THE ALLEGED RELATION OF THE EPINEPHRIN SECRETION OF THE ADRENALS TO CERTAIN EXPERIMENTAL HYPERGLYCEMIAS

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The view has been expressed by various writers that some, perhaps most, of the forms of experimental hyperglycemia (asphyxia (1), piqûre (2), etc.) are dependent primarily upon excitation of the adrenal glands to increased secretion of epinephrin, which then causes an accelerated mobilization of sugar in the same way as adrenalin does when artificially introduced. It is against this idea that subcutaneous injection of adrenalin is more effective in producing hyperglycemia and glycosuria than intravenous injection. However, it has been cited as evidence that the adrenal intervenes as an essential factor in the hyperglycemias in question, that a given experimental hyperglycemia which can be shown to occur while the adrenals are intact cannot be obtained after their removal. Further, some observers have stated that even the normal sugar content of the blood is not maintained after adrenalectomy, hypoglycemia being present; that glycogen cannot be normally stored by the liver, and so on.

The experimental basis for these conclusions seems to us to be quite unsatisfactory. Observations in which the production of hyperglycemia by a given procedure before removal of the adrenals are compared with similar observations after removal of the glands, suffer from two serious defects. In the first place, the animal has been deprived of organs essential to life. The period of survival is very brief in the absence of accessory glands, and when an animal is going to die within twenty-four hours it is surely a matter of difficulty and risk to fix the point up to which such a function as the regulation of the sugar content of the blood may still be regarded as normal. Secondly, the animal has been subjected to a major operation; it has been anesthetized for a time; possibly it has been prepared for the operation by a
period of fasting; certainly it does not eat after the operation. All these things cannot fail to complicate greatly any observations upon changes in the blood sugar dependent upon an increase or diminution of the rate of mobilization of the liver glycogen.

We have put the question of the relation of epinephrin to some of the forms of experimental hyperglycemia to the test by a method which eliminates all these disturbing factors. The epinephrin liberation was abolished, within the limits of sensitiveness of the methods used for its detection, or reduced to an insignificant fraction of its normal amount by dividing in cats the nerve supply of one adrenal and excising the other gland. After recovery from the operation the sugar was estimated from time to time in samples of blood obtained under the various conditions which it was desired to study, these being compared with normal samples previously collected.

TECHNIQUE

The sugar was estimated by the method of Lewis and Benedict (with Pearce's modification). Blood was collected from a vein, usually the femoral, by puncture with a hypodermic needle attached by a rubber tube to a 2 cc. pipette, in the point of which were a few crystals of potassium oxalate. The skin over the vein was shaved, usually the day before the blood collection, so that the animal might have recovered from the disturbance due to the shaving. Generally both legs were shaved at the same time so that blood could be obtained from either vein. Occasionally the jugular, and once or twice the external saphenous were employed. The needle was pushed directly through the skin into the vein. After the blood samples desired had been collected, a little tincture of iodine was placed on the skin over the site of the puncture, and a considerable interval, usually about a week, was allowed to elapse before any further samples of blood were taken. This refers to the observations in which such procedures as asphyxia and anesthetization had been resorted to. The blood was drawn up into the pipette a little above the mark, the pipette disconnected from the needle and the blood after being allowed to flow back to the mark, immediately discharged into a large test-tube containing 8 cc. of distilled water. The test-tube was then shaken. After hemolysis had occurred 15 cc. of saturated solution of picric acid was added and the contents filtered, after being well shaken. Duplicate quantities (7 cc.) of the filtrate were measured into test-tubes graduated in 0.1 cc. from 1 to 20 cc. Two cubic centimeters of saturated solution of picric acid and 1 cc. of 10 per cent solution of anhydrous sodium carbonate were added to each test-tube. After removal from the autoclave, any loss of fluid was replaced up to 10 cc. After filtration the color was compared with the picramic acid standard recommended by Lewis and Benedict, and also with a solution of dextrose of known percentage which was carried through the same process as the blood. Before deciding to use Pearce's modification, we compared it with the original method and found a sufficiently close agreement, as illustrated by the
following figures obtained with different quantities of a solution of dextrose. By the polarimeter it was estimated that 100 grams of the dextrose used corresponded to 93.6 grams dextrose. From this a solution was made of such a strength that 1 cc. would contain 0.936 mgm. dextrose.

<table>
<thead>
<tr>
<th>DEXTROSE SOLUTION</th>
<th>LEWIS AND BENEDICT METHOD</th>
<th>AUTOCLAVE MODIFICATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>cc.</td>
<td>mgm.</td>
<td>mgm.</td>
</tr>
<tr>
<td>1.0</td>
<td>0.881</td>
<td>0.890</td>
</tr>
<tr>
<td>1.0</td>
<td>0.910</td>
<td>0.940</td>
</tr>
<tr>
<td>2.0</td>
<td>2.060</td>
<td>2.012</td>
</tr>
<tr>
<td>0.5</td>
<td>0.418</td>
<td>0.411</td>
</tr>
<tr>
<td>0.5</td>
<td>0.461</td>
<td>0.427</td>
</tr>
<tr>
<td>0.75</td>
<td>0.748</td>
<td>0.741</td>
</tr>
</tbody>
</table>

In this paper are reported observations upon two forms of experimental hyperglycemia, that produced by asphyxia and that produced by ether anesthesia. The routine was to obtain a preliminary specimen of "normal" blood and then to subject the animal to a period of asphyxia or anesthesia. Asphyxia was maintained by placing over the nose and mouth of the animal a metal cone covered with a towel. The effect of the asphyxia was controlled throughout the whole period by palpating the heart through the chest wall. When distinct slowing of the heart had been produced the animal was allowed to breathe freely for a few seconds and the asphyxia was then repeated. The object was to produce and maintain with the necessary intervals of free breathing, a distinct asphyxial condition for ten to twenty minutes. The asphyxia was never pushed so far as to endanger life. As regards the anesthesia effect, an ordinary surgical anesthesia was maintained for fifteen to twenty minutes. In a few observations the etherization was less complete, stopping short of disappearance of the corneal reflex. In a number of the experiments when the animal happened to be especially quiet during the collection of the preliminary specimen, it was immediately afterwards subjected to frightening by a dog for twenty minutes to an hour, and another blood specimen then collected. Generally the animal was free in a small cage during the frightening, but in a few observations it was tied on a holder. In no case was it possible for the dog to inflict any physical injury on the cat. In some cases the sugar content of blood specimens, obtained after varying periods during which the animal remained tied down, was compared, although no systematic observations on the so-called "Fesselungs" hyperglycemia were made. The frightening experiments were followed by an asphyxia observation, in order to determine whether the animal was capable of showing a decided hyperglycemia. In this way it was supposed that a negative result due to poverty of the glycogen store of the liver could be taken account of. In a few of the preliminary observations single blood specimens were obtained, either "normal" or during asphyxia or anesthesia. Although the hyperglycemia associated with these conditions can be demonstrated without difficulty by such isolated observations made on different days on the same or on different animals, it is far better to compare a succession of samples taken on the same occasion from one and the same animal.
Control observations were made on normal cats and also on cats on which laparotomy had been performed, with a certain amount of manipulation of the abdominal viscera, in order to imitate the surgical procedure in the adrenal operations, except that the adrenals and their nerve supply were left intact. It was not necessary to estimate the epinephrin output in the control animals, as we have previously shown (3) that epinephrin is invariably present in the adrenal vein blood of normal cats when tested under our experimental conditions, and that the amount liberated per minute in different individuals varies within rather narrow limits. All the animals, those in which the adrenal operation had been performed as well as the controls, were kept on the same diet and housed together (in the open air for a large part of the time). Except when purposely restricted, the diet was such as to favor the accumulation of glycogen in the liver (rice with milk, pig's liver, with fish occasionally). In the observations before July 9, no rice was given. Glycogen in plenty was demonstrated in the liver in animals which were giving off no detectable epinephrin, (e.g., 4.75 and 4.26 per cent. in two operated cats; 1.95, 2.55 and 4.13 per cent. in three control cats).

**EFFECT OF THE OPERATION ON THE OUTPUT OF EPINEPHRIN**

Our previous experiments (3) showed to what insignificant proportions the liberation of epinephrin (as determined by the denervated eye reactions without drawing blood, or on rabbit intestine and uterus segments with shed blood) is reduced by the operation practiced, even when it is not completely abolished. The values for the residual liberation in the seven cats used for survival observations in that investigation are displayed in table 1. In five of the cats the output of epinephrin was only $\frac{1}{5}$ to $\frac{1}{7}$ of the average output for normal cats. In two of the seven animals no epinephrin whatever could be detected, although in one of them (cat 52) the intestine and uterus segments were so sensitive that $\frac{1}{100}$ of the average normal output per kilogram per minute could have been estimated. In the other (cat 46) $\frac{1}{10}$ of the normal output could have been detected by the segments and $\frac{2}{10}$ by the eye reactions, which in this case happened also to be extraordinarily sensitive.

