were injected directly into the cornea or inoculated by combined scarification and instillation of egg albumen once a week. It has been possible to reproduce the corneal changes described above. The rabbits receiving intracorneal injections begin to show corneal changes within 3-4 injections, and eventually develop a very severe reactivity to egg albumen. In the rabbits receiving egg albumen by instillation and scarification, the reaction develops more slowly and never so severely. Rabbits receiving intracorneal injections of sterile physiological salt solution on the other hand show no increasing reactivity to successive injections.

While it is possible, therefore, to stimulate pannus formation in the cornea of rabbits under various conditions, it is clearly shown that vascularization of the cornea may accompany direct sensitization to live bacteria, or native proteins such as egg albumen.

6460

Effect of Acid Extract of Anterior Pituitary on Heart Rate, and Nervous Irritability of Guinea Pigs.*

PAUL O. HAGEMAN AND HOWARD ANDERSON MC CORDOCK.

From the Department of Pathology, Washington University School of Medicine, St. Louis.

It has been observed that the injection of acid extract of cattle anterior pituitary produces in guinea pigs a condition comparable to that seen in Graves' disease in human beings. Loeb[1] has found a marked hypertrophy of the thyroid gland with diminution in the amount of colloid and a marked increase in the number of mitoses in the epithelial cells. Under the action of this substance the animals lose weight, and Siebert[2] has shown that there is an increase in their metabolic rate. Other effects corresponding to conditions found in Graves' disease have been established in subsequent investigations carried out in this laboratory. Inasmuch as tachycardia and nervous irritability are very prominent symptoms of Graves' disease in man it was of interest to study the effect of this extract of anterior pituitary on the heart rate and the reflex irritability of guinea pigs.

* These investigations were in part carried out with the aid of a grant for research in science made to Washington University by the Rockefeller Foundation.

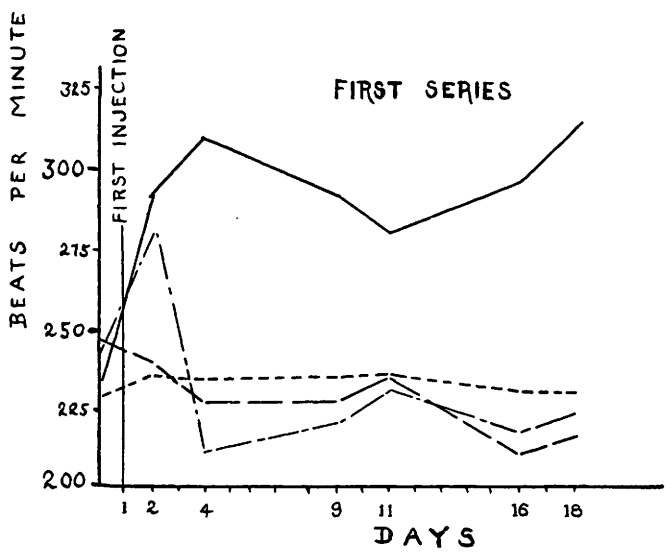
1 Loeb, Leo, and Bassett, R. B., PROC. SOC. EXP. BIOL. AND MED., 1929, **26**, 860.

2 Siebert, W. J., and Smith, R., *Am. J. Physiol.*, 1930, **95**, 396.

Cursory observations of Loeb seemed to indicate that such an effect might possibly exist.

Twelve young male guinea pigs, approximately 200 gm. each, were chosen and normal records were obtained under a standard procedure using the electrocardiograph (lead one) to record the cardiac pulsations. Daily injections of 1 cc. of the acid extract were given to each of 6 guinea pigs, 3 of which had been thyroidectomized. The remaining 6 guinea pigs, 3 of which had been thyroidectomized, were used as controls, and were not injected. Records of the heart rate of all the animals were obtained on the 2nd, 4th, 9th, 11th, 16th, and 18th days. The heart rate of every animal in the group of injected non-thyroidectomized animals was faster on each occasion, after the second day of the injections, than that of any of the animals in the other groups. The rate of the thyroidectomized non-injected (control) animals dropped considerably below normal. In the case of the injected thyroidectomized animals the rate also went below normal, but not as low as that of the thyroidectomized control group, except at the time of the last observation after the death of several animals had rendered the averages used in plotting the graph unreliable. (First series in Fig. 1.) Since some of these animals died and because the number originally used was small, the experiment was repeated to check the accuracy of the results.

In the second series, 17 animals were used and 3 determinations of their heart rates were made before the injections of acid extract



