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Aside from its value as a test for pregnancy, the discovery of a gonad-stimulating hormone in the urine of pregnant women by Aschheim and Zondek has acquainted us with a new fact of the deepest interest to those concerned with the physiology of reproduction. The source of this hormone is still unknown to us, though it has been generally assumed that it comes from the hypophysis. Its effect on the ovary of an infantile animal resembles strikingly the effect produced by "implants" of anterior hypophyseal tissue and of no other tissue known to us save the placenta. It was hence natural to assume that this urinary constituent originates in the hypophysis.

As this laboratory has been concerned for some years with hypophyseal hormones, we early instituted comparisons between the gonad-stimulating hormone from the urine and that which can be extracted from the hypophysis.[2] There are some striking differences between these substances. When prolan is administered in increasing doses, the response of the ovary corresponds to the dosage only up to a certain limit. When doses are given exceeding this limit, no matter what the dose or how often it is administered, the effect on the ovaries is practically the same. If, on the other hand, one gives increasing dosage either in the form of implants of hypophysis, or of extracts of hypophysis, the weights of the ovaries produced increase with the dosage. As an example of the effect of increase in implant dosage we cite an experiment from a previous paper.[3]

A single implantation of half of a rat's anterior lobe caused fifty per cent of the animals to mature and the average ovary weight was 21 mgm. With all higher im-

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[2] For convenience in this paper we will use Zondek's term "prolan" to designate the hormone from urine. By "gonad-stimulating" hormone we do not necessarily imply a single substance. We do not wish to discuss the problem of multiplicity of gonad-stimulating hormones in the hypophysis at this time.

[3] Evans, H. M. and M. E. Simpson. This Journal, 1929, lxxxix, 381.

plant "dosage" practically all animals matured in 100 hours and the ovary weights varied with the dose level as follows: a dose of one anterior lobe gave ovaries weighing 68 mgm.; 4 lobes gave ovaries weighing 105 mgm.

In the following chart (fig. 1) the effect of increasing the dosage of extracts from the anterior hypophysis is contrasted with the effect of increasing the dosage of prolan. The response of the ovary of the immature test animal to different dose levels of these substances was measured by the weight of ovaries produced within 100 hours. It can be seen that

