175.

Dingemanse, E., de Jongh, S. E., Kober, S., and Laquer, E. 1930 *Ueber Kristallinisches Menformon.* Deut. Med. Wochr., Bd. 56, S. 301.

Donahue, J. K. 1934 *Studies on Experimental Lactation in the Rabbit* Physiol. Zool., Vol. 7, p. 479.

Dragstedt, L. R. 1927 *The Physiology of the Parathyroid Gland.* Physiol. Rev., Vol. 7, p. 499.

Dragstedt, L. R., Phillips, K., and Sudan, A. C. 1924 *Tetany of Estrus, Pregnancy and Lactation.* Am. J. Physiol., Vol. 69, p. 477.

Elden, C. A. 1936 *The Pituitary-Ovarian Relationship in Human Hypophysectomized Female.* Endocrinology, Vol. 20, p. 679.

Evans, E. I. 1933 *Diabetogenic Principle of the Anterior Pituitary.* Proc. Soc. Exper. Biol.. Vol. 30, p. 1370.

Evans, E. I. 1933 *Initiation of Copious Milk Secretion in Virgin Goats by Anterior Pituitary.* Proc. Soc. Exper. Biol., Vol. 30, p. 1372.

Evans, E. I. 1934 *Virgin Animals Secrete Milk after Injection of Pituitary Hormone.* U. S. D. A., Yearbook, p. 360.

Evans, H. M. 1934 *Present Position of our Knowledge of Anterior Pituitary Function.* J. A. M. A., Vol. 101, p. 425.

Fischer, R. E., and Pencharz, R. I. 1936 *Carbohydrate Oxidation in Hypophysectomized Rats.* Proc. Soc. Exper. Biol. and Med., Vol. 34, p. 106.

Foster, G. L., and Smith, P. E. 1926 *Hypophysectomy and Replacement Therapy.* J. A. M. A., Vol. 87, p. 2151.

Folley, S. J., and White, P., 1936 *The Effect of Thyroxine on Milk Secretion and the Phosphatase of the Blood and Milk of Lactating Cow.* Proc. Roy. Soc. B., Vol. 120, p. 346.

Freud, J., and de Jongh, S. E. 1935 *The Effect of Progestin upon the Mammary Gland of the Rat.* Acta Brev. Neerl. Physiol. Vol. 5, p. 47.

Friedgood, H. B., and McLean, R. 1937 *The Effect of an Anterior Pituitary Hypophyseal Extract upon the Serum Calcium and Phosphorus.* Am. J. Physiol., Vol. 118, p. 588.

Fry, E. G. 1937 *The Effect of Adrenalectomy and Thyroidectomy on Ketonuria and Liver Fat Content of the Rat Following Injections of Anterior Pituitary Extract.* Endocrinology, Vol. 21, p. 283.

Funk, C., and Zeffrow, P. O. 1932 *The Hormone of Fat Metabolism of the Anterior Pituitary Gland.* Verhandl. 14th Int. Congr. Physiol., Vol. 89.

Fujimoto, Y. 1932 *Ueber den Einfluss der Hypophysenverletzung auf den Blutzuckerspiegel.* Folia Pharmacol. Japon, Bd. 15, S. 10.

Gardner, W. U. 1935 *The Effect of Ovarian Hormones and Ovarian Grafts upon the Mammary Glands of Male Mice.* Endocrinology, Vol. 19, p. 659.

Gardner, W. U.. Gomez, E. T., and Turner, C. W. 1935 *Further Studies of the Effects of the Estrogenic and the Galactopoietic Hormones upon the Mammary Gland of the Rabbit.* Amer. J. Physiol., Vol. 112, p. 673.

Gardner, W. U., and Hill, R. T. 1936 *Effect of Progestin upon the Mammary Glands of the Mouse.* Proc. Soc. Exper. Biol. and Med., Vol. 34, p. 716.

Gardner, W. U., Smith, G. M., and Strong, L. C. 1935 *Stimulation of Abnormal Mammary Growth by Large Amounts of Estrogenic Hormone.* Proc. Soc. Exper. Biol. and Med., Vol. 33, p. 148.

Gardner, W. U., and Turner, C. W. 1933 *The Function, Assay and Preparation of Galactin, a Lactation Stimulating Hormone of the Anterior Pituitary and an Investigation of the Factors Responsible for the Control of Normal Lactation.* Mo. Agr. Exper. Sta. Res. Bul. 196.

Gaunt, R. 1936 (cited by Nelson), 1936, Physiol. Rev. Vol. 16, p. 488.

