ADRENAL CORTICAL STEROIDS AND THE METABOLISM OF GLUTAMIC ACID IN GLUCONEOGENESIS.

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The adrenal cortex is the pivot of the body's reaction to stress. To this gland converge most of the varied effects of shock and stress, and from this gland diverge the steroids which, in ways but partly known, enable the body to withstand the stress. No apology is therefore needed for a study which contributes to an elucidation of the actions of these steroids.

It has been known since the work of Long, Katzin and Fry (1940) that adrenal cortical steroids are necessary for normal gluconeogenesis, for the fasting adrenalectomized rat is unable to convert its protein stores to glycogen and the liver becomes depleted. The process, and the liver-glycogen, can, however, be fully restored by giving some of the adrenal cortical steroids, especially those which are hydroxylated or ketonic at C–11 and hydroxylated at C–17 (Olson, Thayer and Kopp, 1944). But the chain of metabolic processes from protein to glycogen is long, and in this chain the site of action of the steroids is unknown.

In order to localize their actions more narrowly, we have supplied the adrenalectomized rat with substances which occur at some stages of gluconeogenesis in order to see whether the animal can complete the process. Glutamic acid was tested as it is of special interest. It holds a key position in the processes of deamination; for it links those amino-acids which can be converted to glutamic acid (of which histidine, arginine, proline, and lysine are already known) to glutamine and α-ketoglutaric acid, substances known to be active in the Krebs' cycle.

In addition, both glutamic acid and the adrenal cortical steroids have been reported to possess anticonvulsant properties (McQuarrie, Anderson and Ziegler, 1942; Selye, 1942; Spregel and Wycis, 1945; Kerman, 1947; but denied by Goodman, Swinyard and Toman, 1946). These observations are at present of uncertain significance, but they add to the many lines of evidence which suggest that the adrenal cortical steroids and glutamic acid play an important part in brain metabolism.

Method.

Adult white rats of Wistar strain, of weights between 150 and 200 gm., male to avoid possible disturbances by oestrus, were used throughout.

Their diet was maintained carefully constant in composition to avoid disturbance by the "protein effect": that glycogen stores from a diet high in

protein decrease in fasting at a rate different from those formed from a low-protein diet (Todd, Barnes and Cunningham, 1947). The diet was composed of: 50 per cent. (w/w) rat cube meal (N.E. Agricultural Co-operative Society), 10 per cent. meat meal, 10 per cent. wheat germ, 10 per cent. maize meal, 10 per cent. soya flour, 5 per cent. crushed oats, and 5 per cent. full cream dried milk. Each rat received each day 0·5 gm. of a mixture of cod-liver oil and Yeastex in equal parts.

The rats were adrenalectomized by the lumbar route under ether anaesthesia. After operation, the animals were kept in a separate room thermostatically maintained at 70° F., and the drinking water changed to 0·9 per cent. (w/v) sodium chloride with 5 per cent. (w/v) glucose; but from the second day onwards the glucose was omitted. Food of the composition described was given freely until the evening of the fourth day, when all food was removed in accordance with the demonstration by Eggleston, Johnston and Dobriner (1946) that at least six hours' starvation is necessary if the liver is to be almost entirely depleted of glycogen. Throughout the fifth day, 0·9 per cent. (w/v) sodium chloride was freely available, but there was no food, and particular care was taken to ensure that none was obtained from any irregular source. During the fifth day, the substances were administered by a course of seven injections, given intraperitoneally in a volume of 0·6 or 0·8 ml., at 45-minute intervals.

At 45 minutes after the final injection, the animal was anaesthetized by an intraperitoneal injection of 5 per cent. sodium amytal, and the body was weighed. As preliminary trials had shown that the glycogen content of the liver varied considerably from lobe to lobe, the whole liver was removed; it was snipped piecemeal into 30 ml. of 30 per cent. (w/v) KOH in a boiling water bath, and, when digestion was complete, the glycogen was estimated by the method of Good, Kramer and Somogyi (1933). The glycogen contents have been expressed throughout the paper as mg. of glycogen per 100 gm. of whole body weight.

