

who have not had digitalis (Fig. 1). Ritchie⁶ believes that such electrocardiograms indicate a combination of auricular fibrillation and flutter. The same explanation has been more recently given by Schrumph.⁷ Lewis⁸ emphasizes the close relation between the mechanism of these two conditions and publishes a curve from a dog in which auricular fibrillation was changed to flutter by vagus stimulation. He, however, does not believe in the simultaneous presence of the two disorders. This conception does not exclude the possibility of one mechanism changing to the other even at short intervals. That would possibly furnish a plausible explanation for some of the clinical electrocardiograms.

It is a pleasure to acknowledge my indebtedness to Dr. James B. Herrick for his suggestions and for the privilege of studying some of the cases covered in this report.

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THE RELATION OF THE ENDOCRINE SYSTEM TO THE GLYCEMIC REACTION FOLLOWING THE INJECTION OF HOMOLOGOUS PROTEIN.

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WE have previously shown¹ that variations in blood-sugar concentration follow the injection of a relatively large number of substances, and that when proteins from different sources are

¹ Rohdenburg, Bernhard and Krehbiel: *Am. Jour. Men. Sc.*, 1920, *clix*, 577.

injected into rabbits,² the variations observed in blood-sugar concentration follow a definite course and occur concomitantly with the development of antibodies. Variations in the blood-sugar content of normal rats and mice injected with homologous and heterologous protein were also observed,³ and in a small series of spontaneous malignant tumor-bearing mice a large proportion of the animals showed, in contrast to normal animals, no variations in the blood-sugar concentration when injected with homologous protein.

With the intention of investigating the hypothesis that the occurrence of tumors is due to some endocrine disturbance, we have studied the effect of ablation of one or more of the glands of internal secretion upon the glycemic reaction following the injection of homologous protein. As has just been stated, tumor-bearing mice and rats differ from normal animals in their reaction to the injection of homologous protein as shown by the blood-sugar content. If the removal of one or more of the endocrine glands bring about a condition in which injections of homologous protein are not followed by a glycemic reaction, and if absence of a glycemic reaction under these conditions be considered as giving evidence of a predisposition to neoplasia, then the condition underlying predisposition to neoplasia might be subject to further experimental analysis. The term glycemic reaction in this paper has reference only to the variations in blood-sugar concentration which follow the injection of homologous protein.

Albino rats were employed, 0.05 c.c. of blood being obtained from the tail vein after the animal had fasted all night, and the blood-sugar concentration was determined by the method of Epstein.⁴ The animal was then injected subcutaneously with 0.05 c.c. of an extract of homologous protein. This protein was prepared by cutting the tissues of another animal of the same species into small fragments and extracting it in approximately three times its bulk of normal saline for a period of about ten minutes. Sterile precautions are not essential. The opalescent, bloody, supernatant fluid was used. One hour after the injection of the protein extract the blood-sugar value was again determined. In the experiments recorded in this paper, spleen was used, but other tissues are equally effective. While it is true that all tissues contain glucose or glycogen the amount of either of these substances in the fluid injected was so minute that it could not be chemically estimated nor qualitatively identified.

After an injection of homologous protein one of three things may happen to the blood-sugar: It may rise, fall or remain relatively or actually stationary. No explanation can be offered for

² Rohdenburg and Pohlman: *AM. JOUR. MED. SC.*, 1920, clix, 853.

³ Rohdenburg: *Jour. Cancer Research*, 1920.

⁴ *Jour. Am. Med. Assn.*, 1914, lxiii, 1667.

the fact that in some instances the injection of homologous protein is followed by an increase in blood sugar while in other instances it is followed by a decrease. It is possible that the injection is always followed by an increase, but that this increase occurs with different degrees of rapidity in different animals; it may be that the time interval is so chosen that from some animals blood is drawn at a phase when the blood sugar, after having increased, falls below the normal before again returning to its preinjection level.