Gaunt, R., and Parkins, W. M. 1933 *The Alleged Interelationship of the Adrenal Cortical Hormone and the Gonads.* Am. J. Physiol., Vol. 103, p. 18.

Gaunt, R., and Tobin, C. E. 1936 *Lactation in Adrenalectomized Rats.* Anat. Rec., Vol. 64, p. 18.

Gaunt, R., and Tobin, C. E. 1936 *Lactation in Adrenalectomized Rats.* Am. J. Physiol., Vol. 115, p. 558.

Geesink, A., and Koster, S. 1929 *Untersuchung ueber den Gehalt an Wirksamen Hypophysenstoff in der Cerebrospinalflussingkeit des Hundes. Weitere Experimenteller Beitrag zur Kenntniss der Hypophysenfunktion.* Zntrbl. ges. Exp. Med.. Bd. 65, S. 163.

Gerschman, R. 1931 *Calcio y Fosforo de Plasma Sanguineo de los Perros Hipofisoprivos.* Rev. soc. Argent. de Biol., T. 7, p. 302.

Gerschman, R. 1931 *Calcium et Phosphore du Plasma Sanguin des Chiens Hypofisoprives.* Compt. rend. Soc. de Biol., T. 108, p. 494.

Gerschman, R., and Marenzi, A. D. 1936 *Action de l'Extrait Alcalin Ante-hypophysaire sur les Substances Minerales du Plasma.* Compt. rend. Soc. de Biol., T. 120, p. 817.

Gomez, E. T., and Turner, C. W. 1936 *Non-Effect of Estrogenic Hormone on Mammary Gland of Hypophysectomized Guinea-pig.* Proc. Soc. Exper. Biol. and Med., Vol. 34, p. 320.

Gomez, E. T., and Turner, C. W. 1936 *Effect of Hypophysectomy on Development and Function of the Mammary Gland.* J. Dairy Sci., Vol. 19, p. 450.

Gomez, E. T., and Turner, C. W. 1936 *Initiation and Maintenance of Lactation in Hypophysectomized Guinea-pigs.* Proc. Soc. Exper. Biol. and Med., Vol. 35, p. 365.

Gomez, E. T., and Turner, C. W. 1937 *The Adrenotropic Principle in Relation to Lactation.* Proc. Soc. Exper. Biol. and Med., Vol. 36, p. 78.

Gomez, E. T., and Turner, C. W. 1937 *Effect of Thyroxine and Galactin on Lactation in Hypophysectomized Guinea-pigs.* Proc. Soc. Exper. Biol. and Med., Vol. 36, p. 80.

Gomez, E. T., Turner, C. W., Gardner, W. U., and Hill, R. T. 1937 *Oestrogenic Treatment of Hypophysectomized Male Mice.* Proc. Soc. Exper. Biol. and Med., Vol. 36, p. 287.

Gomez, E. T., Turner C. W., and Reece, R. P. 1937 *Mammary Gland Growth of Hypophysectomized Guinea-pigs.* Proc. Soc. Exper. Biol. and Med., Vol. 36, p. 286.

Graham, W. R., Jr. 1934 *The Effect of Thyroidectomy and Thyroid Feeding on the Milk Secretion and Milk Fat Production of Cows.* Jour. Nutrition., Vol. 7, p. 407.

Grollman, A. 1936 *The Adrenals.* William and Wilkins, Baltimore.

Grollman, A., and Firor, W. M. 1935 *Experimental Studies on Replacement Therapy in Adrenal Insufficiency.* Bul. John Hopkins Hosp., Vol. 57, p. 281.

Grueter, F. 1928 *Contribution a l'Etude du Fonctionnement du Lobe Anterieur de l'Hypophyse.* Compt. rend. Soc. de Biol., T. 97, p. 1215.

Grueter, F. 1931 *Hormonale Beeinflussung der Milchsekretion unter besonderer Brücksichtigung von Kühen und Ziegen.* Proc. 2nd Internat. Congr. Sex. Res. (1930), p. 443. Edinburg, Oliver and Boyd.

Grueter, F., and Stricker, P. 1929 *Ueber die Wirkung eines Hypophysen-vorderlappenhormones auf die Auslosung der Milchsekretion.* Klin. Wochr., Bd. 8, S. 2322.

Hammond, J., and Marshall, F. H. A. 1914 *The Functional Correlation between the Ovaries, Uterus and Mammary Glands in the Rabbit, with Observation on the Estrus Cycle.* Proc. Roy. Soc. B., Vol. 87, p. 422.

