XLV. THE INFLUENCE OF THE HYPOPHYSIS ON METABOLISM, GROWTH AND SEXUAL ORGANS OF MALE RATS AND RABBITS.

II. INFLUENCE OF EXTRACTS OF HYPOPHYSIS ON THE BODY WEIGHT, WEIGHT OF FAT, OF SEXUAL ORGANS AND OF ENDOCRINE ORGANS OF RATS.

By VLADIMIR KORENCHEVSKY.

From the Lister Institute, London.

(*Received February 28th, 1930.*)

Historical.

The hypophysis and fat deposition.

It is an established fact that patients with hypopituitarism and animals after the removal of the hypophysis develop a pronounced adiposity, while young animals in the same circumstances show, in addition, retardation of growth [see bibliography in Biedl, 1916; Barker, Hoskins and Palmer, 1922; Schaefer, 1926; Geiling, 1926].

In agreement with these observations the gaseous metabolism is found to be decreased [Narbut, 1903; Wolf and Sachs, 1910; Aschner, 1912, 1913; Aschner and Porges, 1912; Benedict and Homans, 1912; Foster and Smith, 1926]. It was, however, unexpected that injections of pituitary extract into animals should also produce adiposity [Delille, 1909; Evans and Long, 1922] and lowering of metabolism [Bernstein and Falta, 1912; Korenchevsky and Dennison, 1929].

The influence upon growth of the administration of the anterior lobe of the pituitary.

In spite of the large number of experiments which have been performed on mice, rats, guinea-pigs, rabbits, puppies and chicks, either fed or injected with extracts and preparations of whole hypophysis or of the anterior lobe, the most contradictory results have been obtained.

Increased growth of the animals was observed by Schaefer [1909], Goetsch [1916], Gudernatsch [1918], Klinger [1919], Marinus [1919] and Robertson and his co-workers [1916–1925]. Retardation or cessation of growth was obtained by Thompson and Johnston [1905], Cerletti [1907], Sandri [1907, 1909], Cushing [1909], Aldrich [1912, 1913], Wulzen [1914], Pearl [1916] and

Maxwell [1916]. Administration of pituitary preparations had practically no influence upon growth in the experiments of Lewis and Miller [1913], Drummond and Cannan [1922], Smith [1927, 2] and Reichert [1928]. Extremely elaborate experiments were performed by Evans and his co-workers [1921–1928]. They state that when an extract of the anterior lobe of the hypophysis is injected intraperitoneally into young rats from the day of weaning, acceleration of growth is produced, some of the treated rats being from 100 to 250 g. heavier than their normal litter mate controls at eight months of age. Oral administration is ineffective. No detailed account of these experiments has been published.

Smith, Walker and Graeser [1923], Smith and Graeser [1924], and Smith [1927, 1, 2, 3, 4] produced dwarfism by destroying the pituitary body in rats, and found that growth was resumed after injections of anterior hypophysial substance prepared by the method of Evans and Simpson [1926].

Van Wagenen [1927, 1928], using similar extracts of the anterior lobe, increased the body length and the weight of castrated rats up to those of the normal controls.

Putnam, Teel and Benedict [1928], who also used alkaline extracts of hypophysis, produced acceleration of growth in growing rats and one dog, but not in rabbits. The injections were given to the dog for many months before any effect was observed. In the hypophysectomised dog and the rats, restoration of growth was produced. They concluded that the growth of hair and the healing of wounds is much delayed after hypophysectomy, but these processes may be restored to normal by administration of the hormone.

The latest experiments by Larson, Bergeim, Barber and Fisher [1929] do not show any influence of similar extracts, injected intraperitoneally, on the growth of rats, whilst Hewitt [1929] confirmed the results obtained by Evans.

The influence of the administration of the hypophysis on the sexual organs of male animals.

A stimulating effect of pituitary feeding or injections of pituitary extracts on the sexual development or the size of testes was noticed by Wulzen [1914], Goetsch [1916], Klinger [1919] and Marinus [1919]. No such influence, however, was observed in the experiments of Pearl [1916], Maxwell [1916], Kross [1922] and Smith [1927, 3].

