STUDIES ON THE CONDITIONS OF ACTIVITY IN ENDOCRINE GLANDS

XIII. A SYMPATHETIC AND ADRENAL MECHANISM FOR MOBILIZING SUGAR IN HYPOGLYCEMIA

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When insulin in an amount which reduces blood sugar below the common physiological percentage is administered to persons suffering from diabetes, characteristic symptoms occur which have been called "hypoglycemic reactions." They include pallor, rapid pulse, dilatation of the pupils and profuse sweating (1). These are indications of activity of the sympathetic division of the autonomic system and, as is often the case when that system is excited, there are tremors in skeletal muscle. Similar signs—dilatation of the pupils, erection of the hair, salivation—have been reported as occurring in cats after insulin injections (2), and these too are explicable as results of sympathetic nervous discharge. A natural inference from this evidence that the reduction of blood sugar by insulin involves sympathetic impulses is that adrenal secretion, known to be subject to such impulses, might be increased. Stewart and Rogoff have reported that in three cats, in which the influence of insulin on adrenal secretion was investigated, "no definite effect of any moment could be made out" (3). Since their method, however, gives negative results in their hands and positive results in other hands (4), their failure should not be regarded as significant.3

1 A preliminary report of this work was published in the Boston Medical and Surgical Journal, 1923 (July 26), clxxxix, 141.
2 Medical Fellow of the National Research Council.
3 Stewart and Rogoff continue to trust their "straightforward way" of estimating adrenal secretion (collecting blood from a "cava pocket" and assaying its adrenin content outside the body), although other methods, in the hands of different observers, have yielded concordant results quite contrary to theirs. An illuminating commentary on the reliability of their procedure is afforded by Kodama (4) who has used it carefully and in many tests. Stewart and Rogoff declare that the average output from the adrenals of the cat under their experimental conditions is between 0.00021 and 0.00025 mgm. per k. per minute (5); Cannon and Rapport (6) measured it and found approximately 0.0007 mgm. (an amount not very different from the average of 0.0006 mgm. which Stewart and Rogoff (7) had estimated previously by use
If adrenal secretion is increased as blood sugar falls after injection of insulin, the increase would indicate special activity of the sympathetic division of the autonomic system, and the two together—extra adrenin and sympathetic impulses—would have as a natural consequence a mobilization of sugar from the liver. Thus an automatic recovery of a disturbed equilibrium would be provided for. The interesting possibilities in these conjectures led us to put them to experimental test.

THE METHOD. As an indicator of adrenal secretion we have used the heart denervated by removal of both stellate ganglia and section of both vagus nerves. Sympathetic supply to the thyroid gland is thereby eliminated. Previous studies have shown that in animals with the hepatic nerves severed, the acceleration of the heart thus denervated, when an afferent nerve is stimulated or asphyxia is produced, is due solely to increased discharge of adrenin into the blood stream (6) (9), and that, if animals are fasting, the hepatic factor is so slight compared with the adrenal that it may be disregarded (10). The observations on the denervated heart of cats have been confirmed by Searles in studies on the denervated heart of dogs (11).4

of methods which they later repudiated; now Kodama, using the "cava pocket" method, reports the average figure as 0.00065 mgm.

Stewart and Rogoff affirm that 0.00025 mgm. is the steady unvarying secretion, not changed by afferent stimulation (8); Cannon and Rapport quantitated the secretion when increased by afferent stimulation and observed that it ranged between 0.0032 and 0.0049 mgm. per k. per minute; Kodama's assays under the same conditions, but on "cava pocket" blood, though averaging considerably less, ranged as high as 0.0047 mgm.

Rogoff, in a recent comment on Kodama's paper, declares that Kodama, "although he has evidently spent much time" on the method, has not mastered it, and that one of the best proofs that he does not know how to make the assay is the "excessive outputs obtained by him" (28). There is assumption here that Stewart and Rogoff's low figures are correct, and that Kodama's higher figures are not. The same method brought forth the two kinds of results, and therefore cannot be used to decide which is correct. Stewart and Rogoff have had no support from other observers for their constant assays and for their conclusions therefrom. The first observer to employ their method outside their laboratory obtains results which agree, quantitatively, with the results found by Cannon and his collaborators.

4 In a recent paper (12) Stewart and Rogoff have reiterated that they have caused reflex acceleration of the denervated heart after suppression of the adrenal output. We call attention again to the proof a, that the residual increase of rate after adrenalectomy is due to a discharge from the liver, and b, that if the liver nerves have been severed the effect from the adrenals persists, but disappears as soon as these glands are removed (6). Furthermore, as Cannon and Carrasco-Formiguera showed (9), after section of the hepatic nerves the denervated heart can be reflexly accelerated when the blood flow from the adrenals is free, cannot be accelerated when the flow is blocked, and can be accelerated again when the block is removed. These two lines of evidence for reflex increase of adrenal secretion, which are irreconcilable
Our experiments, which were performed on cats, were at first done under anesthesia—a condition which may profoundly affect the metabolism of sugar in the body. Griffith (15) has proved, however, that chloralose anesthesia is satisfactory for studies of physiological factors influencing the glycemic (i.e., the blood sugar) concentration. Deep chloralose anesthesia, in our experience, though not preventing a fall of the glycemic percentage after insulin, does greatly reduce or abolish the hypoglycemic reactions. Since they are signs of disturbance in nerve centers, their absence under deep anesthesia is readily comprehensible. A satisfactory dose of chloralose for our purposes was found to be 0.1 gram per kilo by mouth. We have followed Griffith's method of administering it in milk which the animal drank, or we have given it in solution through a stomach tube (10 cc. of 1 per cent per kilo). During the short time while the chest was opened for removal of the stellate ganglia, ether was commonly administered to a degree which prevented reflex movements. Only a slight amount of ether was needed, and it was discontinued as soon as the operation was ended. The animals were well nourished and had been fed meat the previous afternoon.

