THE EFFECT OF CERTAIN ANTERIOR PITUITARY PREPARATIONS ON MILK SECRETION

THESIS FOR DEGREE OF M. OF S.
MICHIGAN STATE COLLEGE

WILLIAM LOUIS MEULEMAN
1941

THE EFFECT OF CERTAIN ANTERIOR PITUITARY PREPARATIONS ON MILK SECRETION

by

William Louis Meuleman

A THESIS

Submitted to the Graduate School of Michigan State College of Agriculture and Applied Science in partial fulfilment of the requirements for the degree of

MASTER OF SCIENCE

Department of Physiology and Pharmacology

1941

THESIS

ACKNOWLEDGEMENTS

The writer wishes to express his deepest appreciation to Dr. J. F. Sykes for guidance in the work reported in this paper, and for his constructive criticism in the preparation of this manuscript. He also wishes to acknowledge the courtesy of Dr. C. F. Huffman, of the Dairy Department, for placing at his disposal the experimental animals used in this experiment. To Mr. C. C. Lightfoot and Mr. C. W. Duncan of the Chemistry Experimental Station, the writer wishes to express appreciation for their cooperation and assistance in carrying out the various chemical analyses, and to the members of the Department of Physiology and Pharmacology, he wishes to express his indebtedness for their assistance and advice given in the course of this work.

TABLE OF CONTENTS

INTRODUCTION

The physiological activities of the mammary gland, as governed by the hormones, may be divided into two phases. The first phase is concerned with the development of the mammary gland from puberty through pregnancy. It has been proved that this phase is primarily controlled by an ovarian-pituitary relationship.

The second phase of mammary activity is concerned mainly with the secretory processes which occur following parturition. That this part of the cycle is not dependent upon the ovaries is shown by the fact that castration does not terminate lactation. In fact, lactation sometimes follows oophorectomy.

From the studies upon the anterior pituitary, it has been found that there are certain hormones which are responsible for initiating and continuing lactation. The presence of a lactogenic hormone, prolactin, has been well established, and therefore no attempt was made to show that this hormone does initiate lactation.

This investigation was undertaken in an attempt to determine the effect of prolactin and the fat-metabolism hormone, whether given separately or combined, upon total milk yield and fat production.

REVIEW OF THE LITERATURE

The phenomenon of lactation has been known from antiquity, but it has only been in recent years that the causes have been investigated. Prior to 1928, lactation was generally accepted as a catabolic process, ensuing after parturition, and was without an active stimulus. However, since the historic work of Grüter and Stricker (1929), more and more evidence has accumulated showing that the anterior hypophysis and its secretions play a very important part in lactation. Grüter and Stricker found that in mature, normal dairy cows, crude pituitary extracts were capable of initiating lactation. With the knowledge that anterior pituitary extracts would stimulate lactation, many workers sought methods of extracting the lactogenic fraction of the pituitary. Riddle, Bates and Dykshorn (1932) prepared the first prolactin by iso-electric precipitation. From five insoluble fractions prepared, the pH 5 fraction was found to be most potent in the lactogenic principle. The other insoluble fractions contained varying amounts of the gonadotropic fraction with little or no lactogenic potency. In 1935, the same workers improved the original method of preparation so that the follicle-stimulating and thyreotropic fractions were eliminated. The amount of inert protein and other contaminants was reduced and the lactogenic potency was considerably increased by these procedures. On the basis of biological tests, it was shown that the lactogenic hormone was a distinct entity which stimulated milk secretion. This preparation was likewise shown to stimulate the growth and secretion of the crop gland of pigeons, and these latter effects

are used as a basis for assaying the potency of various lactogenic preparations. Evidence concerning the specificity of this hormone was also presented by Lyons and Catchpole (1933) and Lyons, et al., (1933, 1937) who called the hormone mammotropin. Gardner and Turner (1933) have likewise prepared a lactogenic hormone, galactin, and fully described the mammary changes following its administration. At the present time it is recognized that mammotropin, galactin and prolactin have similar effects.

Riddle, Bates and Dykshorn (1932) first prepared prolactin by aqueous extraction at pH 2. By repeated iso-electric precipitation in an acid medium, it was believed that the growth hormone would be eliminated. Later (1933), an aqueous alkaline extraction at pH 9 was adopted because it was more complete. Gardner and Turner (1933) followed a similar extraction procedure. Lyons and Catchpole (1933) used 66% acetone extractions. Lyons (1937) has prepared prolactin, free from the adrenocorticotropic hormone, by an aqueous-acetone precipitation. Bates and Riddle (1935) published a method whereby a 60% to 70% ethanol extraction at pH 9 gave still more satisfactory yields. A 90% ethanol precipitation from this extract yielded a precipitate which contained 80% to 90% of the prolactin. The contaminants, mainly thyreotropic and gonadotropic hormones, were present in smaller proportions; subsequent iso-electric precipitations at pH 3-4 removed all detectable traces of the thyreotropic and gonadotropic fractions. The pH 3-4 insoluble fraction was further

concentrated to a unitage of 5-10 units per mg. by repeated precipitations at pH 6 from 70% alkaline ethanol. Extracts containing prolactin have also been made by Young (1938) after a modification of the Bates and Riddle procedure (1935). Young's procedure is mainly a cold saline extraction at pH 8 and precipitation at pH 5.5. By using these various extracts, the effect of the anterior pituitary on lactation, as first observed by Grüter and Stricker, has been further investigated and substantiated; not only for dairy cows, but also for many other species as well (Corner, 1930; Allan and Wiles, 1932; Asdell, 1932; Catchpole et al., 1933; Evans, 1933; Gardner and Turner, 1933; Graham, 1934; De Fremary, 1936; Azimov and Krouze, 1937; Folley and Young, 1938; Bergman and Turner, 1940).

The effect of the anterior pituitary on lactation has likewise been determined by removal of the gland and by replacement therapy. In all species, if ablation is effected during the second half of pregnancy, there is only a very brief period in which lactation occurs following birth or abortion (Selye, et al., 1933; McPhail, 1935, 1935a; Pencharz and Lyons, 1934; Gomez and Turner, 1936). If animals are hypophysectomized during lactation, secretion generally stops within a very short time (2 days to one week) (McPhail, 1935; Hill, et al., 1935; Gomez and Turner, 1937). Administration of crude pituitary extracts to pregnant hypophysectomized animals will initiate lactation and maintain it following parturition (Gomez and Turner, 1936; Lyons, et al., 1933; Nelson

and Gaunt, 1936). However, the partially purified pituitary extract (prolactin, galactin) by itself is incapable of either continuing lactation or initiating milk secretion in hypophysectomized animals (Gomez and Turner, 1936, 1936a; Nelson and Gaunt, 1936, 1937; Reece, 1939). In all cases, prolactin is only capable of initiating secretion when given in conjunction with other hormones which may affect lactation in an indirect manner and which are lacking in the hypophysectomized animal.