Evans and his co-workers [1921–1928] found that an extract of the anterior lobe of the hypophysis delays sexual maturity in the male rat. Autopsy of two treated male rats, when compared with their controls, showed great diminution of testis weights. Histologically, smaller tubules were found, but no evidence of any other degenerative changes [Evans and Simpson, 1926]. The authors succeeded in separating the principle in the anterior lobe affecting ovulation in rats from that controlling general body growth by the addition of alcohol to the extract until the concentration of alcohol reached 50 %. The supernatant fluid, after centrifugation, still possessed the principle

affecting the gonads, but not that accelerating growth. Probably the latter principle had been carried down with the protein precipitate or been destroyed. Both hormones, however, are still active after bubbling oxygen through the extract for one hour or after adding alcohol to 8 % and keeping at room temperature for one week.

In a later paper Evans and Simpson [1928], concluded that there is present in the hypophysis not an ovary-depressing, but an ovary-stimulating hormone, which is required for the normal development of the ovaries, thyroid and suprarenal cortex. Their previous contradictory results are explained by the fact that the growth-promoting hormone completely nullified the effect of the gonad-stimulating hormone, when simultaneously administered. Alkaline aqueous extracts contain a growth-stimulating hormone and acid extracts of the anterior lobe a hormone provoking sexual maturity in young female rats. By these different methods of extraction these two hormones can be separated. Unfortunately, up to the present time no detailed account of these experiments has been published.

Zondek and Aschheim, in a series of papers [1927–1928] describe an ovary-stimulating hormone in the urine of pregnant women which they believe to be secreted by the anterior lobe of the hypophysis. These authors state that although a single injection of this substance is without effect on male mice, repeated injections, during a period of about 2 weeks, caused the secondary sexual glands, but not the testes, to become hypertrophied. The seminal vesicles were about five times larger than those of the control animals. This did not occur in castrated animals.

Fels [1927], injected infantile male mice with the serum from pregnant women. The testes were smaller than normal, but histologically the amount of the interstitial cells was increased. The prostate and seminal vesicles were hypertrophied, which latter changes were similar to those which occurred in the experiments of Zondek and Aschheim. In Biedl's [1927] experiments injections of hypophysial extract or emulsion retarded the development of the sexual system of male rats and mice, especially of the testes and seminal vesicles. The amount injected was from 0·05 to 0·25 g. of fresh glandular substance. Smith [1927, 3] injected rats with an extract prepared by the method of Evans and Simpson [1926] from ox pituitaries. These injections not only did not cause the restoration to normal of the atrophied testes of hypophysectomised rats, but even prevented their restoration by pituitary homotransplants, which were made simultaneously with the injections.

Larson, Bergeim, Barber and Fisher [1929] found that the weights of the testes of the rats receiving injections were less than those of the controls. However, they suggest that this may be attributed to the lower weights of these rats. The figures were not given.

The influence of the injections on the thyroids and adrenals.

Smith [1927, 3] observed in hypophysectomised rats atrophy of the suprarenal cortex, thyroids and parathyroids. Partial or complete repair was produced by daily homotransplants of fresh pituitaries, but intraperitoneal injections of extracts prepared by the method of Evans and Long, made from ox pituitaries, did not repair the atrophied thyroid or suprarenal cortex or the atrophied testes of hypophysectomised rats.

Loeb and Bassett [1929] prepared acid and alkaline extracts from dried and powdered anterior lobe of the pituitary of cattle and injected these intraperitoneally into guinea-pigs. After 7 to 19 days the injections resulted in hypertrophy of the thyroids. During the period of the injections the animals lost weight. The method of preparation suggests that probably the response of the thyroids was the typical reaction to injections of any emulsion of animal tissue contaminated with bacteria and prepared without sterile precautions.

The analysis of the papers, mentioned in this historical review, shows a perplexing discrepancy in results. It may be that the difference in the results obtained by different investigators is to be explained (1) by the presence in the anterior lobe of more than one hormone, some of these hormones being antagonistic in action; (2) by the different methods of preparation of the extracts; (3) by deficiencies in general technique in some investigations, especially the use of control animals not of the same litter and lack of control of the diet (its uniformity and its sufficiency in respect of all the factors necessary for growth of the animal in general and of some organs in particular).

The present experiments.