Observations were made also on unanesthetized animals in which the heart had been denervated aseptically, and which were living normally in the laboratory.

The insulin (Lilly's) was injected into the jugular vein, 4 units per kilo being the usual dose. The blood for assay of its sugar concentration was taken from a carotid artery in the anesthetized and from a jugular vein in the unanesthetized animals, and was tested by the Folin-Wu method (14).

The temperature of the anesthetized animals was kept uniform within a degree centigrade throughout each experiment.

The heart rate was recorded in blood-pressure records in the observations on anesthetized animals (see fig. 5); it was counted by use of the stethoscope, for 15-second intervals, in the unanesthetized animals.

with Stewart and Rogoff's denial of that secretion, they have not mentioned, though published many months before the appearance of their paper.

The method used by Cannon and Carrasco-Formiguera to prove reflex control of adrenal secretion was so close a repetition of Stewart's method of proving direct splanchnic control that Zunz and Govaerts (13) overlooked the difference and stated that Cannon confirmed Stewart. The identity of methods and results raises the question whether the proof for direct nervous control of adrenal secretion is to be rejected or the evidence for reflex control is to be accepted.

Rogoff has just published the observation that asphyxia of bulbar centers induces an increased rate of adrenal secretion, but there is "no evidence," he states, "that asphyxia of the entire animal is capable of augmenting the output of epinephrin" (26). May we call attention again to the paper by Cannon and Carrasco-Formiguera (9) which reported observed facts contradictory to this conclusion and which Rogoff does not mention.
RESULTS. We shall report first the observations on animals under chloralose anesthesia, and thereafter, at each point, the corresponding observations on unanesthetized animals.

1. As the glycemic concentration falls after injection of insulin, it reaches a critical point, at which the rate of the denervated heart begins to be accelerated, and as the sugar percentage continues to fall the heart rate continues to rise until a maximum is reached (13 cases). This observation is illustrated in figures 1, 2 and 5. As shown in figures 1 and 2, the acceleration is usually sharp when the standard dose, stated above, is given. When a smaller dose is given (see fig. 7) the rise of rate is more gradual. The maximal increase in the anesthetized cases was 48 beats per minute, the minimum, 24. The faster rate may continue for 2 hours or more.

In unanesthetized animals the acceleration was more marked for the standard dose than in the anesthetized. For example, in the case illustrated in figure 2, the increase was as much as 80 beats per minute, and the rate continued high (more than 30 beats per minute above the basal rate of 112) for more than three hours. With smaller doses the increases were smaller—as low as 20 beats per minute. The protocol of the observations illustrated in figure 2 is as follows:

Cat 232, with heart denervated. November 30, 1923, brought from animal room; 12:00 noon, heart rate (h.r.) while quiet in lap, 112 per minute. 12:15, jugular bared under ethyl chloride, cat quiet; blood sample no. 1 taken, 129 mgm. sugar. 12:24, 10 units insulin (4 units per k.) injected subcutaneously on one side. 12:27, squatting on floor, h.r. 112. 12:40, quiet in lap, h.r. 112, resp. 13 per minute. 1:00, squatting on table, h.r. 112, resp. 16 per minute; nictitating membrane (denervated) one-fourth over eye. 1:20, squatting, h.r. 172, resp. 41 per minute, 1:30, blood sample no. 2 taken. 65 mgm. sugar. 1:40, squatting, h.r. 140. 1:50, h.r. 176. 2:00, h.r. 176. 2:10, h.r. 160. 2:20, h.r. 156 to 164, varying up and down during observation. 2:30, h.r. 164. 2:40, h.r. 172. 2:50, h.r. 160-172, varying fast and slow. 3:00, h.r. 160-172. 3:10, h.r. 148. 3:20, blood sample no. 3 taken, 34 mgm. sugar. 3:25, lying on side, pupils dilated, tail hairs lifted, h.r. varying between 176 and 192. 3:30, weak, unable to stand. 3:40, lying on side, resp. 140 per minute, pupils dilated, nictitating membrane disappeared, salivation. 3:45, h.r. 164, with frequent dropped beats. 4:00, lying on side, legs stretched out, tail curved over back, h.r. 164. 4:10, same state, occasional stretching out, very rapid breathing, h.r. 188. 4:20, same state, h.r. 140 (?) paired beats. 4:23, convulsion; blood sample no. 4 taken, 32 mgm. sugar. 4:28, glucose injected under skin left side. 4:30, h.r. 158.