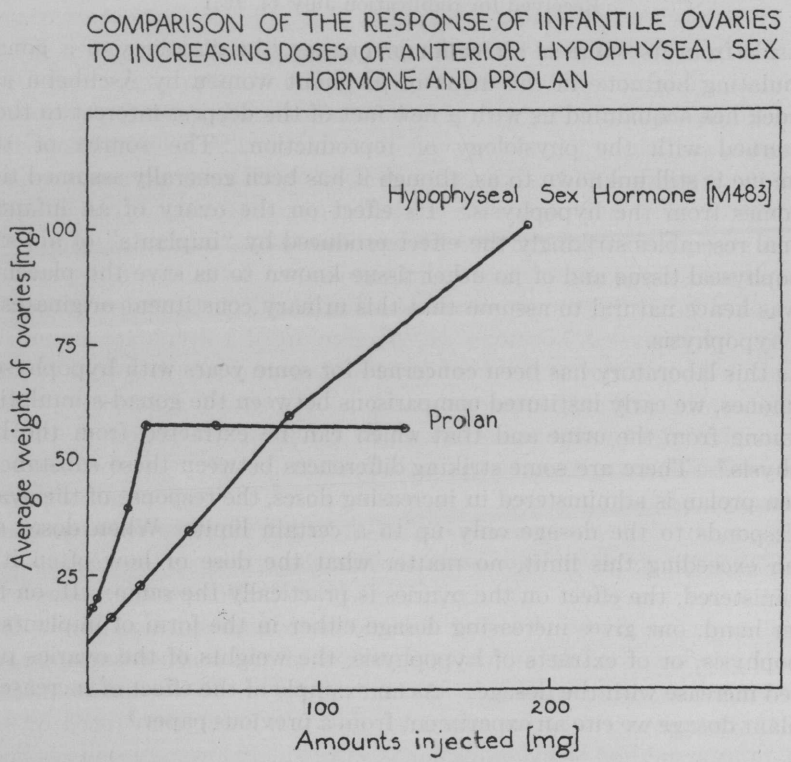

Fig. 1

in the case of prolan administration, the curve in which ovary weight is plotted against dose level quickly reaches a plateau and that a further fivefold or even tenfold increase in dose does not cause a further increase in ovary weight. In the case of administration of the anterior hypophyseal gonad-stimulating hormone, the curve expressing the relation between dose and ovary weight continues a steep ascent as higher dose levels are reached. It has been possible by injection of hypophyseal sex hormone to provoke ovaries weighing up to 190 mgm. within 100 hours. On the other

hand, it is seldom possible even with 200 or more units[4] of prolan to stimulate the production of ovaries weighing over 70 mgm. in this time interval. In cases of administration of other preparations of the hypophyseal sex hormone we have found the ascent of the curve to be even steeper than in the case recorded here. All of our extracts of the gonad-stimulating hormone from the hypophysis itself whether crude or purified were alike in showing this type of curve.

Perhaps the most important reason for assuming that prolan and the hypophyseal gonad-stimulating hormone are different is the relative inefficacy of prolan in hypophysectomized animals as reported in the preceding paper of this series. In contrast to these findings it has been shown by Smith, Reichert and others that the infantile reproductive systems of such hypophysectomized animals can be stimulated to complete development and function by the administration of anterior hypophyseal material.[5]

Briefly then, though prolan and anterior hypophyseal hormone appear to have the same qualitative effect on the ovary of the normal infantile animal, they are dissimilar in that prolan is embarassed in its action in the hypophysectomized animal and is limited in the effects it can provoke in the normal infantile animal. On the other hand the gonad-stimulating anterior hypophyseal hormone is effective in the hypophysectomized animal, and does not suffer the curious limitation of its effect on the ovaries of the normal immature animal. We must assume, it would appear, that the hypophysis itself plays a rôle in the action of prolan. Since prolan appears to have the same qualitative effect on the ovary as has the hypophyseal hormone, it would seem that we must think of prolan either as *provoking the production of the gonad-stimulating hormone of the hypophysis, or as converting some inactive component in the hypophysis into an active substance.* The limited effect of prolan on the normal infantile ovary would be clear for it could be considered as a direct consequence of low content or low production of the mother substance in the hypophysis ofthe immature animal. Even in the sexually mature animal the amount of active substance that can be demonstrated by the implantation method or by extraction is surprisingly low, especially in beef.

Since we can extract directly from the hypophysis the gonad-stimulating hormone, we must assume, if the previously expressed theory is correct, either that this amount of hormone had been previously activated within

[4] The unit of prolan to which reference is made is the minimum dose which administered in three daily subcutaneous doses to immature rats produces in 96 hours the development of ovaries containing a corpus or corpora. Only one of the three test animals needs respond.

[5] Smith, P. E. The disabilities caused by hypophysectomy and their repair. Journ. Amer. Med. Assoc., 1927, lxxxviii, 158. Reichert, F. L., The results of replacement therapy in an hypophysectomized puppy: four months of treatment with daily pituitary heterotransplants. Endocrinol., 1928, xii, 451.

the hypophysis by a prolan-like substance; or that it had been activated by a substance freed during preparation, or finally, that the inert substance or prohormone had been activated at the site of injection. The assumption that an activator is present in the hypophysis itself seems more probable to us.

A direct proof of this theory of the activation of an inert substance in the hypophysis would be furnished if by adding the activator to a given preparation from the hypophysis we could produce effects not secured from the hypophyseal preparation alone. The experimental confirmation of the theory was surprisingly satisfactory. The combination of prolan and hypophyseal extract gave a far greater effect than could be obtained from the administration of either component alone or than could be expected on the basis of an additive effect.