The substances tested were: dl-alanine, l(+)-arginine, l(−)-cystine, l(+)-glutamic acid, glycine, l(+)-histidine, l(+)-lysine, l(−)-proline, dl-serine, l(−)-tyrosine, dl-valine, and glucose. The amino-acids were first brought to pH 7·4 by the addition of sodium hydroxide, and 0·2 gm. was injected either in solution or in suspension. The animals tolerated the injections well, and signs of shock were rare and slight.

Results.

Absorption of Glucose.

In order to test whether the substances given intraperitoneally were being adequately absorbed, the presence or absence of any unabsorbed fluid or material was always noted when the abdomen was opened for removal of the liver. Usually the peritoneal space was empty, but when any fluid was present it was carefully collected, its glucose content was estimated chemically, and the amount unabsorbed was thus determined. Usually the absorption of glucose was complete, but when incomplete, the quantity absorbed was never less than 70 per cent. of the injected quantity.

Absorption of Amino-acids.

Some of the amino-acids failed to dissolve completely in the fluid available, and were injected in suspension. Incomplete absorption occurred with some of the amino-acids, especially with the less soluble ones like cystine and tyrosine. Precise details cannot be given since the animals varied among themselves, but most of the amino-acids were absorbed completely. The question will be referred to again below.

Formation of Glycogen from Glucose.

When glucose was given, at doses varying from 0·105 gm. to 1·68 gm., to the adrenalectomized rat, it was found (Fig. 1) that glycogen deposition occurred

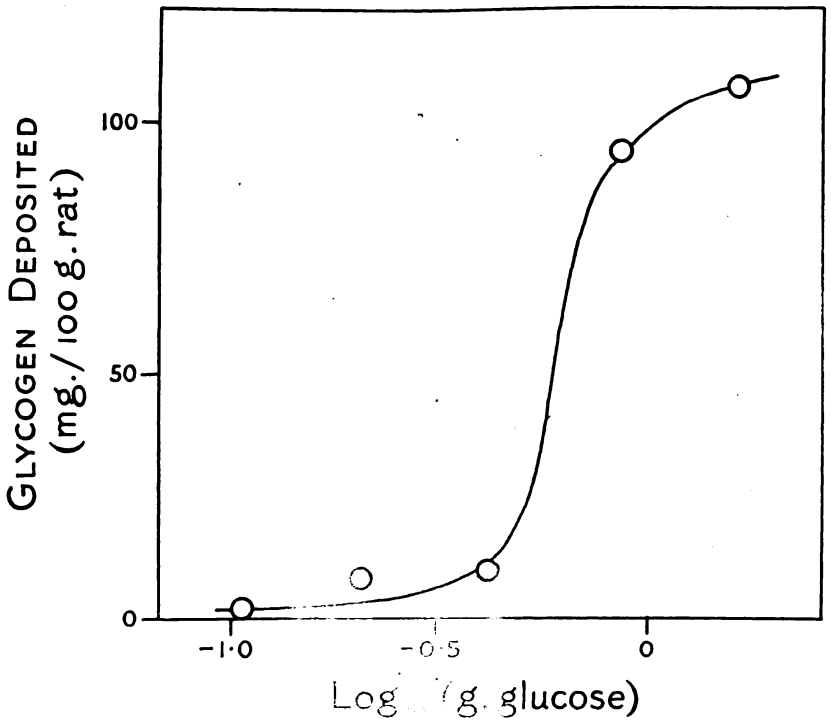

FIG. 1.—Glycogen deposited in the liver of the adrenalectomized rat after the intraperitoneal injection of glucose at doses ranging from 0·105 to 1·68 gm. Each circle is the mean of 5 animals.

freely provided that the dose exceeded about 0·5 gm. (−0·3 on the logarithmic scale). This occurrence of a threshold confirms the experience of Venning, Kazmin and Bell (1946) in the mouse. (Their work has been repeated here in order to provide a proper control for the experiments described below.) A plausible explanation is that the animal, fasting during the administration

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of the test-substance, expends about 0·5 gm. of glucose in its metabolism so that only if an excess over 0·5 gm. is provided can glycogen be deposited. Be that as it may, Fig. 1 shows that, in the adrenalectomized rat, glucose can be converted easily to glycogen.