Hartman, F. A., Lockwood, J. E., and Brownell, K. A. 1933 *Further Studies on the Adrenal Cortex.* Am. J. Physiol., Vol. 46, p. 46.

Haterius, H. O. 1928 *Effect of Placental Extract on Mammary Glands of Male Guinea-pigs.* Proc. Soc. Exper. Biol. and Med., Vol. 25, p. 471.

Hertz, S., and Kranes, A. 1932 *Parathyreotropic Action of the Anterior Pituitary: Histologic Evidence in the Rabbit.* Endocrinology, Vol. 16, p. 350.

Hill, R. T., Turner, C. W., Uren, A. W., and Gomez, E. T. 1935 *Hypophysectomy of the Goat.* Mo. Agri. Exper. Sta. Res. Bul. 230.

Hoffman, F., and Anselmino, K. J. 1934 *Ueber die Wirkung von Hypophysenvorderlappenextrakten auf den Blutkalkspiegel.* Klin. Woschr., Bd. 13, S. 45.

Hogben, L., and Charles, E. 1932 *Studies on the Pituitary. IX. Changes in Blood Calcium Following Injections of Anterior Lobe Extracts and Sexual Excitement in Female Rabbits.* J. Exper. Biol., Vol. 9, p. 139.

Holl, G. 1930 *Zwei Männliche Fälle von Nebennierenrindentumoren.* Deut. Ztschr. f. Chir., Bd. 226, S. 277.

Houssay, B. A. 1921 *Les Surrenale n'ont aucun Role dans la Production des Effets Vasculaires de l'Extrait d'Hypophyse.* Compt. rend. Soc. de Biol., T. 65, p. 35.

Houssay, B. A. 1932 *Hypophyse et Thyroide. Reaction de la Thyroide du Rat en Parabiose ou Injecte avec l'Extrait Ante-Hypophysaire.* Compt. rend. Soc. de Biol., T. 111, p. 459.

Houssay, B. A. 1932 *Hypophyse und Stoffwechsel der Eiweiskorper und Kohlehydrate.* Klin. Woschr., Bd. 11, S. 1529.

Houssay, B. A. 1933 *Diabeteserregende Wirkung des Hypophysenvorderlappenextraktes.* Klin. Woschr., Bd. 12, S. 773.

Houssay, B. A. 1935 *Influencia de la Hipofisaria sobre la Prenez y la Secrecion Lactea en las Perras.* Rev. Soc. Argent. de Biol., T. 11, p. 196.

Houssay, B. A. 1935 *Provocacion de la Secrecion Lactea en Perras y Perros por Medio del Extracto Anterohipofisario.* Rev. Soc. Argent. de Biol., T. 11, p. 240.

Houssay, B. A. 1935 *Secrecion Lactea Provoque por l'Extrait Antehypophysaire chez le Chien.* Compt. rend. Soc. de Biol., T. 120, p. 502.

Houssay, B. A. 1935 *Action de l'Hypophysectomie sur la Grossesse et la Secretion Lactea chez la Chiene.* Compt. rend. Soc. de Biol., T. 120, p. 496.

Houssay, B. A., and Biasotti, A. 1930 *Le Diabete Phlorrhizinique des Chiens Prives d'Hypophyse.* Compt. rend. Soc. de Biol., T. 105, p. 126.

Houssay, B. A., and Biasotti, A. 1931 *Hypophysis, Carbohydrate Metabolism and Diabetes.* Endocrinology, Vol. 15, p. 511.

Houssay, B. A., and Biasotti, A. 1931 *Phlorrhizin Diabetes beim Hypophysektomierten Hund.* Arch. f. d. ges. Physiol. Bd. 227. S. 657.

Houssay, B. A., and Biasotti, A. 1931 *Sobre la Substancia Hipofisaria que Refuerza la Diabetes Pancreatica.* Rev. soc. Argent. de Biol., T. 7, p. 3.

Houssay, B. A., Biasotti, A., and Magdalena, A. 1932 *Hypophyse et Thyroide. Action de l'Extrait Antehypophysaire sur l'Histologie de la Thyroid du Chien.* Compt, rend. Soc. de Biol., T. 110, p. 832, 834.

Houssay, B. A., Biasotti, A., and Mazzocco, P. 1932 *Hipofisis y Tiroides. XVI. Accion del Extracto Anterohipofisario sobre la iodemia de los Tiroprivos y de los Hipofisoprivos.* Rev. Soc. Argent. de Biol., T. 8, p. 554.