2. The critical point at which the denervated heart of the animal under chloralose begins to beat faster appears to lie within a range between 110 and 70 mgm. of glucose per 100 cc. of blood. We say "appears" because in judging the matter we have to assume that the initial drop in the glycemic concentration follows a fairly straight line between the first two assays. It has been reported that after insulin injection in rabbits the blood sugar falls at a practically uniform rate (18).
shown in figure 1B and in figure 5, the first three assays lay in a line, and in those cases we may be fairly sure of the critical range; in figure 1B the faster rate started at a level of 70 mgm. and in figure 5 at a level of about 110 mgm. Other cases (e.g., fig. 1, A and C, and fig. 7) lie within these limits.

Fig. 1. Increase of rate of the denervated heart (solid line), in animals under chloralose anesthesia, when the falling blood-sugar concentration (dash line) passed a critical point. In case A, the insulin was injected into the jugular vein at 11:33; in B, at 11:08; and in C at 9:30. In each case 4 units per kilo were injected.
Figure 2 shows well the remarkable change which occurs at the critical point in the unanesthetized cat. At 1 o'clock the heart rate was 112 and the respiration 16 per minute; at 1:20 the heart rate was 172 and the respiration 41. If we may assume that the blood sugar was dropping uniformly the concentration was between 70 and 80 mgm. per 100 cc. when the heart began to speed. This coincides with clinical observation, for Fletcher and Campbell testify that "when a reaction has already been experienced the onset of a subsequent one is usually recognized by

![Graph showing heart rate and blood sugar levels over time.](image)

Fig. 2. Increase of the rate of the denervated heart (solid line) in an unanesthetized animal, when the falling blood-sugar percentage reached about 0.07. The rate continued high and after an hour or two was spasmodically increased still further, as represented by the height of vertical lines at several counts. A convulsive seizure (see arrow) occurred at 4:23, 4 hours after the subcutaneous insulin injection (4 units per k.), and about 3 hours after the quick rise in the heart rate.

the patient when the blood-sugar percentage falls to some point between 0.08 per cent and 0.07 per cent." And again, "when the blood sugar percentage falls to 0.07 per cent under the influence of insulin, the patient becomes aware of it" (1).

Why, in our cases, the critical level was higher as a rule in animals under chloralose than in the unanesthetized animals is not clear. One would suppose that the anesthetic would decrease rather than increase sensitiveness. If the anesthetic is given to excess, it may decrease the sensitiveness to the extent of wholly abolishing the reaction, as already
noted. Animals lightly chloralosed, however, are hypersensitive to sounds and jars, as indicated by spasmodic response to such stimuli, and they frequently jerk in an incoordinate manner. These are signs of instability in motor centers. Possibly in some instances the nerve cells responsible for the hypoglycemic phenomena are rendered hypersensitive to effects of insulin by chloralose.

In our experience the acceleration of the denervated heart appears before other striking signs of sympathetic activity. In the case illustrated in figure 2, for example, the faster rate was first noted at 1:20; for the first time at 3:25 were the pupils seen to be dilated and the hairs of the tail standing erect. Unfortunately we have no convenient means of recording early effects on the hairs; and the iris is subject to control by way of the short ciliary nerves, even though the stellate ganglia have been removed—a control which may overcome the action of an opposing factor. The absence of obvious changes in the hairs and in the iris need not be regarded, therefore, as very significant. It is possible, however, to obtain evidence of sympathetic activity in the heart itself. In two cases under chloralose the adrenal glands were removed and the vagi cut, but the stellate ganglia were left intact so that the heart could be influenced directly by sympathetic impulses. In both cases the heart accelerated when the glycemic concentration fell below 80 mgm.—in one case 10 beats and the other 16 beats per minute. These results show that the cardioaccelerator nerves themselves may be stimulated at the critical range.

3. If the adrenal glands have previously been removed, or if one has been removed and the other denervated, a fall of the glycemic percentage below the critical range is not accompanied by an increased rate of the completely denervated heart (4 cases). This observation is illustrated in figure 3. It represents graphically the conditions under chloralose in an animal from which the left adrenal gland had been removed and in which the right splanchnic and the hepatic nerves had been cut 19 days previously. The concentration of blood sugar fell to 50 mgm. with no increase in the heart beats per minute as the critical range was traversed. In other instances, levels of 40 and 44 mgm. have been reached under such conditions, or with both adrenals absent, without calling forth any noteworthy acceleration of the pulse.