Formation of Glycogen from Glutamic Acid.

Because this basal quantity (0·5 gm.) of glucose is needed before glycogen deposition can begin, it was considered advisable to give all the animals glucose sufficient to cause a small but definite deposit of glycogen; then the action of other substances was tested by seeing whether they increased the amount of glycogen deposited. Accordingly, all the experiments were duplicated, half of the rats receiving 0·42 gm. and half 0·63 gm. of glucose.

To test the ability of the adrenalectomized rat to convert glutamic acid to glycogen, eight sets of four rats were used, each set of four receiving one of the eight combinations of (1) glucose at doses of 0·42 gm. or 0·63 g.m, (2) adrenal cortical extract (Eschatin) at doses of 0 or 0·1 ml. (a dose just sufficient to give a detectable effect), and (3) glutamic acid at doses of 0 or 0·2 gm.

As the rats responded to all four combinations of glucose and Eschatin similarly, the results have been combined to give Table I.

TABLE I.—*Glycogen Deposited with and without Glutamic Acid.*

Treatment.	No. of rats.	Glycogen deposited (mg./100 gm. rat).	S.D.
Basic . . .	16	42·3	±3·2
Basic + glutamic acid	16	36·3	±3·9
Difference		6·0	±5·0

(The standard deviations (S.D.) are those of the quantities on their left.)

It will be seen that the addition of glutamic acid has resulted in no increase in the deposit of glycogen; in fact, the deposition is reduced, though not significantly.

Partly to repeat the experiment, and partly to encourage glycogen deposition, the experiment was repeated, but using 0·2 ml. of Eschatin throughout. This dose had been found by previous trial to be able to cause only a small deposition of glycogen. Glutamic acid was again given with glucose at doses of 0·42 and 0·63 gm. The results are given in Table II.

TABLE II.—*Glycogen Deposited with and without Glutamic Acid.*

Treatment	No. of rats.	Glycogen deposited (mg./100 gm. rat).	S.D.
Basic + Eschatin .	16	63·4	±5·5
Basic + Eschatin + glutamic acid	16	55·3	±3·2
Difference		8·1	±6·4

Again the administration of glutamic acid has caused no increase in the amount of glycogen deposited; in fact the deposit is smaller, though not significantly so. Evidently a quantity of Eschatin just sufficient to cause some deposition of glycogen from glucose is insufficient to convert the glutamic acid to glycogen.

The two experiments agree in showing that, in the adrenalectomized rat, glutamic acid is not readily converted to glycogen.

Formation of Glycogen from Other Amino-acids.

Ten other amino-acids (listed above) were given similarly. Half the animals received 0·42 gm. of glucose and half received 0·63 gm. These batches were again split, half receiving 0·1 ml. of Eschatin and half receiving none. Control groups were given treatments similar to these except that no amino-acid was given.

The results (Table III) showed that in general, the supplying of amino-acids did not result in glycogen deposition. On the contrary, the tendency was for the deposition to be inhibited.

TABLE III.—*Glycogen Deposited with and without Amino-acids.*

Treatment.	No. of rats.	Glycogen deposited (mg./100 gm. rat).	S.D.
Basic . . .	40	41·8	±2·3
Basic + amino-acid	40	24·2	±1·8
	Difference	17·6	±2·9

The difference between the two means is significant, for it exceeds six times its standard error ($P<0·001$).

In the adrenalectomized rat, therefore, amino-acids are not readily converted to glycogen.

Effects of Individual Amino-acids.