Houssay, B. A., Biasotti, A., di Benedetto, E., and Rietti, C. T. 1933 *Action des Extrait Anterohypophysaire sur le Diabete Phlorrhizinique.* Compt. rend. Soc. de Biol., T. 112, p. 497.

Houssay, B. A., Biasotti, A., and Rietti, C. T. 1932 *Action Diabetogena del Extracto Anterohipofisairo.* Rev. soc. Argent. de Biol., T. 111. p. 479.

Houssay, B. A., di Benedetto, E., and Mazzocco, P. 1933 *Hypophyse et Glycogene chez la Crapaud.* Compt. rend. Soc. de Biol., T. 113, p. 465.

Houssay, B. A., di Benedetto, E., and Mazzocco, P. 1933 *Hipofisis y Glucogeno en el Sapo.* Rev. soc. Argent. de Biol., T. 9, p. 4.

Houssay, B. A., di Benedetto, E., and Rietti, C. T. 1933 *Action de l'Extrait Anterohypophysaire sur le Diabete Phlorrhizinique.* Compt. rend. Soc. de Biol., T. 112, p. 497.

Houssay, B. A., and Magenta, M. A. 1925 *Sensibilite des Chiens Hypophysectomize a l'eggard de l'Insuline des Chiens Prives d'Hypophyse.* Compt. rend. Soc. de Biol., T. 192, p. 429.

Houssay, B. A., and Mazzocco, P. 1922 *Composition de l'Urine et du Sang des Chiens Prives d'Hypophyse.* Compt. rend. Soc. de Biol., T. 86, p. 409.

Jack, E. L., and Bechdell, S. I. 1935 *A Study of the Influence of Thyroxine in Milk Secretion.* J. Dairy Sci., Vol. 18, p. 195.

Kabak, J., and Kisilstein, M. 1934 *Der Einflus Endokriner auf die Laktation beim Schafen.* Z. Züchtg.: B. Tierzuchtg. u. Züchtgsbiol., Bd. 29, S. 165.

Kabak, J. M., and Margulis, Z. S. 1935 *The Influence of Endocrine Preparations on Lactation in Sheep.* Trans. Dynamics of Dev., Vol. 9, p. 167.

Kaufman, M., and Magne, H. 1906 *Sur la Consommation du Glucose du Sang por le Tissu de la Glande Mammaire.* Compt. rend. Acad. Sci. (Paris), T. 143, p. 779.

Kobayashi, K. 1931 *On the Effect of Extract of Some Endocrine Organs on Carbohydrate Metabolism of Normal and Hypophysectomized Dogs.* Japan. J. M. Sc., Tr. IV. Pharmacol., Vol. 5, p. 56.

Koster, S., and Geesink, A. 1929 *Experimentelle Untersuchung der Hypophysenfunktion beim Hunde.* Arch. f. d. ges. Physiol., Bd., 22, S. 293.

Lane-Claypon, J. E., and Starling, E. H. 1906 *An Experimental Inquiry into the Factors which Determine the Growth and Activity of the Mammary Glands.* Proc. Roy. Soc. B., Vol. 77, p. 505.

Laqueur, E., and de Jongh, S. E., and Tausk, M. 1927 *Ueber Weibliches Sexual Hormon, Menformon. V. Ueber den Feminisherenden Einfluss des Menformons auf die Unentwickelte Brustdruse.* Deut. Med. Woschr., Bd. 53, S. 867.

Lee, M., and Ayres, G. B. 1936 *The Composition of Weight Loss and the Nitrogen Partition of Tissue in Rats after Hypophysectomy.* Mem. Found. for Neuro-Endocrine Res., Harvard Med. School, Boston, Mass.

Levenstein, I. 1936 *The Histology of the Mammary Gland of Adrenalectomized Lactating Rats.* Anat. Rec., Vol. 67, p. 7 (Suppl.).

Lisser, H. 1936 *A Case of Adrenal Cortical Tumor in an Adult Male Causing Gynecomastia and Lactation.* Endocrinology, Vol. 20, p. 567.

Loeb, L., and Basset, R. B. 1929 *Effect of Hormone of the Anterior Pituitary on Thyroid Gland in the Guinea-pig.* Proc. Soc. Exper. Biol. and Med., Vol. 26, p. 860.

Loeb, L., and Hesselberg, C. 1917 *The Cyclic Changes in the Mammary Gland under Normal and Pathological Conditions. I. The Change in the Non-pregnant Guinea-pig. II. The Changes in the Pregnant Guinea-pig, the Effect of Lutein Injections and the Correlation between the Cycle of the Uterus and Ovaries and the Cycle of the Mammary Gland.* Jour. Exp. Med., Vol. 25, p. 285.