Most of the experimental data presented have been obtained from studies involving small animals. The usual laboratory animal, however, does not allow any very exact quantitative estimation of milk. Allan and Wiles (1932) and Evans (1933) reported increased secretion as a result of injections of anterior pituitary extracts in goats. Evans, however, noted that virgin goats will not respond to the lactogenic principle. This has been found, by the Russian and English workers, to apply to heifers. In fact, in all species so far examined, prolactin will only stimulate secretion in the mature gland (Asdell, 1931; Catchpole, et al., 1933; Lyons, et al., 1933; Nelson and Pfiffner, 1931). Recently the effects of anterior pituitary preparations have been studied on established lactation in cattle. The Russian workers headed by Azimov and his associates (1937) produced increased lactation with single and repeated injections of alkaline extracts of the anterior pituitary. Their results indicated that the injections were more effective given during early lactation, or more particularily, at the peak of lactation. They also showed that crude extracts were more effective than purified prolactin.

Folley and Young likewise studied the effects of anterior pituitary extracts in cattle. It was found that single injections of crude pituitary extracts, as well as partially purified prolactin preparations, temporarily increased milk production (Folley and Young, 1937, 1938, 1939). Young's preparation (1938) of prolactin, modified from the Bates and Riddle procedure (1935), produced increased secretion upon single injections in cattle. He showed that this preparation contained, in addition to prolactin, significant amounts of thyreotropic, diabetogenic and glycotropic hormones. When these materials were separated from prolactin, it was found that the purified prolactin was much less active, as judged by secretion, than when it was combined with these other fractions, although an intense crop gland response was obtained. On the other hand, injections of extracts containing thyreotropic, diabetogenic and glycotropic activity showed little crop gland response but stimulated milk secretion to some extent. In view of these results, it seems probable that prolactin may not be a specific lactogenic hormone and that the lactogenic properties of anterior pituitary preparations may be due to a number of anterior lobe hormones. These important facts were noted by both the Russian and English workers. They likewise have shown that although milk production is stimulated by the injections, the degree of stimulation can only be carried to a certain maximum, as continued treatment will not cause a greater increase. In fact, a decrease in production sets in, which can not be offset despite continued injections. This

latter effect may be due to the formation of antihormones which have been shown to develop during continued injections (Young, 1938). The type of milk secreted, in so far as was determined, indicated that the milk produced under this stimulation was essentially normal. Only minor variations in fat and other constituents were noted.

At present, there are two general methods of assaying prolactin: the crop gland method and the lactation, or mammary gland method. The latter method of assay (lactation) is rather difficult to carry out and is seldom used, as the usual laboratory test animals do not allow a quantitative estimation of the milk. Further, the amount of milk secreted depends not only on the prolactin dosage, but also upon the amount and maintenance of mammary tissue.

The crop-sac stimulation method may be further subdivided into, (a) the weight method (Riddle, Bates and Dykshorn, 1933), (b) minimum stimulation method (McShan and Turner, 1936) and (c) local stimulation method of Lyons and Page (1935). The weight method of bioassay consists of giving four daily intramuscular injections to pigeons 2 to 3 months old. Ninety-eight hours after the first injection the glands are excised, all fat removed and the glands weighed. Because of the large number of factors which must be controlled, the weight method is unsatisfactory for use in a small laboratory where it is not possible to keep large pigeon colonies. Other variables, too numerous to discuss adequately at this point, also affect the results (Bates and Riddle, 1936, 1939; Bates, et al., 1939).

The minimum stimulation method of McShan and Turner (1936) is more generally used because of its simplicity. Approximately 20 common pigeons, weighing from 260 to 340 grams, are used for one assay. Prolactin, at different dosage levels, is injected once daily for 4 days into the breast muscle. On the fifth day, the crop glands are examined for proliferation. A pigeon unit is defined as the total amount of hormone injected during a period of 4 days which will cause a minimum but definite proliferation of the crop glands of 50% ± 11% of 20 common pigeons weighing 300 ± 40 grams (McShan and Turner, 1936). The local stimulation method of Lyons and Page (1935) is a very valuable test because of the small amounts of hormone used. The results can be obtained in 48 hours. One injection is made over the crop gland on one side. The injected fluid must cause a blister or raised area on the skin. The examination is made 48 hours later, and only the area directly under the site of injection is stimulated.

The Fat-Metabolism Hormone

In 1930, Burn and Ling showed that subcutaneous injections of anterior lobe preparations caused an increase of the urinary acetone bodies in fasting rats. In the rat, dog, and man, it has also been noted that accompanying the urinary acetone increase, the blood acetone bodies also increased (Anselmino and Hoffmann, 1931; Mirsky, 1936; Best and Campbell, 1936). Furthermore, it was noted by Best and Campbell (1936, 1938) that this fat-metabolism hormone also possessed a "liver fat" activity. The liver fat activity caused an intense fatty

infiltration of the liver in normal, fasting, white rats. The evidence concerning the fate of the blood lipids following injections of anterior pituitary preparations has not been ascertained too definitely as some workers report an increase, while others note a decrease in blood lipids (Evans, 1933; Strauber, 1937; Houchin and Turner, 1939). On the basis of fractionation, Campbell and Keenan (1940) reported that the liver fat activity of anterior pituitary preparations is not necessarily associated with ketogenic activity, liver fat activity being stable to heat at pH 3.5 while ketogenic activity is stable at pH 8. These preparations are relatively free from other known anterior pituitary hormones.

The preparation of the fat-metabolism fraction as made by Best and Campbell (1936) is a modified procedure of the Burn and Ling (1930) and Anselmino and Hoffmann (1931) methods. Very briefly, this procedure is as follows: the acetone-dried pituitary powder is extracted with 20 volumes of N/20 NaOH and precipitated at pH 5.2. After two such treatments, the filtrates are combined and the fraction precipitated by adding 2 volumes of absolute alcohol. The precipitate is dissolved in faintly alkaline aqueous solution and brought to neutrality before injection.

Three methods of assay have been proposed for fat-metabolism preparations. Shipley and Long (1938) used male rats weighing from 120 to 150 grams. The rats were fasted for 48 hours previous to the test. At that time, the degree of ketonemia was determined. The extract was then given intraperitoneally

and a second blood sample taken 4 or 5 hours later. A positive test was indicated when there was an increase of 4 mg.% in the blood acetone body content. Campbell (1938), in assaying the potency of the anterior pituitary extracts which increase liver fat, used mice. The mice were fasted before the injection, and 7 hours after the injection, the livers were excised and analyzed for liver fat. The method of Houchin and Turner (1939) assays for liver fat activity as well as fat-metabolism activity. Healthy female guinea-pigs, weighing from 160 to 210 grams, are given a single intraperitoneal injection. Six hours later the pigs are sacrificed. The livers are removed, freed of blood, adipose and connective tissue and analyzed for liver fat. Blood samples are analyzed for plasma-fat.