In figure 4 are represented the changes in an unanesthetized cat with denervated heart and with one adrenal previously removed, and the opposite splanchnic nerves severed, occurring after intravenous injection of 4 units of insulin per kilo. This figure should be compared with figure 2. As the blood sugar fell through the critical range, and further to less than 50 mgm., the heart rate increased 6 beats per minute—an insignificant change—instead of increasing 60 or more beats per minute, as in figure 2. The protocol of the observations illustrated in figure 4 is as follows:
Cat 239, with heart denervated, and with right adrenal removed and left splanchnics cut. Weight, 3.1 k. January 11, 1924. 10:40, h.r. 144. 10:50, h.r. 144. 10:52, blood sample no. 1 taken from jugular vein under ethyl chloride, 200 mgm. sugar. 10:54, 12 units insulin in jugular. 11:00, h.r., 144. 11:10, h.r. 144. 11:20, h.r. 144. 11:26, blood sample no. 2 taken, 95 mgm. sugar. 11:30, h.r. 148. 11:40, h.r. 148, intestinal gurglings heard. 11:50, h.r. 148. 11:58, blood sample no. 3 taken, 57 mgm. sugar. 12:00, h.r. 148, hairs of back and tail lifted. 12:10, h.r. 148, hairs very fuzzy, animal restless (change from previous quiet). 12:20, h.r. 150, cat weak, staggered while walking. 12:26, blood sample no. 4 taken, 51 mgm. sugar, cat weaker, unable to stand. 12:30, lying on side. Periodic rapid panting, mouth wide open, h.r. 144. 12:33, convulsion. 12:38, blood sample no. 5 taken, 46 mgm. sugar; 10 cc. 10 per cent glucose injected under skin on one side. 12:45, h.r. 144, still panting.

Fig. 3. Failure of increase of rate of the denervated heart (solid line) in an animal under chloralose when the falling blood sugar concentration (dash line) passed the critical range. The left adrenal had been removed and the right splanchnic and the hepatic nerves severed 19 days before. Insulin (4 units per k.) was injected intravenously at 12:19.

Fig. 4. Failure of noteworthy increase of rate of the denervated heart (solid line) in an unanesthetized animal when the falling blood-sugar concentration (dash line) passed the critical range. The left adrenal had been removed and the right splanchnic nerves severed months before. Insulin (4 units per k.) was injected intravenously at 10:54. A convulsive seizure (see arrow) occurred at 12:33, an hour and 39 minutes after the insulin injection.
These observations indicate that the cardiac acceleration reported in previous sections and shown in figures 1 and 2 is not due to direct action of insulin, or to the effects of any changes due to insulin, on the heart itself. This evidence coincides with the observations of Hepburn and Latchford on the excised heart (17). Further, neither insulin, nor any disturbance produced by it, acts directly on the adrenal gland for, as shown in figures 3 and 4, one adrenal may still be present, and, if denervated, it does not exercise any notable influence on the heart rate. Moreover, in the case represented in figure 4 as well as in other similar cases, although one adrenal gland had been removed and the other denervated, hepatic nerves were still existent. As the falling glycemic percentage passed the critical range, however, the rate did not become markedly faster, i.e., nothing was given off from the liver that accelerated the pulse to any considerable degree.

The conclusion which we feel justified in drawing from the combined results thus far reported is that the acceleration of the denervated heart is due to a discharge of adrenin from the adrenal glands in response to nervous impulses. In other words, these glands take part in the hypoglycemic reactions; the sympathetic discharges which cause dilatation of the pupil, acceleration of the heart, etc., also evoke increased adrenal secretion.

4. If the rate of the denervated heart has been increased because of hypoglycemia, intravenous injection of glucose promptly reduces the rate. This observation is illustrated in figure 1, A and C, and in figure 5. In the case represented in figure 5, a record was taken during the intravenous injection of 15 cc. of 5 per cent glucose, and at one-minute intervals thereafter. In 65 seconds after starting the injection the rate had fallen from 174 to 160 beats per minute, after another minute it had dropped to 144, and two minutes later it was down to 141—a rate below the original. In 20 minutes it had recovered from this depression and was beating 152 per minute. In our experience, the injection of glucose (1 gram) into an animal without adrenal secretion does not thus promptly decrease the heart rate. Since the faster rate is ascribable to increased adrenal secretion called forth by disturbed cells in the central nervous system, the restorative action of glucose can be accounted for on the assumption that it provides material which these cells need, or modifies factors which have come into play because the glucose supply has been reduced. The cells, no longer excited or rendered hyperexcitable, cease to discharge impulses via the sympathetic. Extra adrenal secretion quickly stops and the heart rate therefore quickly falls.

5. As the rate of the denervated heart increases (indicating adrenal secretion), the rate of drop in the glycemic percentage decreases, i.e., the sugar curve tends to flatten. This observation is illustrated in the
Fig. 5. Increase of rate of the denervated heart (solid line) in an animal under chloralose anesthesia, when the falling blood-sugar concentration (dash line) passed a critical point. Insulin given intravenously at 10:01. At 12:15, 15 cc. of 5 per cent glucose were injected intravenously. Portions of the original records show the method of recording the heart rate in this and in other experiments under anesthesia; the numbers above the base line (zero blood pressure; 5-second intervals) represent the heart rates per minute and the figures below, the time and rectal temperature.
three cases represented in figure 1 and in figures 2, 5 and 7. In clinical cases (27) and in laboratory experiments (16) tests of blood sugar after injection of insulin have shown that the flattening of the curve, following an initial drop, is a characteristic feature. As shown in figure 1C, the sugar percentage may remain fairly constant at a given level; or it may remain constant or nearly constant for a time and later fall, as in figures 1A and 1B; or it may continue falling but at a slower rate than before, as in figures 2 and 5. These variations in the blood-sugar curves are probably to be explained chiefly by differences in the glycogen stores in the different animals, for Macleod and his co-workers have published curves showing similar variations in rabbits that were glycogen-rich and glycogen-poor (16, fig. 6). In our cases, however, the check in the rate of fall in the glycemic concentration was observed when adrenal secretion occurred, as was manifested by an accelerated heart beat.

