In view of the evidence which has been presented that hyaluronidase is not inhibited by salicylates in therapeutic concentrations, it would seem that Guerra's explanation of the anti-rheumatic action of these drugs in terms of hyaluronidase inhibition must be abandoned. The question naturally arises as to whether or no their apparent anti-histamine action can be invoked in this connexion, as well as whether the effect of hyaluronidase on the skin of rheumatic fever patients described by Guerra and its diminution after administration of salicylate can be explained by the same mechanism. A voluminous literature exists regarding the effects of salicylates on immunological reactions, but the evidence, though some is suggestive, seems to be scarcely sufficient to provide a complete answer to these questions. The response to hyaluronidase described by Guerra in rheumatic fever, however, appears to be of the nature of a hypersensitivity reaction, such as was described by Jones & Mote (1934) as occurring in rheumatic fever patients following repeated injections of very small amounts of rabbit serum. It is true that only a single injection of hyaluronidase appeared to have been given (in Guerra's paper no details are provided, nor is any mention made of control tests in normal subjects), but since streptococci of the type believed to be responsible for predisposing to rheumatic fever are hyaluronidase producers, sensitivity to hyaluronidase might well be of general occurrence in rheumatic fever. If such be the case, inhibition of histamine, which is believed to play a part in such hypersensitivity reactions, would account for the reduced response to intradermal injections of hyaluronidase after treatment with salicylate.

SUMMARY

1. Investigation of the effects of sodium salicylate and acetylsalicylic acid on the viscosity-reducing activity of hyaluronidase has shown that they are inhibitory only in relatively enormous concentrations (more than 3 % for sodium salicylate and 0·33 % for acetylsalicylic acid).

2. Such inhibition is apparently due to lowering of pH and, in the case of sodium salicylate, to increased salt concentration.

3. Heparin, on the other hand, is completely inhibitory in a concentration of 0·0066 %.

4. The significance of these findings in relation to the claims made by Guerra (1946*a*) of the association of hyaluronidase with rheumatic fever, and the anti-rheumatic action of salicylates is discussed.

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The Influence *in vitro* of Deoxycorticosterone on Glycogen Formation in Muscle

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The main action of the adrenal cortical hormone is generally believed to be the regulation of glycogen metabolism. In a series of experiments from this Institute the phosphorylation of glycogen was investigated in normal and adrenalectomized animals. This reaction, by which glucose-1-phosphate is formed from glycogen, is the first phase in the breakdown of glycogen. It was shown that the phosphorylation of glycogen *in vitro* was greatly reduced in tissue from adrenalectomized animals (Verzár & Montigel, 1942; Doetsch, 1945).

In order to study directly the synthesis of glycogen, we chose the technique of Gemmill (1940, 1941) and Gemmill & Hamman (1941) by which the synthesis of glycogen from glucose can be observed in the surviving diaphragm of the rat. The rat's diaphragm is suitable for this experiment because, owing to its thin structure, it can be kept alive in Ringer solution for a few hours if saturated with oxygen. If glucose is added to this solution, the diaphragm synthesizes glycogen. Gemmill (Gemmill, 1940, 1941; Gemmill & Hamman, 1941) and also

Hechter, Levine & Soskin (1941) have shown that this glycogen synthesis can be considerably increased by the addition of insulin. The diaphragm of adrenalectomized rats, too, synthesized glycogen from glucose, and insulin gave the same increase as in normal rats (Koepf, Horn, Gemmill & Thorn, 1941). It thus appeared that the main disturbance in glycogen metabolism in adrenalectomized animals is not in the synthesis of glycogen.

We therefore investigated the effect of the addition of deoxycorticosterone (DOC) on the glycogen formation in the diaphragm of normal and adrenalectomized rats. We found that DOC inhibits completely the formation of glycogen and also the effect of insulin on this formation.