Experiments were performed on 87 male rats, of which 76 were young growing rats and 11 were old rats. All the rats belonged to litters, bred at the Lister Institute, of which the day of birth was known. Each litter was divided into two groups, a control group, into which 20 % glycerol in saline solution was injected, and an experimental group into which extract of the anterior lobe of the pituitary was injected. The extracts were injected intraperitoneally, daily, or with some intervals of one, or rarely, of two, days. Seven litters were used for the injections without previous operation, a total of 23 rats; 27 rats belonging to another 7 litters were castrated and 37 from 9 litters were made cryptorchid. The experiments on cryptorchid rats are recorded in Tables I–III in two separate groups A and B, since the experiments on the rats of group B were performed later and with a different batch of the extract. Castrated and cryptorchid rats, having a decreased and an increased sexual inner secretion respectively, were used as well as normal rats since there is a close relationship between the pituitary and the sexual glands. Castration was performed in the usual manner. The animals were made cryptorchid by pushing back the testes from the scrotum into the abdominal cavity, occluding the inguinal canal and putting a suture on the basal portion of the *tunica vaginalis*. The method of preparation of the extract was almost

the same as that used by Evans and Simpson [1928], which produced increased growth in rats. The only difference was that the glands were first ground with glycerol, instead of with water, since a glycerol extract is more easily kept in a fresh condition in the cold store than is a watery extract.

The detailed method of preparation of the extract is as follows. The anterior lobes of pituitary glands, obtained from the slaughter house on the day of killing of the cattle, were, with sterile precautions, chopped to a thin paste and then finely ground with sand in a mortar. Two volumes of glycerol were added and the glycerol paste was kept in the cold store at $-5°$. On the day of injection one part of the paste was mixed with $1\frac{1}{2}$ parts of distilled water and to this mixture $N/5$ NaOH was then added in an amount equal to three-eighths of the volume of the mixture. This was left to stand for about 20 minutes and then neutralised or made slightly acid with $N/5$ acetic acid. By the addition of a few drops of $N/5$ NaOH the p_H was brought to 7·4. The solution was then centrifuged and the supernatant fluid was used for the intraperitoneal injections, in amounts of from 0·5 to 2 cc. per rat. No abscesses or any toxic symptoms due to the injections were observed.

The animals were fed on a special paste, consisting of a normal synthetic diet, so as to ensure that the quality of the diet was identical for each animal. The amount of the diet consumed was recorded. The composition of the diet was the same as of that used for vitamin experiments and was described elsewhere [Korenchevsky, 1922, p. 7]. The duration of the experiments varied, for different litters, from 6 to 11 weeks.

In order to save space the detailed tables of the weights of each individual rat or organ are not given. The influence of the injections was in every case in the same direction as shown by the average figures, given in Tables I, II and III.

Table I. *The influence of intraperitoneal injections of anterior lobe extract on the weight of young and old rats.*

	Controls, glycerol-injected					Anterior lobe extract-injected				
	No. of rats for averages	Weight in g.		Gain + or loss − in weight in g.		No. of rats for averages	Weight in g.		Gain + or loss − in weight in g.	
Groups		Init.	Final	Total	Per week		Init.	Final	Total	Per week
Young rats:										
Normal	8	61·3	233·8	+172·5	+19·6	9	55·9	223·4	+167·5	+19·1
Castrated	12	80·8	214·8	+134·0	+17·0	15	78·6	202·9	+124·3	+15·8
Cryptorchid A	8	74·5	211·1	+136·6	+16·9	12	71·7	205·7	+134·0	+16·6
Cryptorchid B	4	53·5	228	+174·5	+18·6	8	53·0	240	+187	+19·8
Old rats:										
Normal	3	405·0	397·0	−8·0	−0·9	3	366·0	355·0	−11·0	−1·3
Cryptorchid	2	418·0	396·0	−22·0	−2·3	3	451·0	423·0	−28·0	−2·9

The influence on growth and appetite.

The results in relation to growth are given in Table I. The figures represent averages. These figures show no well-defined influence on the growth of the

male rats injected with the extract, as compared with that of the controls injected with glycerol solution. The final weight and the gain in weight per week of both groups of young rats varied only slightly. Old rats, in both groups, lost weight a little during the period of the experiment. The variation in consumption of paste between the two groups was slight, being from 1 to 2 g. per day greater in about 65 % of the groups of the pituitary-injected rats.