EXPERIMENTAL PROCEDURE

The lactogenic hormone, prolactin, and the fat-metabolism hormone were prepared by the methods of Bates and Riddle (1935) and Best and Campbell (1936) respectively. As both preparations have been outlined rather briefly in the preceding paragraphs and as the detailed procedures may be found in the references cited, no method of preparation will be given here. The preparation outlined by Best and Campbell for use on rats has been used in this work.

The hormone preparations were examined for the lactogenic and fat-metabolism activity, as well as to determine the presence of other principles. The prolactin was assayed for lactogenic activity according to the method of McShan and Turner (1936). Assays to determine the glycogropic and diabetogenic activity of the prolactin preparations were run by the method of Young (1938). Prolactin was also assayed for fat-metabolism activity according to the method of Houchin and Turner (1939), and by determining the liver-fat activity. The plasma-fat, liver-fat and lactogenic activity of the fat-metabolism hormone were also determined. Plasma-fat activity was demonstrated by the method of Houchin and Turner (1939). Liver-fat activity was determined by the procedure of Campbell (1938), but, in this case guinea-pigs were used instead of mice. Lactogenic potency was determined by the method of McShan and Turner (1936).

The hormones were prepared for injection by solution in weak alkali. The solution was adjusted to pH 7.0-7.5. All

pH adjustments were made by a Beckman pH Meter. Solutions were made up as needed and then only a few hours before the injection period during which time they were stored at 6° C. The cows were injected subcutaneously just previous to milking. Care was taken so that all injections were given at approximately the same time of day over the injection period. In a few instances the injections raised a welt at the site of injection, but there were no other detectable reactions. The injection period in all instances was five days, during which time one injection per day was given.

Four cows in declining lactation were selected for the injections of these hormones. The effect of each hormone was first determined in separate injection periods, and following this the preparations were combined and their effects in this form ascertained. All cows received a ration of alfalfa hay and corn silage, and were fed liberally at a level in excess of the Morrison standards. The animals were milked twice daily.

Records on these animals were obtained for periods up to 10 days preceding the first injections and for varying periods following treatment. Daily milk records were obtained and the fat per cent determined on 3 day composite samples. From this data the daily amount of 4% fat-corrected milk was calculated to facilitate comparisons.

Blood and urine samples were obtained on the day previous to the initial injection, just before feeding time each day during the injection period and for several days following

the injections. Blood sugars were determined by the Somogyi modification (1926) of the Shaffer and Hartman method (1921), plasma-fat by the method of Allen (1930, 1933) and blood ketones by the method of Van Alyke (1917). Urinary ketones only were determined on the urine samples.

EXPERIMENTAL RESULTS

1. Results of Assay of the Hormone Preparations

The assay of prolactin showed this preparation to be a very strong lactogenic stimulant having a potency of 2 units per mg. Glycotropic and diabetogenic assays were negative for this preparation. The prolactin showed no fat-metabolism properties as judged by the liver-fat or plasma-fat activities on guinea-pigs. The fat-metabolism preparation proved to possess a potent liver-fat activity, while plasma-fat activity was present to a slight degree. This preparation did show very mild lactogenic properties as determined by the crop-sac stimulation method. The results of the assays are tabulated in Table I.

2. Effect of Hormone Preparations on Lactation

When prolactin was given alone at a level of 500 mg. (1000 units) per day, 3 of the 4 cows showed a rise in milk production. The fourth cow did not show the expected rise. The increase in production was not great in any case. A maximum increase of 2.5 pounds was observed in #D5 while #A23 and #285 showed increases of 2.0 and 2.5 pounds, respectively. The increase in production was observed, in all cases where such increases occurred, on the second day of injection, but did not reach the maximum until about the third day of injection. In 2 cows (#A23 and #285) the effect of the hormone did not disappear even when the injections were stopped. Number A23 returned to approximately the pre-injection level of production in about 30 days, and #285 was

TABLE I. HORMONE CONTENT OF THE TWO ANTERIOR PITUITARY PREPARATIONS

ACTIVITY	PROLACTIN PREPARATION	FAT-METABOLISM PREPARATION
Lactogenic	+ + + + (2 units/mg.)	+
Glycotropic	-	Not Determined
Diabetogenic	-	Not Determined*
Plasma Fat	-	+ ?
Liver Fat	-	+ + + +

* A similar preparation has been shown to have only very slight diabetogenic activity (Campbell and Keenan, 1940).
The degree of activity is indicated by plus signs, one for some activity and four for high activity. A negative sign indicates no activity.

still above the pre-injection level 30 days later when another series of injections were given. The production calculated as 4% fat-corrected milk paralleled the actual production. These results are shown in Fig. 1.

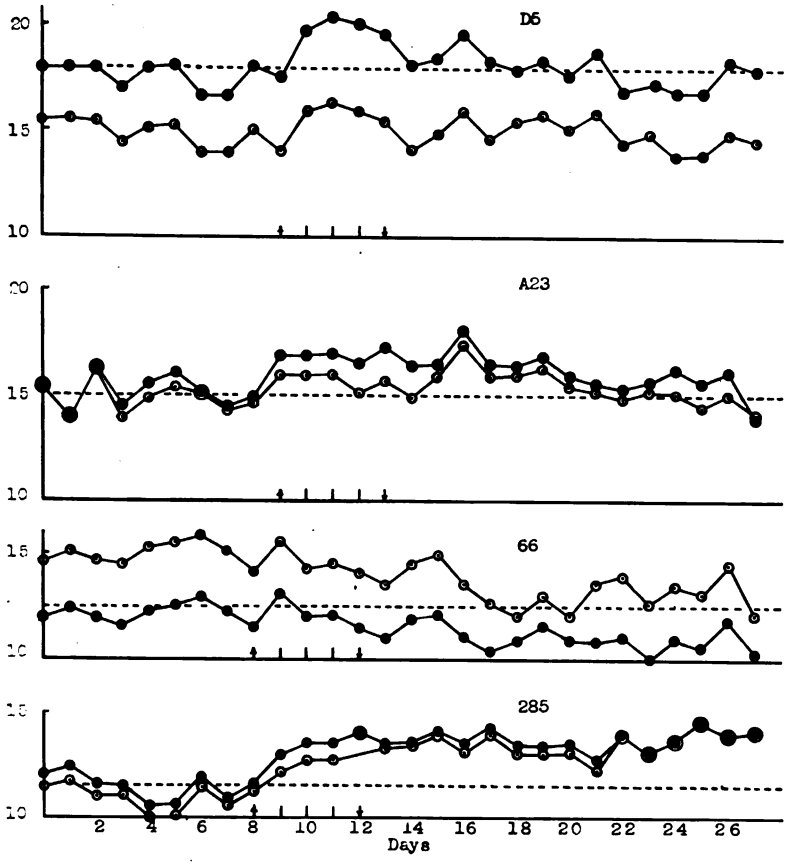


Fig. 1. Effect of prolactin on milk secretion.
---- pre-injection level of milk volume.
●—● daily milk yield in pounds.
⊙—⊙ 4% fat corrected milk.
↑ signifies the first injection.
↓ signifies the last injection.