6. If the adrenal glands have previously been removed, or if one has been removed and the other denervated, the rate of drop in the glycemic percentage after the standard dose in animals under chloralose is usually not checked at the critical level; it may be retarded slightly or not at all at that point, or it may flatten at a very low concentration. These observations are shown graphically in figure 3 and in figure 6, A, B and C. An exception to the general statement is illustrated in figure 6D. In that case the curve flattened between 70 and 80 mgm., although the adrenal glands were absent. The heart rate, however, had been unaccountably high (never less than 254 beats per minute) throughout the experiment, the blood pressure when the curve flattened was only 70 mm. Hg, and the animal became so asphyxiated that artificial respiration had to be started. It is quite possible that in this case, as well as in others (cf. fig. 3, with hepatic nerves cut) asphyxia played a rôle in setting free sugar from the liver, for we have noted not infrequently on taking the late samples in the course of an experiment that the arterial blood was surprisingly venous. The asphyxial condition might act either by stimulation of the hepatic cells from the sympathetic nerve centers (tail hairs were erected in 6D at 12:30) or by direct action on the hepatic cells.

The conditions in unanesthetized animals with inactivated adrenals but with hepatic innervation, when insulin has lowered the blood sugar, will be considered later.

7. If not too much insulin has been given, the increased rate of the denervated heart may be followed by an increase of the glycemic percentage, and an attendant fall in the heart rate. This reaction is shown in the record reproduced in figure 7. It has often been observed that the decrease of blood sugar due to insulin is followed fairly promptly, or after remaining at a low level for a time, by a rise to normal (16). We have frequently had occasion to note the reciprocals of such changes
in counting the rate of the denervated heart in unanesthetized animals which had been given sub-convulsive doses of insulin. The rate has continued uniformly for a varying time—from 50 to 105 minutes after the injection—whereupon it has gradually increased between 20 and 36 beats per minute, as in figure 7, and then gradually subsided again to its former level. The increased rate has not lasted longer than 120

![Graph](http://ajplegacy.physiology.org)
minutes, and in one instance was over in 60 minutes. The drop in the heart rate as the blood sugar increases is just what would be expected from the observation, already noted, that introduction of glucose into the blood stream promptly brings the pulse down (see p. 54).

**The Rôle of Adrenal Secretion in the Hypoglycemia from Insulin.** The considerations presented in the foregoing pages raise the question as to the part taken by adrenal secretion in resisting the fall of blood sugar. The evidence in hand clearly indicates that this resistance and the recovery from insulin injections depend largely on the glycogen available in the liver (16). Glycogenolysis can be produced, as is well known, by direct stimulation of the hepatic nerves. Also, as Griffith has shown, it can occur reflexly in consequence of afferent stimulation, but if the adrenal glands have been excluded from action the glycemic percentage averages lower and the effect of reflex stimulation averages less than in normal animals (15). Furthermore, Trendelenburg has presented evidence that such outpouring of adrenal secretion as can readily occur in physiological states is quite capable of causing a mobilization of sugar (18). When in the early stage of an experiment the denervated heart becomes accelerated, the faster rate indicates not only that adrenal secretion is being augmented, but also by that fact that splanchnic impulses are being discharged. It is altogether probable that the liver receives these impulses just as the adrenals do. The liver, however, would be subject to both nervous and humoral stimuli—the nervous impulses and the secreted adrenin cooperating to influence it. Under chloralose anesthesia the humoral factor seems to be a necessary complement of the nervous, for, as comparison of figures 1 and 6 indicates, the course of the descent of the glycemic concentration is as a rule strikingly different, after standard doses of insulin, according to whether adrenal activity is present or absent. The character of this difference has already been pointed out (pp. 54, 56).

In the unanesthetized animal, likewise, the secreted adrenin appears to be important for the process of mobilizing sugar from the liver. In table 1 are presented the results of injecting insulin into healthy animals, with and without active adrenal glands, that had been kept under the same conditions of feeding and temperature and freedom from disturbance. (In our experience with a few cats which had just previously been subjected to prolonged excitement or which were suffering from infection, they were more sensitive to insulin than are serene and healthy animals.) In a number of cases injections were repeated in the same animal. Rabbits seem to become less sensitive to insulin as doses are repeated (25). In cats with intact adrenals this possibility was met by a larger dose at the second injection. In cats with adrenals inactivated a second dose equal to the first should have been less likely to produce convulsions,
if a sort of immunity develops. This result was perhaps indicated in cats 107 and 112, and in other cases in which the convulsive seizure occurred after a longer delay in the second than in the first test. The seizures occurred, nevertheless, and strikingly differentiated this group from the other. Among animals with normally innervated adrenals doses of insulin varying from 2 to 3 units per kilo caused convulsions in only one instance. This was in cat 228 which had had a major operation 10 days previously, and had not fully recovered from it. A dose of 2.5

### TABLE 1

**Presence or absence of convulsions after subcutaneous injections of insulin into (A) cats with normally innervated adrenals, and (B) cats with one adrenal removed and the other denervated**

