The Influence of α-Methyltryptophan and Some Tryptophan Metabolites on Hepatic Glycogenesis*

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ABSTRACT: Studies of the effect of α-methyltryptophan on carbohydrate metabolism showed that this amino acid analog stimulates hepatic glycogenesis, in contrast to tryptophan which inhibits this process in the rat. Other differences in the actions of the two amino acids were found. Whereas tryptophan interferes with the conversion of exogenous pyruvate into glycogen, α-methyltryptophan does not. Tryptophan promotes an increase in the assayable level of phosphoenolpyruvate carboxykinase in the liver, but α-methyltryptophan causes a decrease. The maximal effects of tryptophan on these parameters are observed in about 4 hr, but those of α-methyltryptophan take about 28 hr to develop. The actions of tryptophan on glycogenesis are independent of the adrenal gland, but α-methyltryptophan acts only in the presence of this organ. Some metabolites of tryptophan were also examined. 3-Hydroxyanthranilic acid mimicked the effect of tryptophan with respect to inhibition of glycogen formation from pyruvate as well as enhancement of phosphoenolpyruvate carboxykinase activity. From a statistical comparison of the curves relating log (dose) and response to tryptophan and 3-hydroxyanthranilic acid, respectively, it was concluded that both compounds may act by the same mechanism and, hence, that 3-hydroxyanthranilic acid could account, in part, for the effect of tryptophan.

Foster *et al.* (1966b) have shown that the administration of L-tryptophan to fasted rats, either adrenalectomized or intact, greatly elevates the PEP[1] carboxykinase activity of the liver. Because various metabolic and endocrine factors causing an increase in the activity of this enzyme also bring about an increase in gluconeogenesis (Foster *et al.*, 1966a; Henning *et al.*, 1966; Lardy, 1965; Lardy *et al.*, 1965), it would be expected that L-tryptophan would do the same. Paradoxically, the concentration of liver glycogen is subnormal after giving tryptophan (Foster *et al.*, 1966b; Rosen and Nichol, 1964). The mechanism involved here seems to be an action of tryptophan in rendering the enzyme nonfunctional *in vivo* (Ray *et al.*, 1966).

The Wisconsin group has suggested that the compound active in elevating PEP carboxykinase, if not L-tryptophan itself, must be very closely related to tryptophan in its structure (Foster *et al.*, 1966b). In our laboratory an analog of tryptophan, α-methyltryptophan, has been used for many years in studies of the function of tryptophan pyrrolase (Moran and Sourkes, 1963; Sankoff and Sourkes, 1962; Sourkes and Townsend, 1956) because this substance brings about a very prolonged increase in the activity of the enzyme in the liver, qualitatively similar to the action of tryptophan itself. The long-lasting action of α-methyltryptophan is presumably due to its structural similarity to the natural amino acid, combined with its long residence in the body and limited metabolic alteration (Madras and Sourkes, 1965). In the present experiments α-methyltryptophan was tested to determine whether it mimics the action of tryptophan also with respect to glycogen deposition and the activity of PEP carboxykinase. When, in the course of this work, it was found that α-methyltryptophan and tryptophan have divergent actions on the systems tested it was decided to extend the work of Lardy and his colleagues (Foster *et al.*, 1966b) by testing some intermediates in the metabolism of tryptophan for their effects on glycogen concentration of the liver as well as on glycogenesis from administered pyruvate.

Experimental Procedure

Male Sprague–Dawley rats weighing approximately 150 g were starved for varying periods of time as indicated below, injected intraperitoneally with the test compound at a specific time, and finally given sodium pyruvate by gavage 4 hr before death. The particulars of each experiment are included with the tables. Control rats received doses of the appropriate vehicles. The solutions containing the test compounds were always adjusted to pH 7–7.5.