Table II. *The influence of intraperitoneal injections of anterior lobe extract on the weight of "retroperineal fat" and "testicular fat" of rats.*

	Controls, glycerol-injected			Anterior lobe extract-injected		
Group of rats	No. of rats for averages	Actual weight in g.	Per 200 g. of rat weight in g.	No. of rats for averages	Actual weight in g.	Per 200 g. of rat weight in g.
			"Retroperineal fat."			
Normal	11	8·26	5·83	12	5·56	4·20
Castrated	12	6·60	5·99	15	4·37	4·02
Cryptorchid A	10	5·31	5·26	15	7·89	5·28
			"Testicular fat."			
Normal	11	8·01	5·61	12	5·58	4·24
Cryptorchid A	10	7·55	5·61	15	5·72	4·22
			Total "intra-abdominal fat" (*i.e.* Retroperineal + testicular fat).			
Normal	11	16·27	11·44	12	11·14	8·44
Cryptorchid A	10	12·86	10·87	15	13·61	9·50
Cryptorchid B	4	12·38	10·84	8	9·98	8·29

Table III. *The influence of intraperitoneal injections of anterior lobe extract on the average weight of the sexual organs and of the glands of inner secretion.*

		Controls, glycerol-injected		Anterior lobe extract-injected	
Organ	Group of rats	Actual weight of organ in g.	Weight in g. per 200 g. of rat weight	Actual weight of organ in g.	Weight in g. per 200 g. of rat weight
Testis	Normal	2·826	2·126	2·346	1·876
	Cryptorchid A	1·037	0·926	0·669	0·572
	„ B	0·784	0·689	0·628	0·528
Penis	Normal	0·310	0·222	0·284	0·222
	Cryptorchid A	0·262	0·222	0·179	0·144
	„ B	0·289	0·254	0·223	0·186
	Castrated	0·074	0·071	0·078	0·077
Prostate with seminal vesicles	Normal	1·960	1·386	1·389	1·068
	Cryptorchid A	1·214	0·999	0·386	0·321
	„ B	1·282	1·124	0·598	0·499
	Castrated	0·080	0·076	0·093	0·095
Adrenals	Normal	0·0636	0·0474	0·0620	0·0494
	Cryptorchid A	0·0616	0·0523	0·0639	0·0528
	„ B	0·0509	0·0449	0·0703	0·0589
	Castrated	0·0690	0·0644	0·0679	0·0682
Thyroids	Normal	0·0382	0·0271	0·0278	0·0218
	Cryptorchid A	0·0336	0·0272	0·0264	0·0215
	„ B	0·0216	0·0190	0·0201	0·0167
	Castrated	0·0241	0·0227	0·0217	0·0217
Hypophysis	Normal	0·0114	0·0085	0·0097	0·0077
	Cryptorchid A	0·0115	0·0098	0·0102	0·0085
	„ B	0·0086	0·0075	0·0095	0·0079
	Castrated	0·0126	0·0115	0·0116	0·0116

The influence on fat deposition.

Since the separation and weighing of the whole of the fat is very difficult in rats, the weight of the intra-abdominal fat was taken as an indication of the general development of the fat in the body. In rats part of this fat is easily separated from the retroperineal region ("retroperineal fat" in the tables). In castrated rats only this fat was taken for weighing. In normal and cryptorchid animals, in addition, the fat attached to the testes was separately weighed ("testicular fat" in the tables). Both fats taken together will be referred to as "intra-abdominal fat." The average results are given in Table II. In this table the weight of fat of young growing rats is not separated from that of old rats, since the fat deposition was similar in both groups. Comparison of these average figures, especially taking into consideration those reduced to 200 g. of rat weight, shows a decreased amount of fat in the rats injected with the extract of the anterior lobe. Detailed figures of the weight of fat of each individual rat (which are not given in order to save space) as well as the average figures in Table II show that this decrease was absent only in the case of the retroperineal fat of Group A of cryptorchid rats. The testicular fat of cryptorchid rats was, however, decreased; so that the total amount of intra-abdominal fat, calculated per 200 g. of rat weight, was decreased by the influence of injections of anterior lobe extract in all three groups of rats, normal, castrated and cryptorchid. Per 200 g. of rat weight, the average amount of intra-abdominal fat in the injected rats was less by 12·6 to 33 % as compared with that in the respective groups of control animals.

The influence on sexual organs.