The effect of prolactin on fat production was not marked. In only one animal (#285) was an increase shown. This increase was accompanied by increased milk production, and the per cent fat in the milk did not change. In the other 3 cows the results were not conclusive, although a slight decrease in per cent fat occurred with cows #D5 and #A23 during the injection period. These results are presented graphically in Fig. 2.

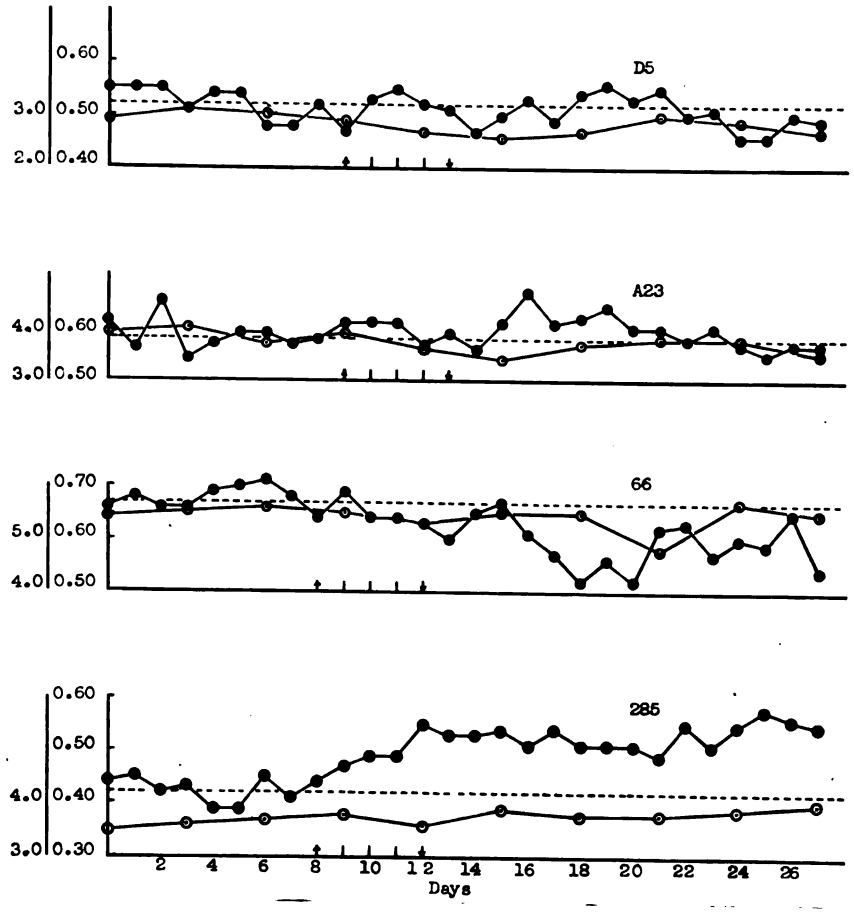


Fig. 2. Effect of prolactin on fat production.
---- pre-injection level of fat production.
●—● daily fat production in pounds.
⊙—⊙ milk-fat percentage.
↑ signifies the first injection.
↑ signifies the last injection.

When the fat-metabolism hormone was given alone at a level of 500 mg. per day only a transitory increase in daily milk volume was observed in 3 cows. In one animal (#66), however, the increase was much more noticeable. Cow #66 showed a maximum increase of 3.5 lbs. on the third day of injection, but secretion continued to rise and 2 days after the injection period an increase of 5 pounds was observed. Thereafter milk volume decreased to the pre-injection level in about 10 days. The other 3 cows showed an increase on

the third day of injection, but as it has been pointed out, this effect did not persist for more than a day or two, and production returned very rapidly to the pre-injection level or lower. The 4% fat-corrected milk values, however, show that production was increased. The results are plotted in Fig. 3.

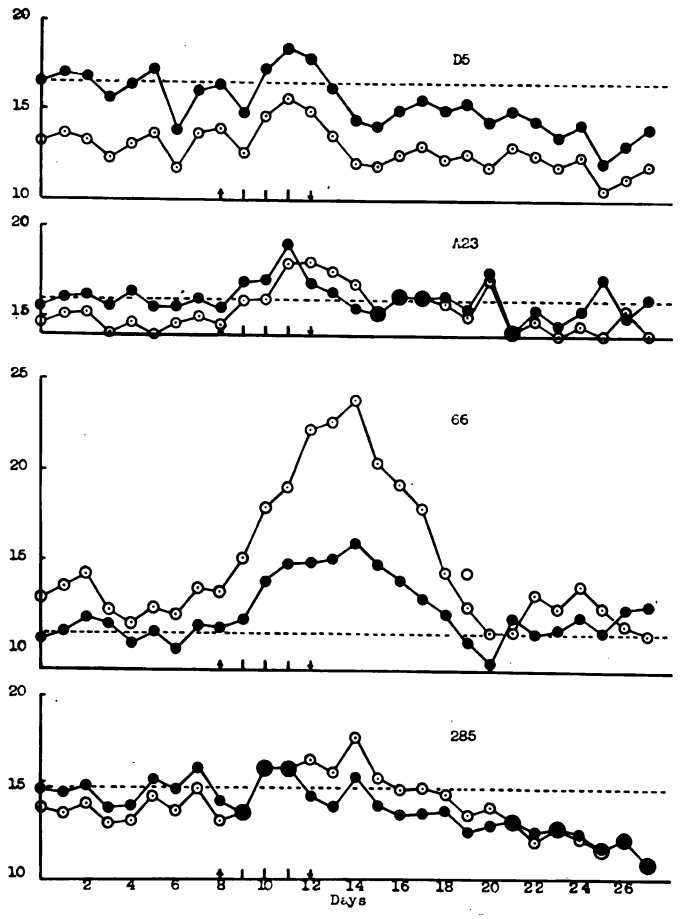


Fig. 3. Effect of fat-metabolism hormone on milk secretion.
---- pre-injection level of milk volume.
daily milk yield in pounds.
4% fat corrected milk.
signifies the first injection.
signifies the last injection.

