THE RELATION OF THE HYPOPHYSIS TO ANTIBODY PRODUCTION.

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The important part that many of the endocrine organs play in development and function suggests the possibility that they may also enter into the mechanism of the body which develops resistance to infection. Up to the present time there is little positive evidence that such is the case. It was felt, however, that certain of the more inaccessible glands of internal secretion, whose function in relation to immunity had not been studied, might yield valuable information, and this study of the pituitary gland was made as part of a general investigation into this field (6).

The experimental work so far has been chiefly of a physiological nature concerning the part the pituitary plays in growth, metamorphosis, sugar tolerance, and sexual activity, and its relation to acromegaly, dyspituitarism, and diabetes mellitus. The functions of the two parts of the gland have been elaborately defined (13, 16). More recently the interrelationship of this gland to the other ductless glands has been much discussed. That total hypophysectomy is incompatible with life[1] would seem to be proved by the work of Cushing and his collaborators (3, 4) and Paulesco (17). The striking physical changes in growth, development, nutrition (3), and general metabolism (2) with partial hypophysectomy (1) and by feeding pituitary gland extract (9, 10, 20) are now accepted. We have not been able to find, however, any direct, quantitative, experimental studies of the relation of the hypophysis to infectious diseases. Aschner (1) thought his partially hypophysectomized dogs succumbed more easily to disease than normal dogs, and certainly many contracted pneumonia. Cushing's experiments (3) seem to bear this out. There are, however, no clinical observations that would seem to indicate a lessened or increased resistance to infectious disease

[1] The work of Gemelli (8), Aschner (1), and Handelsmann and Horsley (10) disputes this, but their extirpation methods were so injurious and uncontrolled as to appear unreliable. Our own studies on the guinea pig agree with those of Paulesco and Cushing.

with pituitary disorders.[2] Gay and Rusk (7) in a study of the locus of antibody production draw no definite conclusion, but the evidence would seem to indicate that the spleen and the lymphatic system play the most important part. The work of Murphy and his associates (15), Hektoen (11), Hektoen and Curtis (12), and Morris and Bullock (14) appears to corroborate this view. The experiments of Topley (19) appear to disprove the contention that antibodies are formed at the point of inoculation.

Problem and Methods.

Guinea pigs were selected as the experimental animal because of their already standardized reactions in immunological studies. An operative technique was elaborated for partial hypophysectomy. In such animals the serum reactions of typhoid agglutination, hemagglutination, and hemolysis were studied. These studies were completed in the following series.

Series 1. The Production of Antibodies after Hypophysectomy.—Partial hypophysectomized, control operation animals and normal controls were immunized to *Bacillus typhosus*. Subsequent titers indicated the degree of immunity produced.

Series 2. The Effect of Hypophysectomy on Antibody Production.—This was measured by the change in height of the antibody curve. Guinea pigs were immunized to (1) *Bacillus typhosus* and (2) hen red blood corpuscles, and then submitted to partial hypophysectomy. Normal and control operation animals were used. Titration of agglutinins and hemolysins was carried out at intervals before and after operation.

Series 3. The Effect of Feeding and Injecting Pituitary Extract on the Production of Antibodies.—Animals were immunized, then fed continuously pituitary extract or submitted to repeated injection of the extract, and their subsequent titers determined.

Operative Technique.

Adult guinea pigs weighing from 300 to 400 gm. were used. In such animals the pituitary is relatively large, weighing about 0.025 gm. (Vanderburgh (21)). It lies in a shallow dural pocket and has the characteristic histologic appearance of other mammalian hypophyses. After some difficulties the following operative attack was chosen as giving an approach through which a definite portion of the

[2] Personal communication from Dr. Harvey Cushing.

