Influence of Cortisone on Rate of Absorption and Formation of Liver Glycogen by Alanine.* (21074)

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Gluconeogenesis from protein in the fasting animal is believed to be mediated by the adrenal cortex(1-4). Hess and Shaffran(5) found that cortisone did not increase the production of liver glycogen after feeding glycine above the additive effect of the individual action of the 2 compounds. Glycine forms liver glycogen rather slowly, reaching a peak value at 14 hours(6) and it was possible that the slow rate of glycogen formation may have been responsible for the failure to demonstrate any effect by cortisone. Alanine has been reported to form liver glycogen more rapidly than glycine(7-9) and its rate of absorption has been found to range from 50 to 94 mg per 100 g per hour for DL alanine, 78 to 82 mg for L(+) alanine and 62 mg for D(—) alanine(8-12). The rate of absorption of glycine was somewhat faster, averaging 102 mg per 100 g per hour(6). Since alanine is more glycogenic than glycine and appears to be absorbed more slowly it was decided to repeat the study on the effect of cortisone on glycogen formation using alanine. Preliminary to this the rate of absorption of the several isomers of alanine and also the rate of liver glycogen formation were restudied.

Experimental. Determination of rates of absorption and glycogen formation. The same procedure was used as previously described (6), DL, D(—), or L(+)-alanine, dissolved in water, was administered at levels of 100, 150, 300, and 450 mg per 100 g body weight for 0.5-, 1-, 2-, and 3-hour periods, respectively. For the longer time periods 450 mg/100 g was used. Alanine was determined in the extracts of the gastrointestinal tract by the method of Alexander and Seligman(13), and liver glycogen by the method of Good, Kramer, and Somogyi(14). The absorption rates are corrected for 4.5% loss of alanine that occurred in the procedure. Absorption rates were not determined after the 3-hour period since the maximum dose given, 450 mg/100 g body weight, would have been completely absorbed. The glycogen values for periods of time longer than 6 hours are averages of determinations on from 4 to 6 animals.

TABLE I. Rate of Absorption of and Glycogen Formation by Alanine.

Isomer	No. of animals	Time, hr	Rate, mg/100 g/hr	Glycogen, %
DL	6	2	108	.30†
	5	3	110	.88
	4	4	112	2.00
	4	6		3.33
			Avg 109.0 ± 10.4*	
L(+)	4	2	109	.08
	4	3	112	.55
	4	6		3.50
			Avg 110.0 ± 4.3	
D(−)	5	2	82	.10
	4	3	92	1.00
	5	6		3.90
			Avg 85.3 ± 9.0	

* Stand. dev.
† Glycogen content of livers of 12 control rats averaged 0.04%.

DL-alanine was absorbed (Table I) at a faster rate than found by Lewis(10-12) but at approximately the same rate reported by Wingo and Smith(9). The rate of absorption of D(—) is somewhat less than that of L(+)-alanine, an observation also made by Lewis(10,11). Gibson and Wiseman(15), measuring the absorption of alanine from isolated loops of rat intestine and using about one tenth of the amount of alanine used in our experiments, found that the absorption rate of L(+)-alanine was twice that of D(—)-alanine. Alanine is more rapidly glyconeogenic than glycine, the peak with DL-alanine (Fig. 1) is reached at 6 hours and with glycine(6) at 14 hours. The rate of absorption of alanine is not greatly different from that of glycine, certainly not enough to explain the

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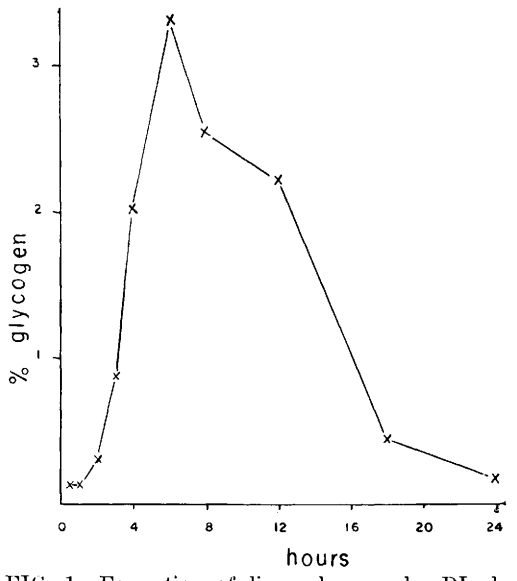

FIG. 1. Formation of liver glycogen by DL-alanine.

difference in the rate of liver glycogen formation. Reid(8) found a similar difference in rate and suggested that the metabolic pathways of the 2 amino acids are different. Both D(—)- and L(+)-alanine produced approximately the same amount of glycogen at the 6-hour period.

Administration of cortisone and alanine. The same procedure was employed as in the glycine experiments(5), 450 mg of DL-alanine dissolved in 2.5 ml H_2O were fed. Cortisone acetate in saline suspension (Cortone Merck) was given intramuscularly. It was found that cortisone in none of the dosage levels used had any effect on the absorption rates of the alanines tested. When alanine and either 2.5 or 5 mg of cortisone were given at the same time and the animals sacrificed after 6, 18, or 24 hours no significant increases in liver glycogen content above the sum of the amounts produced by DL-alanine and cortisone alone were found (Table II). This is the same result found when both glycine and cortisone were given together(5).