On the other hand, fat production was markedly affected by the fat-metabolism preparation as 3 of the 4 cows showed marked increases. Number 66 and #285 did not reach their maximum fat production until 2 days after the last injection, and then both animals showed a decrease in production which in

some instances was below the pre-injection level. Number A23 reached her peak production on the fifth day of injections, and although the fat production values were not as high as the other two animals, (#66 and #285) production declined at a less rapid rate. Number D5 increased slightly in fat production, but production began to decrease on the fifth day of the injections, and within 2 days the fat values were as low as the pre-injection values. It will be noted that the per cent fat in the milk following injections in 3 of the 4 cows markedly increased. These results are shown in Fig. 4.

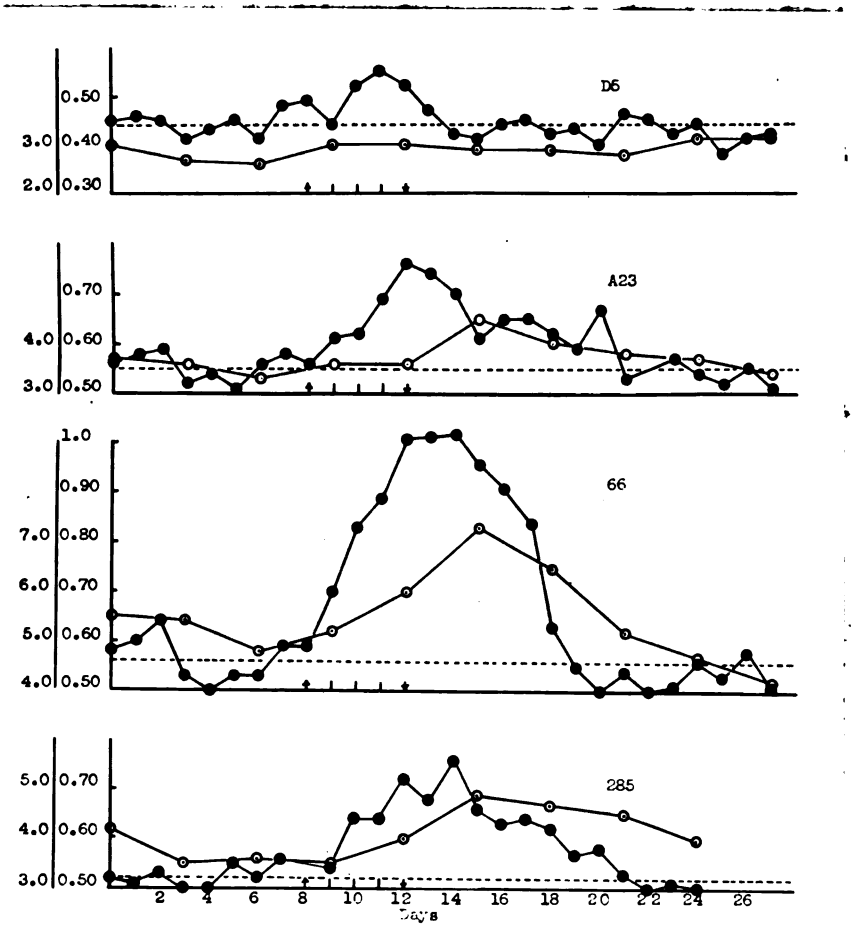


Fig. 4. Effect of fat-net boiler hormone on fat production.
---- pre-injection level of fat production.
●—● daily fat production in pounds.
⊙—⊙ milk-fat percentage.
↑ signifies the first injection.
↓ signifies the last injection.

The effect of 500 mg. of prolactin combined with 500 mg. of the fat-metabolism preparation caused an increase in the volume of milk secreted in 3 of the 4 cows which persisted for about 8 days after the injections were stopped. One cow (#A23) came in heat during the injection period and production was markedly decreased. The milk production of the other 3 cows (#D5, #66 and #285) showed an increase at the time of the second injection and continued to increase until the peak of production was reached on the fifth to the seventh day. The maximum increases over the pre-injection levels for #D5, #66 and #285 were 4.1, 4.6 and 4.3 pounds, respectively. All 4 animals responded by an increased production, expressed at 4% fat-corrected milk, which parralleled the actual increase in volume. These results are shown in Fig. 5.

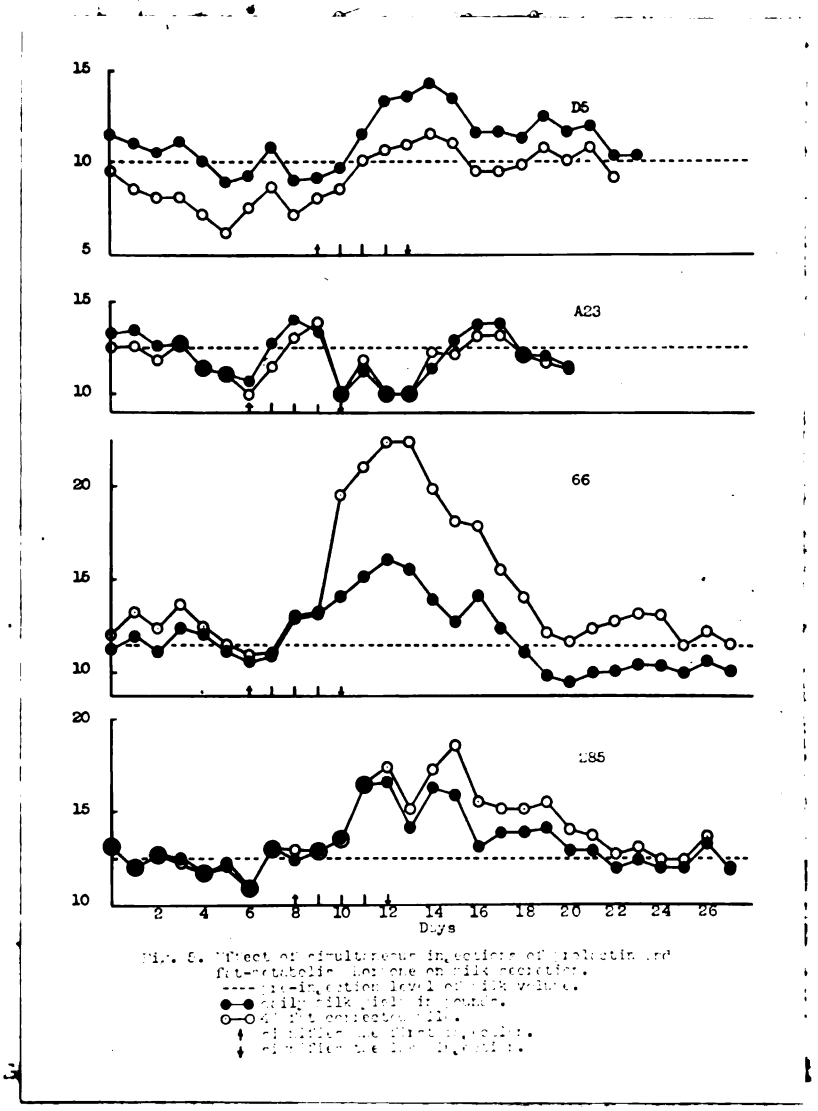
Simultaneous injections of prolactin and the fat-metabolism hormone caused in 2 animals, #D5 and #285, an increase in milk production which was 47% and 23% greater than the combined increases resulting when the hormones were given separately. The increase in milk yield of #66 over a similar period was 29% below the combined increases which resulted from the separate injection of prolactin and the fat-metabolism preparation. Number A23 can not be considered in this part of the experiment because of the onset of estrus during the last injection period. These latter results tend to show that the effect of prolactin on milk secretion may be enhanced by the addition of the fat-metabolism preparation. The results are shown in Table II, in which the values represent the average