gland could be removed and in which success was obtained in about 75 per cent of the animals used. The animal was placed on an electrically heated pad, kept at a fixed temperature, and fastened down to a small raised table. Anesthesia was induced by means of a fine rubber tube passed into the posterior pharynx through which a small pump forced ether vapor (5). Morphia 0.002 gm. and atropine 0.004 gm. were given 15 to 30 minutes before anesthesia. After shaving the head and cleaning the scalp with 1:1,000 bichloride a wet gauze covering was placed over the head and a sterile towel over the body of the animal. The gauze over the vertex of the skull was cut and an incision in the skin was made from just behind the eyes to the occipital protuberance and carried through to the bone. The tissue flaps on both sides were then dissected up and held away by the weight of a single hemostat. The parietal bone was perforated and all the bone over both hemispheres was removed as far anteriorly as the fronto-parietal synostoses and posteriorly to the occipital bone. On the right side the decompression was carried to the temporoparietal juncture and on the left well into the temporal bone. The dura was left intact and the longitudinal sinus which lies in it, free from the bone, remained uninjured. The dura was then incised low down in the left temporal region, a small spatula introduced beneath the temporal lobe, and the brain elevated and slightly dislocated towards the right side. (The removal of bone on the right side was done to permit this.) A view was thus obtained of the pituitary fossa, the small pinkish body lying surrounded on both sides and posteriorly by large venous sinuses. The stalk enters at the anterior end of the gland, running slantwise to the posterior region. The dura over the middle of the hypophysis was then nicked with a small hook knife and with a small double spoon forceps fragments of the gland were removed. Unless the sinus was injured, which was fatal, there was little hemorrhage. Warm normal saline solution on cotton pledgets was used for sponging and hemostasis. After removal of the gland fragment, the brain was allowed to return to its position and the skin carefully sutured with two layers of fine interrupted silk sutures. This was covered by a cocoon dressing. The operation consumed between 30 and 45 minutes.[3] Control animals in which the same operation, without removal of the pituitary, was performed, were prepared. The above procedure was carried out through the step in which the dura over the sella was cut and then closure completed, thus subjecting the control animals to approximately the same amount of trauma and anesthesia.

Total hypophysectomy proved fatal, the animals almost at once entering into a lethargic, partly comatose state, refusing food and water and dying in 24 to 48 hours. All fragments removed were

[3] The chief complication in recovered animals was an ocular palsy, the result apparently of injuring the third cranial nerve which lies practically on the hypophysis but which we attempted to push to one side before the final step of enucleation.

studied histologically, and no animals are reported in which there was not this proof that at least one-half of the gland, which always consisted of fragments of both pars anterior and posterior, was removed. Autopsy controls were used to establish further the efficacy of this method of attack. The surviving animals within 24 hours made excellent recoveries, but lost 50 to 100 gm. in weight in the first few days. After this they regained weight and appeared, except for a rare ocular palsy, as active and vigorous as the normal controls. In many cases the gain in weight was rapid and even above that of the normal animals.

Technique of the Serum Reactions.

Typhoid Agglutinins.—The Sen strain of *B. typhosus* was used.[4] It had been grown on artificial media for years but still combined a ready agglutinability with a high toxicity for guinea pigs. It was carried along throughout these experiments by repeated daily transplants on agar and bouillon with occasional plating to determine its purity. A vaccine was prepared by suspending the 24 hour growth in six Blake bottles in salt solution, killing the organisms by adding chloroform, drying them in a vacuum, and then grinding up the bacteria in a sterile mortar. Weighed quantities were then suspended in normal salt solution and tricresol was added for maintaining sterility. Experimentation with vaccine and living organisms determined that the greatest reaction was produced by the following inoculations given at 3 and 4 day intervals: first injection, 0.002 gm. of vaccine subcutaneously; second injection, 0.002 gm. intraperitoneally; third injection, $\frac{1}{10}$ of a 20 hour growth of living organisms on an agar slant intraperitoneally; and fourth injection, $\frac{1}{10}$ of a 20 hour growth of living organisms on an agar slant intraperitoneally. By the 10th day after the last injection agglutinin titers ranged from 1:1,000 to 1:6,000. In Text-figs. 1 and 2 the normal control curve represents the average of over twenty-five animals. In performing agglutination tests 1 drop of a salt suspension of a 24 hour growth of the bacteria on agar was added to 1 cc. of successive dilutions of serum. Serum was obtained by cardiac puncture, 4 cc. of blood being withdrawn, and the serum inactivated. Titration was recorded after 2 hours at 37°C., after 2 hours at room temperature, and after the tubes had been in the ice box over night. 4 cc. of blood proved sufficient for both agglutination and complement fixation tests. The latter tests, however, although completed with almost all animals used, are not reported since the concentration of complement fixation bodies was not sufficient to ensure reliable comparative results.

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[4] This strain was obtained from Dr. Carroll G. Bull.