The results are given in Table III and represent the average weights in g. of testis, prostate with seminal vesicles, and of penis. The number of rats used for the average was in each group the same as that in Table II. The most suitable figures for comparison are those calculated per 200 g. of rat weight. They show a striking decrease in weight of the sexual organs in cryptorchid animals under the influence of anterior pituitary injections. For example, the weight of the prostate and seminal vesicles was about 68 % less than that of control rats in group A and about 56 % less in group B. The corresponding figures for penis and testis were 35 % and 38 % less than the controls in group A; in group B of cryptorchids the respective figures were 27 and 23 % less than those of the controls. In normal animals the decrease in the weight of the prostate with seminal vesicles and of the testes of rats injected with pituitary was much less pronounced than those of cryptorchid rats. The respective figures for the decrease of weight of these organs, compared with the controls, were about 23 and 12 %. There was no change in the weight of the penis.

In castrated animals the influence of pituitary injections was not clear. Although the penis, prostate and seminal vesicles were on the average heavier

than in the controls, it is impossible to draw definite conclusions from these figures, since the individual weights of these organs, included in the average, varied considerably. This was due, firstly, to the difficulty of accurate separation of fat from the very atrophied and degenerated sexual organs of castrated rats; and secondly, probably to the absence of considerable changes in the organs of castrated animals under the influence of pituitary injections. That the injections had the maximum effect on cryptorchid animals shows that the extracted hypophyseal substances are closely correlated with a special testicular tissue. However it is not possible from the present data to conclude whether this effect was due to the atrophy of the generative tissue or to the hypertrophy of the interstitial tissue.

The influence on the adrenals, thyroids and hypophysis.

It is possible to see from Table III definite decrease only in the weight of the thyroids of normal and cryptorchid animals injected with anterior lobe (on the average, of about 20 % in normal rats and in group A and about 12 % in group B below that of the controls). In castrated rats the thyroids are already atrophied, as a result of castration. The injections of anterior lobe extract did not increase this atrophy in all the castrated rats. Of the 15 castrated rats there was a decrease in weight of the thyroid in only 7 rats, in 5 rats there was no change and in 3 the gland had increased in weight as compared with the castrated controls. The divergence of these results, therefore, renders them inconclusive in considering castrated rats. Similar divergent results were obtained in the weights of the hypophysis in the individual rats used in this experiment. On the average, however, there was a slight decrease in the weight of this gland in the groups of normal and group A of cryptorchid rats (about 9 and 13 % respectively below that of the controls).

The weights of the adrenals did not show any definite change under the influence of anterior lobe injections in the normal and castrated rats and in group A of cryptorchid rats. However, there was an increase by about 31 % in the weight of the adrenals of the rats of group B, injected with a different batch of the pituitary extract.

Discussion.

Taking into consideration our previous paper [Korenchevsky and Dennison, 1929] as well as the results obtained in the present experiments, it is possible to state that from the anterior lobe of the hypophysis an extract was prepared which depressed nitrogen metabolism, produced atrophy of the sexual organs (testis, prostate, seminal vesicles and penis) and of the thyroid gland in male rats. This extract did not affect the growth of rats but it decreased the amount of body fat, as judged by the amount of intra-abdominal fat. The atrophy of the sexual organs was more pronounced in the cryptorchid group than with the normal and castrated rats. With the exception of a considerable decrease

of body fat, the smallest changes were observed in the group of castrated rats. These results, therefore, agree with those of Evans and Simpson [1926], Smith [1927, 3] and Biedl [1927], whose extract depressed the development of the testes. Smith, alone, made accurate weighings of the glands of the few male rats used in his experiments.

In the numerous experiments on feeding with, or the injection of, pituitary preparations performed by other investigators no observation apparently was made of the amount of the body fat of the experimental animals. In no case, including the experiments of Evans and his co-workers, was a direct weighing made of the fat in the body of the animals.

