Brucer, M., PROC. SOC. EXP. BIOL. AND MED., 1951, v76, 278.

2. Brucer, M., *The Merck Report,* 1952, v61, 9.

3. Gofman, J. W., *J. Lab. Clin. Med.,* 1949, v34, 297.

4. Dobson, E. L., Gofman, J. W., Jones, H. B., Kelly, L. S., and Walker, L. A., *Ibid.,* 1949, v34, 305.

5. Hart, H., and Greenberg, J., *Proc. Am. Assn. for Cancer Research,* 1953, v1, 23.

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Effect of Cortisone Upon Formation of Liver Glycogen from Glycine.* (20497)

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Gluconeogenesis from protein in the fasting animal has been established as being mediated by the adrenal cortex(1-4). Hess and Shaffran(5) have studied the rate of liver glycogen formation from glycine in fasted normal rats. The present paper is concerned with the effect of cortisone upon the rate of glycogen formation from glycine in the fasted normal rat.

Experimental. White rats, weighing 100 to 150 g, fasted for 24 hours were fed, by stomach tube, 450 mg of glycine dissolved in 2.5 ml of water. Cortisone acetate in saline suspension (Cortone Merck) was given intramuscularly at the same time as the glycine at 2 dosage levels, 2.5 and 5.0 mg. Controls receiving only cortisone were also studied. At the end of the experimental periods the animals were sacrificed and liver glycogen determined by the method of Good, Kramer, and Somogyi(6). The animals were kept in metabolism cages during the fasting and test periods and urine was collected for total nitrogen determination by the micro-Kjeldahl method. The results are given in Tables I, II, and III; each value is the average of the results from 4 to 6 animals. Duplicate determinations were made on each sample. For comparison the data for the formation of glycogen from glycine alone(5) are included.

Cortisone alone produced the increase in liver glycogen observed by many other investigators. The 5 mg dose induced the maximum deposition at 40 hours, the period from 31 to 48 hours giving a fairly uniform value. A peak was found at 16 hours followed by a slight decline and a subsequent marked increase; a similar effect was noted when cortisone plus glycine were given. The 2.5 mg dose produced a maximum at 24 hours, the amounts found at 31 and 48 hours were slightly less. The maximum amount of glycogen formed following the 5 mg dose of cortisone was almost twice that produced by feeding glycine alone and the maximum formed following the 2.5 mg dose was just slightly less than the peak for glycine alone. However, the maximum glycogen value following glycine was reached earlier.

When 5 mg of cortisone was given at the

TABLE I. Concentration of Liver Glycogen Following Glycine, 5 mg Cortisone, and Glycine plus Cortisone.

	1	2	3*	4
Time, hr	Glycine	Cortisone	Cortisone + glycine	Change
	%			
2	.25	.23	.025	—.455(<.01)†
4	.95	1.80	2.50	—.25 (<.10)
6	1.35	1.60	2.60	—.35 (<.01)
12	2.40	1.80	4.60	+.40 (<.01)
14	2.50	2.35	5.15	+.30
16	2.55	2.40	5.20	+.25
24	1.80	1.90	3.70	.0
31	.40	3.80	4.10	—.10
40	.20	4.00	4.40	+.20
48	.20	3.70	3.90	.0

* Column 3—(Column 1 + column 2).

† P values for the change based upon the calculation for t. From 14 hr to 48 hr the changes are not significant.

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TABLE II. Total Liver Glycogen and Urine Nitrogen, 5 mg Cortisone Dose.

Period and treatment	Liver wt, g	Glycogen, %	Glycogen total, mg	Urine nitrogen, mg
2 hr				
Glycine (7)*	5.3	.25	13.2 (3.7)†	12
Cortisone (4)	5.2	.23	12.0	13
Glycine + cortisone (4)	5.5	.25	1.4	10
6 hr				
Glycine (6)	4.8	1.35	64.8 (17.8)	38
Cortisone (6)	5.2	1.60	83.2	28
Glycine + cortisone (6)	5.1	2.60	132.6 (14.7)	35
24 hr				
Glycine (5)	4.3	1.80	77.4 (21.5)	170 (100)‡
Cortisone (4)	5.0	1.90	95.0	120 (110)
Glycine + cortisone (4)	5.6	3.70	207.2 (28)	200 (110)

* No. of animals used to obtain the avg value given.

† % of glycine converted into glycogen. For the glycine plus cortisone period calculation is based on the assumption that the cortisone would have formed glycogen to the same extent as when administered alone.

‡ Nitrogen excretion for the 24 hr fasting period prior to experimental period.

TABLE III. Concentration of Liver Glycogen Following Glycine, 2.5 mg Cortisone, and Glycine plus Cortisone.

Time, hr	1 Glycine	2 Cortisone	3* Cortisone + glycine	4 Change
	%			
6	1.35	.10	1.20	—.25 (<.01)†
12	2.40	.02	1.90	—.56 (<.01)
18	2.25	.60	2.20	—.65 (<.01)
24	1.80	2.10	3.10	—.80 (<.01)
31	.40	1.70	1.80	—.30 (<.10)
48	.20	1.60	2.40	+.60 (<.10)

* Column 3—(Column 1 + column 2).