Thus in Fig. 1, *A* represents the preinjection level, *B* the maximum rise, *C* the fall below the normal and *D* the return to the normal. If this curve be either stretched out or compressed, as in Fig. 2, where the time intervals are shown by the upright lines, it is at once evident that in those animals in which the entire

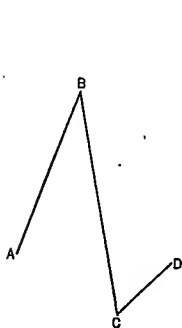


FIG. 1

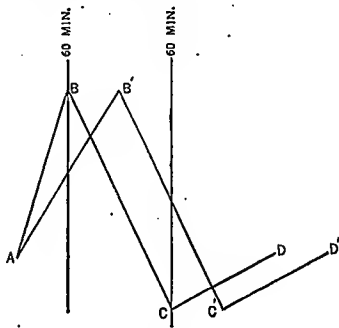


FIG. 2

reaction is over quickly we might easily get a lower value one hour after injection than in those in which the reaction takes a longer period for completion.

A variation between the first and second estimations of blood sugar of 16 or more mgm. when the Epstein method is used, or of 10 or more mgm. when the Meyers-Bailey⁵ modification of the Lewis-Benedict⁶ method is employed, has arbitrarily been defined as a positive glycemic reaction. Reversely a negative reaction is one which shows less than these variations. It is immaterial to our present problem whether the values rise or fall.

In previous publications^{2,3} we have shown that the alterations in blood-sugar concentration following protein injection are not

⁵ Jour. Biol. Chem., 1916, xxiv, 147.

⁶ Ibid., 1915, xx, 61.

due to the excitability of the animal, to the operative procedure, to the influence of a few seconds of anesthesia or to the injection of 0.5 c.c. of normal saline solution.

It is impossible to obtain from a rat twice within an hour the amount of blood necessary for the Meyers-Bailey modification of the Lewis-Benedict blood-sugar method, and it was for this reason that the Epstein method was chosen. We have previously commented upon the limitations of the Epstein method for determining sugar values and have pointed out the possible sources of error. Whatever the error may be, however, it is common to every determination made by that method and in comparative estimations may be disregarded if the results seem well correlated.

When 0.05 c.c. of blood is used the Epstein method gives values which are extremely high and which do not represent the actual blood-sugar content of the rat, as shown by other procedures; the figures are therefore merely relative.

A check experiment will prove the truth of this contention. Five guinea-pigs were tested according to the method outlined in a previous paragraph, the blood being obtained twice within an hour from the heart. On the specimens thus obtained blood-sugar determinations were made according to the Lewis-Benedict and the Epstein methods, 0.05 c.c. of blood being used for the Epstein method. If the assumption made be correct, then an equal percentage of positive reactions should occur, irrespective of the method employed, the double standard of comparison being kept in mind. The blood-sugar values, however, would be much higher with the Epstein than with the Lewis-Benedict method. The observations, as is shown in Table I, are in accord with our premise.

TABLE I.

Epstein method, 60.			Lewis-Benedict method.		
Zero hour.	60-minute interval.	Variation.	Zero hour.	60-minute interval.	Variation.
No. 1 440	408	-32	No. 1 138	132	-6
No. 2 448	433	-15	No. 2 132	126	-6
No. 3 416	528	+112	No. 3 162	212	+50
No. 4 504	489	-15	No. 4 150	126	-24
No. 5 472	406	+24	No. 5 153	165	+12

Percentage giving positive reaction: Epstein method, 60; Lewis-Benedict method, 60.
Averaged variation in mgm.: Epstein method, 40; Lewis-Benedict method, 20.

All figures indicate mgm. per 100 a.c. of blood.

+ = increase in blood sugar.

- = decrease in blood sugar.

The glycemic reaction may be estimated in two ways: by the percentage of positive reactions in a given group, or by the averaged variation in sugar concentration. If we judge only by the per-

centage of positive reactions, then we have no means of estimating the degree of reaction, for with the standard as laid down a variation of 20 mgm., which is but 4 mgm. higher than what we designate a negative reaction, has the same significance as a variation of 64 mgm. It seems more accurate, therefore, to use the averaged variation as a standard of comparison.

In a series of seventy normal full-grown, healthy rats the blood-sugar values were determined before and after injection of homologous protein. As a result of these examinations it was found that 73 per cent. of the animals gave a positive reaction and that the averaged difference between the determinations at the zero hour and at the sixty-minute period was 29 mgm.; this figure has been adopted as the variation in normal animals.