It is out of the question to assume that in this experiment epinephrin was present in the adrenal vein blood in a concentration just below the threshold of detectability for both rabbit segment and eye reactions. It is therefore as certain as anything can be which has not been actually demonstrated that if any epinephrin whatever was being given off in this animal, it represented much less than $\frac{1}{10}$ of the normal output.
We might then have assumed with confidence that the cats operated upon in a similar way for the experiments on hyperglycemia would be practically incapable of liberating epinephrin from the adrenals, either under normal circumstances or in conditions which have been supposed to cause increased liberation through the adrenal nerves. Nevertheless, in each animal the epinephrin output was determined at the end of the series of observations by the methods and under the experimental conditions previously employed for the normal cats. The results of the epinephrin estimations on the seven cats used for the blood sugar experiments after interference with the epinephrin output

* Since one of our objects in the previous investigation was to determine whether the whole secretion of epinephrin is dependent upon the integrity of the nerves, the results were expressed not only in fractions of a milligram per minute per animal, but also as fractions of a milligram per minute per kilogram of animal on the supposition that the animal still had two adrenals secreting at the same rate as the remaining gland. This showed when compared with the normal output of cats with both adrenals intact the extent to which the output of the remaining adrenal had been reduced by the operation. The residual liberation from the one adrenal expressed as a fraction of the normal liberation by one adrenal is obtained from the table by halving the denominators of the fractions in the fifth and sixth columns.

† There was no evidence that any epinephrin was being given off in these animals.
are given in table 2. The adrenalin solution employed for the blood assay was always itself freshly assayed by the method of Folin, Cannon and Denis.

### TABLE 2

<table>
<thead>
<tr>
<th>NUMBER</th>
<th>WEIGHT</th>
<th>EPIINEPHRIN OUTPUT</th>
<th>FRACTION OF NORMAL LIBERATION</th>
<th>EYE REACTIONS</th>
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<tbody>
<tr>
<td></td>
<td>kgm.</td>
<td>mgm.</td>
<td>mgm.</td>
<td>Per minute</td>
</tr>
<tr>
<td>90</td>
<td>1.5</td>
<td>0.000003</td>
<td>0.000002</td>
<td>$\frac{1}{2}$</td>
</tr>
<tr>
<td>108</td>
<td>2.2</td>
<td>0.000005</td>
<td>0.000002</td>
<td>$\frac{1}{2}$</td>
</tr>
<tr>
<td>100</td>
<td>3.22</td>
<td>0.00001</td>
<td>0.000003</td>
<td>$\frac{1}{2}$</td>
</tr>
<tr>
<td>107</td>
<td>1.27</td>
<td>0.00013</td>
<td>0.00001</td>
<td>$\frac{1}{2}$</td>
</tr>
<tr>
<td>91</td>
<td>2.06</td>
<td>0.0001</td>
<td>0.00005</td>
<td>$\frac{3}{4}$</td>
</tr>
<tr>
<td>92</td>
<td>1.93</td>
<td>0.00002</td>
<td>0.00001</td>
<td>$\frac{3}{4}$</td>
</tr>
<tr>
<td>121†</td>
<td>1.7</td>
<td>0.000015</td>
<td>0.00001</td>
<td>$\frac{3}{4}$</td>
</tr>
</tbody>
</table>

* There was no evidence that any epinephrin was being given off.
† Left adrenal excised; nerves of right adrenal cut.

It will be seen that the results are precisely the same in these seven cats as in the seven reported in table 1. In two of them no epinephrin whatever was detected in the adrenal vein blood by the segment tests. No attempt was made to fix the minimum concentration of epinephrin which the segments could detect but it was shown that good reactions were still given with concentrations which with the observed blood flows through the adrenals would have corresponded to an output per kilogram of body weight per minute one hundred and twenty times less than the average for normal cats. There is no doubt that the quantity which could possibly have been present was still smaller. In cat 108 the eye tests gave as the possible maximum output the same fraction, $\frac{1}{2}$ of the normal, as the intestine tests. Again, it is extremely unlikely that had the reactions been a little more sensitive they would have detected epinephrin. It is much more probable that just as in the series shown in table 1, the fortunate coincidence...
of specially sensitive test objects and blood specimens from animals in which the
secretory nerves had been severed with unusual completeness would have enabled
us to drive down the limit of the possible epinephrin output far beyond that
actually obtained. The question, however, is of no consequence for our pur-
pose. For an animal which cannot be liberating \( \frac{1}{3}\) of the normal amount of
epinephrin, owing to section of secretory nerve fibers, is certainly no more cap-
able of responding to stimulation of any remaining fibers by an outburst, bring-
ing the output far above the normal, than if it had been shown that the rate of
liberation had been reduced to \( \frac{1}{2}\) or \( \frac{1}{3}\) of the normal. It must always be
remembered that no evidence was obtained in these animals that any epinephrin
was being given off. It is obvious that in connection with the problem whether
an experimental hyperglycemia depends upon increased epinephrin secretion,
experiments in which no epinephrin has been detected with sensitive test ob-
jects are more important than those in which a small residual liberation is still
present. In a third cat of this series (cat 92) the result of the epinephrin assay
was also negative. But here there were only the eye reactions to go by, not
enough blood having been obtained for satisfactory segment tests. Still, the eye
reactions were quite sensitive and showed that the output per kilogram per
minute could not have been \( \frac{1}{6}\) of the normal average.\(^1\)

In one animal of the series (cat 91) a substantial fraction of the normal aver-
age output of epinephrin was found, \( \frac{1}{5} \) or \( \frac{1}{6} \) by the intestine segments and \( \frac{1}{5} \) by
the eye tests. There was no question that a marked diminution in the output
per minute had been effected by the operation in this animal. For the concen-
tration of epinephrin in the adrenal vein blood was far less than is ever seen in
a normal cat under our experimental conditions, for the corresponding rates of
adrenal blood flow. As the epinephrin assay was made sixty-one days after the
nerve section, the possibility of some regeneration of the secretory fibers might
be considered but we have no evidence as to this. If any regeneration had
occurred in this time, the output of epinephrin determined at the end of the
period would be greater than it was after section of the nerves. However, since
this animal yielded precisely the same results as the others in the blood sugar
observations, the question is of no significance for our present purpose.

To sum up, if tables 1 and 2 are compared it will be seen that the
results of the operation, as practiced by us, upon the residual epinephrin
secretion are of the same general character for the two series. In each
group of seven cats two gave no evidence with either test of any epine-
phrin output whatever. In each group one cat showed a somewhat
substantial residual liberation, although only a mere fraction of the

\(^1\) Generally the eye reactions, as stated in a previous paper (3), will not de-
tect such small outputs of epinephrin as the segment tests with shed blood.
This depends, however, not only upon the threshold concentration and quan-
tity of epinephrin which yield a just detectable reaction, but also upon the
length of time during which it is feasible to continue the collection of the ad-
renal vein blood in the cava pocket, and this in turn depends upon the rate of
blood flow through the gland.
normal. The remaining cats in each group were proved to be giving off a very small amount of epinephrin (1/4 down to 1/8 of the normal), except cat 92 in the second group (table 2), which by the only tests applied, the eye reactions, yielded a negative result. If we reflect that to ensure the severing of the secretory innervation of the adrenal a far larger number of fibers which have nothing to do with the epinephrin secretion must be cut, it will readily be seen that the completeness with which the fibers in question are divided may vary in the different operations. Let this be as it may, the tables demonstrate conclusively that to all intents and purposes the epinephrin secretion by the adrenals may be considered as non-existent in cats after this operation and that any effect produced upon the blood sugar content by given conditions cannot be mediated through the nervous mechanism which normally governs the liberation of epinephrin from these glands.