In order to investigate adequately the differences between the individual amino-acids, a "factorial" design of experiment was used. Each amino-acid was tested as before on four basic treatments; two levels of glucose (0·42 or 0·63 gm.), both with and without Eschatin 0·1 ml. The ten amino-acids and two batches of glutamic acid were used, and each was tested on 8 rats.

When the results were examined, the analysis of variance showed that the second-order interaction (Table IV) was almost significant when compared with the residual variance; for this reason, and especially as it is the variance between amino-acids in which we are interested, the variance of the second-order interaction has been used as the estimate of error.

The result marked (1) in the right-hand column of Table IV shows that the amino-acids are significantly different from one another in their effects on glycogen deposition; evidently the amino-acids have specific as well as general effects.

Table V shows, for each amino-acid, the mean over the four basic treatments of the differences between the amounts of glycogen deposited when the amino-

TABLE IV.—*Analysis of Variance.*

Variance.		D.F.	Sum sq.	Mean sq.	Var. ratio.	P.	Sigce.
Amino-acids	(A)	11	42729·	3888·	7·76	0·001	** (1)
Cortical extr.	(C)	1	29601·	29601·	59·0	<0·001	** (2)
Glucose level	(G)	1	884·	884·	1·76	>0·05	0
Interaction :	AC	11	12535·	1140·	2·27	>0·05	0 (3)
,,	AG	11	9506·	864·	1·72	>0·05	0 (4)
,,	CG	1	2670·	2670·	5·32	0·04	*
,,	ACG	11	5514·	501·	—	—	—
Total		47	103440·	—	—	—	—

0 = not significant.
* = significant at 5% level.
** = significant at 1% level.

TABLE V.—*Inhibition of Glycogen Deposition Caused by Amino-acids.*

Amino-acid.	No. of rats.	Change in glycogen (mg./100 gm. rat).
Glycine	8	−32·6
l(−)-Tyrosine	8	−31·3
l(−)-Cystine	8	−30·6
l(+)-Lysine	8	−23·3
l(+)-Histidine	8	−22·4
l(+)-Arginine	8	−21·9
dl-Alanine	8	−18·8
dl-Serine	8	−12·2
l(−)-Proline	8	−7 5
l(+)-Glutamic acid	8	−6 0
dl-Valine	8	+18·7
(Controls)	(48)	(0)

acid was given and the amounts when it was not given. (A negative sign implies that the glycogen deposit was less when the amino-acid was given.) Thus, the administration of 0·2 gm. of glutamic acid tended, other things being equal, to lower the deposit of glycogen by 6·0 mg./100 gm. rat. (It may be noted that this result with glutamic acid confirms those of Tables I and II.) The tendency of the amino-acids to inhibit glycogen deposition is clearly shown, and the deviation of the mean from zero is significant ($t = 3·80$, $n = 10$, and $P < 0·01$.)

DISCUSSION.

As shown by Fig. 1, the adrenalectomized rat can convert glucose to glycogen without difficulty if more than a minimal quantity is supplied. Similarly it has been found that the adrenalectomized mouse can deposit glycogen from glucose which has been given by injection (Venning *et al.*, 1946), and that the adrenalectomized rat can deposit glycogen from glucose given by stomach tube (Deuel,

Hallman, Murray and Samuels, 1937). In the processes of gluconeogenesis, therefore, the adrenal cortical steroids seem to act only slightly, if at all, in the conditions of our experiment, in the stages in which glucose is converted to glycogen.

Amino-acids, however, seem unable to proceed to glycogen in the absence of these steroids. But before discussing this inability, a possibility must be excluded. Could this failure to deposit glycogen be due to a simple failure in the absorption of the amino-acids? Its likelihood seems small: the completeness of absorption was in most cases shown to be complete by inspection of the peritoneal cavity, and was, for glucose, confirmed by chemical assay; and there is the fact that the amino-acids have demonstrated a definite effect (Table V), which, though one of inhibition, shows that a sufficient absorption has occurred. It seems, therefore, that the chief difficulty in gluconeogenesis without steroids must lie in those processes which convert amino-acids to glucose and its related substances.