<table>
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<th>A. ADRENALS BOTH INNERVATED</th>
<th>B. ADRENALS, 1 OUT, OTHER DENERVATED</th>
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* Panting, weakness and vomiting after 1 hour and 24 minutes.
† Salivation, mewing, weakness and muscular twitching, beginning after 1 hour and 25 minutes, and continuing for about a half hour.

units per kilo brought on a convulsive seizure after three and a half hours; later, after complete recovery, this animal twice withstood doses of 3 units per kilo without any such disturbance. On the other hand, among animals with one adrenal removed and the other denervated, insulin doses lying between 1 and 2 units per kilo induced convulsions within two hours and ten minutes in all but three cases. In the first of these (cat 235) a dose of 3 units was later effective. In the other two cases the premonitory signs appeared, but the animals passed the crisis without a seizure. The liver
was still innervated, it should be remembered, and therefore some protection against hypoglycemia still existed. It should be noted that a dose of 2 units per kilo had no convulsive effect in cat 107 when the adrenal nerve supply was intact——indeed the only influence was an increased rate of the denervated heart by 24 beats per minute for about sixty minutes during an observation lasting three and a half hours; later, after one adrenal gland had been removed and the other denervated and the animal had recovered and was well nourished, 2 units of insulin per kilo twice produced a convulsion, in seventy and in eighty-three minutes respectively, and on a third trial caused panting and vomiting in eighty-four minutes. Comparison of figures 2 and 4 brings out similar testimony. In each case 4 units of insulin per kilo was the dose. In each case the nerves to the liver were intact, but the case of figure 4 differed from that of figure 2 in having only one adrenal and that denervated. In the animal with active adrenal glands the glycemic percentage fell more slowly, the descent began to flatten just below 70 mgm., and the convulsion appeared much later, as compared with the animal lacking active glands.

The only difference between the animals in the two groups of table 1 was presence of active adrenals in one and absence in the other. All the numbered animals had undergone a major operation (denervation of the heart), and all were living together, eating the same food and manifesting the same good health. It could be objected, nevertheless, that inactivation of the adrenals might lower the glycogen storage and therefore, in spite of other similarities, the animals without adrenals might be more sensitive to the action of insulin. According to Stewart and Rogoff, however, the formation and storing of glycogen in the liver in cats is not affected by removal of one adrenal and section of the nerves of the other (19).

From observations on rabbits which had had adrenals removed (for intervals varying from 28 days to more than 8 months) Stewart and Rogoff concluded that the action of insulin does not differ in animals with and without these glands (20). They report tests on only three adrenalectomized animals: one was given 2.7 units, a second 8.8 units of insulin per kilo and a third was given a crude preparation which caused strange and unaccountable symptoms. Only one control was reported. Opposed to this slight and frail support for a rather large inference are the results reported by Lewis (21). He made tests on 27 normal rats and on 11 rats without adrenals, that had received the same food before and since the operation, 12 days earlier. The fatal dose of the insulin he used proved to be 10 mgm. per 100 grams for the normal, and 1 mgm. per 100 grams for adrenalectomized animals. A dose of 2 mgm. per 100 grams, twice the lethal amount for the latter group, did not even produce the typical hypoglycemic symptoms in the normal animals. Similar testimony has come from Sundberg, who studied the effects of insulin on normal rabbits and
on rabbits deprived of the adrenal medulla (29). He found that a given
dose caused a larger reduction of blood sugar and a more certain
appearance of nervous symptoms (asthenia and convulsions) in the
animals without medulla than in the normal controls. It is clear that
all the evidence which we have presented above as to the importance of
adrenal cooperation in protecting the body against the dangers of a too
great hypoglycemia is in harmony with the results reported by Lewis
and by Sundberg and offers no support for the view expressed by Stewart
and Rogoff.5

Discussion. The typical symptoms seen after an overdose of insulin
are probably due almost entirely, if not entirely, to depriving the tissues
of a necessary supply of sugar. That they are not dependent on the action
of insulin as such is proved by their occurrence in hypoglycemic states
produced by other means. About ten years ago Fischler and his collabor-
orators called attention to a group of symptoms—excitement, convul-
sions, collapse and coma—accompanying the low blood sugar produced by
phlorhizin, and Fischler designated the condition as "glycoprival intoxica-
tion" (22). More recently Mann and Magath have reported that the
progressive decrease of blood sugar resulting from removal of the liver is
attended by characteristic symptoms, prominent among which are, first,
muscular weakness and, later, exaggerated reflexes, twitchings and convul-
sions (23). The very rapid soothing of the disturbed nerve cells by
intravenous injections of glucose, whether the disturbance has been
occasioned by phlorhizin, by removal of the liver, or by insulin, points
to a sugar shortage as its cause.

