The effects of insulin and anterior pituitary extract on the blood amino nitrogen in eviscerated rats

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It is well recognized that the intact animal maintains the concentration of blood amino acids at a fairly constant level and that in only very few circumstances do the values vary significantly from normal. Since the liver is the chief organ of deamination in the body, it seemed that observations on the blood amino acid levels in the liverless animal should afford a good opportunity for a study of the regulation by different agents of amino acid metabolism.

The first successful hepatectomies were reported in dogs by Mann (1921); and Bollman, Mann and Magath (1926) showed that such an operation was followed within a few hours by a marked rise in the blood amino acid nitrogen level. This observation has been confirmed for the monkey by Maddock and Drury (1938), and for the rabbit by Svedberg, Maddock and Drury (1938). The effects of evisceration on blood amino acid levels were studied by Svedberg et al. (1938) in the rabbit, and by Mirsky (1938) in the dog, and in both studies the values were found to reach higher levels than in hepatectomized animals. The present paper gives the first detailed report on changes in blood amino acid nitrogen in the rat after evisceration.

Experimental

Albino male rats of the Sprague-Dawley strain, weighing 150-225 gms., were used. After a 24-hour fast they were eviscerated in a single-stage operation according to the procedure outlined by Russell (1942). Such an animal retains its liver but the hepatic blood supply has been cut off, so that the result in a "functional" hepatectomy as indicated by the finding that the total carbohydrate content of the liver is essentially unchanged during the period succeeding the operation. Immediately following evisceration the animals were given glucose to maintain the blood sugar level. This was ad-
ministered either by subcutaneous injection of 100 mg. per 100 gm. body weight as a 10 per cent solution, or by constant intravenous infusion of 15 mg. in saline per 100 gm. body weight/hour.

In the experiments involving the use of insulin the hormone was given as a solution of zinc insulin crystals (Lilly). In 6 experiments, 1.0 unit per 100 gm. body weight was given subcutaneously immediately after evisceration, followed by 400 mg. glucose per 100 gm. body weight. Of these rats, 5 developed hypoglycemic convulsions within 3 to 4 hours and died. In 2 animals the amount of insulin was reduced to 0.5 unit, with the same amount of glucose as before, and here convulsions did not occur. Seven rats were infused intravenously with glucose and insulin together, the proportions of the 2 compounds being adjusted to avoid hypoglycemia; 0.2 unit insulin and 50–60 mg. glucose per 100 gm. body weight per hour were found to result in blood sugars closest to normal levels.

The pituitary extract used was a crude saline extract of beef pituitary glands, and was kept frozen until shortly before use. One ml. of the extract represented 200 mg. of the original gland. Amounts varying between 2 and 4 ml. were administered subcutaneously to rats during periods of 1 to 6 hours prior to evisceration. Treatment with glucose with or without insulin was similar to that already described.

The animals remained under nembutal anaesthesia throughout the experiment. Samples of blood were drawn from the tail for amino nitrogen determinations immediately after evisceration and at hourly intervals thereafter for as long as the animal survived. The survival period varied considerably, but in most cases was from 6–8 hours, with 9 hours in 1 animal being the longest survival period attained. The final blood sample in each case was taken from the heart after respiration had ceased but while the heart was still beating. Sugar determinations were performed in some experiments on the final blood sample.

Blood amino nitrogen determinations were made according to the method of Frame et al. (1943); blood sugar according to the procedure of Somogyi (1937), on copper-tungstate filtrates; and plasma uric acid by the method of Benedict (1922).