To my surprise I was unable to obtain the stimulation of body growth mentioned by Evans and Simpson [1928], although my alkaline extract was prepared in a manner nearly identical with theirs. It is improbable that the use of glycerol in place of water in the first stage of the preparations (see p. 387) was responsible for the divergence. Since the influence on the sexual organs was a depressing and not a stimulating one, an antagonistic action of the sexual hormone upon the growth-promoting hormone cannot be the explanation as was suggested by Evans and Simpson for their results [1928]. Neither from the papers of Evans and his co-workers, nor from the papers of other authors, can indisputable evidence be obtained of the presence of hormones in extracts of hypophysis which stimulate the growth of male sexual organs. The chief difficulty in interpreting many of the experiments is that the authors do not always give figures for the weight of the testes and do not calculate their results per unit of body weight, nor do they use for their controls in their experiments brothers from the same litter. These deficiencies in technique may lead to the gravest mistakes, since I have observed that different litters have different weights of organs and of body fat.

It is important to stress, however, that on one hand Smith's [1927, 3, 4] implantation experiments showed a stimulating influence of the anterior lobe on the male sexual organs, adrenals and thyroids, and Evans and his co-workers obtained extracts which were growth-stimulating. On the other hand, extracts can be obtained, as my experiments show distinctly, which depress nitrogen metabolism, diminish the size of the testes, the prostate, the seminal vesicles, the penis and the thyroid and decrease the deposition of fat. Nevertheless, these extracts do not influence body growth. It may, therefore, be suggested that probably the pituitary contains antagonistic substances for all these functions. It would be unjustifiable, as Evans and Simpson [1928] do, to suggest that there are only two hormones, one required for the normal development of the gonads, thyroid and adrenals and a second for the promotion of growth, and that the latter can be completely nullified by the simultaneous administration of the first.

The extract which I isolated seems to differ from that obtained by Evans and his co-workers, since in my experiments a growth-promoting substance was absent or nullified and at the same time evidence was lacking of the

presence of a hormone which stimulates the activities of the gonads. On the contrary, the extract possessed, except any influence on body growth, a general depressing action.

Summary.

1. A slightly alkaline extract, p_H about 7·4, was prepared from the anterior lobe of the pituitary of oxen which had been ground with glycerol, kept at − 5° and injected intraperitoneally into normal, cryptorchid and castrated young and adult male rats in amounts of 1 to 2 cc.

2. No decided influence was noticed on the growth of the animals as judged by the weight of the rats.

3. Body fat as judged by the intra-abdominal fat was decreased on the average by about 24 %.

4. Under the influence of injections of the extract the weight of penis, testes, and prostate with seminal vesicles was decreased to the greatest extent in cryptorchid animals (on an average for these organs by 31, 30 and 62 % respectively). This effect was less noticeable in normal and absent in castrated rats, showing that a correlation exists between the extracted hypophyseal substances and a special testicular tissue.

5. The weight of the thyroids was decreased by about 17 % in normal and cryptorchid animals, but not significantly in castrated rats.

6. It is concluded that the anterior lobe contains substances diminishing the weights of the penis, prostate, seminal vesicles, testis and thyroids and decreasing fat deposition and nitrogen metabolism.

7. It is suggested that these substances are antagonistic to the growth- and male gonad-stimulating hormones, made evident by implantation experiments; and to the growth-promoting hormone, prepared by Evans and his co-workers.

A grant from the Medical Research Council and the hospitality of the Lister Institute have enabled me to carry out this work, and to them my thanks are due. I wish also to express my sincere gratitude to Sir Charles Martin for his continuous support and criticism of this paper.

REFERENCES.

Aldrich (1912). *Amer. J. Physiol.* **30**, 352.
—— (1913). *Amer. J. Physiol.* **31**, 94.
Aschner (1912). *Arch. ges. Physiol.* **146**, 5.
—— (1913). *Arch. Gyn.* **97**, 200.
—— and Porges (1912). *Biochem. Z.* **39**, 200.
Barker, Hoskins and Palmer (1922). Endocrinology and metabolism.
Benedict and Homans (1912). *J. Med. Res.* **25**, 409.
Bernstein and Falta (1912). *Verh. Congr. inn. Med.* **29**, 536.
Biedl (1916). Innere Sekretion (Berlin).
—— (1927). *Arch. Gyn.* **132**, 175.
Cerletti (1907). *Arch. Ital. Biol.* **47**, 123.
Cushing (1909). *J. Amer. Med. Assoc.* **53**, 251.
Delille (1909). Quoted by Biedl (1916), p. 152.