METHODS

The method of Gemmill (1940, 1941) was used with slight modification, but concentrations of insulin and glucose were kept within physiological limits. Rats of 100–150 g. body weight of both sexes and of our own breed were killed by a blow on the head and decapitation and were bled as much as possible. The diaphragm was rapidly dissected out and cut into three parts of almost equal weight (from 100 to 150 mg.). The posterior thicker part of the diaphragm was immediately weighed on a torsion balance, dropped into 20% (w/v) NaOH (1 ml.) and hydrolyzed on a boiling water bath for the estimation of the initial glycogen content. The two remaining pieces of diaphragm were also weighed immediately, washed in phosphate buffer Ringer solution and dropped into 50 ml. Erlenmeyer flasks containing 10 ml. of the phosphate buffer Ringer solution, which was prepared according to Hastings, Muus & Bessey (1939) and contained 8 g. NaCl, 0·2 g. KCl, 0·2 g. $CaCl_2$, 0·1 g. $MgCl_2$, 0·8 g. $Na_2HPO_4.2H_2O$ and 0·15 g. KH_2PO_4/l. (pH *c.* 7·2–7·4 as measured by Bayer pH papers nos. 7 and 8). The following substances were then added to the Ringer solution: 100 mg. glucose/100 ml., 1 unit insulin/100 ml., 0·01–10 mg. DOC/100 g. and 0·25–0·50% ethanol. A solution of insulin (Eli Lilly) with an activity of 40 units/ml. was diluted with Ringer solution to a concentration of 1 unit/ml. Usually 0·1 ml. of this solution was dropped into the flasks containing 10 ml. of the phosphate buffer Ringer solution. The final insulin concentration was therefore 1 unit/100 ml. DOC (100 mg.) was dissolved in 5 ml. ethanol and 5 ml. water were added; 0·1 ml. of this solution was added to the flasks if a concentration of 10 mg./100 ml. was required. According to Miescher & Meystre (1943) DOC has a solubility of 0·012% in water and so all the DOC in our experiments was dissolved by the Ringer solution. Solutions of 0·1 and 0·01% DOC were used if lower concentrations were needed. If diaphragm is permeable to insulin and DOC, the hormone concentrations in the muscle and in the Ringer solution should be identical. One of the lateral pieces of diaphragm was always kept as a control without the addition of the hormone. The flasks containing the pieces of diaphragm were at once filled with oxygen, closed with rubber stoppers and shaken in a water bath at 37°. From time of death to the beginning of shaking only 4–5 min. elapsed. It was therefore not necessary to prepare the diaphragms at 0°. In the majority of experiments the shaking in the water bath was continued for 1·5 hr., and the pieces of diaphragm were then dropped into 1 ml. of 20% (w/v) NaOH and hydrolyzed on a boiling water bath until the solution was clear (10–15 min.). The glycogen was then precipitated from the hot solution with 2 ml. of ethanol and the precipitation completed by standing in ice water for 0·5 hr. The glycogen was centrifuged off, the supernatant poured away, the glycogen stirred up with 2 ml. of ethanol, again centrifuged at 3000 r.p.m. for 3–5 min. and dried on a boiling water-bath. According to the amount of precipitate 1–2 ml. 2N-H_2SO_4 were added and the glycogen hydrolyzed on a boiling water bath for 1·5 hr. The hydrolysate was washed into a 50 ml. measuring flask and made alkaline with 2·3 ml. of 2N-Na_2CO_3. Depending on the amount of glycogen 2–10 ml. were taken from the flask and the sugar content estimated by the Hagedorn-Jensen method, according to Hallmann (1939). The glycogen contents are given as mg. glycogen/100 g. wet muscle.