EXPERIMENTAL. The procedure was as follows: groups of three animals 24–26 days of age were injected daily, subcutaneously, for three successive days (Monday, Tuesday, Wednesday) with 1 cc. doses of the different aqueous preparations. The animals were observed twice daily after the 50th hour (Wednesday) for rupture of the vaginal membrane. After 96 hours, i.e., on the fourth day (Friday), the animals were sacrificed and the genital tract was examined under a binocular microscope. The ovaries were dissected free of bursa and oviduct and weighed on an analytical balance to an accuracy of 1 mgm. The size and vascularity of the genital systems and in particular the size and number of follicles and corpora and the presence of blood points were noted. The ovaries were sectioned when occasion demanded.

The methods of preparation of extracts from hypophysis and urine will be reported in detail in another paper. We are concerned in this paper with more purely biological investigations. The source of the hormone from the urine of pregnant women used by us was a crude prolan kindly furnished to us by the I. G. (Elberfeld)[6]. The crude prolan powder was extracted with acidulated water and poured into alcohol. The precipitate was washed with alcohol and ether and quickly dried in vacuo. The minimal potent dose of this powder when redissolved in water was 1.5 mgm. (0.5 mgm. daily for 3 days). Beef hypophyses were the source of the anterior lobes used by us in the preparation of the hypophyseal extracts reported here. Stable dry powders were prepared by acetone precipitation and were used in most of the experiments reported in this paper. Unless otherwise stated, they contained both the gonad-stimulating and the growth hormone. Other more purified preparations in which growth and sex hormones had been separated were also used and will be indicated in the appropriate place in the text and tables. All preparations were brought to neutrality (pH 7.4) before injection.

[6] In particular we wish to thank Dr. Werner Schulemann and Dr. Fritz Lacquer.

TABLE 1

Effects on the ovary of the immature rat of prolan and hypophyseal hormones given separately and in combination

	DESIGNATION OF EXPERIMENT																
	486	502	509	307	332	308	328	329	456	502	459	429	431	430	419	426	421
Dose* prolan, mgm.	54.2	0	54.2 (#486)	136.3	0	136.3 (#307)	54.5	54.5 (#328)	54.5	0	54.5 (#456)	136.3	0	136.3 (#429)	13.6	0	13.6 (#419)
Dose A. H. H., mgm.	0	65.0	65.0 (#502)	0	65.0	65.0 (#332)	0	65.0 (#332)	0	65.0	65.0 (#502)	0	65.0	65.0 (#431)	0	65.0	65.0 (#426)
Average weight of ovaries, mgm.	62.0	21.3	156.3	59.7	22.7	131.3	59.3	136.3	60.0	21.3	117.0	56.7	21.3	117.7	37.3	26.7	74.7
Activation			151%			120%		121%			95%			107%			100%

* By "dose" we mean the weight in mgm. of dried material dissolved in 3 cc. of water and administered to each animal in 1 cc. doses for 3 days.

Examples of activation of hypophyseal preparations by prolan. In table 1 we present examples of the effect on the immature ovary of combining prolan with hypophyseal preparations. Given amounts of prolan and hypophyseal preparations were administered, separately and after combination. The combination was made in vitro. The total volume was equal to that of the individual constituents when injected alone. The dose levels of hypophyseal preparations were chosen carefully so that they were barely able to provoke precocious maturity (with corpora formation) or fell just below the minimum dose level. The prolan preparations were usually administered at a dose level at which they already provoked the maximum effect possible with prolan. They stimulated numerous corpora lutea, large follicles and follicular cysts. It can be seen in the table that the injection of the combination of these constituents, administered at these dose levels, stimulated the development of ovaries weighing far more than the ovaries provoked by the individual constituents alone—also larger ovaries than could be expected from any additive effect resulting from the combination of the two constituents. The ovaries stimulated were usually characterized by great numbers of corpora though large follicles and follicular cysts were often present.

The percentage activation was calculated as the relation between ovary weight stimulated by prolan and the increased weight due to the combination. The ovary weight of the hypophysis-injected rats was neglected in our calculation of activation because the weight of these ovaries was seldom more than the weight of infantile ovaries.