The animals were killed by decapitation. For glycogen determination, the left lobe of the liver was quickly removed, blotted with filter paper to remove excess blood, and then placed in a petri dish embedded in

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[1] Abbreviations used: PEP, phosphoenolpyruvate; ACTH, adrenocorticotropin; ATP, adenosine triphosphate.

cracked ice. A piece of tissue weighing 50–100 mg was weighed immediately on a microtorsion balance, placed in 1.0 ml of 30% KOH, and the glycogen content was measured by the anthrone procedure of Seifter *et al.* (1950). For estimation of PEP carboxykinase, the left lobe of the liver was stored temporarily in 0.25 M sucrose in a beaker kept cold on ice. A 10% homogenate (in 0.25 M sucrose) was prepared and then centrifuged at 105,000*g* for 1 hr at 0–1°. PEP carboxykinase activity in the supernatant was determined by the method of Nordlie and Lardy (1963) as employed by Foster *et al.* (1966b). The phosphate from PEP was cleaved by the addition of a solution of mercuric chloride (Lohmann and Meyerhof, 1934) and the liberated inorganic phosphate was estimated by the method of Sumner (1944). Blood sugar was determined by the glucose oxidase method, using reagents prepared by Worthington Biochemical Corp., Freehold, N. J. (Glucostat). Blood was collected in beakers containing oxalic acid and sodium fluoride.

DL-α-Methyltryptophan was a gift from Merck Sharp and Dohme, Inc., Rahway, N. J. The following compounds were purchased from the indicated sources: DL-kynurenine, xanthurenic acid, glutaric acid, and 3-hydroxyanthranilic acid, Sigma Chemical Co., St. Louis, Mo.; L-tryptophan, Matheson Coleman and Bell, Inc., East Rutherford, N. J.; kynurenic acid and quinolinic acid, Nutritional Biochemicals Corp., Cleveland, Ohio; oxalacetate and serotonin creatinine sulfate (B grade), Calbiochem, Los Angeles, Calif.; inosine triphosphate disodium and glutathione, Schwartz BioResearch, Inc., Orangeburg, N. Y.; and anthranilic acid and tryptamine, Eastman Organic Chemicals, Rochester, N. Y.

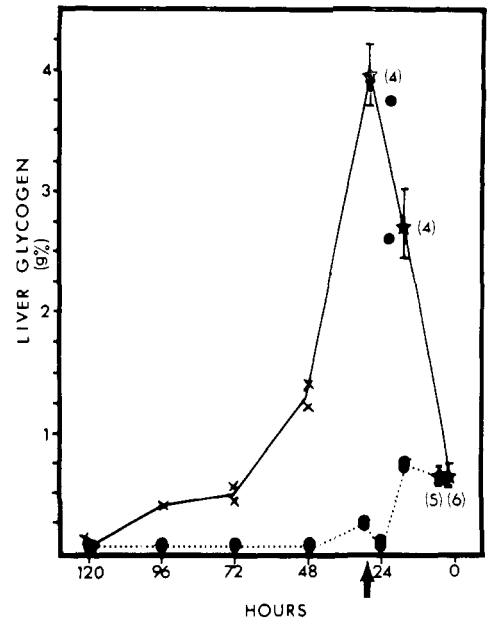

FIGURE 1: Effect of DL-α-methyltryptophan on endogenous glycogenesis of rat liver. Animals received 750 mg of DL-α-methyltryptophan/kg body weight by intraperitoneal injection for varying periods, as indicated by the abscissa, before they were killed for the determination of liver glycogen concentration. All animals, including those in which the test compound was to act for less than 28 hr, were fasted for 28 hr before death. Controls were treated similarly except for the administration of the amino acid analog. Number of rats, in parentheses; otherwise, each point represents a single animal. Solid line, α-methyltryptophan; broken line, control animals; vertical bars, standard error. Arrow indicates start of fasting period.