One other remark may be made before passing from the consideration of these tables. It will be noticed that in table 2, the fraction of the normal output represented by the residual liberation expressed per kilogram of body weight is in general greater than the fraction expressed for the whole animal, while the opposite is the case in table 1. The reason for this, or at least the main reason, is a purely artificial one, namely that in table 2 the body weight of most of the cats is less than the body weight of the majority of those in table 1. This is partly a matter of accident but partly due to the fact that the cats in table 1, after the loss of weight which always occurs in the first weeks after the operation, remaining undisturbed by further interference, rapidly regained their original weight and in several instances became considerably heavier than before the operation. The animals in table 2 were used for the blood-sugar observations, samples of blood being repeatedly taken from them; they were subjected to periods of asphyxia or anesthesia, and naturally most of them lost some weight.

THE NORMAL BLOOD-SUGAR CONTENT IN THE CAT

It has already been stated that in determining whether an increase in the sugar content of the blood was caused by the conditions investigated, comparison was not made with an average "normal" content deduced from observations made at other times on the same or on other animals, but successive samples collected at the time of each experiment were compared. In testing out the technique, however, a number of normal sugar estimations were first made. Some of these are given in table 3, with the duplicate estimations; in the rest of the paper only the average of the duplicate observations is given. For convenience, estimations on two dogs are included in the table.
TABLE 3

<table>
<thead>
<tr>
<th>NUMBER OF ANIMAL</th>
<th>PERCENTAGE OF DEXTROSE IN BLOOD</th>
<th>REMARKS</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Duplicates</td>
<td>Average</td>
</tr>
<tr>
<td>1</td>
<td>0.106</td>
<td>0.115</td>
</tr>
<tr>
<td>2</td>
<td>0.081</td>
<td>0.083</td>
</tr>
<tr>
<td>3</td>
<td>0.108</td>
<td>0.108</td>
</tr>
<tr>
<td>4</td>
<td>0.117</td>
<td>0.116</td>
</tr>
<tr>
<td>5</td>
<td>0.107</td>
<td>0.111</td>
</tr>
<tr>
<td>7</td>
<td>0.103</td>
<td>0.103</td>
</tr>
<tr>
<td>11</td>
<td>0.081</td>
<td>0.081</td>
</tr>
<tr>
<td>19*</td>
<td>0.094</td>
<td>0.097</td>
</tr>
<tr>
<td>15†</td>
<td>0.113</td>
<td>0.114</td>
</tr>
<tr>
<td>16†</td>
<td>0.105</td>
<td>0.104</td>
</tr>
</tbody>
</table>

* This cat was given no liver for two days before the blood samples were obtained, and no food at all for twenty-four hours, two days before the blood experiment. All the others were on the liver diet with milk daily, but no rice.
† Dogs: no. 15, a large female hound; no. 16, a small female fox terrier

Scott (4) has published numerous blood-sugar estimations on cats. He worked with large quantities of blood obtained by decapitation, precipitating the proteins by a special method and estimating the sugar by a method described by Munsen and Walker (5). Our "normal" results for cats agree fairly well with those given by him but are on the whole somewhat higher. We have not employed dogs for the adrenal operations because the nerve paths whose section abolishes or greatly lessens the epinephrin output are better known in the cat than in the dog.

Experiments on cats in which the epinephrin output was interfered with

Cat 90. Condensed protocol. Weight, 1.82 kgm. Right adrenal excised and nerves of left adrenal cut on May 9.

<table>
<thead>
<tr>
<th>BODY-WEIGHT</th>
<th>PERCENTAGE OF BLOOD-SUGAR</th>
</tr>
</thead>
<tbody>
<tr>
<td>km.</td>
<td></td>
</tr>
<tr>
<td>May 22</td>
<td>1.025</td>
</tr>
<tr>
<td>June 11</td>
<td>1.035</td>
</tr>
<tr>
<td>June 30</td>
<td>1.495</td>
</tr>
<tr>
<td>June 28</td>
<td>1.085</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table of Percentage of Blood-Sugar

<table>
<thead>
<tr>
<th></th>
<th>Preliminary specimen</th>
<th>Ether</th>
<th>Asphyxia</th>
</tr>
</thead>
<tbody>
<tr>
<td>June 21</td>
<td>1.515</td>
<td>0.20*</td>
<td>0.233</td>
</tr>
<tr>
<td>June 27</td>
<td>1.67</td>
<td>0.087</td>
<td>0.151</td>
</tr>
<tr>
<td>July 2</td>
<td>1.78</td>
<td>0.07</td>
<td>0.168</td>
</tr>
<tr>
<td>July 7</td>
<td>1.635</td>
<td>0.089</td>
<td>0.164</td>
</tr>
</tbody>
</table>

* During collection of this specimen the blood flowed very slowly and was very dark.

July 13. Weight, 1.5 kgm.
10.00 a.m. 3 grams urethane by stomach tube.
10.30 a.m. Prepared cava pocket, tying all arteries, i.e., renal, coeliac and mesenteric arteries and abdominal aorta.
11.20 a.m. Pocket experiment, 2 minutes occlusion; no eye reactions.
11.30 a.m. Pocket experiment, 4 minutes occlusion; no eye reactions.
11.35 a.m. Pocket experiment, 6 minutes occlusion; no eye reactions.
11.38 a.m. 0.5 cc., 1:1,000,000 adrenalin injected; very slight pupil, no nictitating reaction.
11.45 a.m. Repeated last observation with same result.

Then collected the following specimens of adrenal blood:
First specimen, 1.5 grams in 1½ minutes, blood flow 1 gram per minute.
Second specimen, 4.5 grams in 5 minutes, blood flow 0.9 gram per minute.
Third specimen, 5.8 grams in 7 minutes, blood flow 0.83 gram per minute.
Right adrenal weighed 0.206 gram, and contained 0.18 mgm. epinephrin.
Left adrenal weighed 0.186 gram, and contained 0.17 mgm. epinephrin.

Some of the tracings illustrating the epinephrin assay are reproduced in figures 1 to 3. Figure 1 shows that the second adrenal specimen diluted with three volumes of Ringer's solution caused no inhibition of the intestine (observation 21), and that the undiluted blood could not have contained 1:150,000,000 epinephrin since indifferent blood containing this amount caused a distinct inhibition (observation 23).

That the limit must have been decidedly lower than 1:150,000,000 is shown by the fact that in reducing the concentration from 1:90,000,000 (fig. 2, observation 13), to 1:150,000,000 (fig. 1) only a moderate reduction takes place in the inhibitory effect, whereas the difference between the effect of 1:30,000,000 (fig. 2, observation 11) and 1:90,000,000 is very great. In figure 3, it is proved that indifferent blood containing a concentration of 1:200,000,000 adrenalin (observation 41) causes a much greater increase of tone of a uterus segment than the second adrenal blood specimen (observation 37), or than the third specimen (observation 39). The comparison of observations 36 and 42...
shows that this uterus segment could detect a concentration of 1:300,000,000. We can conclude that the second adrenal specimen does not contain 1:200,000,000, probably not 1:300,000,000. It is certainly quite safe to assume that it could not have contained 1:250,000,000. Therefore, the output of epinephrin could not have been 0.0000035 mgm. per minute, i.e., 0.000002 mgm. per kilo of body weight per minute, or $\frac{1}{35}$ of the normal average output per kilogram, as determined by rabbit segments on shed blood. There was no evidence that any epinephrin was being given off.

Fig. 1. Intestine tracings. Bloods from cat 90. At 20 Ringer was replaced by indifferent (arterial) blood, and this at 21 by the second adrenal blood specimen. Both bloods were diluted with three volumes of Ringer's solution. At 22 Ringer's solution was replaced by indifferent blood diluted with three volumes Ringer, and this at 23 by the indifferent blood made up with adrenalin to a concentration of 1:150,000,000, the mixture being then diluted with three volumes Ringer before application to the segment. (Reduced to two-thirds.)

Cat 108. Condensed protocol. Female. Weight, 2.75 kgm. Right adrenal excised and nerves of left adrenal cut July 6.

<table>
<thead>
<tr>
<th>WEIGHT</th>
<th>PERCENTAGE OF BLOOD-SUGAR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Preliminary specimen</td>
</tr>
<tr>
<td>July 17</td>
<td>2.4</td>
</tr>
<tr>
<td>July 26</td>
<td>2.475</td>
</tr>
<tr>
<td>August 6</td>
<td></td>
</tr>
</tbody>
</table>

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Fig. 2. Intestine tracings. Bloods from cat 90. At 10 and 12 Ringer's solution was replaced by indifferent blood diluted with three volumes Ringer, and this at 11 and 13 by the indifferent blood made up with adrenalin to a concentration of 1:30,000,000 and 1:90,000,000, respectively, the adrenalin bloods being then diluted with three volumes Ringer before application to the segment. (Reduced to two-thirds.)