RESULTS

The results are reported in Tables 1 and 2. Only the mean values of results obtained with several animals are given. The glycogen contents are given at the beginning of the experiment (initial glycogen content) and after 1·5 hr. with additions of the different substances as glucose, insulin, DOC and ethanol. The tables show the differences between the glycogen contents before and after incubation with a given addition. More important, however, are the differences between the results with different additions, i.e. glucose and glucose + insulin, or no glucose and glucose + insulin, or those with DOC, which give the true effect of the addition. From Tables 1 and 2 these values can easily be calculated. Since all values are arithmetic mean values the standard error for the differences in each series has been calculated. One example of a series with 10 single experiments is given in Table 2. Here the differences between the results with different additions are also shown.

1. *Action of glucose*

The experiments on 25 animals (Table 1, series 1) show that the glycogen content of the diaphragm in phosphate buffer Ringer solution without glucose decreased in 1·5 hr. by a mean value of 57 mg./100 g. Addition of glucose (100 mg./100 ml.) (without insulin) produced glycogen in normal rats (series 2) and also in rats which had been starved for 2 days (series 6). In the former series the glycogen content increased by 46 mg./100 g. compared with the initial value and in the latter by 126 mg./100 g. If these values are compared with those of series 1 (without glucose), where the glycogen content decreased by 57 mg./100 g., the total glycogen production can be calculated to be 103 and 183 mg./100 g. respectively. In single experiments differences of 50 mg./100 g. are not regarded as real, but in a series of several experiments the mean error becomes much smaller, as is shown in the tables.

Table 1. *Changes in the glycogen contents of diaphragms of normal, starved and adrenalectomized rats on incubation with and without the addition of glucose, insulin and deoxycorticosterone*

Series no.	No. of rats	Mean initial glycogen content (mg./100 g. of muscle)	Additions to suspending fluid: Glucose (mg./100 ml.)	Insulin (units/100 ml.)	DOC (mg./100 ml.)	Ethanol (%)	Mean glycogen content after 1·5 hr. (mg./100 g. of muscle)	Mean differences (mg./100 g. of muscle)	S.E. of mean differences
1	25	239	—	—	—	—	182	− 57	12
2	60	280	100	—	—	—	326	+ 46	13
3	13	296	100	0·1	—	—	397	+101	20
4	14	265	100	0·5	—	—	494	+229	17
5	102	233	100	1	—	—	363	+130	8
6	13*	100	100	—	—	—	226	+126	8
7	13*	100	100	0·5	—	—	422	+322	19
8	8	170	—	0·5	—	—	136	− 34	23
9	11	165	—	1	—	—	142	− 23	9
10	8	129	100	100	—	—	279	+150	17
11	8	270	100	—	10	0·50	185	− 85	22
12	14	305	100	—	5	0·25	298	− 7	14
13	12	301	100	—	1	0·50	297	− 4	16
14	11	261	—	—	5	0·25	203	− 58	13
15	14	326	100	—	—	0·50	377	+ 51	20
16	10	175	100	1	—	0·50	321	+146	24
17	12	187	100	—	10	—	155	− 32	14
18	12	277	100	1	10	—	245	− 32	24
19	13	157	100	1	10	0·50	88	− 69	17
20	30	246	100	1	5	0·25	244	− 2	17
21	20	321	100	1	1	0·50	470	+149	20
22	6	203	100	1	0·5	0·25	318	+115	31
23	15	183	100	1	0·1	0·50	291	+108	28
24	6	131	100	1	0·05	0·25	290	+159	25
25	12	265	100	1	0·01	0·25	399	+134	25
26*a*	7	297	100	1	10	0·50	166	−131	39
26*b*	7	297	100	100	10	0·50	191	−106	32
27*a*	23†	225	100	—	—	—	288	+ 63	18
27*b*	23†	225	100	0·5	—	—	407	+182	15
28*a*	16†	164	100	1	—	—	282	+118	20
28*b*	16†	164	100	1	10	0·50	106	− 58	12

* After a starvation period of 2 days.

† Adrenalectomized animals.