TABLE II. THE INCREASE IN MILK VOLUME RESULTING FROM HORMONE INJECTIONS

ANIMAL	INCREASES WHEN PROLACTIN AND FAT METABOLISM HORMONE GIVEN SINGLY			INCREASES WHEN PROLACTIN AND FAT METABOLISM HORMONE GIVEN SIMULTANEOUSLY	COMPARISON OF EFFECTS OF THE SIMULTANEOUS INJECTIONS WITH TOTAL EFFECTS OF THE SINGLE INJECTIONS
	PROLACTIN	FAT METABOLISM HORMONE	TOTAL INCREASE		
D5	1.7 lbs.	-0.07 lbs.	1.63	2.4 lbs.	+0.77 lbs. (47% Inc.)
66	-0.3 lbs.	3.4 lbs.	3.1	2.2 lbs.	-0.9 lbs. (29% Dec.)
285	2.0 lbs.	-0.12 lbs.	1.94	2.3 lbs.	+0.44 lbs. (23% Inc.)

The values indicated for single and simultaneous hormone injections represent the average daily increase observed for a 6 day period, which included the 5 injection days, and are not the maximum increases which were observed.

daily yield over a 6 day period including the 5 day injection period.



The fat production values as affected by the combined hormones are shown by Fig. 6. Number 66 and #285 showed an appreciable increase in per cent fat, as well as in fat production. The per cent fat of #D5 did not increase to any extent, but her fat production did rise. The results obtained on #A23 were irregular.

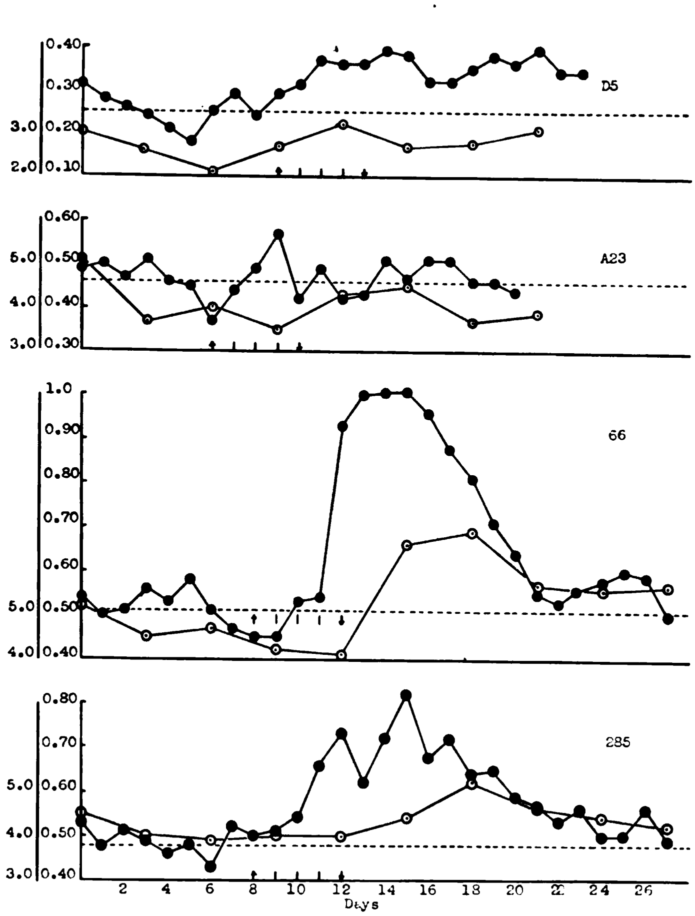


Fig. 6. Effect of simultaneous injections of prolactin and fat-metabolism hormone on fat production.
---- pre-injection level of fat production.
●—● daily fat production in pounds.
○—○ milk-fat percentage.
↑ signifies the first injection.
↓ signifies the last injection.

3. Results of Blood and Urine Analysis

The analytical data of the blood and urine samples are in no way conclusive. It seems probable that before this data can be considered significant a more extensive analysis must be made. The blood sugar and plasma-fat values for all the injected animals were well within the accepted normal standards, and showed no variations which could be correlated in any way with the hormone treatment. However, in several animals over both injection periods, there is some indication that blood and urinary ketones tend to rise after the injection period.

DISCUSSION OF RESULTS

The administration of the prolactin prepared in this laboratory produced results which are essentially in agreement with those obtained by Folley and Young (1938), and Azimov and Krouze (1937). The increase in production was not great in any instance and no significant variations in the per cent fat occurred. The results obtained in this experiment indicated that prolactin primarily affects the volume of milk produced. It is true that total fat production was increased in 3 of the cows (#D5, #A23 and #285, Fig. 2) but this was altogether due to the increased volume since the per cent fat in the milk did not change. In 2 of the cows, prolactin produced a rather prolonged effect, a fact which is somewhat at variance with the observations of previous investigators. The failure of cow #66 to respond to prolactin is unexplained. However, the workers noted above have shown that certain animals fail to respond even when very large doses of prolactin are given. The reasons for such failures are obscure.

The effect of injections of the fat-metabolism preparation as presented in Fig. 3 showed that this preparation had no effect on milk volume in 3 of the 4 cows. Cow #66, although not responding to the prolactin injections, (Fig. 1) showed a very substantial though temporary increase in milk yield. The explanation of this latter observation is not clear. However, Folley and Young (1938) suggest that repeated prolactin injections may cause some regeneration of the involuted mammary tissue. As only one month elapsed from the

last prolactin injection to the beginning of the fat-metabolism injections, and as this latter preparation did contain some prolactin, the small amount of prolactin might have been sufficient to cause the increased milk yield. If this were true for this one animal, it seems that it would also hold for the other 3 cows. However, variations in the degree of involution, the sensitivity of the animal or the possible production of an antiserum (Young, 1938) are all possible explanations for the different responses noted in these animals. When production was calculated on a 4% fat-corrected basis, however, all 4 cows showed an increase in response to the fat-metabolism hormone. This was particularily marked in 3 of the cows. The increase in production when expressed in this manner was altogether due to the increase in the per cent fat in the milk, since the volume secreted per day did not change appreciably except in the case of cow #66.