Fig. 3. Uterus tracings. Bloods from cat 90. At 36 Ringer was replaced by indifferent (arterial) blood; at 37 by the second adrenal specimen; at 39 by the third adrenal specimen; at 40 by the indifferent blood to which adrenalin had been added to make up a concentration of 1:100,000,000; at 41 by the indifferent blood to which adrenalin had been added to make up 1:200,000,000; at 42 by the indifferent blood to which adrenalin had been added to make up 1:300,000,000. All the bloods were undiluted. (Reduced to one-half.)
ADRENALS AND EXPERIMENTAL HYPERGLYCEMIA

August 2. Excised left superior cervical ganglion.
August 10. Weight, 2.2 kgm. Made cava pocket under urethane (4 grams), tying all arteries.
1.10 p.m. Pocket experiment 4 minutes: no eye reactions.
1.16 p.m. Pocket experiment 6 minutes: no eye reactions.
1.25 p.m. 0.5 cc. adrenalin, 1: 1,200,000 injected: very good eye reactions in 7.2 seconds.
1.28 p.m. 0.5 cc. adrenalin, 1: 2,300,000 injected: good eye reactions in 8 seconds.
1.34 p.m. 0.5 cc. adrenalin, 1: 4,500,000 injected: slight eye reactions in 10.2 seconds.
1.38 p.m. 0.5 cc. adrenalin, 1: 7,000,000 injected: small retraction of nictitating in 10.2 seconds.

Now collected the following specimens of adrenal blood:
First specimen, 3.2 grams in 1 minute, blood flow 3.2 grams per minute.
Second specimen, 7.8 grams in 3.5 minutes, blood flow 2.2 grams per minute.
Third specimen, 3.0 grams in 3 minutes, blood flow 1.0 gram per minute.
Then obtained blood from abdominal aorta.
Right adrenal weighed 0.176 gram, and contains 0.23 mgm. epinephrin.
Left adrenal weighed 0.278 gram, and contained 0.26 mgm. epinephrin.

Some of the tracings of the epinephrin assay are reproduced in figures 4 to 6. In figure 4 it is shown that the second adrenal specimen (observation 4) gave no inhibition of the intestine when diluted with an equal volume of Ringer's solution. Another observation, not reproduced, proved that even when undiluted it caused no inhibition, which was also true of the third adrenal specimen (observation 14). Observation 16 (fig. 5) indicates that even the third specimen could not have contained nearly 1: 200,000,000 epinephrin. Comparison of observations 10 and 16 suggests that the limit of sensitiveness of the intestine segment had not been nearly reached with a concentration of 1: 200,000,000, since the inhibitory effect at 16 is far from insignificant as compared with that at 10. In figure 6 it is demonstrated by uterus tests that the second adrenal specimen (observation 21) contained less epinephrin than 1: 230,000,000 (observation 20). Indeed, the effect of this sample on the uterus was not greater than that of the indifferent blood (observation 19). Taking the rate of blood flow through the adrenals during collection of the third specimen as 1 cc. per minute, it follows that the output of epinephrin per minute could not have been nearly as much as 0.000005 mgm. per minute for the animal, or 0.000002 mgm. per kilogram of body weight per minute, i.e., less than 1/10 of the average output of normal cats, as determined on rabbit intestine and uterus segments. If any epinephrin at all was being given off, of which there was no evidence, the amount must have been decidedly
less than this. The eye reactions, which in this animal were very sensitive, also gave a completely negative result, even for a six-minute occlusion of the pocket. From these reactions it can also be calculated that the possible output could not have been \( \frac{1}{10} \) of the normal as determined by the eye tests.

---

**Fig. 4.** Intestine tracings. Bloods from cat 108. At 3 Ringer's solution was replaced by indifferent (arterial) blood, and this at 4 by the second adrenal blood specimen. Both bloods were diluted with one volume Ringer before application to the segment. At 13 Ringer was replaced by the indifferent blood undiluted, and this at 14 by the third adrenal blood sample also undiluted. After observation 4 was completed the drum and writing point were lowered. (Reduced to two-thirds.)

**Cat 98.** Condensed protocol. Female. Weight, 2.525 kgm. Right adrenal excised and nerves of left cut May 9.

May 21. Weight 2.075 kgm. Blood sugar after light ether anesthesia, 0.154 per cent.

May 29. Excised left superior cervical ganglion.

June 20. Weight, 2.285 kgm., tied on board 3 minutes. Blood sugar, 0.098 per cent. After being on board 28 minutes, blood sugar, 0.108 per cent.
ADRENALS AND EXPERIMENTAL HYPERGLYCEMIA

<table>
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* Very slow collection and serum separated in the pipette.

July 9. Weight, 1.93 kgm., cava pocket formed under urethane (4 grams) with all arteries tied.
12.50 p.m. Pocket experiment, 3 minutes: no eye reactions.
12.55 p.m. Pocket experiment, 5 minutes: no eye reactions.
1.05 p.m. 0.5 cc. adrenalin, 1:1,000,000 injected; very good eye reactions in 10.2 seconds.
1.08 p.m. 0.25 cc. adrenalin, 1:1,000,000 injected: good eye reactions in 10.4 seconds.
1.10 p.m. 0.5 cc. adrenalin, 1:3,000,000 injected: small pupil; no nictitating reaction, 11.6 seconds.
1.18 p.m. 0.5 cc. adrenalin, 1:4,000,000 injected: small pupil and nictitating reaction, 10.8 seconds.
1.20 p.m. 0.5 cc. adrenalin, 1:5,000,000 injected: small pupil and nictitating reaction, 11.4 seconds.

A small specimen of adrenal blood was obtained, but not sufficient for assay, as the cat died shortly after collection was begun.
Left adrenal weighed 0.250 gram, and contained 0.19 mgm. epinephrin.
Right adrenal was crushed in removal, no assay.

In this animal the eye tests showed that a reaction could be gotten with 0.0001 mgm. epinephrin, whereas the adrenal vein blood collected for five minutes and then released produced no reaction whatever. Accordingly, the output of epinephrin could not have been as great as 0.00002 mgm. per minute, i.e., 0.00001 mgm. per kilogram of body weight per minute. This is not \( \frac{1}{6} \) of the average output in normal cats, as determined by eye reactions. There was no evidence that any epinephrin was being given off.
Fig. 5. Intestine tracings. Bloods from cat 108. At 9 and 15 Ringer's solution was replaced by the indifferent blood (undiluted), and this at 10 and 16 by the indifferent blood made up with adrenalin to a concentration of 1: 115,000,000 and 1: 200,000,000 respectively. (Reduced to two-thirds.)

Fig. 6. Uterus tracings. Bloods from cat 108. At 19 Ringer's solution was replaced by the indifferent blood; at 20 by the indifferent blood made up with adrenalin to a concentration of 1: 230,000,000; at 21 by the second adrenal specimen. All the bloods were undiluted. (Reduced to one-half.)

<table>
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<th>WEIGHT</th>
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August 2. Excised left superior cervical ganglion.

August 14. Weight, 3.22 kgm., cava pocket formed under urethane (5 grams), all arteries tied.

12.28 p.m. Pocket experiment, 3 minutes: no eye reactions.
12.30 p.m. Pocket experiment, 6 minutes: no eye reactions.

0.5 cc. adrenalin, 1:2,300,000 injected: slight nictitating reaction in 11.2 seconds.
0.5 cc. adrenalin, 1:1,150,000 injected: good pupil and nictitating in 8.2 seconds.

Now collected the following adrenal blood specimens:
First specimen, 2.1 grams in 1 minute, blood flow 2.1 grams per minute.
Second specimen, 6.9 grams in 6 minutes, blood flow 1.15 grams per minute.
Third specimen, 3.4 grams in 5 minutes, blood flow 0.7 gram per minute.

Obtained blood from abdominal aorta.
Right adrenal weighed 0.365 gram and contained 0.38 mgm. epinephrin.
Left adrenal weighed 0.331 gram and contained 0.34 mgm. epinephrin.

Some of the tracings of the epinephrin assay are given in figures 7 to 9. The second adrenal specimen diluted with three volumes of Ringer's solution (fig. 7, observation 2) gave no inhibition of the intestine, while indifferent blood containing 1:35,000,000 adrenalin similarly diluted gave a good inhibition. In figures 8 and 9 it is proved that the undiluted second adrenal blood specimen, while causing distinct inhibition of the intestine, could not have contained 1:85,000,000 epinephrin though somewhat more than 1:115,000,000. Taking the concentration as the average of these two observations, i.e., 1:100,000,000, we get 0.00001 mgm. as the output per minute or 0.000003 mgm. per kilogram of body weight per minute. This is only 2 of the average output in normal cats, as estimated by rabbit segments in drawn blood.