Specificity of the activation. We next wish to give the evidence for considering that the activation of hypophyseal preparations is a specific reaction. We tested the specificity of this reaction in three ways: first, we combined prolan with active and inactivated hypophyseal hormone; secondly, we combined prolan with extracts from other organs prepared in a manner identical with that used in preparing hypophyseal extracts; thirdly, we substituted for prolan extracts from tissues prepared in the same way prolan is prepared.

The hypophyseal hormone was inactivated by being heated for ten minutes in vigorously boiling water, or for 30 minutes at 70–80°C. The hypophyseal hormone heated for 30 minutes at 70–80°C., when administered in combination with prolan, no longer produced large ovaries but only those of the weight provoked by prolan alone.[7] The shorter treatment at 100°C. apparently did not completely inactivate the hypophyseal component so that the result of combination of the heated product with prolan while less than that in cases where active hormone was used (see

[7] Since the visible physical properties of the hypophyseal hormone preparation when heated to 70–80°C. were unchanged, the probability that the activation phenomenon is due to some physical and hence non-specific effect is excluded.

table 2 A) was still greater than the effect of administration of prolan alone.

The second method of testing the specificity of the activation was as follows: calf and pig liver were extracted and treated in a manner similar

TABLE 2A

Specificity of the activation effect obtained by combining prolan and hypophyseal hormones; (A, Effect of the destruction of the specific substances by heat)

	DESIGNATION OF EXPERIMENT								
	328	332	329	334	330	307	308	310	309
Dose prolan, mgm.	54.5 (*307)	0	54.5 (*307)	0	54.5 (*307)	136.3	136.3 (*307)	0	136.3 (*307)
Dose A. H. H., mgm.	0	65.0 (Active solution)	65.0 (Active solution *332)	65.0 (Inactivated 34′ at 70–80° C.*332)	65.0 (Inactivated 34′ at 70–80° C.*332)	0	65.0 (Active solution *332)	65.0 (Inactivated 10′ at 100° C. *332)	65.0 (Inactivated 10′ at 100° C. *332)
Average weight of ovaries, mgm.	59.3	22.7	136.3	13.7	62.3	59.7	131.3	16.7	79.0
Activation			121%		None		120%		Inconsiderable

TABLE 2B

Specificity of the activation effect obtained by combining prolan and hypophyseal hormones; (B, Substitution of a non-specific substance, liver, for the hypophyseal component)

	DESIGNATION OF EXPERIMENT						
	486	505	503	509 (control)	546	549	556
3 days prolan dosage, mgm.	54.5	0	54.5 (*486)	54.5 (*486)	54.5	0	54.5 (*546)
3 days liver dosage, mgm.	0	54.5	54.5 (*505)	65.0 (A. H. H. *502)	0	65.0	65.0 (*549)
Average weight of ovaries, mgm.	62.3	17.3	55.3	156.3	53.7	17.0	47.5
Activation			None	150%			None

to that used in making hypophyseal preparations. This product was administered separately and in combination with prolan as in the usual procedure. In table 2 B it can be seen that the potency of the prolan was not increased by combination with the liver preparations. But the

potency of the same prolan was increased when combined with a hypophyseal preparation. This is shown in the table for contrast.

It would seem from the above data that the hypophyseal component of our combination is certainly a specific one. We have endeavored to examine similarly the specificity of prolan. Since the activation we describe resembles a ferment reaction, we tried to substitute certain ferments in place of prolan. We chose liver as the source of the ferment partly because the liver is well known to be rich in these substances, but chiefly because we suspected that the reaction described in this paper might

TABLE 2C

Specificity of the activation effect obtained by combining prolan and hypophyseal hormones; (C, Substitution of a non-specific substance for the prolan)