Results

The Effect of α-Methyltryptophan on Glycogen Metabolism of the Liver. The graph in Figure 1 illustrates the influence of DL-α-methyltryptophan on the glycogen content of the liver of rats that had been fasted for 28 hr before being killed for the estimation of liver glycogen. Rats injected with saline 120, 96, 72, 48, or 24 hr before death had almost negligible concentrations of liver glycogen while control animals injected at 28 or 16 hr before death had higher levels in their livers (Figure 1, broken line). On the other hand, animals receiving α-methyltryptophan 28, 24, or 16 hr before death showed much higher concentrations of hepatic glycogen than the corresponding control rats. When α-methyltryptophan was injected at longer intervals (48, 72, or 96 hr before death) excess liver glycogen accumulated also, but not in as great amounts as in the experiments of short duration (Figure 1, solid line). There is no effect of α-methyltryptophan at 5 days (Figure 1). Tryptophan administered at any time during this fasting period had little or no effect on the hepatic glycogen concentration, the mean change being 0.02 g/100 g of liver (standard error 0.06, $n = 17$) below control values.

Among the mechanisms that could account for these elevated levels of glycogen are (a) enhanced glycogenesis in the presence of α-methyltryptophan, (b) inhibition of breakdown of endogenous glycogen in fed rats or rats fasted for short periods (up to 12 hr in our experience), or (c) a combination of these. In addition, the action of α-methyltryptophan on carbohydrate metabolism could be an indirect one, mediated by an action on the anterior pituitary gland (release of ACTH) or the adrenal cortex, with corticosterone effecting the metabolic changes. To ascertain which of these mechanisms might be responsible, further experiments were performed. In the first of these, rats that had been fasted for 24 hr before receiving the α-methyltryptophan were used. Such rats have a glycogen concentration in the liver below 0.5 g/100 g. Six animals were used, all receiving α-methyltryptophan after having been without food for 24 hr. Three of the rats were maintained on fast for 16 hr more, at which time the liver contained 4.87, 4.78, and 5.00 g of glycogen/100 g of liver, respectively, in excess of controls. The other three were fasted 28 hr longer (total of 52 hr); their liver glycogen then amounted to 3.96, 3.04, and 3.90 g/100 g of liver. These results show

that despite the 24-hr fast prior to the injection of the amino acid, the level of hepatic glycogen in α-methyltryptophan-treated animals was high. This finding suggests that the analog does, in fact, promote the formation of glycogen, probably by stimulating gluconeogenesis. The mean rates of glycogenesis can be calculated as 0.30 and 0.13 μmole of carbohydrate/g of liver per min in the two groups of fasted rats. Sixteen hours after the administration of α-methyltryptophan to fasted, intact rats, *i.e.*, at a time when there is substantial net glycogen deposition in the liver, the blood glucose level was found to be 80 mg % (standard error 1.3, $n = 5$); a comparable group of control animals had 59 mg % (standard error 1.0, $n = 4$). This represents an elevation of 36%.

Because of the apparently prolonged effect of α-methyltryptophan on glycogen metabolism (Figure 1), the time relations were also examined with respect to glycogenesis from intragastrically administered pyruvate. The rats were all fasted for 28 hr, receiving pyruvate 4 hr before being killed for the determination of liver glycogen. The α-methyltryptophan was injected at the beginning of the fasting period, at the same time as the pyruvate, or at an intermediate time, as shown in Table I. When the amino acid analog was injected at the same time as the pyruvate or 12 hr before this, it had no significant influence on the hepatic glycogen concentration. But when it was injected 24 hr before the pyruvate was given there was a substantial increase in the liver glycogen. These results indicate that the effect of α-methyltryptophan on the formation of glycogen may be exerted independently of pyruvate, and that α-methyltryptophan does not interfere with the formation of glycogen from administered pyruvate.

TABLE I: Effect of α-Methyltryptophan on Pyruvate-Induced Glycogenesis in Fasted Rats.[a]

Treatment	n	Hepatic Glycogen Concn (g/100 g of liver)
None	8	0.62 ± 0.08[b]
Pyruvate	8	2.19 ± 0.21
Pyruvate; α-methyltryptophan given simultaneously	6	1.86 ± 0.28
Pyruvate; α-methyltryptophan given 12 hr previously	4	2.97 ± 0.34
Pyruvate; α-methyltryptophan given 24 hr previously	3	4.07 ± 0.39

[a] Rats were given water, but no food, for 28 hr, at which time they were killed for determination of liver glycogen. Four hours before this they were given 2.2 g of sodium pyruvate intragastrically/kg of body weight. Some rats received in addition 750 mg of α-methyltryptophan/kg of body weight at the times indicated in the table. Control rats were given an injection of 0.9% NaCl instead of the α-methyltryptophan. [b] Mean plus or minus standard error.