Hemagglutinins and Hemolysins.—Another series of guinea pigs with normal controls, partially hypophysectomized controls, and operation controls was immunized to hen red blood corpuscles for the study of hemagglutinins and hemolysins. At 4 day intervals all animals were given intraperitoneally 1 cc. of washed hen corpuscles made up to the original blood volume. At the end of 1 week serum was obtained and the quantity of reaction determined. In testing for hemagglutinins 1 drop of a 10 per cent suspension of washed hen corpuscles was added to 1 cc. volumes of successively diluted, inactivated serum. Readings were made as with the typhoid studies. For hemolysins the usual technique of the Wassermann reaction was followed with 0.25 cc. volumes of inactivated serum, fresh guinea pig complement 1:10, a 5 per cent suspension of hen corpuscles, and 0.5 cc. of saline solution. The tubes were then incubated for 1½ hours, being shaken after 30 minutes and 1 hour. Readings were made after 1½ hours and after standing in the ice box over night. The usual controls were performed with each test.

The several series were performed with the above methods and complement deviation studies were also made. The latter, however, are not reported because of the low and variable nature of the titers resulting; it appears probable that for this test insufficient antibodies are manufactured. Operative difficulties now and then caused the loss of valuable animals but a sufficient number was always used in each experimental unit to ensure a satisfactory number of complete studies. As a rule, the experimental unit consisted of two normal animals, two partially hypophysectomized animals, and two operative control animals for Series 1. Series 2 experiments, however, were usually started with eight guinea pigs, since the operation was completed only when an immunity had been established and titers determined, and unless sufficient animals were provided the loss of a guinea pig at operation would vitiate that particular experimental unit. Before commencing with typical experimental units, the operative technique was perfected and a supply of hypophysectomized and control operation animals provided. The agglutinin, hemolysin, and hemagglutinin reactions were studied in normal guinea pigs and the quantitative reaction was well standardized. The immunity production in partially hypophysectomized and operative control animals showed no appreciable difference in these preliminary studies. In the actual experimental units guinea pigs as near of a size and kind as possible were chosen. Because of the reported interrelation of the

pituitary body with the sex glands (9) and the reported increased reaction in menstruating women to typhoid vaccine, preliminary studies were made which ruled out any appreciable influence that such sexual periods might have on antibody production. Neither menstruation[5] nor the pregnant state appeared to have any influence on the antibody curve.

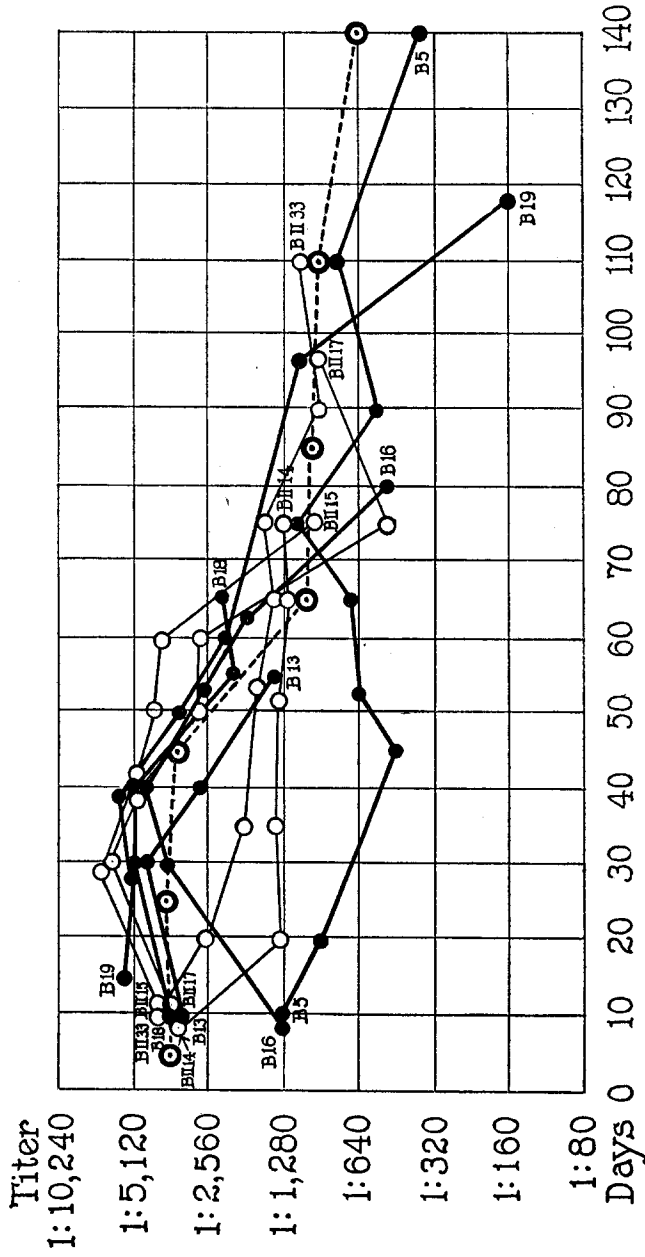
EXPERIMENTAL.