RESULTS

Evisceration

Table 1 and Figure 1 illustrate the effects of evisceration on whole blood amino acid nitrogen levels. Of the 17 animals in this series, 14 were given glucose subcutaneously while the remaining 3 were given constant intravenous infusions of glucose. The results with the 2 procedures agreed so closely that the data have been combined. The grouping of the data under intervals of time in the later periods after evisceration was required by the fact that the final samples were obtained at irregular intervals. From the initial mean of 15 mg. per cent, the nitrogen rose steadily to twice this value in 4 hours and to 45 mg. per cent in 6 hours. In 3 animals surviving longer than 8 hours a mean value of 65 mg. per cent was reached, with a value of 76 mg. per cent in 1 case. Figure 1 shows that the rate of rise increased gradually up to 3 hours and that after this time there was an increase
TABLE 1. EFFECTS OF EVISCERATION, INSULIN AND APE† ON BLOOD AMINO NITROGEN* IN RATS

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of Experiments</th>
<th>Hours after Evisceration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Evisceration</td>
<td>17</td>
<td>0</td>
</tr>
<tr>
<td>Evisceration plus Insulin</td>
<td>7</td>
<td>15.3</td>
</tr>
<tr>
<td>Evisceration plus APE</td>
<td>7</td>
<td>13.2</td>
</tr>
<tr>
<td>Evisceration plus Insulin plus APE</td>
<td>6</td>
<td>15.0</td>
</tr>
</tbody>
</table>

A. ABSOLUTE LEVELS

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of Experiments</th>
<th>Hours after Evisceration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Evisceration</td>
<td>17</td>
<td>0</td>
</tr>
<tr>
<td>Evisceration plus Insulin</td>
<td>7</td>
<td>0.64</td>
</tr>
<tr>
<td>Evisceration plus APE</td>
<td>7</td>
<td>3.8</td>
</tr>
<tr>
<td>Evisceration plus Insulin plus APE</td>
<td>6</td>
<td>-0.92</td>
</tr>
</tbody>
</table>

B. CHANGES FROM INITIAL LEVELS

* Means and standard errors of the means are expressed in mg. per cent.
† Anterior pituitary extract.

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FIG. 1. The Effect of Insulin and Anterior Pituitary Extract (APE) on the Blood Amino Nitrogen in Eviscerated Rats.
to a rate which remained very nearly constant throughout the period. Data from the individual animals showed no relationship between survival time and either the rate at which the blood amino nitrogen rose or the amino nitrogen levels achieved. Terminal values obtained between 2 and 7 hours after evisceration were in general agreement with observations made at intermediate periods on animals surviving longer times.

In Table 1 the results are expressed also in terms of change from the initial level, in mg. per cent. This method of expression permits account to be taken of individual variations in the initial levels, which ranged from 13.5 to 17 mg. per cent and especially allows a more accurate comparison with the animals treated with pituitary extract, to be described later.

Plasma amino nitrogen levels were determined in 6 additional animals. Samples of blood were drawn from the tail at 2 and 4 hours after evisceration and in 1 instance also at 5 hours. In order to avoid further withdrawal of the relatively large amounts of blood necessary for determinations on plasma, all subsequent values were obtained from terminal heart blood samples. Starting from a mean value of 8.2 mg. per cent the average plasma amino nitrogen levels rose to 14, 26, 35, 49 and 60 mg. per cent in 2, 3, 4, 5, and 8 hours respectively. One animal which survived for 9 hours had a plasma value of 75 mg. per cent, equalling the highest value obtained on whole blood. In the initial samples the amino nitrogen concentrations were evidently lower in the plasma than in the cells, but at 4 hours or more after evisceration the plasma levels were within the range of values obtained on whole blood. Without information on plasma and whole blood values in the same animals, it is not possible to decide whether the increased amino nitrogen content is due to an increase chiefly in the plasma as Engel, Winton and Long (1943) found to be the case after hemorrhage, or to an increased concentration in both plasma and cells.

Glucose determinations were performed on 5 of the terminal blood samples, the values of 33, 65, 71, 76, and 91 mg. per cent being obtained. No relationship between blood sugar and blood amino acid levels was apparent.

The uric acid content of the plasma was determined in 5 animals. Starting from an initial value of about 2 mg. per cent the average uric acid level rose to 14 mg. in 4 hours, 28 mg. in 7 hours and 34 mg. in 8 hours after evisceration. The results indicate that uric acid is formed in the peripheral tissues, and accumulates in the blood when uricase, the enzyme of the liver which normally converts uric acid into allantoin, is lacking from the system. It has been shown by Frame et al. (1943) that in the procedure employed in the present study for the determination of amino acid nitrogen, 28 per cent of uric acid nitrogen, or about 9 per cent of uric acid, is determined as amino nitrogen.
Even the high levels of uric acid cited above do not interfere seriously with the values as given here for amino acid nitrogen, since they can have contributed only a few mg. per cent to the high amino nitrogen figures obtained in these experiments.