In many conditions favoring gluconeogenesis there is an associated increase in PEP carboxykinase activity (Lardy *et al.*, 1965). To determine whether this occurs when α-methyltryptophan is given, estimates of PEP carboxykinase activity were made in rats that had been treated in the same way as for determination of liver glycogen. The results in Table II show that α-methyltryptophan, acting for 4 hr, results in no change in the activity of this enzyme. However, when it was given at greater intervals preceding the death of the animal there was a decrease in enzyme activity. This result indicates that just as in its effect on liver glycogen (with and without the administration of pyruvate), α-methyltryptophan has an action on PEP carboxykinase activity of the liver that differs from the effect of tryptophan.

With the glycogenetic effect of α-methyltryptophan established, it was necessary to determine whether this was mediated through corticosterone, and for the next experiments adrenalectomized rats were used. The following procedure was used. The animals, adrenalectomized 5 days beforehand, were fasted for 24 hr except for the provision of 1% NaCl to drink; they were then injected intraperitoneally with 250 or 500 mg of α-methyltryptophan/kg of body weight, fasted 16 hr more, and killed. At this time the concentration of liver glycogen was 0.03–0.05 g/100 g ($n = 6$), as against control values of 0.01–0.03 g/100 g ($n = 3$). In regard to the concentration of PEP carboxykinase in the liver, α-methyltryptophan had no effect in adrenalectomized rats (Table II). Thus, the adrenal gland is needed for the effects of the amino acid on carbohydrate metabolism.

Effect of Tryptophan and Its Metabolites. The glycogen-decreasing effect of tryptophan was studied as a function of the concentration of this amino acid, and the results are shown in Table III. At some dose levels (particularly at 75 and 750 mg/kg of body weight) the tryptophan effected a decrease; other concentrations tested had no effect. In parallel experiments rats were given sodium pyruvate (2.2 g/kg of body weight) by intragastric tube. This resulted in a mean glycogen level of 2.07 ± 0.07 g/100 g of liver ($n = 43$). But when tryptophan was given intraperitoneally at the same time as the pyruvate, there was an inhibition of formation of glycogen, and this inhibition was intensified in degree in going from 37.5 to 150 mg of tryptophan/kg of body weight. The latter dose provided the maximum effect.

Because of the difference in effect of tryptophan and α-methyltryptophan there was the possibility that the former is not acting *per se* but through a metabolite formed from it. Some of these metabolites were tested for their effect on the liver glycogen of fasted rats; the results are shown in Table III. In regard to the glycogen stores in the liver under the experimental conditions of fasting, the concentration was depressed below that found in the controls not only by tryptophan but also by 3-hydroxyanthranilic acid and anthranilic acid in the same dose range. Xanthurenic acid and tryptamine tended to give somewhat higher glycogen values than

TABLE II: The Effects of Tryptophan, α-Methyltryptophan, and Related Compounds on the PEP Carboxykinase Activity of Liver of Intact (I) and Adrenalectomized (A) Rats.[a]