2 The first small sample is collected apart, in order to get rid of any epinephrin which may have been liberated by manipulation when the upper end of the pocket is being clipped off. The much greater apparent rate of blood flow sometimes seen in the collection of this sample is partly due to the inclusion of some blood already in the pocket when the clamp is applied.
Fig. 7. Intestine tracings. Bloods from cat 109. At 1 Ringer's solution was replaced by indifferent blood and this at 2 by the second adrenal blood specimen. Both bloods were diluted with three volumes Ringer. At 3 Ringer's was replaced by the indifferent blood (diluted with three volumes Ringer), and this at 4 by the indifferent blood made up with adrenalin to a concentration of 1:35,000,000, the mixture being then diluted with three volumes Ringer before application to the segment. (Reduced to two-thirds.)

Fig. 8. Intestine tracings. Bloods from cat 109. At 5 Ringer's solution was replaced by the indifferent blood, and this at 6 by the second adrenal blood sample. At 7 Ringer was replaced by the indifferent blood, and this at 8 by the indifferent blood made up with adrenalin to a concentration of 1:115,000,000. All the bloods were undiluted. (Reduced to two-thirds.)
The eye reactions were negative even for a six-minute collection of the adrenal vein blood although a reaction was obtained when as little as 0.0002 mgm. adrenalin was injected. It can be calculated from these data that the output per kilogram of body weight per minute could not have been $\frac{1}{4}$ of the average output in normal cats, as determined by the eye reactions.

**Fig. 9.** Intestine tracings. Bloods from cat 109. At 15 Ringer's solution was replaced by the indifferent blood, and this at 16 by the second adrenal blood sample. At 17 Ringer was replaced by the indifferent blood, and this at 18 by the indifferent blood to which adrenalin had been added to make up 1: 85,000,000. All the bloods were undiluted. The weight was less than in figure 8. (Reduced to two-thirds.)

*Cat 107.* Condensed protocol. Female. Weight 1.82 kgm. Right adrenal excised and nerves of left cut, July 6.
August 2. Excised left superior cervical ganglion.

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August 9. Weight 1.27 kgm. Under urethane (3 grams) cava pocket formed with all arteries tied. Positive pupil reactions were obtained on collecting adrenal vein blood in the cava pocket and then releasing it, but there was no movement of the nictitating membrane. The following samples of adrenal blood were collected.

First sample, 1.1 grams in 1 minute, blood flow 1.1 grams per minute.
Second sample, 3.8 grams in 5 minutes, blood flow 0.8 gram per minute.
Third sample, 3.3 grams in 7 minutes, blood flow 0.47 gram per minute.
Indifferent blood was obtained from the abdominal aorta.
Right adrenal weighed 0.168 gram and contained 0.23 mgm. epinephrin.
Left adrenal weighed 0.152 gram and contained somewhat more than 0.12 mgm. epinephrin.

Figure 10, in which a few of the tracings of the epinephrin assay are reproduced, shows that even the third adrenal specimen, in spite of the relatively small flow of blood during its collection, had scarcely $1: 35,000,000$ epinephrin and much less than $1: 17,000,000$. The epinephrin output was therefore less than $0.000015$ mgm., i.e., about $0.000001$ mgm. per kilogram of body weight per minute. This is not more than $\frac{1}{2}$ of the average output of normal cats per kilogram. It is only because of the slow blood flow that the concentration is even as great as $1: 35,000,000$.

Cat 91. Condensed protocol. Female. Weight, 2.23 kgm. Right adrenal excised and nerves of left out, May 9.
May 29. Weight 2.00 kgm., dextrose in blood, 0.105 per cent.
May 29. Excised left superior cervical ganglion.
June 20. Weight, 1.95 kgm. Tied on board 3 minutes, blood-sugar, 0.108 per cent. Still tied on board 12 minutes, blood-sugar, 0.108 per cent.

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<th>WEIGHT</th>
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July 9. Weight 2.065 kgm. Under urethane (4 grams) cava pocket formed with all arteries tied.
4.23 p.m. Pocket experiment, 2 minutes: slight pupil reaction in 9 seconds.
4.25 p.m. Pocket experiment, 2 minutes: slight pupil reaction in 11.6 seconds.
4.28 p.m. Pocket experiment, 4 minutes: small pupil and nictitating reaction in 10 seconds.
4.35 p.m. 0.5 cc. adrenalin, 1:1,000,000 injected: good pupil, slight nictitating in 9.2 seconds.
Fig. 10. Intestine tracings. Bloods from cat 107. At 24 Ringer was replaced by indifferent (arterial) blood, and this at 25 by the third adrenal blood sample. Both bloods were diluted with one volume of Ringer. At 26 Ringer was replaced by the indifferent blood diluted with an equal volume of Ringer, and this at 27 by the indifferent blood made up with adrenalin to 1: 17,000,000, and then diluted with an equal volume of Ringer. At 28 Ringer was replaced by the indifferent blood diluted with an equal volume of Ringer, and this at 29 by the indifferent blood made up with adrenalin to a concentration of 1: 35,000,000 and then diluted with an equal volume of Ringer. (Reduced to two-thirds.)
4.40 p.m. 0.5 cc. adrenalin, 1:2,000,000 injected: small pupil in 12.2 seconds (like observation at 4.28).
4.45 p.m. Pocket experiment, 4 minutes; pupil reaction in 8.2 seconds, greater than at 4.40, more like that at 4.35.
4.48 p.m. 0.5 cc. adrenalin, 1:1,000,000: slight pupil reaction in 12.4 seconds.
4.57 p.m. Pocket experiment, 2 minutes: small pupil reaction in 8.6 seconds.
5.00 p.m. 0.5 cc. adrenalin, 1:1,000,000 injected; good pupil reaction in 10.8 seconds (larger than in observation at 4.57).
5.07 p.m. Pocket experiment, 3 minutes; pupil reaction nearly the same as that in observations at 5.00.

A number of additional observations on the eye reactions with adrenalin injection, and adrenal vein blood released from the pocket were made. Then the following specimens of adrenal blood were drawn through a cannula in the cava and tested on rabbit intestine and uterus segments.

First specimen, 1.1 grams in 2 minutes, blood flow, 0.55 gram per minute. 
Second specimen, 2.5 grams in 15 minutes, blood flow 0.17 gram per minute. 
Third specimen, 1.8 grams in 23 minutes, blood flow 0.08 gram per minute.
The blood flowed very slowly.
Right adrenal weighed 0.232 gram and contained 0.26 mgm. epinephrin.
Left adrenal weighed 0.265 gram and contained somewhat more than 0.16 mgm. epinephrin.

In figures 11 and 12 are reproduced some of the tracings of the epinephrin assay. It gave much the greatest concentrations of epinephrin in the adrenal vein seen in the series of cats whose adrenal innervation had been interfered with. The second adrenal specimen had a concentration of epinephrin about equal to 1:1,500,000 (fig. 11). It was distinctly greater than 1:2,000,000. In the third specimen the concentration was greater than 1:800,000, and less than 1:500,000 (fig. 12). The blood had an unusually high proportion of plasma, not far from 90 per cent, and the flow during collection was very small. For both reasons the concentration is high. We have stated elsewhere (3), (6) that a concentration of more than 1:1,000,000 is unusual in cats' adrenal vein blood, but the proportion in serum can be considerably higher since the erythrocytes contain practically no epinephrin. The output of epinephrin calculated for the second adrenal specimen was 0.0001 mgm. per minute for the animal and 0.00005 mgm. per kilogram per minute, or about \( \frac{1}{2} \) of the normal output in the cat as determined on drawn blood by rabbits' intestine segments. Taking the concentration in the third specimen at 1:650,000, we get 0.00012 mgm. per minute, or 0.00006 mgm. per kilogram of body weight per minute.