The deficiency of sugar is probably local, and is not necessarily a con-
sequence of a low concentration in the blood. A number of observers
have called attention to a hypoglycemia which follows an abundant inges-
tion of sugar (24). Folin and Berglund have noted a glycemic concen-
tration as low as 54 mgm. per 100 cc. of blood shortly after the ingestion of
glucose. This low level which is considerably under the critical range
described above, they explain by assuming that because the local stores
are well filled there is no need for sugar to be in transport, and consequently
the blood as a carrier is lightly loaded. It seems probable that the

5 In an article which appeared while the present paper was in galley proof, Stewart
(Physiological Reviews, 1924, iv, 183) remarks on the "flimsy foundation" of Zuel-
zer's theory that "epinephrin is the physiological antagonist of the pancreatic
hormone and that when the pancreas is removed diabetes follows owing to the un-
checked action of epinephrin given off from the adrenals." He later states that
"Cannon, McIver and Bliss have put forward the hypothesis, which may be con-
sidered as an elaboration of the theory of Zuelzer, that when the blood sugar tends
to fall the output of epinephrin is stimulated." Readers of the present paper are in
a position to judge whether our evidence is properly described as an "hypothesis,"
and whether in any sense it can be correctly considered as an elaboration of Zuelzer's
theory.
condition of hypoglycemia after insulin is quite the opposite. The effect of insulin is to increase the utilization of sugar by active tissues; we may reasonably suppose that the local supply is the first to be reduced; the sugar in the blood probably soon passes into the tissues depleted of their sugar stores until what may be called the sugar pressure in the blood is so greatly diminished that it no longer meets the demands. In these circumstances the hypoglycemia would be caused, not by an oversupply in the local stores, but by an undersupply and an excessive demand in them. The main source of the needed material is in the liver. If the sugar is not called forth promptly from that reserve and conveyed by the blood, the organism may be in serious peril.

The observations detailed in the foregoing pages have revealed a mechanism, or set of mechanisms, having the function of maintaining the physiological percentage of blood sugar when there is danger of deficiency. If that percentage falls below a critical point in consequence of deficiency, splanchnic neurones of the sympathetic system are set in action, as indicated by increased adrenal secretion. This is a reaction which, as seen in figure 2, may occur hours before a convulsion occurs, or may occur without a subsequent convulsion (see fig. 7). It coincides with the appearance of subjective feelings of anxiety reported by patients, and shortly thereafter with objective signs of increased sympathetic innervation. Both the nerve impulses and the secreted adrenin have the effect of liberating sugar from the liver into the circulation, and thus tending to restore the disturbed equilibrium. This may be regarded as a first line of defense against a falling glycemic concentration.

Whether this first defense is effectual or not will depend in part on the reserves which can be called upon. Macleod and his collaborators have pointed out that in fasting animals with a meager glycogen store a given dose of insulin produces convulsions more frequently and earlier than in well-fed animals with an abundance of this material (16). Since adrenin is an important factor in the humoral and nervous cooperation which influences the liver, however, it is quite possible that a low adrenin content may be the occasion of weakness in the first defense. In one of our animals, already noticed on p. 59, which received a dose of 2.5 units of insulin before it had fully recovered from a major operation, the denervated heart began to beat faster within sixty minutes after the injection and eighteen minutes later it had increased 62 beats per minute; two hours later still the rate had slowly fallen to the original level, indicating that the adrenal glands had ceased to be especially active. One might suppose that this implied that the call for sugar had been answered; but a very bushy tail revealed great activity of sympathetic neurones, and the animal, weak and panting, had a convulsion a few minutes thereafter. There may have been, of course, a using up of the glycogen reserve in this case, as well as
the adrenin reserve, but if so, the two must have run down simultaneously. On the other hand, in the case illustrated in figure 2, adrenal secretion was still being poured forth in extra amount, as shown by the accelerated heart rate, when the convulsion occurred. It is clear, then, that there may be a separation of the adrenal and hepatic factors. Unfortunately it is difficult in any case to discriminate between the two, for the conditions which might lead to depletion of the adrenal medulla would be likely to lessen the glyco
gen content of the liver. The possibility must be kept in mind, however, that variation in the amount of available adrenin may affect the efficiency of the first defense.

If the first defense fails to prevent the glycemic concentration from falling, there is evidence of more widespread and more effective sympathetic discharges, characterized in cats by dilatation of the pupils, erection of hairs and salivation. As shown in figure 2, this stage is associated with spasmodically increased adrenal secretion (manifested by the marked temporary increases of the rate of the denervated heart). The culmination of this stage of agitation is the convulsive attack. Such an attack is accompanied by increased adrenal discharge (in one of our cases the denervated heart increased 33 beats per minute during the muscular spasms), and it is the occasion for further liberation of sugar into the circulation (25, p. 43). In well-fed rabbits a convulsion may be followed by temporary or permanent recovery without injection of glucose (16).

It is probable, therefore, that the convulsion, and the agitation immediately preceding and attending it, form a secondary defense against the damage from an insufficient glucose supply for the needy tissues.

Still another factor which may be at work, though we have little evidence regarding the role it may play, is the asphyxial state (i.e., the venosity) of even arterial blood in the later stages of insulin hypoglycemia. It is quite conceivable that asphyxia, which can in itself produce powerful convulsions, either co-operates at some stage with lack of glucose, or with changes induced thereby, to excite activity in the nerve cells. This possibility has been suggested also by Olmsted and Logan (2).