In our first experiments the reaction was studied in animals from which one internal secretory gland had been removed. The glands removed individually were the spleen, thymus, both testes, both ovaries, both adrenals, all entire, approximately 90 per cent. of the thyroid and embedded parathyroids and approximately 90 per cent. of the pancreas. There were six full-grown normal animals in each group; and, with the exception of the adrenal-free group, in which owing to the short life of the animals the examinations were carried out after twenty-four hours; from fourteen to one hundred and twelve days elapsed after removal of the gland before tests were made. In a second series of animals double ablations were performed in such rotation that all possible combinations were obtained. For example: testes-spleen, testes-thymus, testes-thyroid, testes-adrenal, testes-pancreas. In each of these groups there were six animals and the averaged time of testing after gland ablation was fourteen days, again excepting the adrenal-free group, which was tested twenty-four hours after operation. A total of 162 animals were used in the experiment.

The effect of the various gland ablations upon the glycemic reaction is presented in chart form. The solid color indicates the averaged variation in milligrams for each group of 6 animals, the double vertical line the adopted normal variation of 29 mgm.

It is interesting to note that ablation of the sex glands alone influences the glycemic reaction in a different manner in each sex; thus, removal of the testes slightly inhibits, while ablation of the ovaries slightly stimulates the reaction. The removal of the spleen with either set of gonads markedly inhibits the reaction, while excision of the thymus and testes or of the thymus and ovaries causes no change from the normal. Ablation of the testes and pancreas causes a marked stimulation of the reaction; but removal of the ovaries and pancreas brings about a directly opposite result, that is, inhibition. An analogous difference in reaction is noted when the gonads are removed with the thyroid, for with this combination in the male inhibition follows, while in the female stimu-

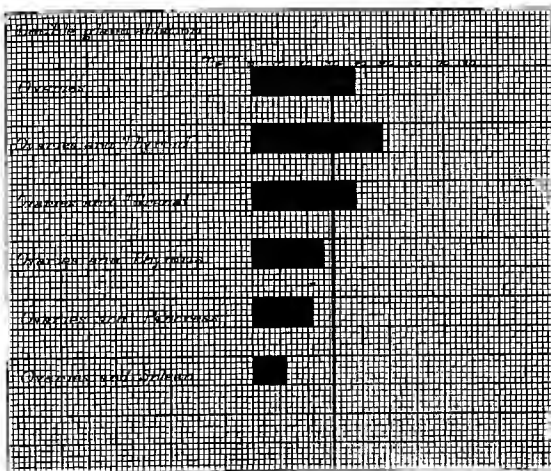


CHART I.

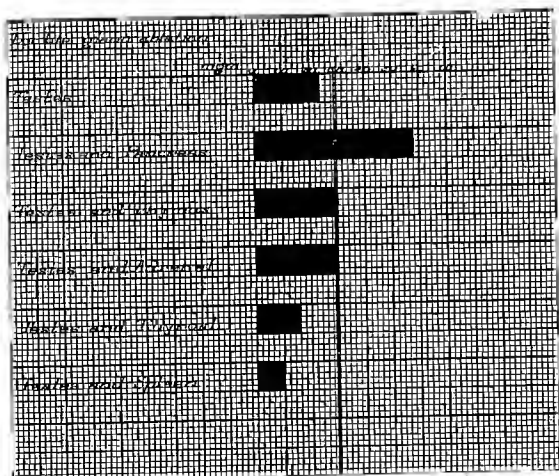


CHART II.

lation occurs. Extirpation of the adrenals in combination with the sex glands exerts little or no influence on the reaction (Charts I and II).

In rats almost complete removal of the pancreas has no effect upon the type of glycemic reaction under discussion (Chart III). However, when the thyroid, spleen or testes are removed in combination with the pancreas there is a very marked stimulation. In contrast to these results, removal of the ovaries or thymus and the pancreas shows a very slight inhibition of the reaction, while when the adrenals are ablated in combination with the pancreas there occurs the most marked inhibition encountered in the entire series of experiments.