The eye reactions gave about 0.00015 mgm. per minute, or 0.00007 mgm. per kilogram of body weight per minute as the output, i.e., about \( \frac{1}{3} \) or \( \frac{1}{4} \) of the normal as determined by the eye tests.
Fig. 11. Intestine tracings. Bloods from cat 91. At 10 and 17 Ringer's solution was replaced by indifferent (arterial) blood, diluted with five volumes Ringer, and this at 11 and 18 by the indifferent blood made up with adrenalin to concentrations of 1: 1,500,000 and 1: 2,000,000 respectively, the adrenalin bloods being then diluted with five volumes Ringer before application to the segment. At 19 Ringer's solution was replaced by the indifferent blood and this at 20 by the second adrenal blood specimen, both bloods being diluted with five volumes Ringer. (Reduced to two-thirds.)
Fig. 12. Intestine tracings. Bloods from cat 91. At 25 and 31 Ringer's solution was replaced by the indifferent blood diluted with five volumes Ringer, and this at 26 and 32 by the indifferent blood made up with adrenalin to concentrations of 1:800,000 and 1:500,000 respectively, the adrenalin bloods being diluted with five volumes Ringer before application to the segment. At 29 Ringer's solution was replaced by the indifferent blood and this at 30 by the third adrenalin blood sample, both bloods being diluted with five volumes Ringer. In observation 32 the writing point went below the drum and stayed down for a time. (Reduced to two-thirds.)
ADRENALS AND EXPERIMENTAL HYPERGLYCEMIA

It is commonly stated that in the rabbit the left splanchnic is much more important than the right for the production of some of the experimental hyperglycemias. Although there is no reason to believe that such a difference exists in the cat, the left adrenal was removed in one of the animals (cat 121) and the nerves of the right adrenal, including of course the right splanchnic, cut. The results differed in no way from those obtained on the other cats.

Cat 121. Condensed protocol. Female. Weight, 1.975 kgm. Left adrenal excised and nerves of right cut, July 31.

August 13. Weight, 1.71 kgm. Preliminary blood specimen, 0.111 per cent dextrose. Asphyxia, 0.163 per cent dextrose.

August 16. Excised left superior cervical ganglion.

August 24. Weight, 1.70 kgm. Under urethane (3 grams) cava pocket formed, with all arteries tied.

11.35 a.m. Pocket experiment, 34 minutes: no eye reactions.

11.40 a.m. Pocket experiment, 6 minutes: no eye reactions.

11.50 a.m. 0.5 cc. adrenalin, 1:1,150,000: excellent eye reactions in 11.8 seconds.

0.5 cc. adrenalin, 1:2,300,000: good eye reactions in 17.4 seconds.

Now collected the following specimens of adrenal vein blood:

First specimen, 0.8 gram in 1 minute, blood flow 0.8 gram per minute.

Second specimen, 3.6 grams in 0½ minutes, blood flow 0.5 gram per minute.

Third specimen, 5.2 grams in 11 minutes, blood flow 0.47 gram per minute.

Fourth specimen, 2.0 grams in 6 minutes, blood flow 0.33 gram per minute.

During collection of the fourth specimen, a clot had to be squeezed out of the pocket.

Right adrenal weighed 0.242 gram and contained 0.26 mgm. epinephrin.

Left adrenal weighed 0.152 gram and contained 0.16 mgm. epinephrin.

It was shown on the rabbit intestine and uterus segments that the third adrenal blood specimen contained about 1:30,000,000 epinephrin. The rate of blood flow was approximately 0.45 cc. per minute. Accordingly the output of epinephrin per minute was about 0.000015 mgm. per minute for the animal or 0.00001 mgm. per kilogram of body weight per minute, i.e., \( \frac{1}{30,000} \) of the average normal output, as determined on drawn blood with rabbits' segments. The eye reactions were negative even for a six-minute collection although 0.0002 mgm. of adrenalin gave a reaction which was more than minimal. The output of epinephrin was therefore not above 0.000035 mgm. per minute for the animal (about \( \frac{1}{30} \) of the normal average), or 0.00002 mgm. per kilogram of body weight per minute, (about \( \frac{1}{30} \) of the normal average), as determined by eye reactions.

As regards the blood-sugar percentages, it will be seen from the protocols that asphyxia and etherization caused distinct hyperglycemia in the cats whose epinephrin output had been abolished, within the
limits of detection by the methods employed as well as in those in which a small residual output was still present. If the results on these cats are compared with those obtained in the same way on normal cats (tables 4 and 5) it will be seen that no essential difference can be made out. The precise amount of the hyperglycemia has no particular significance for it is well known that this is influenced greatly by the nutritive condition of the animals, particularly by the glycogen content of the liver. The same is true of the occasional failure to obtain the expected hyperglycemia, which is seen in the normal animals as well as in those subjected to the adrenal operation. It is conceivable that in the operated cats the loss of one major splanchnic, in addition to other sympathetic fibers, possibly going to the liver, if these nerves are at all concerned in the hyperglycemia studied, may have prevented the percentage of dextrose in the blood from rising as high after asphyxia or etherization as in the normal cats, although it is not cer-

TABLE 4

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* This was the date of the operation.

tain from the results that there is any decided deficiency in this respect in the former group.

In table 4 are given some observations on two cats in which a dummy operation was performed, as already mentioned, to imitate as far as possible the nutritive consequences of the adrenal operation, except that in these cats the adrenals were intact.

In table 5 are displayed the results of the blood-sugar estimations on a series of normal cats subjected to the same procedures as the cats which had undergone the adrenal operations.

From these results it seems impossible to draw any other conclusion than that the hyperglycemia associated with asphyxia and with etherization is not produced through the intervention of epinephrin liberated from the adrenal glands, or at least that the liberation of epi-
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</table>

* Struggled and micturated during collection of normal specimen.
† The ether was too concentrated (in jar); artificial respiration had to be performed.
‡ The animal had distemper and had lost weight.
§ Blood was obtained with difficulty; finally the femoral artery was cut and sample A collected into 2 per cent oxalate (without laking), and B into water (well laked).
|| These cats were kept tied on the board during the period of frightening; the others were placed in a cage.
* Very much excited during collection of normal specimen.
* A, collected immediately after the animal was tied on the board; B, after it had been tied for eight minutes on the board.
nephrin is not essential to the production of the hyperglycemia. The question whether these experimental hyperglycemias depend upon nervous influences exerted upon the liver so as to hasten the transformation of glycogen into dextrose, or upon a more direct influence, e.g., through changes in the hydrogen-ion concentration of the blood (7) does not concern us here. In one experiment (cat 113) after the right adrenal had been excised and the nerves of the left cut, as in the ordinary operation, the right major splanchnic was also divided after an interval of twenty-seven days. Fourteen days after the second operation, the animal being in excellent condition, the preliminary blood sample collected while the cat was quiet contained 0.089 per cent of dextrose. A second sample, taken after the animal had been frightened by a dog for thirty-eight minutes, contained 0.084 per cent. Urine passed two to three minutes after the end of the frightening period gave a negative test for sugar. A third blood sample obtained after fifteen minutes of asphyxiation, immediately succeeding the period of fright, contained 0.153 per cent. The asphyxiation hyperglycemia was therefore unmistakably produced in this animal after section of both splanchnics. However, our problem was merely to determine whether those hyperglycemias could or could not be produced in the absence of epinephrin. If epinephrin is not necessary for the marked and prompt augmentation of the blood-sugar observed in the conditions studied, then the deductions which have been drawn as to the importance of the epinephrin secretion of the adrenals in the mobilization of sugar can no longer be upheld.

We desire to make it clear that our present conclusions concern solely the hyperglycemia produced by asphyxiation and etherization in cats. Whether such a hyperglycemia as that produced by piqure, in which according to the best evidence the nervous system is essentially concerned, can also be elicited in the absence of epinephrin we have not as yet sufficient data to decide. The hypoglycemia described by certain observers after adrenalectomy was not seen in our animals. The average for 19 "normal" or preliminary blood samples from the control cats was 0.096 per cent. of dextrose. The average for 19 similar samples from the cats which had been subjected to the adrenal operation was the same (0.095 per cent.).

SO-CALLED EMOTIONAL HYPERGLYCEMIA

A question which has been much discussed and which cannot very well be avoided in work on the blood-sugar, is the influence of emotional
disturbances upon the sugar content. Some writers (8) have convinced themselves that emotional hyperglycemia is so easily produced in the ordinary laboratory animals that it is impossible to obtain "normal" sugar percentages unless great precautions are adopted to prevent excitement. Others (9) have found it difficult to convince themselves that a real emotional hyperglycemia exists. We have no desire to enter into this question except insofar as it arises out of our own work. The frightening experiments were merely incidental, advantage being taken, as already mentioned, of opportunities afforded by animals which were particularly quiet during the withdrawal of the preliminary blood specimen, to see whether any marked or constant difference would be found when they were then frightened, usually by a barking dog. Since the adrenals have been supposed by some observers to be concerned also in the production of emotional hyperglycemia, we made such experiments not only on normal cats but also on cats which had undergone the adrenal operations. As will be seen from the results given in the protocols and tables, we have been unable to demonstrate in normal animals any constant increase in the percentage of sugar in the blood which could be considered as associated with emotional excitement. Further, our experiments do not reveal any essential difference between the results of emotional excitement on the blood sugar in the normal and in the operated cats. We do not claim that our observations disprove the existence of a true emotional hyperglycemia, but they do suggest that if it exists it is a rather infrequent phenomenon, not to be elicited at will, in cats at least, as the asphyxia hyperglycemia can be elicited and so insignificant in amount that very numerous observations would be necessary to disentangle it from the uncontrollable variations in the sugar content.