Series 1. The Production of Agglutinins after Hypophysectomy.—Guinea pigs, in which partial hypophysectomy[6] and a control operation[6] had been performed were immunized to *Bacillus typhosus* by the above method. Their agglutinin titers were recorded from the 7th to the 10th day after the last injection and followed every week or 2 weeks. Unoperated control animals were carried along in each experimental unit, but their course ran such a comparatively parallel curve to the large number of normal animals studied previously that an average line has been used for Text-figs. 1 and 2 in order to avoid confusion. Text-fig. 1 represents a group of hypophysectomized animals with operated controls compared to the normal average agglutinin curve. The general curve in the hypophysectomized and control operation animals shows a parallelism to the normal controls and needs no comment. The repetition of such experiments brought the same results. Partial hypophysectomy apparently has no influence on the subsequent production of typhoid agglutinins in guinea pigs.

Series 2. The Effect of Hypophysectomy on Antibody Production.—Three sets of experiments were performed. In Class 1 the agglutinin titer in animals immunized to *Bacillus typhosus* was studied before and after hypophysectomy, in Class 2 the hemagglutinin titer, and in Class 3 the hemolysin production. Normal and operated controls were used. For each experimental unit eight animals were used. All were immunized, two were saved for normal controls and three each submitted to partial hypophysectomy or the control operation.

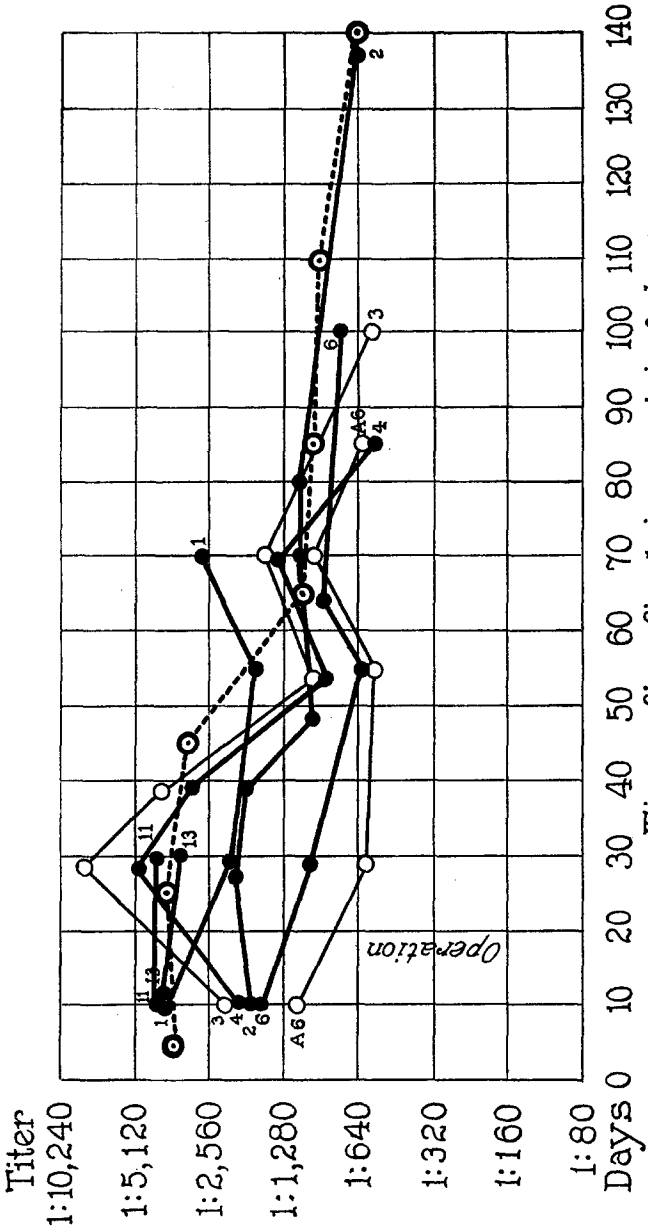
[5] The guinea pig has a definite menstrual cycle (18).

