Glycogen Formation and Glucose Uptake of Isolated Muscle with 11-Deoxycorticosterone and 11-Dehydro-17-hydroxycorticosterone

By ESTHER LEUPIN AND F. VERZÁR
Physiological Laboratory, University of Basel

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The isolated diaphragm of the rat produces glycogen from glucose in oxygenated Ringer solution, especially in the presence of insulin (Gemmill, 1940; Hechter, Levine & Soskin, 1941; Stadie & Zapp, 1947; Corkill & Nelson, 1947). This glycogenesis is inhibited by the addition of small doses of deoxycorticosterone (Verzár & Wenner, 1948*a*) during rest and during work (Mentha, Vögtli & Verzár, 1948). The isolated muscle takes up glucose from the solution (Krahl & Park, 1948; Perlmutter & Greep, 1948) and this also is inhibited by deoxycorticosterone (Leupin & Verzár, 1949). Other steroids were found to have varying effects. The inhibition of glycogen formation (expressed as decrease of glycogen production in mg./100 g. muscle) with 5 mg./100 ml. deoxycorticosterone (DOC) was 136, with corticosterone 66, with 17-hydroxycorticosterone 44 and with 10 mg./100 ml. dehydrocorticosterone acetate 92. Thus DOC proved to be the most active in this respect (Verzár & Wenner, 1948*b*).

It is generally thought that DOC, in contrast to 11-oxygenated corticosteroids, has no action on carbohydrate metabolism in the living animal. We have, however, shown that in the adrenalectomized, otherwise intact, animal, DOC also, if given over a longer period, restores liver and muscle glycogen to normal values (Montigel & Verzár, 1943; Sass-Kortsák, Wang & Verzár, 1949). But there is no doubt that the 11-oxygenated corticosteroids have a more pronounced action, especially in short-term experiments (Olson, Jacobs, Richert, Thayer, Kopp & Wade, 1944; Wang & Verzár, 1949*a*).

The corticosteroid which is most active on carbohydrate metabolism is 11-dehydro-17-hydroxycorticosterone acetate (compound E). Since this was now to hand in large enough quantities, we have done experiments to compare the activity of this compound with that of DOC on the isolated diaphragm. The adrenal cortical extract of the Upjohn Co. was also available for comparison.

EXPERIMENTAL

The method of Gemmill (1940, 1941) was used with slight modifications. The diaphragm of rats was quickly removed after pithing, cut into three parts and weighed on a torsion balance. The middle (vertebral) part was used for the estimation of the initial glycogen content. The other two parts were placed in vessels with 2 ml. of Ringer solution, containing phosphate buffer (Hastings, Muus & Bessey, 1939). Glucose (200 mg.) and 1 i.u. of insulin (Lilly)/100 ml. were added. All experiments lasted 90 min. The muscle was shaken in O_2 at 37°. The controls described in Table 1 show that the three parts of the diaphragm have equal glycogen contents and therefore can be used in parallel experiments, as was also done by Verzár & Wenner (1948*a*) and Mentha *et al.* (1948).

Table 1. *Glycogen content of the diaphragm of the rat*

(Vertebral part = middle part.)

No.	Glycogen content (mg./100 g.)			Glycogen content of side parts as percentage of content of vertebral part	
	Vertebral part	Side part I	Side part II	Side part I	Side part II
1	120	170	130	142	108
2	178	182	191	102	107
3	171	131	126	77	74
4	194	194	157	100	81
5	176	121	110	69	62
6	155	125	160	81	103
7	180	154	167	86	93
8	118	100	148	85	125
9	169	184	169	109	100
			Mean ± S.E.M.	**95 ± 7***	**95 ± 7***

* These values show that the glycogen contents of the two side parts do not differ significantly from one another or from that of the vertebral part.

Deoxycorticosterone or its acetate and compound E were dissolved in that amount of ethanol which would give a final ethanol concentration of 0·5 ml./100 ml. Ethanol in this concentration does not influence glycogen formation (Verzár & Wenner, 1948*a*). The solubility of 11-dehydro-17-hydroxycorticosterone acetate in Ringer solution is very low. Prof. T. Reichstein, to whom we are grateful for help in this matter, informed us that saponification gives a more soluble product. Saponification was done with $KHCO_3$ in methanol-water. About one-third of the hydrolysate was crystalline compound E with m.p. 213–217° (E_1). The residue (E_2) was amorphous and contained an unknown quantity of compound E together with an unidentified steroid. E_1 had a solubility similar to that of deoxycorticosterone; E_2 was easily soluble in water. We used E acetate, E_1 and E_2 and also a mixture of E_1 and E_2 in the original proportion of 1:2, and the action of these was compared with that of deoxycorticosterone and its acetate.