The milk fat records produced as a result of the fat-metabolism injections are most remarkable. Three cows (A23, #66 and #285, Fig. 4) showed marked increases in fat yields. This increased fat production was primarily due to the increase in per cent fat as the fat-metabolism injections did not produce increased milk secretion except in the case of #66 (Fig. 2). This cow showed the greatest fat production, and in view of her milk increase, the increased fat is undoubtedly a result of both per cent fat and milk yield increases. In 3 of the 4 cows, however, rather remarkable increases in the per cent fat in the milk occurred and it would seem that this

hormone primarily affects the fat metabolism of the mammary gland.

When prolactin and the fat-metabolism hormone were administered simultaneously in equal amounts the responses are similar to those obtained by Folley and Young (1938) by their injections of prolactin and the thyreotropic principle. The thyreotropic fraction by itself, like the fat-metabolism hormone, was incapable of causing any significant increases in milk production but when combined with prolactin it enhanced the effect of this hormone. The record of one animal (#A23) of this group can not be considered because of the onset of estrus. The milk production of 2 cows (#D5 and #285) showed substantially greater increases than when either prolactin or the fat-metabolism hormone were given alone, if the maximum increases are taken as a basis of comparison. The average daily increase over a 6 day period was likewise greater as a result of injections of combined prolactin and the fat-metabolism hormone than the total of the average daily increases for a similar period which occurred when these preparations were given alone (Table II). Since one cow (#66) gave the opposite result, more extensive data are necessary before definite conclusions may be made. However, the data presented indicate that the fat-metabolism preparation does enhance the effect of prolactin and further that lactation is not the result of the effect of a single hormone, prolactin, but rather a result of the combined effects of several hormones.

As it has been previously pointed out, neither prolactin nor the fat-metabolism preparation caused any variations in the blood sugar values. These results indicate that the hormone preparations used did not affect carbohydrate metabolism to any detectable extent. The plasma-fat determinations likewise showed no appreciable variations over the injection period. However, it is possible that the blood fat values might decrease following injections of the fat-metabolism hormone if the dosage levels of this preparation were increased, since this preparation was shown to cause a slight decrease in the plasma-fat of guinea-pigs. The values obtained for the blood ketones over the injection period show wide variations. However, in 6 of the 8 injection periods, in which prolactin or the fat-metabolism preparation was given, there was a tendency toward increased blood ketones after the injections were stopped (4 days post-injection). That more pronounced indications of ketosis did not result from the injections of these preparations is somewhat surprising in view of the fact that both have been shown to possess ketogenic activity in other species (Campbell and Keenan, 1940). However, Mirsky (1937) and Peterson and Shaw (1938) have presented data which indicate that part of the milk fat may be derived from ketones and the fact that fat production increased in these cows following injection may have been partly due to utilization of extra ketones which were produced by these hormones with the consequent failure of these extra ketone bodies to appear in the blood and

urine. The appearance of increased amounts of these substances in the blood and urine when fat production was decreasing further indicates that increased utilization for fat production would account for the failure of ketones to increase appreciably during the injection periods.

CONCLUSION

It has been found that a partially purified prolactin preparation will cause increases in milk volume and fat production. The latter effect is entirely the result of the increased milk volume as the per cent fat did not vary.

The fat-metabolism preparation, on the other hand, caused marked increases in fat production and per cent fat, but did not result in an increased milk yield.

Simultaneous injection of prolactin and the fat-metabolism preparation resulted in an increased milk volume and fat production, and this increase was greater than the combined increases affected by the separate injections of prolactin and the fat-metabolism hormone. These data indicate that lactation is not the result of the action of a single hormone, but rather due to the combined effects of at least two hormones.

The analytical data of blood sugar, plasma-fat and blood and urinary determinations could not be correlated with the above findings to any degree. A possible relationship of the ketones to fat production is discussed.

REFERENCES

Allan, N. N.
1930. Blood Fat of Dairy Cattle. I. A Simple Volumetric Method for Determination of Fat in Blood Plasma. II. Factors Influencing the Fat Content of the Blood Plasma.
Minn. Agr. Expt. Station. Tech. Bul. 130.

1933. A Simple Volumetric Method for Determination of Fat in Blood Plasma.
Proc. Soc. Expt. Biol. and Med. 31: 991.

Allan, W., and P. Wiles.
1932. Effect of the Induction of Hypophyseal Extract in Advanced Lactation.
Am. Jour. Physiol. 100: 137.

Anselmino, K. J., and F. Hoffmann.
1931. Das Fettstoffwechselhormon des Hypophysenvorderlappens. I. Nachweis, Darstellung und Eigenschaften des Hormons. (The Fat-Metabolism Hormone of the Anterior Pituitary. I. Identity, Preparation and Properties of the Hormone.)
Klin. Wschr. 10: 2380.

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

1932. The Effect of the Injection of Hypophyseal Extract in Advanced Lactation.
Am. Jour. Physiol. 100: 137.

Azimov, G. I., and N. K. Krouze.
1937. The Lactogenic Preparation from the Anterior Pituitary and The Increase of Milk Yields in Cows.
Jour. Dairy Sci. 20: 289.

Bates, R. W., and O. Riddle.
1935. The Preparation of Prolactin.
Jour. Pharm. and Expt. Therap. 55: 365.

1936. Effect of Route of Administration on the Bioassay of Prolactin.
Proc. Soc. Expt. Biol. and Med. 34: 847.

Bates, R. W., O. Riddle, and E. L. Lahr.
1939. The Racial Factor in the Pigeon Crop-Sac Method of Bioassay of Prolactin.
Am. Jour. Physiol. 125: 722.

Bergman, A. J., and C. W. Turner.
1940. The Specificity of the Lactogenic Hormone in the Initiation of Lactation.
Jour. Dairy Sci. 23: 1229.

Best, C. H., and J. Campbell.
1936. Anterior Pituitary Extracts and Liver Fat.
Jour. Physiol. 86: 190.

1938. The Effect of Anterior Pituitary Extracts on the Liver Fat of Various Animals.
Jour. Physiol. 92: 91.

Burn, J. H., and H. W. Ling.
1930. Ketonuria in Rats on a Fat Diet (a) After Injections of Pituitary (Anterior Lobe) Extract, (b) During Pregnancy.
Jour. Physiol. 69: xixP.

Campbell, J.
1938. A Method of Assaying the Potency of Anterior Pituitary Extracts Which Increase Liver Fat.
Endocrin. 23: 692.