	DESIGNATION OF EXPERIMENT								
	505	502	504	506	549	555	548	550	551
3 days dose liver, mgm.....	54.5 (Pig)	0	54.5 (*505)	27.2 (*505)	54.5 (Calf)	0	54.5 (*549)	54.5 (*549)	54.5 (*549)
3 days dose A. H. H., mgm.....	0	65.0	65.0 (*502)	65.0 (*502)	0	65.0	65.0 (*555)	65.0 (*555) 1 hr. at 37°C.	65.0 (*555) 1 hr. at 37°C.
Average weight of ovaries, mgm.....	17.3	21.3	35.7	28.3	17.0	30.7	37.7	42.7	34.7
Activation..			69%	33%			Inconsiderable	39%	Inconsiderable

bear a relation to the arginine content of the hypophysis. In a later paper we will elaborate on the reasons which led to these deductions. In table 2 C we give some examples showing the effect of administering a liver powder in combination with hypophyseal extracts. Although there was a slight difference in the effects of administering the hypophyseal preparation alone and in combination with liver, the difference may not be significant. To establish such a nonspecific activation would naturally require much further biological and chemical investigation.

Quantitative relations. We have considered two possible mechanisms

by which the activation process may take place. Either a stoichiometric combination occurs between the two components or the conversion of a mother substance, a prohormone, is facilitated by a catalyst. In the first case we should expect the reaction to involve definite proportions of the reacting constituents. If the second supposition were true, we should expect the reaction to be more nearly independent of one of the constituents, namely, the catalyst. We tried to decide between these possibilities by experiments in which we varied the amounts of each constituent, hypophysis and prolan. When we administered constant amounts of prolan

TABLE 3

Effect on ovaries of immature rats of combining different amounts of hypophyseal preparations with a constant prolan

	DESIGNATION OF EXPERIMENT								
	375	220	446	450	328	332	329	333	331
3 days prolan dosage, mgm..	27.2	0	27.2 (*375)	27.2 (*375)	54.5	0	54.5 (*328)	0	54.5 (*328)
3 days A. H. H. dosage, mgm.....	0	112.5 (sex free growth hormone)	45.0 (*220)	22.5 (*220)	0	65.0	65.0 (*332)	6.5 (*332)	6.5 (*332)
Average weight of ovaries, mgm.....	32.7	21.0	85.1	53.0	59.3	22.7	136.3	17.7	60.3
Activation..			160%	62%			121%		None

with decreasing amounts of hypophyseal hormone the ovarian response diminished rapidly (table 3). When on the other hand the hypophyseal component was kept constant and the amounts of prolan were varied the activation values were quite constant after a certain concentration of prolan was reached. The results speak rather for the catalytic theory. When smaller amounts of prolan are used in the reaction there is a considerable decrease in the percentage activation as well as lower absolute values for the ovary weights (table 4).

Constancy of the activation reaction. In tables 5 and 6 we have given the results of several dozen experiments made in an effort to see whether

Effect on the ovary of he immature rat of the combination o

	307	332	308	328	329	429	431	430	369	374
3 days dose prolan, mgm.....	136.3	0	136.3 (*307)	54.5 (*307)	54.5 (*307)	136.3	0	136.3 (*429)	54.5 (*429)	54.5 (*429)
3 days dose A. H. H., mgm.....	0	65.0	65.0 (*332)	0	65.0 (*332)	0	65.0	65.0 (*431)	0	65.0 (*431)
Average weight of ovaries, mgm.....	59.7	22.7	131.3	59.3	136.3	56.7	21.3	117.7	52.0	96.0
Activation.....			120%		121%			107%		85%

the activation reaction was one which could be duplicated quantitatively. This did not prove to be the case. In table 5 are summarized all results from experiments in which samples from the same prolan preparation were combined with a variety of hypophyseal preparations. In table 6 experiments are cited in which samples from a single acetone powder are combined with different prolan preparations. As can be seen from average

TABLE 5

Effect on the ovary of the immature rat of combinations of different hypophyseal preparations with the same prolan

	DESIGNATION OF EXPERIMENT							
	509	459	374	547	581	376	382	384
Dose prolan *108, mgm...	54.5	54.5	54.5	54.5	54.5	27.2	27.2	27.2
Dose A. H. H., mgm.....	65.0 (*120)	65.0 (*120)	65.0 (*107)	65.0 (*16)	65.0 (*120)	65.0 (*107)	65.0 (*97)	65.0 (*93)
Average weight of ovaries, mgm.....	156.3	117.0	96.0	88.5	110.0	63.7	63.0	48.3
Activation.....	150%	95%	85%	65%	77%	95%	84%	48%

ovary weights and from percentages of activation, the results in both sets of experiments varied very considerably. We are aware of the fact that much of this variability can be traced to the small number (three) of animals in our experimental groups.