The possibility must be admitted that different species of animals, perhaps different individuals of the same species, may vary in their susceptibility to emotional excitement in this regard. If this were so, man might be expected to be more susceptible than lower animals. On the other hand, great differences in the effects of such conditions as asphyxia and other anesthesia would scarcely be looked for. However this may be, a survey of the data in the literature as well as our own data, indicates that there is a fundamental distinction between asphyxial and post-anesthetic hyperglycemias, which are well established and easily verifiable, and "emotional hyperglycemia," the existence of which is asserted by some authors on the basis of small and inconstant differences in the blood sugar content, which other writers consider to fall within the limits of variation of the normal. As regards any relation between the epinephrin discharge and emotional hyperglycemia, even granting the existence of the latter,
our experiments seem to show that there is no such association. The so-called “Fesselungs” glycosuria and hyperglycemia perhaps stand on a different footing from emotional glycosuria and hyperglycemia; for there are several factors besides the emotional one which might possibly affect the blood-sugar content of an animal tied down in an abnormal position and struggling to free itself. Such data as have been incidentally accumulated by us on this point seem to indicate a real increase in the percentage of blood-sugar when the animal has been kept for some time on the board, whether or not it has been purposely “frightened.” However, we do not desire to lay stress on these observations, since where the maximum changes in the sugar content are small large numbers of experiments would be necessary to reach a safe conclusion. The three cats included in table 6 had only recently come to the laboratory. If this circumstance had anything to do with the result, we should be inclined to find the explanation rather in their nutritive condition than in greater susceptibility to excitement on account of the strangeness of their surroundings. For cats which

<table>
<thead>
<tr>
<th>NUMBER OF CAT</th>
<th>DATE</th>
<th>TIME ON BOARD</th>
<th>DEXTROSE per cent</th>
<th>REMARKS</th>
</tr>
</thead>
<tbody>
<tr>
<td>23</td>
<td>June 19</td>
<td>3 minutes</td>
<td>0.178</td>
<td>Cried during puncture of vein</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20 minutes</td>
<td>0.216</td>
<td>Cried during puncture of vein</td>
</tr>
<tr>
<td>24</td>
<td>June 19</td>
<td>8 minutes</td>
<td>0.079</td>
<td>Excited</td>
</tr>
<tr>
<td></td>
<td></td>
<td>22 minutes</td>
<td>0.135</td>
<td>Excited</td>
</tr>
<tr>
<td>25</td>
<td>June 19</td>
<td>3 minutes</td>
<td>0.131</td>
<td>Excited</td>
</tr>
<tr>
<td></td>
<td></td>
<td>13 minutes</td>
<td>0.104</td>
<td>Excited</td>
</tr>
</tbody>
</table>

were to all appearance much more strongly excited, but were not kept tied down, showed no definite increase in the blood-sugar.

Observations on animals which were frightened while tied on the board are given elsewhere in the paper (table 5). Where we wished to study the effect of emotional excitement alone, the animals were set free after the preliminary specimen of blood had been obtained, and were frightened in a cage.

IS THE OUTPUT OF EPINEPHRIN INCREASED IN NORMAL ANIMALS BY CONDITIONS WHICH CAUSE OR HAVE BEEN SUPPOSED TO CAUSE HYPERGLYCEMIA?

If the hyperglycemias studied do not depend upon epinephrin, the attempts which have been made to show that the conditions associated with the increased blood-sugar content also are associated with increased output of epinephrin would, even if they were successful, have
no bearing upon the mechanism or significance of the hyperglycemias in question. Even if it were demonstrated that asphyxia, etc., caused a marked increase in the epinephrin output, this could not be used as an argument in favor of the theory that it is a function of the adrenals to aid in the mobilization of sugar, once it was shown that this mobilization could occur all the same in the absence of epinephrin. We have, however, failed to demonstrate any definite increase in the output of epinephrin either through asphyxia or through stimulation of sensory nerves (10).

As for emotional disturbance, no method of satisfactorily testing the question has occurred to us. General anesthesia, of course, cannot be employed and the collection of the unmixed adrenal vein blood is therefore impracticable. The method adopted by Cannon and de la Paz could at most yield information as to the concentration of epinephrin in the inferior cava blood. They do not seem to have made any assay of the concentration in their published work; but the concentration alone, unless the amount of blood passing the point of collection in a given time is known, does not permit the rate of liberation of epinephrin to be calculated. Nevertheless, in order to have first-hand experience of the method we repeated the observations of Cannon and de la Paz in three cats, imitating in every particular their procedure, except that the blood specimens were tested on rabbits' intestine and uterus segments instead of cats' intestine strips. Cannon and Hoskins in their work on asphyxia and sensory stimulation used rabbits' intestine segments for the tests, and state that they are not inferior to cats' intestine strips.

In testing the bloods we did not empty the cylinder when replacing one liquid by another, as apparently Cannon and his associates do, for this produces a distortion of the curve at the critical point owing to the weight of the segment coming on the lever when the cylinder is emptied, and coming off the lever when the new liquid is introduced. A further objection to this method is that a segment which has been immersed in a liquid of given temperature saturated with oxygen is suddenly exposed to air, and then again suddenly immersed in another liquid. This can hardly be done without altering the temperature and oxygenation of the segment, and artificial effects including inhibition may sometimes be produced in this way. As in all our previous work, the contents of the cylinder were changed by displacement from below up, the new liquid, previously well oxygenated, being run in gently from a pipette drawn out to a fine point, and the old liquid overflowing into the bath. The pointed end of the pipette is bent at about a right angle so that it is easily introduced to the bottom of the cylinder without disturbing the preparation. The same quantity of liquid is always introduced when comparative observations are
being made, the pipette being filled to a mark. The amount of admixture with the liquid which is being displaced is small and approximately constant; and all that is necessary to eliminate any error due to admixture, and to make the liquid in the cylinder precisely the same as that in the stock from which it is taken, is to run in a sufficient excess of the liquid beyond the amount corresponding to the capacity of the cylinder. The curve is unaffected by the changing of the liquid in the cylinder except so far as it produces a physiological alteration in the segment. Unless the point at which the change is made is marked on the tracing, it would be impossible to identify it. If it is desired to change the liquid by drawing off the contents of the cylinder, which would be advantageous when it is necessary to save them, this should be managed so that the new liquid enters the cylinder at the same rate as that at which the old liquid is withdrawn.

Cat 25. Condensed protocol. Female. Weight, 2.475 kgm. The cat was secured on a comfortable holder of the same kind as that used by Cannon for his work with the Roentgen rays on the movements of the alimentary canal. Under local anesthesia with ethyl chloride, the left femoral vein was exposed. A flexible catheter oiled inside and outside was introduced with its opening at a level 5 to 6 mm. anterior to the orifices of the adrenal veins. The level of the catheter was verified at the end of the experiment. While it could not be said that the animal showed no discomfort, the degree of disturbance during the "quiet" period was no doubt much less than when the cat was frightened by a barking dog. Specimens of blood were obtained through the catheter with the aid of an aspirator, as follows:

1. While the cat was quiet.
2. After 1½ minutes excitement by the dog.
3. After 3 minutes excitement by the dog.
4. After 13 minutes excitement by the dog. The cat micturated.
5. After 15 minutes rest while the animal was fairly quiet.

The sixth catheter specimen was obtained with the catheter inserted so that its orifice was about 8 cm. below the adrenals. This specimen was taken fifteen minutes after the fifth catheter specimen. A sample of blood was then obtained from the femoral vein through a cannula, and another sample from the carotid artery. The bloods were defibrinated and tested on rabbit intestine and uterus segments. Combined weight of adrenals, 0.38 gram.