Loeb, L., and Kountz, W. B. 1928 *The Effect of Injections of Follicular Extract on the Sex Organs in the Guinea-pig and the Interaction between the Follicular Substance given off by the Corpus Luteum.* Amer. J. Physiol., Vol. 84, p. 283.

Loeb, R. F., and Stahl, J. 1935 *Role of Sodium in Adrenal Insufficiency.* J. A. M. A., Vol. 104, p. 2149.

Long, C. N. H., and Lukens, F. D. W. 1935 *Effect of Adrenalectomy and Hypophysectomy upon Experimental Diabetes in Cat.* Proc. Soc. Exper. Biol., Vol. 32, p. 743.

Lucke, H., Heydemann, E. R., and Berger, O. 1934 *Der Einfluss von Operativen Eingriffen am Hypophysenvorderlappen auf die Stoffwechsellage des Pankreasdiabetischen Hundes.* Ztschr. f. exper. Med., Bd. 92, S. 711.

Lyons, W. R. 1936 *Hormonal Requirements for Lactation in Male Rabbits.* Anat. Rec., Vol. 64, p. 31 (Suppl.).

Lyons, W. R., and Catchpole, H. R. 1933 *Assay with the Guinea-pig of the Lactogenic Hypophyseal Hormone.* Proc. Soc. Exper. Biol. and Med., Vol. 31, p. 299.

Lyons, W. R., Chaikoff, I. L., and Reichert, F. L. 1933 *Experiments with Hypophyseal Lactogenic Hormone on Normal, Ovariectomized and Hypophysectomized Dogs.* Proc. Soc. Exper. Biol. and Med., Vol. 31, p. 303.

Lyons, W. R., and Page, E. 1935 *Detection of Mammotropin in the Urine of Lactating Women.* Proc. Soc. Exper. Biol. and Med., Vol. 32, p. 1049.

Lyons, W. R., and Pencharz, R. I. 1936 *Reactions of Mammary Glands of Normal and Hypophysectomized Guinea-pigs to Female Sex Hormones.* Proc. Soc. Exper. Biol. and Med., Vol. 33, p. 589.

Macchiarulo, O. 1936 *Sui Rapporti Intercorrenti tra l'ipofisi a la Mammella. Effecti della Ipofisetomia in Cavie Allatanti.* Ann. di Ost. e Gynec. Anno 48, p. 3.

MacDonald, G. I. 1936 *The Response of the Mammary Gland to Prolonged Stimulation with Ovarian Hormones.* Surg. Gynec. and Obst., Vol. 63, p. 138.

MacKay, E. M., and Barnes, R. H. 1936 *Fasting Ketosis in the Pregnant Rat as Influenced by Adrenalectomy.* Proc. Soc. Exper. Biol. and Med., Vol. 34, p. 682.

MacKay, E. M., and Barnes, R. H. 1937 *The Effect of Adrenalectomy on liver fat in Fasting and After Administration of Anterior Pituitary Extracts.* Amer. J. Physiol., Vol. 118, p. 525.

Magistris, H. 1933 *Das Stoffwechselhormon des Hypophysenvorderlappens.* Klin. Wochr., Bd. 46. S. 908.

Margulis, Z. S., Skvortzov, V. A., and Poliakov, A. N. 1935 *The Influence of Endocrine Factors on Lactation and Live Weight of Cows.* Trans. Dynamics of Dev., Vol. 9, p. 177.

Marenzi, A. D., and Gerschman, R. 1935 *L'Hypophyse et les Substances Minerales du Sang.* Compt. rend. Soc. Biol., T. 118, p. 488.

McPhail, M. K. 1935 *Hypophysectomy of the Cat.* Proc. Roy. Soc. B., Vol. 117, p. 45.

Moon, H. D. 1937 *Preparation and Biological Assay of Adrenotropic Hormone.* Proc. Soc. Exper. Biol. and Med., Vol. 35, p. 649.

Nakamura, K. 1931 *Experimentelle Beitrage zur Kenntniss des Hypophysenfunktion.* Fukuoka-Ikwadaigaku-Zasshi, Bd. 24, S. 18.

Nelson, W. O. 1933 *Reciprocal Relationship Between Ovaries and the Anterior Hypophysis as Factor in Control of Lactation.* Proc. Soc. Exper. Biol. and Med., Vol. 30, p. 953.