Drummond and Cannan (1922). *Biochem. J.* **16**, 53.
Evans (1923). *Anat. Rec.* **25**, 107.
—— Flower, Forkner, Kellum, Walker and Smith (1923). *Anat. Rec.* **25**, 107
—— and Long (1921). *Anat. Rec.* **21**, 62.
—— —— (1922). *Proc. Nat. Acad. Sci. (Baltimore)*, **8**, 38.
—— and Simpson (1926). *Anat. Rec.* **32**, 206.
—— —— (1927). *Anat. Rec.* **35**, 36.
—— —— (1928). *J. Amer. Med. Assoc.* **91**, 1337.
Fels (1927). *Arch. Gyn.* **132**, 206.
Foster and Smith (1926). *J. Amer. Med. Assoc.* **87**, 2151.
Geiling (1926). *Physiol. Rev.* **6**, 62.
Goetsch (1916). *Johns Hopkins Hosp. Bull.* **27**, 29.
Gudernatsch (1918). *Anat. Rec.* **14**, 35.
Hewitt (1929). *Biochem. J.* **23**, 718.
Klinger (1919). *Arch. ges. Physiol.* **177**, 232.
Korenchevsky (1922). *Med. Res. Council Spec. Rep. Ser. No.* 71.
—— and Dennison (1929). *Biochem. J.* **23**, 868.
Kross (1922). *Amer. J. Obst. Gyn.* **4**, 19.
Larson, Bergeim, Barber and Fisher (1929). *Endocrin.* **13**, 63.
Lewis and Miller (1913). *Arch. Intern. Med. (Chicago)*, **12**, 137.
Loeb and Bassett (1929). *Proc. Soc. Exp. Biol. Med.* **26**, 860.
Marinus (1919). *Amer. J. Physiol.* **49**, 238.
Maxwell (1916). *Pub. Univ. California in Physiol.* **5**, 5.
Narbut (1903). Inaug. Dissert. (St Petersburg).
Pearl (1916). *J. Biol. Chem.* **24**, 123.
Putnam, Teel and Benedict (1928). *Amer. J. Physiol.* **84**, 157.
Reichert (1928). *Endocrin.* **12**, 451.
Robertson (1916, 1). *J. Biol. Chem.* **24**, 385, 397, 409.
—— (1916, 2). *J. Biol. Chem.* **25**, 647.
—— (1916, 3). *J. Exp. Med.* **23**, 409.
—— (1917). *Endocrin.* **1**, 24.
—— (1919). *J. Biol. Chem.* **37**, 377.
—— and Ray (1919). *J. Biol. Chem.* **37**, 443.
—— —— (1925). *Austral. J. Exp. Biol. Med. Sci.* **2**, 175.
Sandri (1907). *Riv. Patol. Nerv. Ment.* **12**. Quoted by Schaefer (1909), p. 458.
—— (1909). *Arch. Ital. Biol.* **51**, 337.
Schaefer (1909). *Proc. Roy. Soc. Lond.* **81**, 442.
—— (1926). The endocrine organs (London).
Smith (1926, 1). *Proc. Soc. Exp. Biol. Med.* **24**, 131.
—— (1926, 2). *Anat. Rec.* **32**, 221.
—— (1927, 1). *Amer. J. Physiol.* **80**, 114.
—— (1927, 2). *Amer. J. Physiol.* **81**, 20.
—— (1927, 3). *J. Amer. Med. Assoc.* **88**, 158.
—— (1927, 4). *Proc. Soc. Exp. Biol. Med.* **24**, 337.
—— and Graeser (1924). *Anat. Rec.* **27**, 219.
—— Walker and Graeser (1923). *Proc. Soc. Exp. Biol. Med.* **21**, 204.
Thompson and Johnston (1905). *J. Physiol.* **33**, 189.
Van Wagenen (1927). *Anat. Rec.* **35**, 51.
—— (1928). *Amer. J. Physiol.* **84**, 468.
Wolf and Sachs (1910). *Proc. Soc. Exp. Biol. Med.* **8**, 36.
Wulzen (1914). *Amer. J. Physiol.* **34**, 127.
Zondek and Aschheim (1927, 1). *Klin. Woch.* **6**, 1321, 1322.
—— —— (1927, 2). *Arch. Gyn.* **130**, 1.
—— —— (1927, 3). *Arch. Gyn.* **132**, 176, 179.
—— —— (1928). *Klin. Woch.* **7**, 831, 1404, 1453.