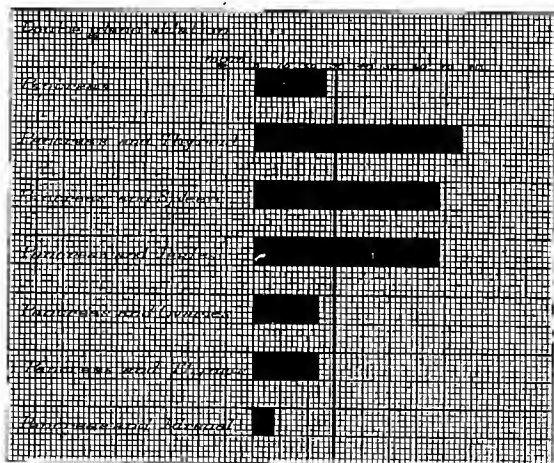


CHART III.

A very slight to moderate stimulation occurs when either the adrenals alone or a combination of adrenals with ovary, thyroid or spleen are removed. Ablation of either testes or thymus in combination with the adrenals shows practically no change from the normal, while, as previously stated, excision of adrenals and pancreas induces a most marked inhibition (Chart IV).

Excision of the thymus singly or in combination with either the testes or spleen exerts practically no influence upon the reaction. When the gland is ablated in combination with either the ovaries, adrenal, pancreas or thyroid there results a varying degree of inhibition, though it is not marked (Chart V).

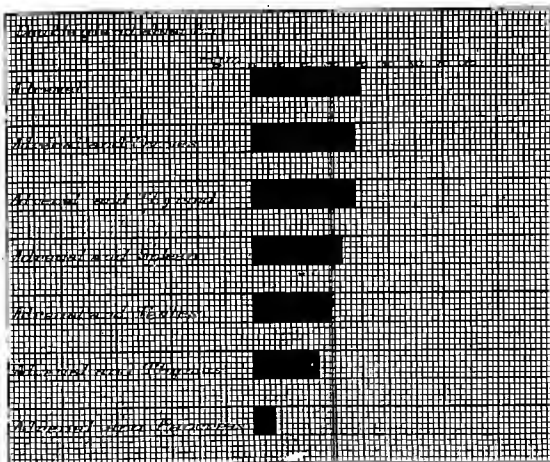


CHART IV.

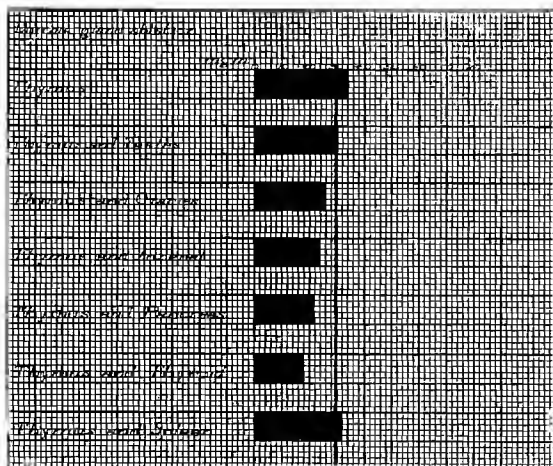


CHART V.

Removal of the spleen, alone or in combination with either gonad, induces a marked inhibition, while excision of the gland in combination with either thyroid or pancreas produces a marked stimulation. When either the adrenals or the thymus are removed in combination with the spleen there is no noteworthy variation from the normal (Chart VI).

Ablation of the thyroid, with such parathyroid tissue as may be embedded in it, results in a marked stimulation of the reaction and a similar condition prevails when the gland is removed in combination with the pancreas, spleen or ovary, and also, though to a

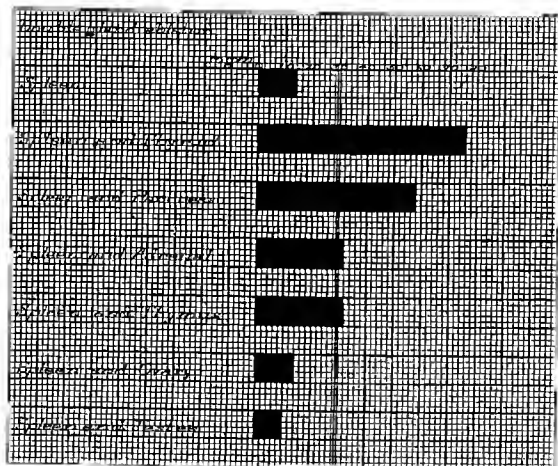


CHART VI.

lesser degree, when it is removed with the adrenals. The removal of the thymus or the testes with the thyroid gives rise to an inhibition (Chart VII).