[6] At least 8 days after the operation, when the animals were entirely recovered.



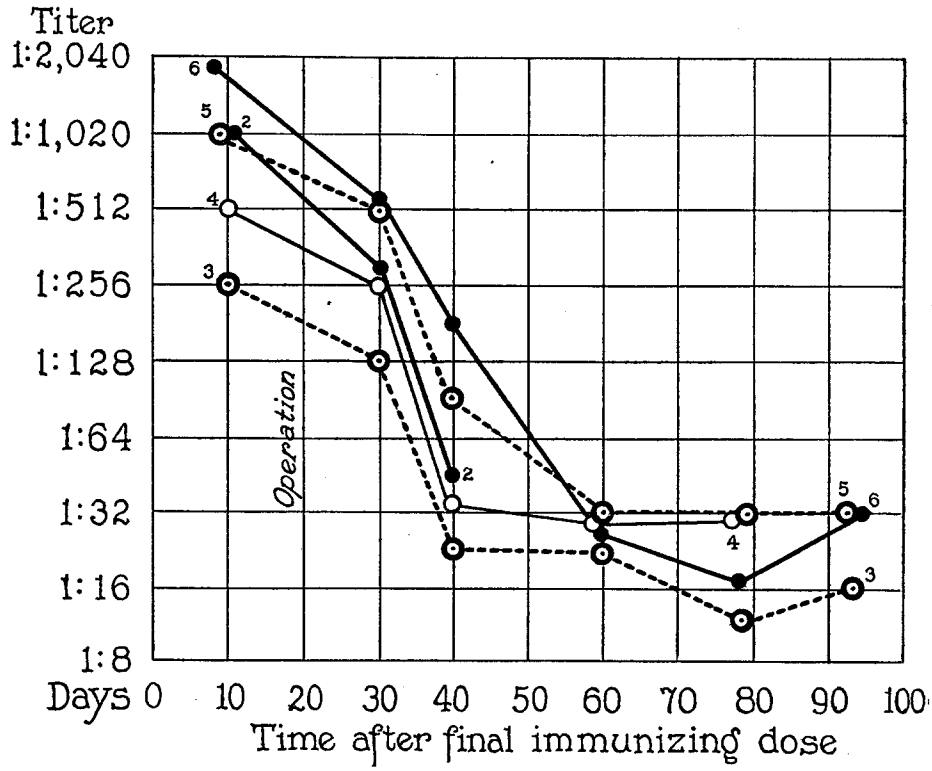
In Text-figs. 1 to 4 the following curves are used. ——— Partially hypophysectomized animals.
——— Control operation animals. - - - - - - - - Normal control animals.

TEXT-FIG. 1. Series 1. Typhoid agglutinin titers of animals immunized after hypophysectomy.



TEXT-FIG. 2. Series 2. Typhoid agglutinin titers before and after hypophysectomy.

Text-fig. 2 represents the study of such an experiment in Class 1, in which the animals were immunized to *Bacillus typhosus*. No special comment is necessary since all titrations fall practically within normal limits.