Since alanine forms glycogen at a faster rate and to a greater extent than glycine it is possible that by the time the maximum effect of the cortisone occurs the peak of glycogen production has been passed by several hours. Consequently an experiment was planned so that the peak effect of both the cortisone and the DL-alanine would occur at approximately the same time. Cortisone alone produced the maximum amount of glycogen after 24 hours and DL-alanine after 6 hours. By giving DL-alanine 18 hours after the cortisone the peaks for both compounds would coincide at 24 hours. In Table III the results of this experiment using 5 and 2.5 mg of cortisone are given and also the effect of feeding DL-alanine 1, 2, 4, and 6 hours before the cortisone peak. The values in column 3 are on the control animals given only cortisone and sacrificed after 24 hours. The values in column 4 are the differences between the values in column 1 and the sum of the alanine values for 1, 2, 4, and 6 hours given in column 2, taken from Fig. 1, and the values in column 3. Significant increases in glycogen content result when the peaks of cortisone activity and glycogen production from alanine are close together, particularly after 5 mg of cortisone. To ascertain whether a similar result could be obtained with glycine this amino acid was given so that its maximum period would occur at the same time as the cortisone maximum. The peak for glycine alone is at 14 hours therefore it was fed 10 hours after the cortisone was injected and the animals sacrificed at 24 hours, no extra liver glycogen was produced.

Total nitrogen determinations were made on the urines of the rats receiving 5.0 mg of cortisone for 24 hours and 450 mg of DL-alanine during the last 6 hours and also on 4

TABLE II. Liver Glycogen Formation by Simultaneous Administration of Cortisone and DL-Alanine.

Time, hr	1 Alanine + cortisone	2 Alanine	3 Cortisone (5)	4 Change
	%			
6 (4)*†	3.5	3.3	.1	+.1
18 (4)*	1.8	.5	.6	+.7
24 (6)*	1.7	.2	2.1	−.6
6 (4)‡	4.5	3.3	1.6	−.4
18 (4)‡	3.3	.5	2.2	+.6
24 (6)‡	2.5	.2	1.9	+.3

* 2.5 mg cortisone.
† No. in parentheses is No. of animals used.
‡ 5.0 mg cortisone.
Column 4 is the difference between column 1 and the sum of columns 2 and 3.

TABLE III. Cortisone Given 24 Hours, DL-Alanine Given Varying Number of Hours following Cortisone, and Subsequent Liver Glycogen Determinations.

	1	2	3	4
Alanine, hr after cortisone	Alanine + cortisone	Alanine	Cortisone (5)	Change
	%			
23 (4)*†	2.7	.1	2.1	+ .5
22 (4)*	3.0	.3	"	+ .6
20 (4)*	4.9	2.0	"	+ .8
18 (6)*	6.6	3.3	"	+1.2
23 (4)‡	2.5	.1	1.9	+ .5
22 (4)‡	4.0	.3	"	+1.8
20 (6)‡	6.3	2.0	"	+2.4
18 (6)‡	7.3	3.3	"	+2.1

* 2.5 mg cortisone.
† No. in parentheses is No. of animals used.
‡ 5.0 mg cortisone.
Column 4 is the difference between column 1 and the sum of columns 2 and 3.

control rats receiving the same amount of DL-alanine for the last 6 hours of a 24-hour fasting period but with no cortisone. The average excretion of nitrogen on DL-alanine alone was 220 mg and for the cortisone plus alanine animals it was 250 mg. The nitrogen excretion of rats fasting for 24 hours was 190 mg and for rats receiving 5 mg of cortisone during the 24 hours fast the nitrogen excretion was 210 mg. There was a slight increase in nitrogen excretion from the DL-alanine when cortisone was administered, the extra nitrogen was equivalent to 40% of the alanine nitrogen when fed alone and 60% when cortisone was also administered.

Apparently there is a stimulation of glycogen formation by cortisone when alanine is fed but not with glycine. This may also point to a difference in the metabolic pathways of these 2 amino acids as suggested by Reid(8) and noted in connection with the rate of liver glycogen formation. It is planned to study this problem further by the use of isotopic carbon labeled alanine and isolation of the liver glycogen to determine if the increase in glycogen represents an increase in the number of carbon atoms from the alanine.

Summary. 1. The rates of absorption of D(—), L(+), and DL-alanine from the gastrointestinal tract of white rats were found to be 85, 110, and 112 mg/100 g of body weight/hour, respectively. All 3 were approximately equal in their ability to form liver glycogen, maximum values were obtained 6 hours after administration. Cortisone administration had no effect upon the rate of alanine absorption. 2. When DL-alanine was fed to rats at the same time as 5 or 2.5 mg of cortisone was injected no increase in liver glycogen was obtained over the sum of the amounts formed when each of the substances was given separately. When DL-alanine and cortisone was administered so that the peak of glycogen formation for each compound was reached at the same time then there was a significant increase in glycogen formation over the sum of the independent actions of the 2 compounds.

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