Component in the hypophysis activated. We have been able to resolve the crude acetone powders of hypophyseal substance employed in these

TABLE 4
Different amounts of prolan with a constant hypophyseal preparation

DESIGNATION OF EXPERIMENT														
376	377	378	379	380	427	428	418	420	419	421	422	424	423	425
27.2 (*429)	13.6 (*429)	13.6 (*429)	2.7 (*429)	2.7 (*429)	136.3	136.3 (*427)	27.2 (*427)	27.2 (*427)	13.1 (*427)	27.2	27.2	27.2 (*422)	13.1 (*422)	13.1 (*422)
65.0 (*431)	0	65.0 (*431)	0	65.0 (*431)	0	65.0 (*431)	0	65.0 (*431)	0	65.0 (*431)	0	65.0 (*431)	0	65.0 (*431)
63.7	32.3	49.3	19.0	27.0	64.0	114.0	44.3	78.7	37.3	74.7	39.0	72.3	34.7	65.0
95%		53%		38%		78%		79%		100%		85%		91%

studies into chemically and physiologically different substances, into the growth hormone and the gonad-stimulating hormone. We have prepared from the acetone powder, growth hormone free of the gonad-stimulating hormone, also the gonad-stimulating hormone free of the growth promoting factor.[8] We were naturally interested to know whether one or both of these two components of the acetone powder was responsible for the activation. Combinations of prolan with the gonad-stimulating hormone of the hypophysis gave no activation whatever (table 7). On the other hand a marked activation resulted in most cases in which prolan was combined with purified growth fractions (table 8). We have proven that our growth hormone preparations are free of gonad-stimulating hormone but we can not naturally be equally certain that other physiologically active substances are absent. Therefore we can not be sure at present whether some unknown substance is concerned. Nevertheless good grounds exist for the belief that the activated substance is the growth hormone. In the first place our various growth preparations were prepared in radically different ways. Secondly, although not crystalline, they are quite pure as judged by the fact that we have found them to be almost free from serum proteins.[9] Further reason for considering that it is indeed the growth hormone itself which is the activated substance is furnished by the following facts. Slight changes in methods of preparation can be made, which though not radical ones, are sufficient to cause destruction of the growth hormone. These slight changes in procedure are also followed by failure of the activation reaction on combination with prolan.

DISCUSSION. It is necessary to bear in mind the following facts which have been ascertained: Prolan does not easily affect the ovary of the

[8] The purification was tested in each case by the largest quantities that could be administered—the limiting factors being solubility and the amount the animal could tolerate.

[9] Mr. W. R. Lyons has performed precipitin tests on these preparations and has shown that no more than 2 per cent of the total protein is beef serum protein.

TABLE 6

Effect on the ovary of the immature rat of combination of different prolan preparations with the same hypophyseal preparation

	DESIGNATION OF EXPERIMENT												
	509	459	581	582	438	428	430	420	424	378	421	425	378
Dose prolan, mgm.....	54.5 (※108)	54.5 (※108)	54.5 (※108)	54.5 (※23)	54.5 (※1)	136.3 (※1)	136.3 (※108)	27.5 (※1)	27.5 (※11)	27.5 (※108)	13.6 (※1)	13.6 (※11)	13.6 (※108)
Dose A. H. H., mgm.....	65.0 (※120)	65.0 (※120)	65.0 (※120	65.0 (※120)	65.0 (※120)	65.0 (※107)	65.0 (※107)	65.0 (※107)	65.0 (※107)	65.0 (※107)	65.0 (※107)	65.0 (※107)	65.0 (※107)
Average weight of ovaries, mgm.....	156.3	117.0	110.0	88.0	107.3	114.0	117.7	78.7	72.3	63.7	74.7	65.7	49.3
Activation	150%	95%	77%	29%	53%	78%	107%	78%	85%	95%	100%	91%	53%

hypophysectomized animal. Prolan has but a limited effect on the ovaries of the normal infantile rat. Hypophyseal hormone stimulates the infan-