Nelson, W. O. 1934 *Studies on the Physiology of Lactation. III. The Reciprocal Hypophyseal-Ovarian Relationship as a Factor in the Control of Lactation.* Endocrinology, Vol. 18, p. 33.

Nelson, W. O. 1935 *The Effect of Hypophysectomy upon the Mammary Gland Development and Function in the Guinea-pig.* Proc. Soc. Exper. Biol. and Med., Vol. 33, p. 222.

Nelson, W. O. 1936 *Endocrine Control of the Mammary Gland.* Physiol. Rev., Vol. 16, p. 488.

Nelson, W. O., and Gaunt, R. 1936 *Initiation of Lactation in Hypophysectomized Guinea-pigs.* Proc. Soc. Exper. Biol. and Med., Vol. 34, p. 671.

Nelson, W. O., and Pfiffner, J. J. 1931 *Studies on the Physiology of Lactation. I. The Relation of Lactation to the Ovarian and Hypophyseal Hormones.* Anat. Rec., Vol. 51, p. 51.

Nelson, W. O., and Smelser, G. K. 1933 *Studies on the Physiology of Lactation. II. Lactation in the Male Guinea-pig and its Bearing on the Corpus Luteum Problem.* Am. J. Physiol., Vol. 103, p. 374.

Nelson, W. O., and Tobin, C. E. 1936 *The Effect of Oestrone and of the Pituitary Extract on the Mammary Gland of Rat.* Anat. Rec., Vol. 67, p. 111 (Suppl.).

O'Donoghue. C. H. 1911 *The Growth Changes in the Mammary Apparatus of Dasyurus and the Relation of the Corpora Lutea.* Quart. J. Micr. Sc., Vol. 57, p. 187.

Parkes, A. S. 1929 *The Functions of the Corpus Luteum. III. Factors Concerned in the Development of the Mammary Gland.* Proc. Roy. Soc. B., Vol. 104, p. 189.

Parkes, A. S. 1930 *The Functions of the Corpus Luteum. IV. The Relation of Estrin to the Luteal Phase of Oestrous Cycle.* Proc. Roy. Soc. B., Vol. 107, p. 188.

Pencharz, R. I., and Long, J. A. 1931 *The Effect of Hypophysectomy on Gestation in the Rat.* Science, Vol. 74, p. 206.

Pencharz, R. I., and Lyons, W. R. 1934 *Hypophysectomy in the Pregnant Rat.* Am. J. Anat., Vol. 53, p. 117.

Pencharz, R. I., and Lyons, W. R. 1934 *Hypophysectomy in the Pregnant Guinea-pig.* Proc. Soc. Exper. Biol., Vol. 31, p. 1131.

Perla, D. 1936 *Effect of Adrenotropic Hormone on the Natural Resistance of Hypophysectomized Rats.* Proc. Soc. Exper. Biol. and Med., Vol. 34, p. 751.

Perla, D., and Sandberg, M. 1936 *The Effect of Complete and Partial Hypophysectomy in Adult Albino Rats on Nitrogen, Calcium and Phosphorus Metalbolism.* Endocrinology, Vol. 20, p. 481.

Phillips, R. A., and Robb, P. 1934 *The Effect of Desiccated Thyroid, Irradiated Ergosterol and Ammonium Chloride on the Excretion of Calcium in Rats.* Amer. J. Physiol., Vol. 109, p. 82.

Reece, R. P., Turner, C. W., and Hill, R. T. 1936 *Mammary Gland Development in the Hypophysectomized Albino Rat.* Proc. Soc. Exper. Biol. and Med., Vol. 34, p. 204.

Reiss, M., Schwarz, L., and Fleischmann, F. 1936 *The Relation Between the Anterior Lobe of the Hypophysis and Protein Metabolism.* Endokrinologie, Vol. 17, p. 167.

Riddle, O., Bates, R. W., and Dykshorn, S. W. 1932 *A New Hormone of the Anterior Pituitary.* Proc. Soc. Exper. Biol., Vol. 29, p. 1211.

Riddle, O., and Polhemus, I. 1931 *Studies on the Physiology of Reproduction in Birds.* Am. J. Physiol. Vol. 98, p. 121.

Ruinen, F. H. 1932 *Über den Angriffspunkt der Mammawirkung von Menformon.* Acta Brevia Neerl. Bd. 2. S. 161

Russell, J. A. 1936 *Carbohydrate Levels in Fasted and Fed Hypophysec-tomized Rats*. Proc. Soc. Exper. Biol. and Med., Vol. 34, p. 279.