That the steroids promote deamination has been suggested by the work of Russell and Wilhelmi (1941*a*), who showed, in kidney slices of the rat, that dl-alanine and l(+)-glutamic acid were deaminated at a rate which was less in slices from the adrenalectomized than in slices from the normal rat. They found, also, that the decreased rate of deamination could be restored to the normal level by the addition of adrenal cortical extract or of desoxycorticosterone. (In the last respect their work does not parallel ours, for it has repeatedly been found (e.g. Reinecke and Kendall, 1943; Olson *et al.*, 1944) that desoxycorticosterone does not promote gluconeogenesis in the adrenalectomized rat.) They also showed (1941*b*) that previous adrenalectomy reduced not only the rate of deamination but also the rate of formation of carbohydrate.

The conversion of alanine to glucose in the adrenalectomized rat has also been measured by the use of the phloridzinized animal (Lewis, Kuhlmann, Delbue, Koepf and Thorn, 1940). They found that this conversion was impaired on adrenalectomy, that impairment was corrected by adrenal cortical extract (Wilson Laboratories) and by 17-hydroxy-11-dehydro-corticosterone, but not by desoxycorticosterone. It may be significant that the potencies of these steroids in the conversion of alanine to glucose are similar to their potencies in gluconeogenesis, for in both processes desoxycorticosterone is inactive (Reinicke *et al.*, 1943) while adrenal cortical extracts and 17-hydroxy-11-dehydrocorticosterone are active (Olson *et al.*, 1944).

Related to this effect is the fact that adrenalectomy reduces, in rat liver, the activity of arginase (Fraenkel-Conrat, Simpson and Evans, 1943). The activity can be restored by some steroids, for corticosterone, 11-dehydrocorticosterone, and 17-hydroxy-11-dehydro-corticosterone are active while desoxycorticosterone is without effect. Again their relative potencies in the activation of arginase match their relative potencies in gluconeogenesis.

The Inhibition of Glycogen Deposition by Amino-acids.

The data of Tables III, IV and V show that the administration of amino-acids inhibits, to some degree, the deposition of glycogen in the adrenalectomized

rat. A review of the technical details has suggested no simple explanation, and we may turn to others' work for comparison.

A possible confirmation of the fact has been given by Chiu and Needham (1948) who found that rabbit liver slices in 0·4 per cent. dl-alanine, in conditions favourable for glycogen deposition, changed in glycogen content: with Eschatin, from 1·09 to 1·18 mg./gm., without Eschatin from 1·09 to 0·79 mg./gm. In their conditions, with no substrate and with no Eschatin, the glycogen is stated to have increased slightly, So the fall in glycogen from 1·09 to 0·79 mg./gm. when alanine was present may be anəlogous to our inhibition. But the interpretation is complicated by uncertainty as to what quantity of steroid has been brought to the experiment by the tissue, and also how long the quantity has continued effective. The work of Vogt (1943) suggests that the quantity would be soon consumed.

The normal animal, supplied with amino-acids, can deposit glycogen without difficulty. In fact, the administration of glycine having C^{13} in the carboxyl group has shown not only that the glycine is converted to liver glycogen, but that the ingested glycine promotes the formation of glycogen from other body constituents (Olsen, Hemingway and Nier, 1943). The normal and the adrenalectomized animals evidently react very differently to the administration of amino-acids.

The comparative powers of the different amino-acids to inhibit glycogen deposition, shown in Table V, give as yet no clue to the underlying reason for the inhibition. The amino-acids have also been arranged in order of their specific dynamic activities (Lusk, 1912; Rapport and Beard, 1937), but no clear correspondence emerges; nor does it when they are arranged in order of their powers to form glucose in the phloridzinized dog (Ringer and Lusk, 1910; Dakin, 1913*a*, *b*, *c*).