The setting into operation of the sympathetic and adrenal mechanism for mobilizing sugar from the liver explains a number of conditions which have been observed in man. As already noted (p. 51), when the mechanism is started by a fall of the glycemic percentage to approximately 0.07, a patient becomes aware of a feeling of nervousness or tremulousness, or weakness, or of a sense of "goneness." These are subjective symptoms which have been described as occurring when adrenalin is injected subcutaneously (26). "The reaction may go no further than this of its own accord"; so Fletcher and Campbell testify (1); this relief is precisely what would occur when the mechanism proves effective. "Or it may be cut short at this stage by the administration of carbohydrate;" the increase of
available carbohydrate (sugar) is the result toward which the mechanism is operating, and which, when it is achieved, automatically cuts short the reaction. If the initial symptoms become worse, they are characterized by pallor and flushing, rapid pulse, dilated pupils and sweating, with experience of anxiety, excitement or vague emotional disturbance; again these are the severer symptoms reported by peculiarly sensitive persons when given an injection of adrenalin (26). In this connection it will be recalled that as the blood sugar falls below the critical point the denervated heart beats with increasing frequency, thus indicating both an increased adrenal secretion and a greater discharge of sympathetic nerve impulses. This relationship between hypoglycemia and adrenal output was surmised by Wilder and Boothby and their collaborators. Because of a sharp change in the rate of decrease of the glycemic percentage in a diabetic patient when the first subjective symptoms of hypoglycemia appeared, they suggested that there might be a spontaneous outpouring of adrenin at that point—a result which would tend to protect against a further decrease. And in a later paper the more rapid metabolism which set in at that point was mentioned as supporting the possibility that adrenal secretion was then called forth (26). These interesting conjectures Wilder and Boothby did not pursue further, and they have brought no evidence to support their view. The experiments presented in the foregoing pages, however, definitely confirm their insight. The use of adrenin in the treatment of insulin hypoglycemia (1) is evidently a physiological procedure; the injected adrenin is added to that normally secreted and thereby augments its efficacy, or if the adrenal medulla is more or less exhausted the injected extract has a natural replacement value. A store of glycogen in the liver is, of course, presumed.

The mechanism described in the foregoing pages acts like many others, already known, that assure stability of the organism. When the equilibrium, which normally indicates a concentration of sugar in the blood sufficient to supply needy tissues, is disturbed by serious lessening of the concentration, this compensatory mechanism is set in action to restore the equilibrium. Sugar is set free from the reserves. If the reserve station is absent, as in the experiments on liver extirpation by Mann and Magath, or if the agency reducing the sugar content of the blood is too potent, as when excessive doses of insulin are given, the mechanism is overwhelmed and the result is disastrous to the organism—convulsions and coma are followed by death. From such consequences of an inadequate sugar supply to the tissues the organism is protected by the combined activity of the sympathetic system and adrenal secretion. From the evidence adduced by Griffith, cited above, it is probable that this mechanism is normally at work well within the danger line, sustaining a requisite sugar concentration in the blood.
The hypoglycemic reactions occurring when an excessive dose of insulin is given include pallor, rapid pulse, dilatation of the pupils and profuse sweating, which are indications of discharge of sympathetic impulses. The question whether adrenal secretion is involved was tested by means of the denervated heart in animals anesthetized with chloralose and in unanesthetized animals.

As the glycemic concentration falls after injection of insulin it reaches a critical point at which the rate of the denervated heart begins to be accelerated, and as the sugar percentage continues to fall the heart rate continues to rise until a maximum is reached (see figs. 1, 2 and 5).

The critical point at which the denervated heart of the animal under chloralose begins to beat faster appears to lie between 110 and 70 mgm. of glucose per 100 cc. of blood. In the unanesthetized the point lies between 70 and 80 mgm. The cardiac acceleration appears before other striking signs of sympathetic activity.

If the adrenal glands have previously been removed, or if one has been removed and the other denervated, a fall of the glycemic percentage below the critical range is not accompanied by an increased heart rate (see figs. 3 and 4). The cardiac acceleration, therefore, is not due to direct action of insulin on the heart or on the adrenal gland, but is due to increased adrenal discharge in response to nervous impulses.

If the rate of the denervated heart has been increased because of hypoglycemia, intravenous injection of glucose promptly reduces the rate (see figs. 1, A and C, and fig. 5).

As the rate of the denervated heart increases (indicating adrenal secretion), the rate of drop in the glycemic percentage decreases, i.e., the sugar curve tends to flatten (see figs. 1, 2, 5 and 7).

If the adrenal glands have previously been removed, or if one has been removed and the other denervated, and the animals are under chloralose anesthesia, the rate of drop in the glycemic percentage is usually not checked at the critical level (see figs. 3 and 6A, 6B and 6C). In unanesthetized animals without active adrenal glands the fall of blood sugar is less retarded at the critical level, and the convulsive seizures are induced sooner and with smaller doses than in animals with active glands (see table 1 and cf. figs. 2 and 4).

If not too much insulin has been given the increased rate of the denervated heart may be followed by an increase of the glycemic percentage and an attendant fall in heart rate (see fig. 7).

It is pointed out that the mechanism protecting the body from dangerous hypoglycemia probably operates in two stages—a primary stage in which sympathetic activity with adrenal secretion occurs and mobilizes sugar...
from the liver; and, if this proves to be inadequate, a secondary stage in which the activities of the first stage are intensified and augmented in convulsive seizures.

The mechanism here described is another remarkable example of automatic adjustments within the organism when there is a disturbance endangering its equilibrium.

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