Glycogen estimations were carried out as in earlier papers (Verzár & Wenner, 1948*a*). Glucose was determined, following deproteinization according to Folin (1919), by the method of Hagedorn & Jensen (1923).

Normal and adrenalectomized rats of 100–200 g. body weight, from the Institute's stock, were used throughout.

RESULTS

Inhibition of glycogen formation

Normal animals. In Table 2 are shown experiments with the addition of DOC and in Table 3 (series II–VII) experiments with compound E. In Table 4 the mean values of all the experiments on normal animals are collected. In Table 4 the initial glycogen contents are not given and 'glycogen formation' is the difference between the glycogen content before and after incubation for 90 min. in oxygenated Ringer solution with 200 mg. glucose and 1 i.u. insulin/100 ml., calculated for 100 g. muscle. 'Glucose uptake' is the difference in glucose content in the same solution at the beginning and the end of a 90 min. period, also calculated for 100 g. muscle.

but there is still an almost equally large glucose uptake which obviously has nothing to do with glycogen formation. This glucose might be used for energy production. Thus it seems that the quantity of DOC used inhibits only the glycogen formation, and so a correspondingly smaller amount of glucose is taken up.

Glycogen formation was decreased by E_1 from 324 to 276, by E_2 from 307 to 210, by the $E_1 + E_2$ mixture from 257 to 187 and the E acetate from 228 to 188 mg./100 g. muscle (Table 4). For comparison DOC acetate was tested. It decreased glycogen formation from 286 to 159 mg./100 g. muscle.

Table 4 contains all mean values from the above series. Whilst it is clear that DOC inhibits glycogen formation and glucose uptake, Compound E was only about one-third of the activity of deoxycorticosterone. The number of experiments is not large enough to distinguish between the activity of the single E fractions. The glucose uptake does not show regular differences. DOC acetate has a smaller action on isolated muscle than has DOC itself. It was for this reason that we formerly used the latter in all experiments on isolated muscle. In Tables 3 and 4 some experiments with adrenal cortical extract (Upjohn Co.) are also shown. No inhibition was found.

Small doses of DOC and compound E tested on diaphragm of normal animals. In all previous experiments on the isolated muscle only an inhibition of glycogen formation has been observed. In the intact animal on the contrary glycogen production is increased with DOC and compound E. However, recently we have been able to show that DOC in large doses, which have in addition an anaesthetic action, decreases glycogen production in the intact animal, just as with the isolated diaphragm (Wang & Verzár, 1949*b*). We therefore tested again whether it might be possible to increase glycogen production in isolated muscle with much smaller quantities of

Table 2. *Glycogen formation and glucose uptake by isolated diaphragm muscle of normal animals without and with addition of* 11-*deoxycorticosterone* (5 *mg.*/100 *ml.*) *and in the presence of insulin*

(Exp. no. 1–31.)

	Initial glycogen (mg./100 g.)	Glycogen formation (mg./100 g.)		Glucose uptake (mg./100 g.)	
		Without DOC	With DOC	Without DOC	With DOC
Mean and S.D.	285±23	279±17	58±8	453±24	195±23

It is obvious that the addition of 5 mg. of DOC/100 ml. gives an almost complete inhibition of glycogen formation (from 279 to 58 mg./100 g. muscle), while the glucose uptake is inhibited for a much lesser degree (from 453 to 195 mg./100 g. muscle). The absolute decrease in glucose uptake is nearly identical with the decrease in glycogen formation, these hormones. In a few experiments of Verzár & Wenner (1948*a*) with smaller doses either inhibition or no action was found. It seemed possible that the latter could be the result of a balancing of glycogenetic and glycogenolytic activity.

We therefore tested the action of small quantities of DOC (0·01–0·1 mg./100 ml.) and of compound E

Table 3. *Glycogen formation and glucose uptake by isolated diaphragm muscle of normal animals without and with different corticosteroids in the presence of insulin*

(DOC acetate, deoxycorticosterone acetate. E acetate, 11-dehydro-17-hydroxycorticosterone (compound E). E_1, crystalline fraction of hydrolysate of compound E. E_2, amorphous fraction of hydrolysate of compound E. E_1+E_2, E_1+E_2 (1:2).)