TABLE 7

Effect on the immature rat ovary of prolan combined with hypophyseal preparations containing the gonad stimulating hormone but free of growth hormone

	DESIGNATION OF EXPERIMENT							
	377	373	372	375	389	388	369	399
Dose prolan, mgm...	13.6	0	13.6 (*337)	27.2	0	27.2 (*375)	54.5	54.5 (*369)
Dose A. H. H., mgm.	0	20.2	20.2 (*373)	0	65.0	65.0 (*389)	0	65.0 (*389)
Average weight of ovaries, mgm.....	32.3	25.7	32.7	32.7	13.7	43.3	52.0	43.7
Activation.....			None			Inconsiderable		None

TABLE 8

Effect on the ovary of the immature rat of combinations of prolan with hypophyseal preparations containing growth but free from the gonad stimulating hormone

	DESIGNATION OF EXPERIMENT								
	375	220	446	450	486	485	499	272	501
Dose prolan, mgm.	27.2	0	27.2 (*375)	27.2 (*375)	54.5	0	54.5 (*486)	0	54.5 (*486)
Dose A. H. H., mgm.....	0	112.5	45.0 (*220)	22.5 (*220)	0	24.0	24.0 (*485)	41.3	41.3 (*272)
Average weight of ovaries, mgm....	32.7	21.0	85.1	53.0	62.3	20.3	92.3	16.5	106*
Activation.....			160%	62%			48%		70%

* These ovaries were composed of 0–3 corpora and 7–20 large cysts. In this experiment the activation can not be explained on the basis of the additive effect of so-called "follicle stimulating" and "luteinizing" hormones.

tile genital system of the hypophysectomized dog and rat to full development. Hypophyseal hormone is not limited in its action on the infantile ovary as is prolan. Prolan can be made to have a maximal effect on the

ovary by combining it with an hypophyseal extract, which by itself is not potent in accelerating sexual maturity.

We offer the following explanation of these facts. The gonad-stimulating hormone is present in the hypophysis in an inactive state. The active substance is formed from this "prohormone" by an activator. This activator is found in the most concentrated form as prolan in the urine of pregnant women. It may also be present in the hypophysis itself, even in the infantile hypophysis. But if the activator is present in the hypophysis it is normally separated from the prohormone somewhat as glycogen is separated from glycogenase in the liver. In the normal infantile animal its effect is limited by the small amount of prohormone present. If we add more prohormone to the infantile animal's own store, we can stimulate by prolan the development of ovaries that exceed normal physiological limits. Our purest growth hormone preparations are activated in this way. Hence the prohormone may be the growth hormone; we have at any rate not been able to separate it from the growth hormone. On the other hand growth-free gonad-stimulating hormone from the hypophysis can not be further activated by prolan, doubtless because it is already completely activated or maximally potent.

These explanations seem to us to best fit the experimental findings though we realize that there are other possible interpretations than those offered. The simplest explanation would seem merely that there is an additive effect when prolan is combined with hypophyseal hormone. We believe that our experimental work has excluded this possibility. The ovary weights stimulated by the combination are larger than what would be expected on the basis of an additive effect. Further, prolan can be combined with potent sex hormone preparation from the hypophysis without showing the activation effect. Most crucial of all, prolan activates hypophyseal growth extracts which by themselves show no gonad-stimulating effect at any concentration.[10]

We believe we have excluded also a simple physical explanation of the activation phenomenon (tables 2 A, B, C). The absorption, excretion, or possible destruction of prolan might be delayed by the combination, and therefore the actual amount of hormone available to the affected organ might be greater. If the hypophyseal component is replaced by a non-specific substance of similar physical make-up—e.g., liver—the activation reaction does not occur. Similar activation fails if the hypophyseal component is destroyed by chemical reaction or mild heat which does not sensibly change its physical character.