Russell, J. A., and Bennet, L. L. 1936 *Maintenance of Carbohydrate Levels in Fasted Hypophysectomized Rats Treated with Anterior Pituitary Extracts*. Proc. Soc. Exper. Biol. and Med., Vol. 34, p. 406.

Sanberg, M., and Perla, D. 1936 *Nitrogen and Sulfur Metabolism in Suprarenalectomized Rats*. J. Biol. Chem., Vol. 113, p. 35.

Sardi, J. L. 1935 *Secrecion Lactea sous l'action de l'Extrait Antehypophy-saire chez les Cobayes Males*. Compt. rend. Soc. de Biol., T. 120, p. 503.

Shaeffer, M. K., and Lee, M. 1935 *The Effect of the Anterior Pituitary Growth Hormone on Protein Metabolism*. J. Biol. Chem., Vol. 108, p. 355.

Schumacker, H. B., and Firor, W. M. 1934 *The Interrelationship of the Ad-renal Cortex and the Anterior Lobe of the Hypophysis*. Endocrin-ology, Vol. 18, p. 672.

Schultze, A. B., and Turner, C. W. 1933 *Experimental Initiation of Milk Secretion in the Albino Rat*. J. Dairy Sci., Vol. 16, p. 129.

Selye, H., Browne, J. S. L., and Collip, J. B. 1936 *Effect of Combined Ad-ministration of Oestrone and Progesterone in Adult Ovariectomized Rats*. Proc. Soc. Exper. Biol. and Med., Vol. 34, p. 198.

Selye, H., and Collip, J. B. 1936 *Fundamental Factors in the Interpreta-tion of Stimuli Influencing Endocrine Glands*. Endocrinology, Vol. 20, p. 667.

Selye, H., Collip, J. B., and Thomson, D. L. 1933 *Effect of Hypophysectomy upon Pregnancy and Lactation*. Proc. Soc. Exper. Biol. and Med., Vol. 34, p. 416.

Smelser, G. K. 1933 *The Response of Guinea-pig Mammary Gland to Injected Sex Hormones and Ovarian Grafts and its Bearing on the Problem of Sex Hormone Antagonism*. Physiol. Zool., Vol. 6, p. 396.

Smith, P. E. 1926 *The Disabilities Caused by Hypophysectomy and Their Repair*. J. A. M. A., Vol. 88, p. 207.

Smith, P. E. 1927 *The Experimental Feeding of Fresh Anterior Pituitary*. Am. J. Physiol., Vol. 81, p. 20.

Smith, P. E. 1930 *Hypophysectomy and Replacement Therapy in the Rat*. Am. J. Anat., Vol. 45, p. 205.

Smith, P. E., Dotti, L., Tyndale, H. H., and Engle, E. T. 1936 *Effect of Hypophysectomy on Blood Sugar of Reshus Monkeys*. Proc. Soc. Exper. Biol. and Med., Vol. 34, p. 247.

Smith, P. E., Greenwood, L., and Foster, G. L. 1917 *A Comparison in Nor-mal, Thyroidectomized and Hypophysectomized Rats of the Effect upon Metabolism and Growth Resulting from Daily Injections of Small Amount of Thyroid Extract*. Am. J. Path., Vol. 3, p. 689.

Smith, P. E., and White, W. E. 1931 *The Effect of Hypophysectomy on Ovulation and Corpus Luteum Formation in the Rabbit*. J. A. M. A., Vol. 97, p. 1861.

Soskin, S., Mirsky, A. I., Simmerman, L. M., and Crohn, N. 1935 *Influence of Hypophysectomy on Glycogenesis in the Normal and Depancrea-tized Dog*. Am. J. Physiol., Vol. 114, p. 110.

Stricker, P., and Grueter, F. 1928 *Action du Lobe Anterieur de l'Hypo-physe sur la Montee Laiteuse*. Compt. rend. Soc. de Biol., T. 99, p. 1978.

Stricker, P., and Grueter, F. 1929 *Recherches Experimentales sur les Fonctions du Lobe Anterieur de l'Hypophyse. Influence des Extraits du Lobe Anterior sur l'Appareil Genital de la Lapine et sur la Montee Laiteuse.* Presse Med., T. 37, p. 1268.

Swingle, W. W., and Pfiffner, J. J. 1932 *The Adrenal Cortical Hormone.* Medicine, Vol. 11, p. 371.

Teel, H. M., and Watkins, O. 1929 *The Effect of Extracts Containing the Growth Principle of the Anterior Hypophysis upon the Blood Chemistry of Dogs.* Am. J. Physiol., Vol. 89, p. 662.