**Insulin**

The effects of the administration of insulin and glucose to the eviscerated rat are shown in Table 1 and Figure 1. Data on only the 7 animals which received glucose and insulin together by constant intravenous infusion are included. The results are qualitatively similar whether the compounds are administered by the subcutaneous or intravenous route, but in the former there is a slight time lag owing to the relative slowness of absorption of insulin. The effect of insulin in slowing the rate and extent of accumulation of amino acid nitrogen is marked, and the differences between the groups of animals with and without insulin are statistically significant for all time intervals after evisceration. Terminal blood sugar values showed marked individual variation. With the exception of 1 animal which had a value of 42 mg. per cent, hyperglycemic levels were observed, varying from 101 to 280 mg. per cent, but there was no correlation between the levels of glucose and of amino acid nitrogen in the blood.

**Adrenal-demedullation**

Five rats were adrenal-demedullated 3 to 4 weeks prior to evisceration and observation as described above. The whole blood amino nitrogen values tended to be slightly lower in the adrenal-demedullated animals as compared with animals having intact adrenals, both initially and in terms of rise above the initial level, but the figures were not markedly different in the 2 groups of animals. It may therefore be concluded that adrenal-demedullation has slight, if any, effect on the increase in blood amino nitrogen content. Two adrenaldemedullated animals were infused with glucose and insulin together. The results were in close agreement with those obtained from normal animals receiving similar treatment.

**Anterior Pituitary Extract**

The blood amino nitrogen levels were followed in 7 normal (non-eviscerate) rats given anterior pituitary extract (APE) during the course of 6 hours, and a progressive and statistically significant decrease from an initial mean value of 16.8 mg. per cent to 13.2 mg. per cent at the end of 6 hours was noted. At this time evisceration was performed and glucose was administered either subcutaneously or by constant intravenous infusion. The results, presented in Table 1, show very close agreement in the effects of evisceration on blood amino nitrogen levels with and without APE, indicating that the factor in APE which causes a lowering of the blood amino nitrogen in
the intact animal must act through some organ which was removed by
evisceration. Additional amounts of extract were given to some of the
animals at intervals after evisceration, demonstrating that the lack
of effect in the eviscerated rat is not due to a wearing off of the action
of the extract after 6 hours.

Nine rats were given APE over the course of 1 hour before evis-
ceration. Of these, 3 received insulin and glucose subcutaneously after
the operation, while the remaining 6 were infused intravenously with
the 2 substances in the ratio previously found to be the most suitable
for maintenance of blood sugar levels. Only the latter group is in-
cluded in the data in Table 1 and Figure 1 in order that a more ac-
curate comparison may be made with the similar group not receiving
APE. The data indicate that the animals receiving APE in addition
to insulin had lower blood amino nitrogen levels at all time intervals
than those receiving insulin alone. At both the 2- and 3-hour intervals
the mean values were still below the initial value, and in 1 animal the
initial level had not been reached after 4 hours. After this time there
was a slow rise in all instances. Statistical analysis of the results from
the 2 groups of animals receiving insulin, with and without APE,
showed that P at all time intervals was less than 0.01, indicating that
the 2 groups differ significantly from each other. The terminal blood
sugar values in the animals receiving insulin and APE varied between
68 and 218 mg. per cent.

Urine Examination

Samples of bladder urine were removed from a number of animals
at the conclusion of the experiment and examined qualitatively. Heating of the urine with ninhydrin solution resulted in the develop-
ment of a deep blue color, indicating the presence of amino acids. These are normally present only in traces in the urine. This observa-
tion indicates the continuation of kidney function in the eviscerated
rat. In all urine specimens a flocculent precipitate formed immediately
after withdrawal from the bladder, and increased in amount as the
sample cooled. This precipitate gave a positive murexide test, indicat-
ing that it was uric acid, a result in keeping with the high blood levels
of uric acid mentioned above. Mann and his collaborators (1925–33)
have previously reported high blood and urinary levels of uric acid
in hepatatecmtized dogs.