Test Compound	Animals Used	Dose (mg/kg)	Hr before Death	Enzyme Act. (nmoles of PEP/min per mg of protein)		Act. Ratio[b]
				Control	Experimental	
Tryptophan	I	750	4	68, 62	173, 190, 164	2.7
Tryptophan	A	750	4	62	118	1.9
α-Methyltryptophan	I	750	4	82, 62	81, 85, 53	1.0
α-Methyltryptophan	I	750	16	80, 76	57	0.7
α-Methyltryptophan	I	750	28	77, 71	37, 43, 41	0.5
α-Methyltryptophan	I	500	28	67	41, 47, 40	0.6
α-Methyltryptophan	A	500	16	78, 75	74, 88	1.1
α-Methyltryptamine	I	170	4	75, 92	71, 72	0.9
3-Hydroxyanthranilic acid	I	750	4	66, 58	126, 140, 178	2.4
3-Hydroxyanthranilic acid	A	750	2.5	62	117	1.9
3-Hydroxyanthranilic acid	I	500	4	71, 70	129, 167, 123	2.0
3-Hydroxyanthranilic acid	A	500	2.5	62	117	1.9
3-Hydroxyanthranilic acid	I	375	4	69, 71	105, 107, 117	1.
3-Hydroxyanthranilic acid	I	250	4	77, 77	112, 106, 99	1.4
3-Hydroxyanthranilic acid	A	250	2.5	62	100	1.6
Serotonin	I	50	4	65, 56	78, 67, 98	1.37

[a] Rats were fasted for 24 hr, and were then killed for the determination of enzyme activity. They received the compounds intraperitoneally in 3.0 ml of 0.9% NaCl at the indicated number of hours before death. [b] Activity with test compound per activity in control liver (mean values used for this calculation).

seen in the controls. Kynurenic, quinolinic, and glutaric acids and serotonin were without effect.

The interference exerted by tryptophan on pyruvate-induced glycogenesis (Foster *et al.*, 1966b) was also shown by glutaric acid, 3-hydroxyanthranilic acid, anthranilic acid, kynurenine, and serotonin (*cf.* column 5, Table III). In this test there was no distinct effect of kynurenic xanthurenic, and quinolinic acids, or of tryptamine.

The glycogen deficit caused by 3-hydroxyanthranilic acid in rats receiving pyruvate increased with the dose of this compound, a phenomenon also observed with tryptophan (Table III). Using the method of Irwin (1937), as described by Pugsley (1946), lines of best fit were calculated for the pertinent data so that the relative potency of the two substances in depressing liver glycogen could be stated precisely. The appropriate parameters are listed in Table IV, and these show that the two regression lines are parallel ($P < 0.01$), indicating that the two compounds have a similar type of action in the rat with respect to the liver glycogen. 3-Hydroxyanthranilic acid has, however, only 22% of the potency (fiducial limits, $P = 0.05$, were 11–41%) of tryptophan in this test.

Because of this action of 3-hydroxyanthranilic acid, the compound was tested in regard to its action on hepatic PEP carboxykinase. It was found (Table II) that it causes a substantial increase in the enzyme activity, although the effect is not as notable as with tryptophan. The effect of 3-hydroxyanthranilic acid, like that of tryptophan itself, persists in adrenalectomized rats (Table II). Anthranilic acid has been previously shown not to affect the concentration of PEP carboxykinase (Foster *et al.*, 1966b).

Serotonin caused some increase in the activity of PEP carboxykinase (Table II).

Discussion

Our results with L-tryptophan agree with the demonstration by Foster *et al.* (1966b) that formation of glycogen from certain of its endogenous precursors is inhibited by this amino acid in the fasted rat, even though the assayable level of PEP carboxykinase is substantially increased. Several metabolites of tryptophan mimicked its actions on glycogen metabolism (Table III) to a greater or lesser extent, and it is therefore conceivable that some of them play a role in the effects attributed to tryptophan. 3-Hydroxyanthranilic acid appeared to be more active than the other compounds and was studied more extensively. Like tryptophan, it inhibits glycogenesis from pyruvate (Table III), at the same time increasing the activity of PEP carboxykinase of the liver (Table II). It exerts its action on this enzyme in both intact and adrenalectomized rats. It seems to interfere with gluconeogenesis by a similar mechanism to that of tryptophan, judged by the parallelism of their regression curves (Table IV). At least some of the effect

TABLE III: Effect of Tryptophan and Some of Its Metabolites on Liver Glycogen and on Pyruvate-Induced Glycogenesis in Fasted Rats.[a]