Thomson, K. W. 1932 *A Technique for Hypophysectomy of the Rat.* Endocrinology, Vol. 16, p. 257.

Turner, C. W. 1930 *The Anatomy of the Mammary Glands of Cattle. I. Embryonic Development.* Mo. Agri. Exper. Sta. Res. Bul. 140.

Turner, C. W. 1931 *The Anatomy of the Mammary Gland of Cattle. II. Fetal Development.* Mo. Agri. Exper. Sta. Res. Bul. 160.

Turner, C. W. 1932 *The Mammary Glands, Chapt. XII, in "Sex and Internal Secretion."* Allen. William and Wilkins, Baltimore

Turner, C. W., and de Moss, W. R. 1934 *The Normal and Experimental Development of the Mammary Gland. I. The Male and Female Cat.* Mo. Agri. Exper. Sta. Res. Bul. 207.

Turner, C. W., and Frank, A. H. 1930 *The Effect of Estrus Producing Hormone on the Growth of the Mammary Gland.* Mo. Agri. Exper. Sta. Res. Bul. 145.

Turner, C. W., and Frank, A. H. 1932 *The Effect of the Ovarian Hormone Theelin and Corporin upon the Growth of the Mammary Gland of the Rabbit.* Mo. Agri. Exper. Sta. Res. Bul. 174.

Turner, C. W., Frank, A. H., Gardner, W. U., Schultze, A. B., and Gomez, E. T. 1932 *The Effect of Theelin and Theelol on the Growth of the Mammary Gland.* Anat. Rec., Vol. 53, p. 174.

Turner, C. W., and Gardner, W. U. 1931 *The Relation of the Anterior Pituitary Hormones to the Development and Secretion of the Mammary Gland.* Mo. Agri. Exper. Sta. Res. Bul. 158.

Turner, C. W., and Gomez, E. T. 1933 *The Normal Development of the Mammary Gland of the Male and Female Albino Mouse.* Mo. Agri. Exper. Sta. Res. Bul. 182.

Turner, C. W., and Gomez, E. T. 1933 *The Normal Development of the Mammary Gland of the Male and Female Guinea-pig.* Mo. Agri. Exper. Sta. Res. Bul. 194.

Turner, C. W., and Gomez, E. T. 1934 *The Normal and Experimental Development of the Mammary Gland. II. The Male and Female Dog.* Mo. Agri. Exper. Sta. Res. Bul. 207.

Turner, C. W., and Gomez, E. T. 1934 *The Experimental Development of the Mammary Gland. I. The Male and Female Albino Mouse. II. The Male and Female Guinea-pig.* Mo. Agri. Exper. Sta. Res. Bul. 206.

Turner, C. W., and Gomez, E. T. 1935 *The Development of the Mammary Glands of the Goat.* Mo. Agri. Exper. Sta. Res. Bul. 240.

Turner, C. W., and Schultze, A. B. 1931 *A Study of the Causes of the Normal Development of the Mammary Glands of the Albino Rat.* Mo. Agri. Exper. Res. Bul. 157.

Van Dyke, H. B. 1936 *The Physiology and Pharmacology of the Pituitary Body.* The Univ. of Chicago Press, Chicago, Ill.

Weichert, C. K., and Boyd, R. W. 1933 *Induction of Typical Pseudo-pregnancy in the Albino Rat by Means of Experimental Hyperthyroidism.* Anat. Rec., Vol. 58, p. 55.

Weichert, C. K., and Boyd, R. W. 1934 *Stimulation of Mammary Gland Development in the Pregnant Rat under Condition of Experimental Hyperthyroidism.* Anat. Rec., Vol. 59, p. 157.

Wells, L. J., and Gomez, E. T. 1937 *Some Effects of Crystalline Androgenic Materials on Spermatogenesis in Normal and Hypophysectomized Male Ground Squirrels.* Anat. Rec., Vol. 67, p. 52 (Suppl.).

White, W. E. 1933 *The Effect of Hypophysectomy of the Rabbit.* Proc. Roy. Soc. B., Vol. 114, p. 64.

Wyman, L. C. 1928 *Effect of Adrenal Insufficiency on Reproduction and the Oestrous Cycle of the Rat.* Am. J. Physiol., Vol. 86, p. 528.

Wyman, L. C. 1929 *Studies on Adrenal Insufficiency.* Am. J. Physiol., Vol. 94, p. 579.

Zum Busch, J. P. 1922 *Gynäkomastie bein Hypernephrom.* Deut. med. Wochr., Bd. 53, S. 323.