Figures 13 to 15 show some of the tracings. Those in figure 13 afford no evidence that blood collected at the level of the adrenals during excitement caused any inhibition of the rabbit intestine segment when it displaced blood collected at the same level with the animal quiet. The bloods were tried both undiluted (observation 2) and diluted with Ringer's solution (observation 27). In figure 14, blood from the same level obtained after a period of great excitement, during which the animal micturated, was caused to displace blood obtained while the animal was quiet (observation 8). Instead of inhibition there was a further increase of tone. This was not due to the so-called "sthenic"
effect of a small concentration of epinephrin, for a similar increase of tone was seen when the "quiet" specimen replaced the "excited" specimen (observation 10). In figure 15, blood collected during excitement from the adrenal level produced also some increase of tone instead of inhibition when it replaced indifferent venous blood from the lower part of the inferior cava (observation 48). The effect was not essentially different from that caused by blood collected from the adrenal level after a period of quiet when it replaced blood previously drawn during excitement (observation 46). The result of this experiment, then, was negative, the adrenal vein blood, which doubtless contained epinephrin, being too much diluted by the general mass of epinephrin-free blood in the cava to yield reactions with the intestine segments (10), (11), (12).

It is surprising how little account has been taken by many writers on this subject of the great dilution which any epinephrin liberated from the adrenals must undergo before it reaches the systemic capillaries and veins, apart from loss due to its oxidation or removal. A little reflection on this point would have made the concentrations of epinephrin sometimes alleged to have been found in venous blood incredible, because they would involve incredible concentrations in the blood leaving the adrenals. In a quite recent paper, e.g., Herring (13) quotes A. Fraenkel (14) and Broking and Trendelenburg (15) as having shown that in Graves' disease the epinephrin content of the blood obtained from an arm vein is increased, as compared with the normal blood. Now Fraenkel reports that he found

Fig. 13. Intestine tracings. Bloods collected by catheter from cat 25. At 1 Ringer's solution was replaced by the first catheter specimen, from the level of the adrenals, collected while the cat was quiet, and this at 2 was replaced by the second catheter specimen collected at the same level after one and one-half minutes of excitement. Both bloods were undiluted. At 26 Ringer was replaced by the first catheter specimen (quiet) and this at 27 by the third catheter specimen, collected after three minutes of excitement. Both bloods were diluted with four volumes Ringer. (Reduced to two-thirds.)
Fig. 14. Intestine tracings. Bloods from same cat used in figure 13. At 7 Ringer's solution was replaced by the first catheter specimen (quiet), and this at 8 by the fourth catheter specimen collected at the adrenal level after thirteen minutes of great excitement. At 9 Ringer was replaced by the fourth catheter specimen, and this at 10 by the first catheter specimen. (Reduced to two-thirds.)

Fig. 15. Intestine tracings. Bloods from same cat as in figures 13 and 14. At 45 Ringer was replaced by the fourth catheter specimen (excitement) and this at 46 by the fifth catheter specimen (collected without excitement). At 47 Ringer was replaced by blood from the lower part of the inferior vena cava, and this at 48 by catheter blood collected at the adrenal level after ten minutes excitement. (Reduced to two-thirds.)
in a case of Graves' disease a concentration of 1: 400,000 in the venous blood. How can a statement of this sort, which according to the best available evidence would imply a concentration in the adrenal vein blood of probably at least 1: 1000 or 1: 2000 be seriously accepted? In cats under experimental conditions which are widely believed to increase the output of epinephrin, a concentration of more than 1: 1,000,000 is rarely seen in the blood coming from the adrenals. In this connection it must be remembered that although some of the blood which passes through the adrenal cortex may come into relation with the cells of the medulla before issuing from the gland, it will be safe to assume that the amount of blood which can have effective interchange with the medulla is only a fraction of the total adrenal blood flow. The concentration of epinephrin in the blood of the medullary sinusoids must therefore be higher, perhaps much higher, than that in the blood of the adrenal veins. Trendelenburg (16) has since come to the conclusion that the method of Laewen (frog perfusion), as he applied it in his earlier work, is unreliable because of the rapid development of pressor substances in shed blood, and he does not now believe that the arterial blood (in rabbits) can contain even 1: 1,000,000,000 or 1: 2,000,000,000 epinephrin. Fraenkel states that he was able to detect epinephrin (1: 20,000,000 or more) in all specimens of normal human blood! Fraenkel's data are absolutely worthless, as he ignored the fact that it was the serum and not epinephrin in it, which produced the effects on the rabbit uterus segments, which he relied upon for estimating epinephrin. When rabbit intestine segments were employed to check the results obtained on uterus segments, no epinephrin was detected in the venous blood either of normal men (17) or of patients suffering from various diseases, including Graves' disease (18).

The result of the other two experiments on emotional excitement (cats 4 and 5) was the same as in the first experiment.

**Cat 4.** Condensed protocol. Female. Weight, 2.0 kgm. The procedure was the same in cat 25. Bloods were obtained from a catheter as follows:
1. From adrenal level while cat was quiet.
2. From adrenal level after ten minutes of intense excitement (by dog).
3. With orifice of catheter withdrawn 7 to 8 cm., in lower part of cava.

Then obtained blood from the femoral artery through a cannula. As in the other experiments, the catheter was removed, cleaned and again oiled before collection of each specimen.

In this experiment some of the blood tests on the intestine segments were carried out in the usual way. Figure 16 shows that the "excited" blood did not cause any definite inhibition when it replaced "quiet" blood. For the sake of comparison, in other tests, the cylinder was emptied when a change of liquid was made (fig. 17). The apparent increase of tone in the segment at 9 is partly due to the mechanical effect of its weight coming on the lever when the Ringer's solution was removed. The same is true of the rise of the curve at 11, when the
"quiet" blood was withdrawn. The drop in the curve at 12 when the "excited" blood was introduced is likewise a purely mechanical effect, due to floating up of the segment. There is no inhibition. At 13 the blood was removed and the weight of the segment carried up the writing point. The drop at 14, when Ringer's solution was introduced into the cylinder, is quite decided, since not only was the segment floated up (mechanical effect), but the increase of tone was removed by the washing out of the blood by the Ringer's solution. There is no possibility of confounding the drop at 12 with a genuine inhibition, at least when the record begins with the segment initially beating in Ringer's solution, and not after blood has been applied to it, for the curve just regains the level it started with at 11. At 14 also there is no inhibition, the segment coming back to the same length which it had before 9.

The third experiment, on a female cat, was performed precisely like that on cat 4 and yielded a similar result.

**SUMMARY**

1. The relation of the epinephrin secretion of the adrenals to experimental hyperglycemias can be investigated under much better conditions in animals whose epinephrin output has been abolished or greatly reduced by removal of one adrenal and section of the nerves of the other, than in animals deprived of both adrenals. For in the first case the animals, after recovery from the operation, remain indefinitely in good health, whereas after total adrenalectomy observations on the blood-sugar are complicated by the fact that they must be made: a, practically on dying animals (unless survivors in species where accessory adrenals are common are employed) and b, on animals suffering from the immediate effects of a major operation and anesthetization.

2. The hyperglycemia associated with asphyxia and ether anesthesia is obtained in cats which have undergone the adrenal operation described, even when no detectable residual liberation of epinephrin is present. No essential difference could be made out in this regard between these animals and control normal cats.

3. Accordingly, the mobilization of sugar, of which these experimental hyperglycemias are the expression, is not mediated through the epinephrin secretion of the adrenals.

4. Such observations as we have made on the effect of fright do not support the view that so-called emotional hyperglycemia is a constant
Fig. 16. Intestine tracings. Bloods from cat 4. At 4 Ringer's solution was replaced by the first catheter specimen obtained from the adrenal level while the cat was quiet. At 5, this was replaced by the second catheter specimen from the same level after the cat had been excited for ten minutes. (Reduced to one-half.)

Fig. 17. Intestine tracings. Bloods from the same cat used for figure 16. At 9 the Ringer's solution was removed from the cylinder; at 10 the first catheter specimen of blood (quiet) was introduced; at 11 the blood was removed; at 12 the second catheter specimen (excitement) was introduced; at 13 the blood was removed; at 14 Ringer's solution was introduced; at 15, the Ringer's solution in the cylinder was displaced by the introduction of Ringer's solution from a fine pipette, with its orifice at the bottom of the cylinder. (Reduced to one-half.)
or even a common phenomenon in cats. If it exists, it does not depend upon an increase in the epinephrin liberated from the adrenals. For a, no essential difference could be detected between the results of emotional disturbance on the blood-sugar content in the cats whose epinephrin output had been interfered with, and in the control normal cats; b, no evidence was obtained that emotional disturbance increases the output of epinephrin in normal cats.

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