Test Compound	Dose (mg/kg)	No. of Rats in Each Group	Concn of Liver Glycogen (g/100 g of fresh liver)	
			Endogenous Glycogenesis	Pyruvate-Induced Glycogenesis
L-Tryptophan	750	3	−0.59 ± 0.13[b]	−1.93 ± 0.31
L-Tryptophan	250	3	−0.07 ± 0.14	−1.74 ± 0
L-Tryptophan	150	3	−0.05 ± 0.02	−1.94 ± 0.08
L-Tryptophan	75	6	−0.33 ± 0.06	−1.23 ± 0.18
L-Tryptophan	37.5	3	−0.04 ± 0.01	−0.48 ± 0.16
DL-Kynurenine	750	3	0.04 ± 0.07	−0.52 ± 0.41
Kynurenic acid	750	5	−0.10 ± 0.12	0.11 ± 0.18
Anthranilic acid	750	5	−0.29 ± 0.05	−1.51 ± 0.25
Xanthurenic acid	750	3	0.58 ± 0.09	0.15 ± 0.22
3-Hydroxyanthranilic acid	750	5	−0.49 ± 0.05	−1.74 ± 0.20
3-Hydroxyanthranilic acid	250	3	0.13 ± 0.03	−1.47 ± 0.23
3-Hydroxyanthranilic acid	150	3	0.02 ± 0.09	−0.60 ± 0.20
Quinolinic acid	750	1, 2	−0.12	−0.06, −0.11
Glutaric acid	750	3	0.08 ± 0.06	−0.67 ± 0.25
Tryptamine	150	3	0.35 ± 0.18	0.05 ± 0.02
Serotonin	150	3	−0.08 ± 0.16	−1.83 ± 0.24
Serotonin	50	3	−0.02 ± 0.01	−1.76 ± 0.00
Serotonin	20	3	0.08 ± 0.00	−0.77 ± 0.00

[a] Rats were fasted for 24 hr prior to death. Four hours before being killed for determination of the concentration of liver glycogen they received the test compound by intraperitoneal injection. Some animals also received sodium pyruvate (2.2 gm/kg) intragastrically at the same time as the test compound. Individual values were corrected for the concentration of glycogen found in the corresponding control animal (no test compound injected) before calculating the means shown. Control rats (intact) in these experiments had a liver glycogen concentration of 0.36 g/100 g of liver ±0.04 std error for $n = 47$. Adrenalectomized control rats had less than 0.02 g/100 g of liver. [b] Mean plus or minus standard error.

of 3-hydroxyanthranilic acid may be attributed to stimulation of glycolysis, observed when the compound is added to liver homogenates (Papa *et al.*, 1962) or inhibition of oxidative phosphorylation in mitochondria (Quagliariello *et al.*, 1964). A continuing supply of ATP is necessary for gluconeogenesis from pyruvate, lactate, and four-carbon precursors, and interference with its formation would be quickly reflected in a diminished rate of gluconeogenesis (Mendicino and Utter, 1962; Krebs, 1964; Newsholme and Underwood, 1966). This process would be further jeopardized by a diminished rate of PEP carboxykinase activity, because an elevated level of adenosine monophosphate inhibits this enzyme (Gevers and Krebs, 1966). Recently Ray *et al.* (1966) have expressed the view that the catalytic effect of PEP carboxykinase *in vivo* is blocked when tryptophan is given, despite the considerable increase in the level of the enzyme. Such a block may also then be involved in the action of 3-hydroxyanthranilic acid *in vivo*. By extension of this argument, it is conceivable that the 3-hydroxyanthranilic acid formed intracellularly from the administered tryptophan contributes to the over-all effect of tryptophan.