Campbell, J., and H. C. Keenan.
1940. The Substance of the Anterior Pituitary Gland Which Increases Liver Fat.
Am. Jour. Physiol. 131: 27.

Catchpole, H. R., W. R. Lyons, and W. M. Regan.
1933. Induction of Lactation in Heifers with the Hypophyseal Lactogenic Hormone.
Proc. Soc. Expt. Biol. and Med. 31: 310.

Corner, G. W.
1930. The Hormonal Control of Lactation.
Am. Jour. Physiol. 95: 43.

De Fremary, P.
1936. On the Influence of Different Hormones on Lactation.
Jour. Physiol. 87: 50P.

Evans, E. I.
1933. Initiation of Copious Milk Secretion in Virgin Goats by Anterior Pituitary.
Proc. Soc. Expt. Biol. and Med. 30: 1372.

1936. The Effect of Prolactin Upon the Milk Yield of Lactating Cows and Goats.
Am. Jour. Physiol. 116: 45P.

Folley, S. J., and F. G. Young.
1938. The Effect of A. P. Extracts on Established Lactation in the Cow.
Roy. Soc. of London, B. 126: 45.

1939. The Effect of Continued Treatment with Anterior Pituitary Extracts on Milk Volume and Milk-Fat Production in the Lactating Cow.
Jour. Biochem. 33: 192.

Gardner, W. V., and C. W. Turner.
1933. The Function, Assay and Preparation of Galactin, A Lactation Stimulating Hormone of the Anterior Pituitary. An Investigation of the Factors Responsible for the Control of Normal Lactation.
Mo. Agr. Expt. Station. Res. Bul. 196.

Gomez, E. T., and C. W. Turner.
1936. Initiation and Maintenance of Lactation in Hypophysectomized Guinea-Pigs.
Proc. Soc. Expt. Biol. and Med. 35: 365.

1937. Effect of Thyroxine and Galactin on Lactation in Hypophysectomized Guinea-Pigs.
Proc. Soc. Expt. Biol. and Med. 36: 80.

Graham, W. R., Jr.
1934. The Action of Thyroxine on the Milk and Milk-Fat Production of Cows.
Biochem. Jour. 28: 1368.

Grüter, F., and P. Stricker.
1929. Über the Wirkung eines Hypophysenvorderlappenshormons auf die Auslosung der Milchsekretion. (Action of a Hormone of the Anterior Pituitary on the Secretion of Milk.)
Klin. Wschr. 8: 2322.

Hill, R. T., C. W. Turner, A. W. Wien, and E. T. Gomez.
1935. Hypophysectomy of the Goat.
Univ. Mo. Res. Bul. 230.

Houchin, O. B., and C. W. Turner.
1939. A Method of Assay for the Fat-Metabolism Hormone of the Anterior Pituitary.
Endocrin. 25: 216.

1939. The Relation of the Pituitary to Blood Lipids. Endocrin. 24: 638.

Lyons, W. R.
1937. Preparation and Assay of Mammotropic Hormone. Proc. Soc. Expt. Biol. and Med. 35: 645.

Lyons, W. R., and H. R. Catchpole.
1933. Assay With the Guinea-Pig of the Lactogenic Hypophyseal Hormone. Proc. Soc. Expt. Biol. and Med. 31: 299.

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

Lyons, W. R., and E. Page.
1935. Detection of Mammotropin in the Urine of Lactating Women. Proc. Soc. Expt. Biol. and Med. 32: 1049.

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

1935a Studies on the Hypophysectomized Ferret. IX. The Effect of Hypophysectomy on Pregnancy and Lactation. Proc. Roy. Soc. B. 117: 34.

McShan, W. H., and C. W. Turner.
1936. Bioassay of Galactin, the Lactogenic Hormone. Proc. Soc. Expt. Biol. and Med. 34: 50.

Mirsky, I. A.
1936. The Source of the Blood Acetone Resulting from the Administration of the Ketogenic Principle of the Anterior Hypophysis. Am. Jour. Physiol. 115: 424.

Nelson, W. O., and J. J. Pfiffner.
1931. Studies on the Physiology of Lactation. Anat. Rec. 51: 113.

Nelson, W. O., and R. Gaunt.
1936. Initiation of Lactation in the Hypophysectomized Guinea-Pig. Proc. Soc. Expt. Biol. and Med. 34: 671.

1937. The Adrenal and Pituitary in Initiation of Lactation.
Proc. Soc. Expt. Biol. and Med. 36: 136.

Pencharz, R. I., and W. R. Lyons.
1934. Hypophysectomy in the Pregnant Guinea-Pig.
Proc. Soc. Expt. Biol. and Med. 31: 1131.

Reece, R. P.
1939. Initiation of Lactation in the Albino Rat with Lactogen and Adrenal Cortical Hormone.
Proc. Soc. Expt. Biol. and Med. 40: 25.

Riddle, O., and R. W. Bates.
1939. Chap. XX, Sex and Internal Secretions.
Williams and Wilkins, Baltimore.

Riddle, O., R. W. Bates, and S. W. Dykshorn.
1931. A New Hormone of the Anterior Pituitary.
Proc. Soc. Expt. Biol. and Med. 29: 1211.

1933. The Preparation, Identification and Assay of Prolactin.
Am. Jour. Physiol. 105: 191.

Selye, H., J. B. Collip, and D. L. Thomson.
1933. Anterior Pituitary and Lactation.
Proc. Soc. Expt. Biol. and Med. 30: 588.

Shaffer, P. A., and A. F. Hartmann.
1921. II. Methods for the Determination of Reducing Sugars in Blood, Urine, Milk and Other Solutions.
Jour. Biol. Chem. 45: 365.

Shipley, R. A., and C. N. H. Long.
1938. CCLXXXVIII. Studies on the Ketogenic Activity of the Anterior Pituitary. I. The Relation of Ketonaemia to Ketonuria in the Rat. II. A Method for the Assay of the Ketogenic Activity. III. The Nature of the Ketogenic Principle.
Biochem. Jour. 32: 2242.

Somogyi, M.
1926. Notes on Sugar Determination.
Jour. Biol. Chem. 70: 599.

Van Slyke, D. D.
1917. Studies of Acidosis. VII. The Determination of -Hydroxybutyric Acid, Acetoacetic Acid and Acetone in Urine.
Jour. Biol. Chem. 32: 455.

Young, F. G.
1938. LXIX. Studies on the Fractionation of Diabetogenic Extracts of the Anterior Pituitary Gland
Biochem. Jour. 32: 524.

1938. LXXXVII. The Production of Antisera to Preparations of Prolactin Containing the Glycotropic (Anti-Insulin) Factor on the Anterior Pituitary Gland.
Biochem. Jour. 32: 656.

ROOM USE ONLY

Aug 31 '48