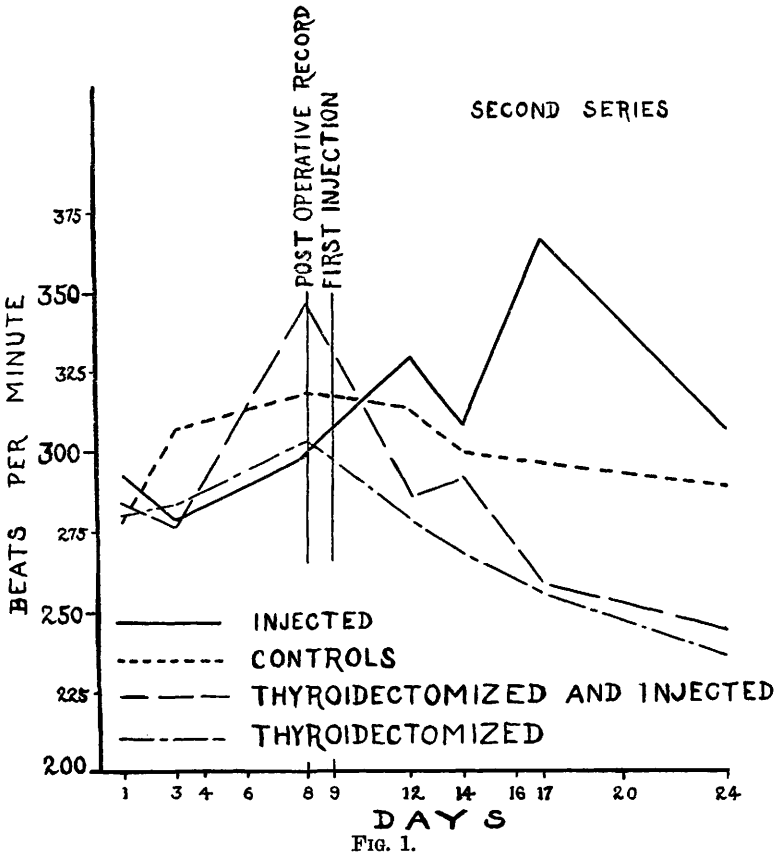


FIG. 1.

Graphic representation of the heart rates of the 4 groups of animals during the course of the experiments. The curves in each case are plotted from the average number of beats per minute of all the animals in a particular group.

were started. The animals were then divided into 4 groups and determinations and observations were made on the 3rd, 5th, 8th, and 15th days after the injections were begun. The results were similar to those obtained in the first series. The thyroidectomized animals, whether injected or not, showed heart rates slower than those of the normal animals, and in each instance the rate was below that observed in the same animal before the experimental procedures were instituted. An increase of 70 or more beats per minute was observed in the case of the normal animals injected with acid extract. The maximum increase in beats per minute in the individual animals was 71, 86, 96 and 105.

In the first series, the rate rose until the fourth day after the injections were started, it then dropped until the eleventh day, after

which it rose steadily. In the second series the initial rise was not as rapid, and the maximum rate was observed on the eighth day, after which it declined. We may conclude that acid extract of the anterior pituitary of cattle is effective in increasing the heart rate of the guinea pig when the thyroid gland is intact. This substance increases the heart rate, in the main, indirectly by acting through the thyroid gland. A slight increase in the heart rate was observed in the injected thyroidectomized animals which may have been due to small remnants of thyroid tissue. However, this increase was, on the whole, much less marked than in the non-thyroidectomized injected animals.

In our first series, the respirations were counted throughout the experiment and the results seemed to indicate that the rate of respiration varies directly as the heart rate. Our observations on the second series were less complete and less convincing. The respirations were counted for a period of one minute by 2 different observers, several counts being made of each animal, but the counts varied so much, even in the case of the same animal, perhaps due to the necessity for handling them, that we regard the results on respiration merely as suggestive.

An attempt was made to determine whether any difference in excitability or in degree of response to a given stimulus was noticeable between the animals of the several groups. The response of an injected and a non-injected animal to the same stimulus was always compared, and in many instances, when graded stimuli were used, the injected animal reacted to a smaller stimulus than the normal animal. We made use of an ear reflex that one of us had studied in connection with an infectious otitis media and interna in the rat.[3] When a sudden noise occurs near a rat or guinea pig the animal moves its ears backward and then forward. This motion usually occurs once, but an unusually loud noise, or one repeated several times in rapid succession, may elicit several such movements. A dull noise was produced by striking the table upon which the 2 animals to be tested were resting. Graded stimuli could be produced by gradually increasing the force with which the table was struck. In comparing the reactions of an injected and a normal animal a stimulus could be found to which the injected animal responded by moving the ears, but which was without effect upon the normal animal. In many cases by the time the stimulus was increased to the point of eliciting only the ear motion in the normal

[3] McCordock, H. A., and Congdon, C. C., PROC. SOC. EXP. BIOL. AND MED., 1924, **22**, 150.

animal, it caused the injected animal to jump and shudder several times before quieting down.

Since many of the animals, even the controls, showed individual variations, an attempt was made in the second series to pair the animals as equally as possible. We selected as a pair 2 animals that showed nearly equal heart rates and as nearly as possible the same degree of response to noise stimuli. One of these was used as a control animal and its partner as an experimental subject. The response of these 2 animals to an identical stimulus was always compared at the same time.

With the elimination of the individual variations that this pairing accomplished, the increase in response to stimuli on the part of injected animals over the controls was more striking. In the first series one of the 3 injected animals always showed an increased response to stimuli regardless of the control animal with which it was compared. However, the increase in irritability of the other 2 injected animals was less marked, and the control guinea pigs showed such individual variations in response to stimuli, that either the control or the injected animal could be made to respond first to a given stimulus, by selecting a certain type of control. In the second series this variability in response was successfully eliminated by pairing the animals in the manner stated above. Observations were made every other day and after the fifth injection the difference in the strength of reactions of the control and injected animals became quite definite in each case. The injected animal of each pair always responded to a weaker stimulus than did its control partner. These results apply to animals with intact thyroid glands. On the other hand, if thyroidectomized control and injected guinea pig were compared no appreciable difference was observed in the reactions of these two kinds of animals.

Because of the individual variations in the nervous excitability of guinea pigs as manifested by their response to noise stimuli it is suggested that in experiments of this nature more uniform results can be obtained by pairing the animals on the basis of observations made before the experimental procedures are started.

The facts established add further data of interest concerning the similarity between the changes caused by the injection of acid extract of the anterior pituitary gland and those observed in Graves' disease in man.