DISCUSSION

The effect of evisceration on the blood amino nitrogen levels in the
rat scarcely requires comment other than to emphasize the magnitude
and constancy in rate of rise. It may also be pointed out that even
when the organism is presumably amply supplied with glucose to meet
its energy requirements there is continuous breakdown of tissue pro-
tein.
The evidence in the present experiments that insulin influences protein metabolism even when hypoglycemia is avoided is in agreement with previous investigations. The depressing effect of insulin on blood urea and non-protein nitrogen in human beings was noted by Janney and Shapiro (1926); on blood urea and amino acids in rabbits by Tashiro (1926); on blood amino acids in rabbits, rats and humans by Luck, Morrison and Wilbur (1928); on blood amino acid nitrogen in dogs by Kerr and Krikorian (1929); and on blood urea and non-protein nitrogen in rats by MacKay et al. (1939). The converse effect has been reported by Luetscher (1942), who found high levels of plasma amino acids in patients with severe untreated diabetes mellitus. Mirsky (1938) found that insulin decreased the extent of the increase in non-protein nitrogen and amino acid nitrogen in the blood in eviscerated nephrectomized dogs. The preliminary report, without detail, by Levine et al. (1945) indicates comparable results in the eviscerated rat. The mechanism whereby insulin exerts its protein-sparing action is not known. That such action, in part at least, is a peripheral one is evident since it can be demonstrated in the absence of the liver. Kiech and Luck (1928) suggested that the decrease in blood amino acids may be due to an increase in their rate of catabolism; but that this is not the only mechanism is demonstrated in the present experiments and in those of Mirsky, since such a device would require the liver for deamination. Whether insulin promotes the synthesis of tissue protein from blood amino acids or inhibits its breakdown is not known. The former alternative is suggested by the observation of Mirsky (1938) that intravenous glycine disappeared more quickly from the blood of the eviscerated dog given insulin than in the control animal.

Our observation that adrenal-demedullation was without significant effect on the blood amino nitrogen levels of the eviscerated rat given insulin is not in agreement with the results of Davis and Van Winkle (1934), who found that insulin had no effect on the blood amino acid level of the adrenal-demedullated rabbit, or with those of Luck and Morse (1933), who attributed the blood amino nitrogen-lowering effect of insulin to epinephrine liberation. The fact that those observations were made on intact rabbits, while those reported here were made on eviscerated rats, may indicate either that a species difference exists, or, more likely, that different mechanisms were in play in the different circumstances.

It is well recognized that the administration of certain anterior pituitary extracts to animals causes growth, which is reflected in a retention of nitrogen. One manifestation of this action is the depression of the non-protein nitrogen of the blood, as shown in the dog by Teel and Watkins (1929) and Teel and Cushing (1930) and by Gaebler (1933) and others for non-protein nitrogen, urea and amino acids. Similar results were obtained on normal rats in the present work.
That insulin plays some part in the mechanism of action of the growth-promoting principle of the anterior pituitary was first indicated in the work of Mirsky (1939). He found that in the nephrectomized dog after either evisceration or pancreatectomy the administration of APE caused a greater, rather than a lesser, increase in the blood amino acids and non-protein nitrogen levels, as compared to the rise observed in control animals not receiving the extract. The subsequent pertinent literature has recently been fully reviewed by Young (1945) and will be only briefly summarized here. Young (1940; 1941; 1944) has found that the administration of APE to dogs may be either diabetogenic or growth-promoting, whereas in rats, which are refractory towards the diabetogenic action, the extracts are only growth-promoting. He considers the response to be dependent on the adequacy of pancreatic function. More recently Young (1945) has stated the view that the secretory activity of the pancreatic islets is an important factor in the nitrogen retention which may be observed simultaneously with the diabetogenic activity of pituitary extract. Gaebler and his collaborators (1941; 1942) observed that the administration of a pituitary preparation to depancreatized dogs receiving constant doses of insulin did not cause nitrogen storage as it did in normal animals, but that if the insulin dosage was increased nitrogen storage resulted.