The ablations, either of single glands or of combinations of glands which have thus far been described, result in one of three conditions: inhibition, stimulation or no change. Those which result in inhibition have, for easy reference, been collected in Chart VIII: the spleen with either set of gonads, and the pancreas and adrenals. Those which induce stimulation are shown in Chart IX: the spleen with either the pancreas or thyroid, the testes and pancreas, the thyroid and ovaries, and, finally, the pancreas and thyroid.

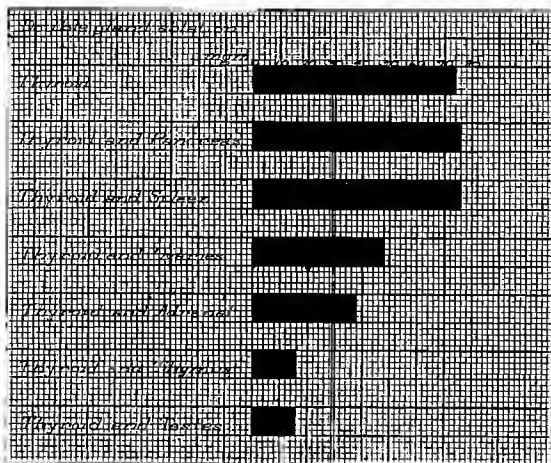


CHART VII.

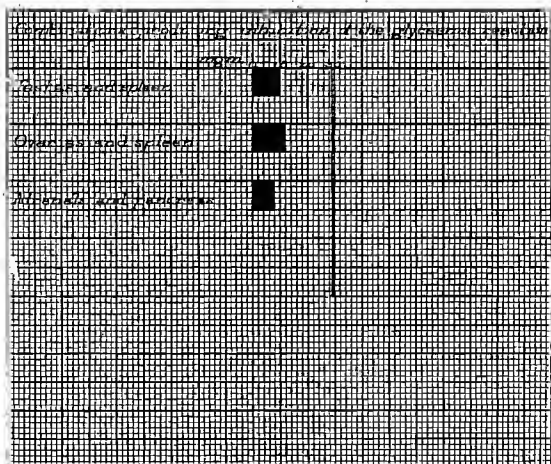


CHART VIII.

In order, if possible, to establish a correlation of glandular activity with respect to the glycemic reaction following the injection of homologous protein, further ablations were performed, three, four and five glands being removed from the same animal. Again, groups of six animals were used and the test was performed as previously described. From the results obtained it appears that the connection is as follows:

There are two combinations of endocrines which when ablated inhibit the reaction; these are (1) spleen and either gonad set, (2) adrenal and pancreas. If one gland from either system be ablated and with it one gland from the other system, there results

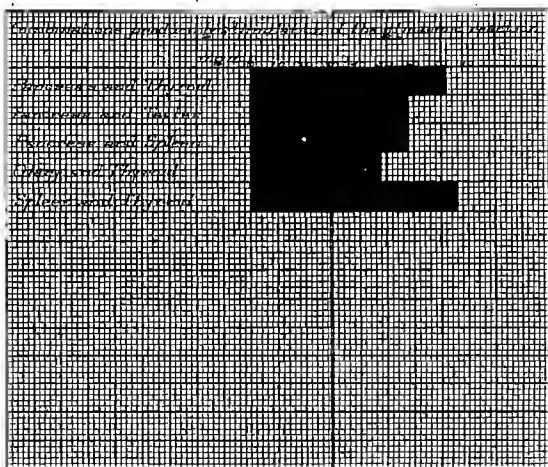


CHART IX.

little or no change from the normal reaction (see 1, 2 and 7, Chart X). If, however, one gland from one system be removed with one gland from the other system, and, in addition, either the thyroid or thymus, then marked inhibition occurs (see 5, 9, 10, 14, 15, Chart X). Removal of one gland from one system and of either the thyroid or the thymus results in practically no inhibition (see 4, Chart X). If both glands be removed from each system, then, in order to induce inhibition, both of the intermediate glands must also be removed, the removal of one intermediate gland is not sufficient (compare 3, 8, 11, 12, 13, Chart X). From this it would appear that there are two systems which control the reaction and

that these systems are connected through two apparently interchangeable glands; the thyroid and the thymus.