Exp. no.	Addition (mg./100 ml.)	Initial glycogen (mg./100 g.)	Glycogen formation (mg./100 g.) Without addition	Glycogen formation With addition	Glucose uptake (mg./100 g.) Without addition	Glucose uptake With addition
32	E_1, 5	186	410	356	550	460
33	E_1, 5	142	—	330	—	357
34	E_1, 5	197	—	400	—	500
35	E_1, 5	217	313	233	—	390
36	E_1, 5	228	—	246	—	280
37	E_1, 5	166	—	210	—	460
38	E_1, 5	85	—	201	—	395
39	E_1, 10	199	249	201	485	340
40	E_1, 10	167	—	373	—	550
41	E_1, 10	246	—	208	—	215
42	E_2, 5	90	307	252	415	408
43	E_2, 5	85	—	163	—	408
44	E_2, 5	225	—	217	—	410
45	E_1+E_2, 5	495	135	120	200	370
46	E_1+E_2, 5	208	212	212	308	334
47	E_1+E_2, 10	450	84	32	70	290
48	E_1+E_2, 10	214	360	244	—	—
49	E_1+E_2, 10	145	189	82	340	133
50	E_1+E_2, 15	141	426	249	609	504
51	E_1+E_2, 15	114	284	103	195	330
52	E_1+E_2, 15	124	190	170	170	170
53	E acetate, 5	148	86	95	360	390
54	E acetate, 5	166	207	61	225	150
55	E acetate, 5	149	306	256	270	375
56	DOC acetate, 5·6	229	351	178	380	210
57	DOC acetate, 5·6	202	163	28	369	145
58	DOC acetate, 5·6	40	260	210	325	350
59	DOC acetate, 5·6	91	370	218	300	300
60	Adrenal cortical extract (0·1 ml./100 ml.)	243	187	187	450	375
61	Adrenal cortical extract (0·1 ml./100 ml.)	253	257	283	360	360
62	Adrenal cortical extract (0·1 ml./100 ml.)	175	140	130	220	250
63	Adrenal cortical extract (0·1 ml./100 ml.)	300	150	170	250	360
64	Adrenal cortical extract (0·1 ml./100 ml.)	250	200	180	450	400
65	Adrenal cortical extract (0·5 ml./100 ml.)	260	222	230	360	340
66	Adrenal cortical extract (0·5 ml./100 ml.)	210	210	250	310	315
67	Adrenal cortical extract (0·5 ml./100 ml.)	205	235	200	322	277

Table 4. *Mean values of glycogen formation and glucose uptake by isolated diaphragm muscle of normal animals with different corticosteroids in the presence of insulin* (*series I–VII*)

(Number of animals in brackets. For abbreviations see Table 3.)

Series	Addition (mg./100 ml.)	Glycogen formation (mg./100 g.) Without addition	Glycogen formation With addition	Glucose uptake (mg./100 g.) Without addition	Glucose uptake With addition
I	DOC, 5	279 (31)	58 (31)	453 (31)	195 (31)
II	E_1+E_2, 5–15	257 (7)	187 (9)	305 (7)	311 (8)
III	E_1, 5–10	324 (3)	276 (10)	517 (2)	394 (10)
IV	E_2, 5	307 (1)	210 (3)	415 (1)	408 (3)
V	E acetate, 1–5	228 (9)	188 (9)	285 (3)	305 (3)
VI	DOC acetate, 5·6	286 (4)	159 (4)	344 (4)	251 (4)
VII	Adrenal cortical extract (0·1–0·5 ml./100 ml.)	200 (8)	186 (12)	340 (8)	347 (8)

(0·05–0·5 mg./100 ml.) (series VIII and X). For comparison we also, at the same time, repeated experiments with larger inhibitory doses (series IX and XI). DOC inhibited glycogen production at 0·5 mg./100 ml. and above, and compound E at 1 mg./100 ml. and above. Table 5 shows the mean values. The P (probability) values (after Fisher) for the difference of glycogen production with or without addition are also shown. Whilst it is obvious that larger doses of DOC inhibit glycogen production in series IX, it is possible, from the mean values of series VIII on thirty-eight single experiments, that small doses of DOC, from 0·01 to 0·1 mg./100 ml., may somewhat increase glycogen production in muscle.

For compound E the slight increase of glycogen production with 0·05–0·5 mg./100 ml. in series X and also the inhibition with large doses of 1·0–5·0 mg./100 ml. (series XI) has only the small P value of about 0·5.

Adrenalectomized animals. The same experiments were then done with the diaphragms of adrenalectomized animals. It seemed possible that the glycogenetic action might be more obvious in the muscle of such animals. Table 6 shows the mean values. Compound E gave, in doses between 1·0 and 5·0 mg. (series XIII), only a slight inhibition, and, in smaller doses of 0·1–0·5 mg. (series XII), a slight increase of glycogen production. No difference was large enough to be significant.

Verzár & Wenner (1948*a, b*) had already shown that large doses of DOC (5 mg./100 ml.) inhibit glycogen production in the muscle of adrenalectomized animals. These experiments were therefore not repeated, but in series XIV twelve experiments with small concentrations of DOC (0·01–0·05 mg./100 ml.) are shown. No significant increase of glycogen production could be detected.