DL-Kynurenine was not as effective as tryptophan and 3-hydroxyanthranilic acid in inhibiting glycogenesis (Table III). In respect to hepatic PEP carboxykinase Foster *et al.* (1966b) found this metabolite much less effective than tryptophan in stimulating an increase in activity. They used racemic amino acid, and it is not known what contribution the D isomer makes to its action on the enzyme; if it has no action, then the natural form of kynurenine must be considered more effective than the reported figures indicate. Moreover, kynurenine possesses a net positive charge at physiological pH unlike tryptophan and 3-hydroxyanthranilic acid which exist there in the zwitterionic form; the net charge may reduce the rate of absorption from the peritoneal cavity.

Anthranilic acid, like its 3-hydroxy derivative and tryptophan, interfered with the deposition of liver glycogen (Table III). But because it differs from these two compounds in not affecting the level of PEP carboxykinase in the liver (Foster *et al.*, 1966b) and, therefore, lacked an important resemblance to the action of tryptophan, it was not investigated further. Of all the metabolites examined by the Wisconsin group only

TABLE IV: Comparison of the Effect of Tryptophan and 3-Hydroxyanthranilic Acid on the Formation of Glycogen from Exogenously Supplied Sodium Pyruvate.

Regression equations[a]	
Tryptophan	$Y = 1.19X + 0.51$
3-Hydroxyanthranilic acid	$Y = 1.24X - 34.24$
M = log (potency of tryptophan/potency of 3-hydroxyanthranilic acid) = 1.3364	
Antilog M = 0.217 (corresponding to 21.7%)	
Standard error of the assay, $S_m = \pm 0.140$	
Fiducial limits ($P = 0.95$): 0.53 and 1.88 (corresponding to relative potencies of 11.5 and 40.8%, respectively)	
Test for parallelism of the regression lines: for 1 deg of freedom, $P = 0.01$, $\chi^2 = 0.005$	

[a] Y = g of glycogen deficit/100 g of liver. X = log (dose, in milligrams per kilogram of body weight).

N-formyl-L-kynurenine proved to be active; it was 48% as effective as tryptophan in provoking an increase in PEP carboxykinase when it was administered at low dosage, but over 80% as effective at higher dosage (Foster *et al.*, 1966b).

Serotonin also interfered with deposition of glycogen in the liver. It is known that this amine can cause glycogenolysis, hyperglycemia, and stimulation of hepatic phosphorylase activity (Levine *et al.*, 1964). Whatever the manner in which serotonin causes glycogen depletion of the liver, the effect is not mediated by inhibition of PEP carboxykinase, for enzyme activity is actually increased slightly by treatment of the animals with serotonin (Table II).

The stimulation of endogenous glycogenesis by xanthurenic acid is contrary to the observation of Kotake and Inada (1954). These investigators state that in a dosage of 200 mg/kg this compound causes a 50% decrease in hepatic glycogen 4 hr after it is given.

Experiments with α-methyltryptophan were performed in an attempt to throw some light on the action of tryptophan on liver glycogen. Because of its structural resemblance to tryptophan and its qualitatively similar effect in stimulating the activity of hepatic tryptophan pyrrolase (Civen and Knox, 1960; Sourkes and Townsend, 1956), it was expected that it would reproduce at least some features of the action of tryptophan. Surprisingly, it had an opposite effect in regard to the activity of PEP carboxykinase and to the concentration of liver glycogen with or without the provision of pyruvate. Estimating from the plotted data for α-methyltryptophan in Figure 1, the net rate of glycogen formation stimulated by this amino acid in the livers of previously fed rats was 0.25 g/100 g of liver per hr, or about 0.15 μmole of carbohydrate/g of liver per min. In rats fasted for 24 hr and then given α-methyltryptophan, the rate of glycogenesis rose to as high as 0.30 μmole/g of liver per min, during a 16-hr period (see above).