† P values for the change based upon the calculation for t.

same time as glycine an increased production of glycogen resulted. In general it appears, from the data in Table I, that the amount of glycogen formed following administration of both glycine and cortisone is equal to the sum of the amounts formed when the two are given separately. This statement may not hold in the first 6 hours during which there appears to be some inhibition of glycogen formation, since the sums of the 2 separate experiments were significantly greater than the amounts formed with the combined administration. Engel, Schiller, and Pentz(7) found that when adrenal cortical extract was administered to rats the amount of glycogen formed during the total experimental period of 6 hours while greater than after the lysine alone was less than after the ACE alone, a result similar to that obtained with glycine and cortisone after 2 hours. For the single period of 12 hours the amount of glycogen formed following both cortisone and glycine was significantly greater than the sum of the 2 separate experiments. For all the other periods there were no significant differences.

Since, during the early hours following cortisone plus glycine, there was inhibition of glycogen formation, based on the per cent formed, the absolute amounts of glycogen were also calculated. These data are given in Table II. The results thus calculated show the same trend; at the end of 2 hours the sum of the values obtained with glycine and with cortisone is 25.2 mg, whereas when the 2 were given together only 1.4 mg of glycogen was formed. At the end of the 24-hour period there was a slight excess of glycogen formed when cortisone and glycine were given together, 207.2 mg compared to 172.4 mg for the sum of the 2 given separately. If the theoretical amount of glycogen that can be formed from glycine is calculated and compared with the total glycogen produced there is a gradual increase in utilization and at the end of 24 hours the liver glycogen accounts for 21.5% of the theoretical amount when glycine is given alone and 28.0% when both glycine and cortisone are given. For the 2- and the 6-hour periods glycine alone gives a higher per cent conversion reflecting the inhibition produced by the hormone at these

periods.

Urinary nitrogen data of a number of the animals are also given in Table II. For the 24-hour period there is a comparison between the nitrogen excretion for the 24-hour control and the experimental periods. When glycine alone is given, the increase in nitrogen excretion accounts for 83.5% of the glycine nitrogen, and for both glycine and cortisone, 95.6%. Cortisone alone produced a slight increase, 10 mg, over the control nitrogen excretion in the 24-hour period. The values for the 2- and the 6-hour periods can only be compared within the series and it appears that some inhibition of excretion occurred. In both periods the nitrogen values for cortisone plus glycine are somewhat less than for glycine alone.

With the 2.5 mg dose of cortisone the effects are, qualitatively, similar to those with the 5 mg dose; in fact, the early inhibitory effect is more apparent. Up to 18 hours the values for glycine plus cortisone are less than those for glycine alone. In Table III the hourly values of the liver glycogen are given. The cortisone plus glycine failed to produce as much glycogen as the sum of the amounts formed by the 2 compounds administered separately up to 48 hours. The changes as calculated in column 4 are significant at the 1% level in every case except the last two.

Neither dosage of cortisone increased the amount of glycogen formed from glycine. Perhaps under the fasting conditions employed maximum utilization was occurring and no further increase could result from the action of the cortisone. This result could also be interpreted as supporting the suggestion of Engel, Schiller, and Pents(7) that the action of the adrenal cortex on nitrogen metabolism may be on protein rather than on amino acids.

Summary. 1. Cortisone produced an increase in liver glycogen of fasted normal rats. After a 5 mg dose the peak production period is from 31 to 48 hours, after a 2.5 mg dose the peak is lower and is reached earlier. 2. Glycine given at the same time as the cortisone increased the liver glycogen content. At the 5 mg cortisone dose, after 12 hours, the amount formed was equal to the sum of the amounts formed when each of the substances was given separately. During the earlier periods there was an inhibition of glycogen formation. At the 2.5 mg cortisone level the same results were obtained but to a lesser degree. 3. The excretion of urinary nitrogen reflected the change in liver glycogen in that during the early periods the nitrogen excretion was somewhat less than during the control periods, but within 24 hours the extra urinary nitrogen accounted for 95.6% of the glycine nitrogen.

1. Britton, S. W., and Silvette, H., *Am. J. Physiol.*, 1932, v100, 701.

2. Long, C. N. H., Katzin, B., and Fry, *Endocrin.*, 1940, v26, 309.

3. Lewis, R. A., Kuhlman, D., Delbue, C., Koepf, G. F., and Thorn, G. W., *Endocrin.*, 1940, v27, 971.

4. Lockett, M. F., and Evans, M. M., *J. Endocrin.*, 1951, v7, 357.

5. Hess, W. C., and Shaffran, I. P., PROC. SOC. EXP. BIOL. AND MED., 1952, v81, 404.

6. Good, C. A., Kramer, H., and Somogyi, M., *J. Biol. Chem.*, 1933, v100, 485.

7. Engel, F. L., Schiller, S., and Pentz, E. I., *Endocrin.*, 1949, v44, 458.

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