Our observation that the administration of APE was without effect on the blood amino acid level, and presumably on muscle protein metabolism, in the eviscerated animal not supplied with insulin is not in agreement with that of Mirsky (1939), who concluded that the direct action of APE on muscle is one of stimulating protein catabolism. The present results substantiate the view that insulin is concerned with the nitrogen-retaining action of the anterior pituitary, but do not support the postulate that APE exerts its nitrogen-sparing effect only through a pancreotrophic action as has been suggested or implied by Mirsky (1939), Young (1941) and Ogilvie (1945). In these experiments the pancreas was not present, so that no additional amount of insulin could have been put out under the influence of the APE. Moreover, insulin was being supplied in large amounts so that the maximum effect which it was capable of exerting on protein metabolism in these conditions was most probably already occurring. (This is indicated in the finding that the blood amino acid nitrogen level remained about the same whether 1 or 2 units of insulin per kg. body weight per hour were given.) The fact that APE caused a further decrease in the blood amino nitrogen level indicates that the effect observed here was independent of the amount of insulin present in the body above a certain undetermined minimum. Our results suggest that a synergistic action may exist between a principle of the anterior pituitary and insulin; that is, that the action of insulin is necessary at some stage in the protein synthesis stimulated by APE. Analogous relationships between hormones of the anterior pituitary and of the adrenal cortex
have been suggested by Russell (1940) and by Long, Katzin and Fry (1940). However, the existence also of a true pancreatrophic effect of APE is not precluded by the results of the present experiments. Reference should be made to the finding of Engel, Winton and Long (1943) that extensive hemorrhage leading to shock in normal rats caused a marked increase in the blood amino nitrogen level. In their series of animals, which were bled slowly up to 3 or 4 per cent of the body weight, the levels rose with time to values approximating those found in animals which were eviscerated and given insulin and glucose in the present study. The possibility is raised that the evisceration *per se* was a shocking procedure and that the insulin had a non-specific effect on amino acid nitrogen levels through improving the status of the animal. Against such a thesis the following points may be raised: (1) The operation was usually accompanied by very little bleeding, and little difficulty was experienced in bleeding the animal from the tail throughout the course of the experiment. (2) Rats in which the extra-hepatic viscera were removed as in the present operation but in which the hepatic artery remained patent were observed in this laboratory by Engel, Harrison and Long (1944). In these animals, the blood amino nitrogen concentration remained very near the original level during the first 4 hours after the operation and then rose only very slowly during the remainder of the 7 hour observation period. Hence, the increase in blood amino nitrogen which was observed in the present experiments most probably resulted from the exclusion of the liver from the circulation and not from shock following the operation *per se*. (3) The administration of insulin had no beneficial effect in prolonging the survival period. It is possible that the constant rate of rise in blood amino nitrogen found subsequent to the 4-hour interval after evisceration, which was not prevented by insulin or insulin plus APE, may have been in part the result of approaching shock. If the changes which occur within the first 4 to 5 hours after evisceration only are considered to be quite independent of the shock phenomenon, they demonstrate the more clearly the effects of insulin and APE administration on the blood amino nitrogen levels.

**SUMMARY**

In eviscerated rats supplied with glucose the amino nitrogen content of the blood rose continuously through the periods of survival which were observed.

The administration of insulin in addition to glucose caused a significant decrease in the rate and extent of rise in the blood amino acid content. This effect was not related to the blood sugar level.

Adrenal-demedullation did not influence the action of insulin on the blood amino acid content of the eviscerated rat.

The administration of whole anterior pituitary extract decreased
the blood amino acid content of the normal rat but was without effect in the eviscerated rat.

The administration of insulin together with anterior pituitary extract had a greater effect in decreasing the rise in blood amino acid levels in the eviscerated rat than did insulin alone.

REFERENCES

DAVIS, B. L., AND W. VAN WINKLE: J. Biol. Chem. 104: 207. 1934.
YOUNG, F. G.: Endocrinology 26: 345. 1940.