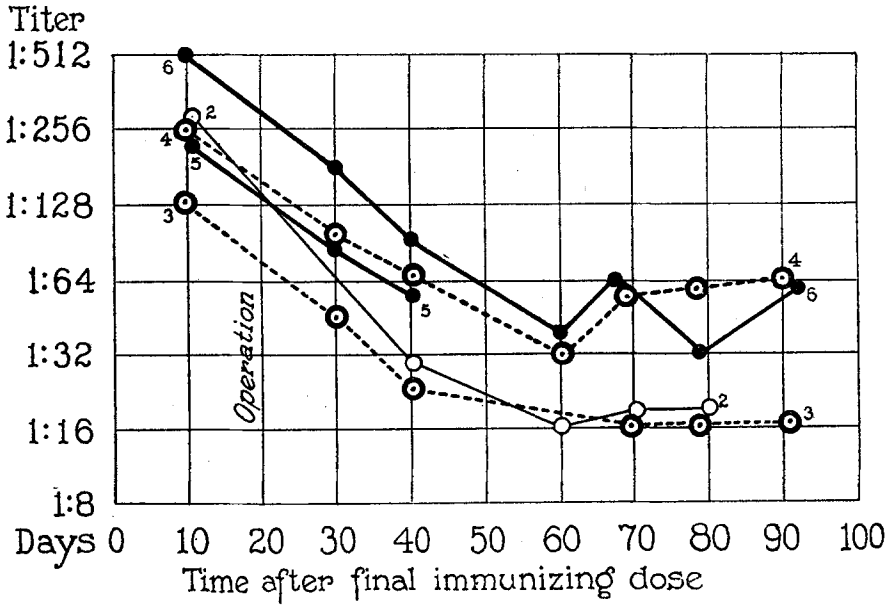
TEXT-FIG. 3. Series 2. Hemagglutinin titers before and after hypophysectomy.

Text-figs. 3 and 4 represent the studies in Classes 2 and 3. Here, too, the parallel of operated animals to normal animals is striking. Repeated experimental units showed no appreciable variation.

These experiments would seem to indicate that partial hypophysectomy has no influence on the maintenance of specific agglutinins for *Bacillus typhosus*, hemagglutinins, or hemolysins in the guinea pig.

Series 3. The Effect of Feeding and Injecting Pituitary Extract on Antibody Production.—In the feeding experiments six animals were

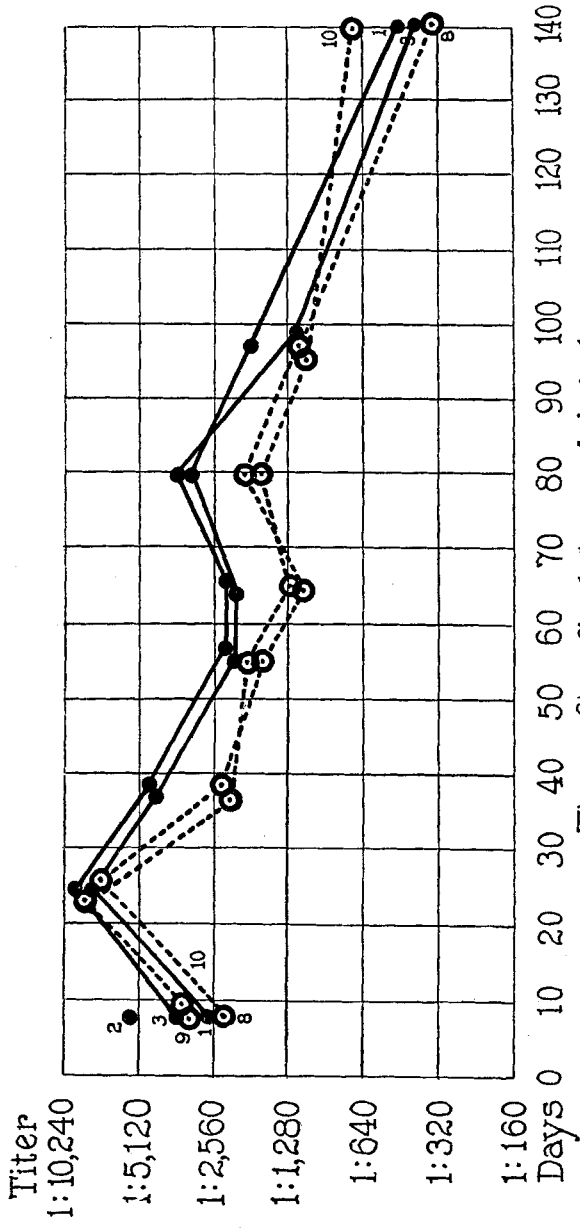
selected and immunization was carried out as described above. Beginning on the day of the first inoculation all animals were fed two medicine droppers full of milk. To the milk of three animals 0.13 gm. of the Burroughs Wellcome whole pituitary gland extract was added. The feeding of milk and of pituitary extract in the same amount of milk was carried out daily until the experiment was finished. This was the method of feeding gland extract adopted by Goetsch (9)



TEXT-FIG. 4. Series 2. Hemolysin titers before and after hypophysectomy.