Furthermore we do not feel that we have grounds for assuming that our findings can be explained on the basis of different proportions of so-

[10] Even at five times the concentration used in the activation experiment.

called "follicle-stimulating" and "luteinizing" hormones in the two components used in the activation reaction.[11] For example it might be assumed that prolan contains more follicle-stimulating hormone and that the hypophyseal extracts furnish an excess of the luteinizing principle and that on combination of the two products a summation of these effects occurs. This assumes that the luteinizing hormone cannot act on an ovary unless it is first stimulated by the follicle-stimulating hormone. Although the large ovaries in the activation reaction are usually composed chiefly of corpora, this is not always true. We have had cases of activation in which the heaviest ovaries produced in a given experiment were composed almost entirely of large follicles and follicular cysts.

We believe that a recent observation of Hill and Parkes on ovulation in decerebrate rabbits can be explained on the same basis as our experiments. Hill and Parkes found that the injection of the urine of pregnant women (prolan) was able only in slight measure to replace the action of the animal's own hypophysis in provoking ovulation in animals decrebrated immediately after copulation. It was effective in only a small per cent of cases "4 inferior results" resulting from 19 trials. The ovulation was delayed longer than usual and less ova were freed. Even this minimal effect was not possible when placental extracts were injected into these decerebrate rabbits. On the other hand the placenta of pregnant rabbits, which had been injected previously with the urine of pregnant women, provoked ovulation in the decerebrate animals. We assume that the development of the gonads of the immature rat is a comparable phenomenon to ovulation in rabbits, and that hypophyseal hormone was necessary for ovulation in the rabbits used in the Hill and Parkes experiments; in other words, that prolan was unable to function except in combination with hypophyseal hormone and that the few positive cases of ovulation reported by them were due to minimal amounts of hypophyseal hormone still circulating in the blood of the decerebrate animals, which, combined with the prolan injected or stored in the placenta of the urine-injected animal, was adequate to provoke ovulation.

CONCLUSIONS

1. Prolan does not easily repair the gonadal deficiencies of hypophysectomized animals (dog, rat).

2. There is always a definite limit to the weight of the ovary which can

[11] After the present communication was written, the paper by H. L. Fevold, F. L. Hisaw and S. L. Leonard on the gonad-stimulating and the luteinizing hormones of the hypophysis (This Journal, 1931, xcvii, 291) appeared, but too late for discussion here. In it activation effects are described as the result of combining two hypophyseal preparations which gave respectively the follicle-stimulating and luteinizing effects. We intend to discuss this matter fully in a later paper.

be stimulated by prolan in the immature rat within a definite time interval (100 hr.).

3. Hypophyseal hormones completely repair the gonadal deficiencies of hypophysectomized animals (dog, rat).

4. The hypophyseal gonad-stimulating hormone does not show the limited effect on the ovary of the immature rat found to be characteristic of prolan. The development of the ovary provoked by the hypophyseal hormone corresponds to the dose level.

5. Only very small amounts of prolan, measured in terms of dry weight, are required to give the minimal effect on the ovary. The amount of hypophyseal hormone needed to give a minimal effect is always much greater. This is in contrast to the fact that higher doses of hypophyseal hormone provoke much greater ovary weights than can be obtained with any amount of prolan.

6. In our earlier experiments the effect of prolan was increased to the maximal effect obtainable by injecting hypophyseal sex hormone simply by combining prolan with small amounts of crude hypophyseal preparations, containing both gonad-stimulating and growth hormones. The combination of prolan was made with doses of hypophyseal preparations which, given alone, were minimal or just subminimal in gonad-stimulating effect.

Later we added prolan to hypophyseal extracts (growth hormone) which, when administered alone, were devoid of any effect on the ovaries of immature animals and in this way also secured maximal effects.

7. This activation effect is a specific reaction. If the hypophyseal extract is destroyed by heating, the combination of heated hypophyseal hormone with prolan is no longer any more effective than prolan alone.

8. Low concentrations of pure hypophyseal sex hormone combined with prolan do not result in activation effects. On the other hand, sex-free growth hormone is typically activated by prolan.