Although it is clear that the action of α-methyltryptophan depends upon the presence of the adrenal gland for its action, nevertheless in the intact rat this amino acid can be expected to contribute an independent effect that is favorable to glycogenesis. It probably increases the supply of gluconeogenic carbon chains derived from amino acids. It could accomplish this by its acceleration of the breakdown of tryptophan (Moran and Sourkes, 1963), resulting in the contemporaneous degradation of other amino acids because of the relative tissue deficiency of tryptophan, an essential amino acid, that now develops (Salmon, 1954, 1958). In the rat, a decline in body weight for 1–2 days after injection of α-methyltryptophan has been observed; this effect is not the result of appetite suppression (Sankoff and Sourkes, 1962) and probably reflects a novel type of situation in which an essential amino acid becomes limiting at the sites of protein synthesis in the tissues, owing to an endogenously derived deficiency. Hepatic glycogenesis is stimulated at the expense of protein. Although tryptophan also causes a large increase in the level of pyrrolase in the liver, a deficiency cannot develop because of the large exogenous supply of the amino acid that is injected and because the elevated pyrrolase activity declines toward normal as the concentration of tryptophan in the liver is restored to normal levels.

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Electron Transport in Relation to Steroid Biosynthesis. Inhibition of Side-Chain Cleavage of Cholesterol by Hyperbaric Oxygen*

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ABSTRACT: Mitochondria from rat adrenals convert [^{3}H]cholesterol to [^{3}H]pregnenolone (side-chain cleavage of cholesterol). Side-chain cleavage was stimulated by succinate but not by other Krebs cycle intermediates; stimulation by succinate was prevented by malonate. Amytal inhibited side-chain cleavage in the presence or absence of succinate and neither L-malate plus amytal nor endogenous substrates plus amytal stimulated side-chain cleavage. Measurement of oxidized diphosphopyridine nucleotide (DPN^+) and reduced diphosphopyridine nucleotide (DPNH) in mitochondria from bovine adrenal cortex indicated that succinate reduced DPN^+ to a greater extent than endogenous substrates or endogenous substrates plus other Krebs cycle intermediates. This effect of succinate was prevented by dinitrophenol, amytal, and arsenate. Fluorimetric measurement of the redox state of pyridine nucleotides in adrenal mitochondria demonstrated that hyperbaric oxygen oxidized reduced pyridine nucleotides reversibly, and that succinate with or without adenosine triphosphate (ATP) protected against this effect of hyperbaric oxygen. Hyperbaric oxygen (a specific inhibitor of reversed electron transport: Chance, B., Jamieson, D., and Coles, H. (1965), *Nature 206, 257*) inhibited side-chain cleavage of cholesterol and succinate protected mitochondria against this inhibition. Finally, mitochondrial or submitochondrial systems in which side-chain cleavage requires or is stimulated by exogenous reduced triphosphopyridine nucleotide (TPNH) were not inhibited by hyperbaric oxygen. These observations suggest that electron transport associated with the side-chain cleavage of cholesterol in adrenal mitochondria follows the pathway: succinate → DPN^+ → TPN^+ → cholesterol.

Steroid biosynthesis involves the conversion of cholesterol to pregnenolone by way of two reactions which successively hydroxylate the side chain of cholesterol at C_{20} and C_{22} (Solomon *et al.*, 1956; Shimizu *et al.*, 1962; Constantopoulos *et al.*, 1962). The conversion of cholesterol to pregnenolone (side-chain cleavage of cholesterol) takes place in mitochondria (Halkerston *et al.*, 1961) and includes the rate-limiting step in steroid biosynthesis (Stone and Hechtor, 1954), a step specifically stimulated by the trophic hormones ACTH[1] (Karaboyas and Koritz, 1965) and ICSH (Hall and Koritz, 1965; Hall, 1966).

Like other steroid hydroxylations, the above reactions require TPNH (Halkerston *et al.*, 1961) and a system of electron carriers, one component of which is inhibited by carbon monoxide (Simpson and Boyd, 1966); presumably this component is the cytochrome

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[1] Abbreviations used: ACTH, adrenocorticotropic hormone; ATP, adenosine triphosphate; BSA, bovine serum albumin; ICSH, interstitial cell stimulating hormone; TPN^+ and TPNH, oxidized and reduced triphosphopyridine nucleotides; DPN^+ and DPNH, oxidized and reduced diphosphopyridine nucleotides.