We have still to consider how inhibition may occur when the various glands are *in situ*, the data so far presented having to do with conditions following their removal. It is probable that if the removal of a gland causes inhibition, then that gland when *in situ* acts as a stimulator, and that the same thing holds true for various combinations. Furthermore, it is possible that inhibition of the reaction may be due either to lack of action of the stimulators or to excessive action of the inhibitors.

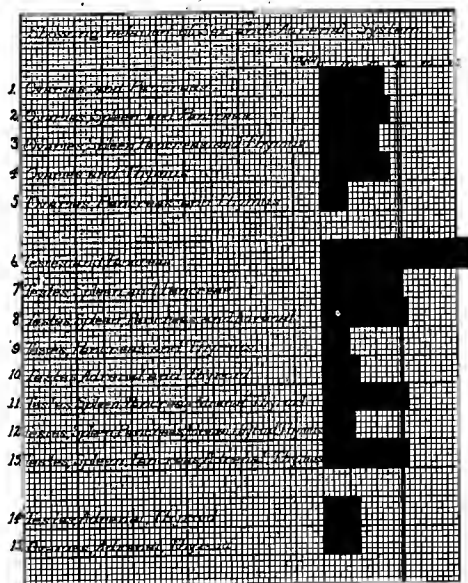


CHART X.

The hypothesis that inhibition of the glycemic reaction following the injection of homologous protein may be an indicator of a state of predisposition to cancer, coupled with the demonstration that various combinations of gland removal bring about inhibition of the reaction, offers a wide field for speculation and suggests numerous other investigations. All that can, at the present time, be

stated as a result of the experiments here recorded is that the glycemic reaction, which follows the injection of homologous protein, appears to be under the control of the endocrine system and that inhibition of the reaction may be viewed as a disturbance of metabolism due either to hypoactivity or to hyperactivity of the endocrines. Whether this disturbance of metabolism is or is not of etiologic significance in neoplasia cannot be stated from the facts at present at our disposal.

THE LEUKOCYTES AFTER HEMORRHAGES.

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DURING the course of some experiments undertaken for the purpose of studying blood regeneration after simple anemia, an unusual opportunity was presented for studying the behavior of the leukocytes after hemorrhage. In this series of experiments the total number of white cells per cubic millimeter were enumerated in some cases before and in all cases immediately after bleeding and a differential count made at the same time. Other counts, both differential and total, were subsequently made at various intervals after the first count until the white cells had reached the level of the preliminary determination or had attained figures which were approximately normal for the dog. Coincidental blood-volume estimations by the dye method afforded a basis for interesting comparisons. In a previous series of forty-eight dogs these normal figures had been found to be as follows: Average count of white cells per cubic millimeter, 15,923; average number of polynuclear neutrophils, 66.66 per cent.¹

Before discussing the results that were attained in the present series the literature of the subject will be briefly reviewed. It seems to be a quite generally appreciated fact that a leukocytosis occurs after hemorrhage. However, in most hematologies or in books dealing with laboratory studies in general this fact either is omitted, or if mentioned but brief mention is made of this occurrence. For example, Simon² merely lists in a long column, among the other causes of leukocytosis, that which follows hemorrhage. Webster³ discusses the matter more fully than do other authors of

¹ John H. Musser, Jr., and E. B. Krumhaar: Studies of the Blood of Normal Dogs, *Folia Haematologica*, 1914, xviii, 576.

² Manual of Medical Diagnosis, 9th ed., Philadelphia, 1918, p. 55.

³ Diagnostic Methods, 6th ed., Philadelphia, 1920, p. 572.