DISCUSSION

In continuation of the work of Verzár & Wenner (1948*a, b*) and Mentha *et al.* (1948), in which it was found that DOC inhibits glycogen formation in isolated muscle, it has now been shown that DOC also inhibits the uptake of glucose to an extent which corresponds to the decrease of glycogen formation. We thus confirm the observations of Perlmutter & Greep (1948) and those of Krahl & Park (1948) that the glucose uptake of a normal diaphragm in Ringer solution is about twice as great as the equivalent of the glycogen produced in the same period. DOC probably does not diminish the amount of glucose taken up and used directly for energy production.

The inhibition of glycogen production by 11-dehydro-17-hydroxycorticosterone (compound E), either as acetate or saponified, was about one-third of that found with deoxycorticosterone; and no regular inhibition of the uptake of glucose was observed.

Thus the corticosteroid 11-dehydro-17-hydroxycorticosterone, which has the greatest influence on glycogen production in the normal or adrenalectomized animal, has a much smaller inhibitory action on glycogenesis in the isolated muscle than has deoxycorticosterone. This is in agreement with the earlier result of Verzár & Wenner (1948*b*) that various 11-oxygenated corticosteroids have a much smaller inhibitory action than DOC. This 'inhibition of glycogen formation' may be rather an acceleration of glycogen breakdown in the isolated muscle. This interpretation would lead to the view that the smaller action of deoxycorticosterone on glycogen production in the living animal is explicable by a relatively stronger action of this substance on glycogen breakdown. The 11-oxygenated corticosteroids on the contrary might have less action in increasing

Table 5. *Mean values of glycogen formation of normal animals in the presence of insulin (series VIII–XI)*

Series	Addition (mg./100 ml.)	No. of animals	Initial glycogen (mg./100 g.)	Glycogen formation (mg./100 g.) Without addition	With addition	P values for the difference without and with addition
VIII	DOC, 0·01–0·1	38	152±13	228±21	264±18	0·2–0·3
IX	DOC, 0·5–5·0	9	159±29	144±11	47±25	0·01
X	Compound E_2, 0·05–0·5	23	168±14	237±21	254±20	0·5–0·6
XI	Compound E_2, 1·0–5·0	9	129±13	223±29	182±34	0·3–0·4

Table 6. *Mean values of glycogen formation of diaphragm of adrenalectomized animals in presence of insulin (series XII–XIV)*

Series	Addition (mg./100 ml.)	No. of animals	Initial glycogen (mg./100 g.)	Glycogen formation (mg./100 g.) Without addition	With addition	P values for the difference without and with addition
XII	Compound E, 0·1–0·5	5	269±42	232±51	238±39	0·9–1·0
XIII	Compound E, 1·0–5·0	4	253±39	243±37	198±46	0·4–0·5
XIV	DOC, 0·01–0·05	12	208±20	254±26	262±23	0·8–0·9

glycogenolysis and have a greater effect on glycogenesis.

We have been impressed by another difference between these two corticosteroids. DOC acetate (10 mg.) injected intraperitoneally into a rat of 100–150 g. produces (as first shown by Selye, 1941*a*, *b*, *c*) a narcosis-like state which lasts for several hours, whereas 10 and even 20 mg. of compound E, injected into similar animals, have no narcotic action whatever. Also, in concentrations above 5 mg./100 ml. in Ringer solution, DOC decreases the work obtainable from the isolated diaphragm muscle (Mentha *et al.* 1948). Compound E showed no such action up to 10 mg./100 ml.

The inhibition of work through DOC might be due to the same cellular process as the narcosis in intact animals. Lately (Wang & Verzár, 1949*b*) we have been able to demonstrate an inhibition of glycogenesis also in intact animals, using doses of DOC large enough to cause narcosis.

We have tried, but failed, to show in a decisive way that very small doses of either DOC or of compound E have a glycogenetic effect on the isolated diaphragm either of normal or of adrenalectomized rats. If such an activity does exist, as series VIII and X on normal and XII and XIV on adrenalectomized rats seem to suggest, the action is very slight and cannot therefore be decisively proved. However, it might be that if such a slight action is continuous in the intact animal it leads to the glycogenesis which is observed there.

SUMMARY

1. Deoxycorticosterone, in concentrations of 5 mg./100 ml. or above, inhibits the glycogen formation of the isolated diaphragm of normal rats in the presence of 200 mg. glucose and 1 i.u. insulin/100 ml.

2. Deoxycorticosterone also inhibits that part of the glucose uptake which corresponds to the glycogen formation. About half of the original glucose uptake remains unaffected.

3. 11-Dehydro-17-hydroxycorticosterone (compound E) decreases glycogen formation only about one-third as much as deoxycorticosterone, and the effect on glucose uptake is variable.

4. Small doses of DOC or of compound E which have no inhibitory action on glycogen formation cause a very slight increase of glycogen production in normal and also in adrenalectomized animals. This effect is, however, so small that it cannot be considered significant.

5. Possible ways are discussed of correlating the different activities of these corticosteroids on the carbohydrate metabolism of isolated muscle with their different activities in the intact animal.

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