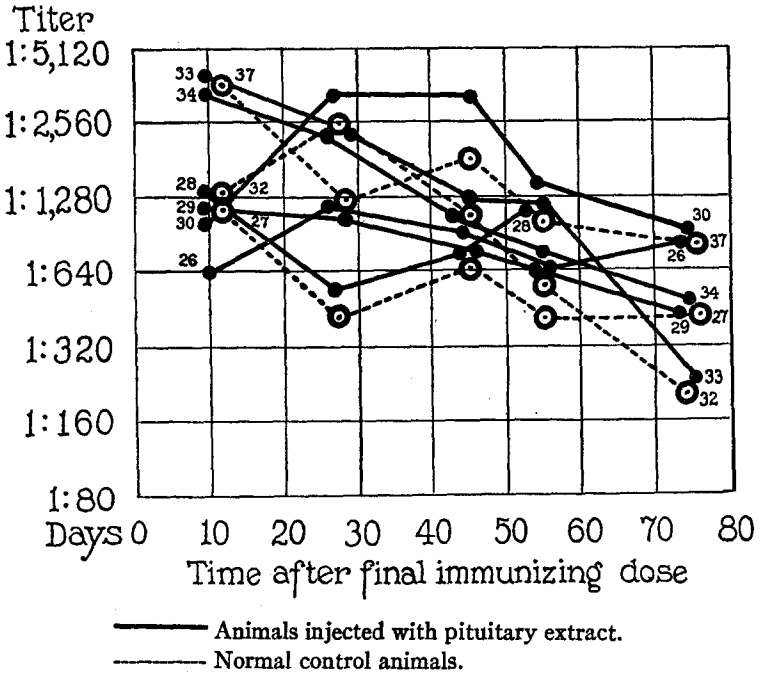
and proved very satisfactory, the animals taking their ration with evident relish. The control animals were fed milk to obviate any criticism of extra nourishment vitiating or influencing the experiments. Beginning 9 days after the last inoculation the agglutinin titer was studied, such studies being then repeated at weekly or biweekly intervals. Text-fig. 5 represents an experimental unit of the above description. The parallelism of normal and pituitary-fed animals is striking.

In another group of animals the gland was given by intraperitoneal injection. 1 gr. of Burroughs Wellcome whole gland extract



TEXT-FIG 5. Series 3. The effect of feeding pituitary extract on the typhoid agglutinin titer. In two animals, only one determination was made because they died from cardiac puncture the day of the first titration.

tablets was used dissolved in 1 cc. of normal saline solution. 1 cc. of normal saline solution was injected at corresponding intervals into the controls. Nine animals were used in these tests, three for controls and six for injection. The animals were first immunized to *Bacillus typhosus*. On the 10th day following the last inoculation their agglutinin titer was determined. This was repeated in 18 days. 10 days later the injection of pituitary extract and salt solution was



TEXT-FIG. 6. Series 3. The effect of repeated intraperitoneal injections of pituitary extract on the typhoid agglutinin titer.

begun and repeated every other day for 3 doses, following which the agglutinin titer was again done. Later the pituitary extract injections were repeated and again subsequent agglutinin titrations carried out. Such an experiment is reported in Text-fig. 6. Further studies varying the amount of extract injected and the intervals between injections were carried out, but no appreciable changes were noticed. As seen in Text-fig. 6 the animals injected with pituitary ran a titer approximate to that of the controls.

SUMMARY.

An operative technique was evolved permitting successful partial hypophysectomy in guinea pigs.

Such animals, when immunized to *Bacillus typhosus*, produced specific agglutinins in the same quantity and at the same rate as unoperated and operation controls immunized at the same time and by the same method.

In guinea pigs previously immunized to *Bacillus typhosus* and hen red blood corpuscles partial hypophysectomy had no effect on the continued production and persistence of typhoid agglutinins, hemagglutinins, and hemolysins.

In guinea pigs immunized to *Bacillus typhosus* both the continued ingestion and the intraperitoneal injection of the whole pituitary gland extract (Burroughs Wellcome) had no effect on the subsepuent agglutinin titers as compared to that of normal animals.

The experiments would appear to show either that the hypophysis does not play an important direct or indirect part in the production of and persistence in the blood of typhoid agglutinins, hemagglutinins, and hemolysins, or that the amount of hypophysis left behind in the operation in order to maintain life is adequate also to exercise the degree of functional influence on these processes which the entire hypophysis conceivably exercises.

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