Conclusion.

The results show that failure of gluconeogenesis in the adrenalectomized rat is due primarily to an inability to convert glutamic acid to glucose and its related substances. Secondary to this is the failure of the other amino-acids to be converted to glycogen; for an inability to convert glutamic acid will obstruct all the amino-acids whose metabolic paths in gluconeogenesis lie through glutamic acid, glutamine, and α-ketoglutaric acid.

Reactions to shock, insofar as they involve the adrenal cortical steroids, may therefore affect in this way the metabolism of glutamic acid.

Summary.

(1) The adrenalectomized rat is unable to convert glutamic acid to glycogen, but the conversion of glucose to glycogen is unimpaired.

(2) Under these conditions, other amino-acids are also unable to proceed in gluconeogenesis.

(3) Tne administration of glutamic acid and of other amino-acids to the adrenalectomized rat causes a partial inhibition of gluconeogenesis.

(4) A mechanism is suggested through which the reaction to stress may affect the metabolism of glutamic acid.

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REFERENCES.

CHIU, C. Y., and NEEDHAM, D. M. (1948), *Biochem. J.*, **42**, Proc., xix.
DAKIN, H. D. (1913*a*), *J. biol. Chem.*, **13**, 513.
Idem (1913*b*), *ibid.*, **14**, 321.
Idem (1913*c*), *ibid.*, **14**, 328.
DEUEL, H. J., HALLMAN, L. F., MURRAY, S., and SAMUELS, L. T. (1937), *J. biol. Chem.*, **119**, 667.
EGGLESTON, N. M., JOHNSTON, B. J., and DOBRINER, K. (1946), *Endocrin.*, **38**, 197.
FRAENKEL-CONRAT, H., SIMPSON, M. E., and EVANS, H. E. (1943), *J. biol. Chem.*, **147**, 99.
GOOD, C. A., KRAMER, H., and SOMOGYI, M. (1933), *J. biol. Chem.*, **100**, 485.
GOODMAN, L. S., SWINYARD, E. A., and TOMAN, J. E. P. (1946), *Arch. Neurol. Psychiat.*, **56**, 20.
KERMAN, E. F. (1947), *Dis. nerv. System*, **8**, 313.
LEWIS, R. A., KAHLMAN, L. F., DELBUE, C., KOEPF, G. F., and THORN, G. W. (1940), *Endocrin.*, **27**, 971.
LONG, C. N. A., KATZIN, B., and FRY, E. G. (1940), *Endocrin.*, **26**, 309.
LUSK, G. (1912), *J. biol. Chem.*, **13**, 155.
MCQUARRIE, I., ANDERSON, J. H., and ZIEGLER, M. R. (1942), *J. clin. Endocrin.*, **2**, 406.
OLSEN N. S., HEMINGWAY, A., and NIER, A. O. (1943), *J. biol. Chem.*, **148**, 611.
OLSON, R. E., THAYER, S. A., and KOPP, L. J. (1944), *Endocrin.*, **35**, 464.
REINECKE, R. M., and KENDALL, E. C. (1943) *Endocrin.*, **32**, 505.
RINGER, A. I., and LUSK, G. (1910), *Z. physiol. Chem.*, **66**, 106.
RUSSELL, J. A., and WILHELMI, A. E. (1941*a*), *J. biol. Chem.*, **137**, 713.
Idem (1941*b*), *ibid.*, **140**, 747.
SELYE, H (1942), *J. lab. and clin. Med.*, **27**, 1051.
SPREGEL, E., and WYCIS, H. (1945), *J. lab. and clin. Med.*, **30**, 947.
TODD, W. R., BARNES, J. M., and CUNNINGHAM, L. (1947), *Arch. Biochem.*, **13**, 261.
VENNING, E. H., KAZMIN, V. E., and BELL, J. C. (1946), *Endocrin.*, **38**, 79.
VOGT, M. (1943), *J. Physiol.*, **102**, 341.