2. *Action of insulin on glycogen production*

In Table 1, series 3, 4 and 5 show the effect on glycogen formation of 0·1, 0·5 and 1 unit of insulin/100 ml. respectively. To 10 ml. phosphate buffer Ringer solution in which was suspended one third of a diaphragm 0·1 unit of insulin was added (series 5). This corresponds to an insulin content of 1 unit/100 ml. of Ringer solution, or 100 g. of muscle. Insulin already at a dose of 0·1 unit/100 ml. increases glycogen production by about 50 mg./100 g. (series 3) as compared with glucose alone (series 2). With 0·5 unit the insulin effect is almost maximal giving an increase of 183 mg./100 g. as compared with glucose alone (series 4). In series 5, 102 experiments with insulin (1 unit/100 ml.) are collected. The mean value for the glycogen production is 130 mg./100 g. The values of this series are also plotted against the number of animals in Fig. 1. In this figure the total number of experiments in which a given increase (± 20 mg./100 g.) in glycogen content

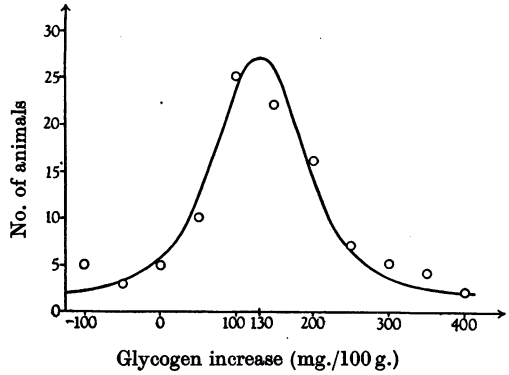

Fig. 1. Glycogen production from glucose (100 mg./100 ml.) by diaphragms of normal rats with insulin (1 unit/100 ml.) in 102 experiments. The ordinate value of each point indicates the number of diaphragms which produced the amount of glycogen (±20 mg./100 g. muscle) given by the abscissa. The solid line is the normal probability equation.

was obtained is plotted, as the ordinate, against the magnitude of the given increase (abscissa).

Nearly 80 % of all experiments fall inside the values of +50 to +300 mg./100 g. There are, however, even in this series a certain number of negative results which are included in the mean value decreasing this to 130 mg./100 g. This seems to be lower than the mean value with 0·5 unit insulin/100 ml. in a series with only 14 experiments, but the difference is not significant.

In the experiments of Gemmill and co-workers (Gemmill, 1940, 1941; Gemmill & Hamman, 1941; and Koepf *et al.* 1941), 100–200 units insulin/100 ml. were used, but these authors observed about the same maximal increase of glycogen production as we found with much smaller quantities of insulin. We have therefore compared the action of different quantities of insulin on two parts of the same diaphragm. (The third part of the diaphragm was always used to obtain the initial glycogen value.)

In a series of 4 experiments we found glycogen productions, with 0·5 unit insulin/100 ml., of 65 mg./100 g. and, with 1 unit/100 ml. of 70 mg./100 g. In another series of 8 experiments we found glycogen productions, with 1 unit/100 ml. of 136 mg./100 g. and, with 100 units/100 ml. of 150 mg./100 g. (series 10).

Thus, increasing the concentration of insulin above 0·5 unit/100 ml. has very little effect, and on the same diaphragm 0·5 unit acts like 1, or 1 like 100 units/100 ml. It must be remembered however that the activity of different doses of insulin cannot be compared on different diaphragms. The largest values for glycogen content, above 400 mg./100 g., were found with 0·5 unit/100 ml. in series 4 and 7, and these are not less than with 100 units/100 ml. in series 10.

We have also measured the time in which insulin has this effect. Insulin (1 unit/100 ml.) was added to both parts of the diaphragm and the incubation was stopped at different times. Glycogen production is especially active in the first 0·5 hr. being about 100 mg./100 g. After that, only about 10–20 mg./100 g. glycogen is formed in each 0·5 hr.

Control experiments were also done to see whether insulin has any action if it is added without glucose. Series 8 and 9 show that no glycogen was formed and the initial glycogen was broken down as in series 1 where neither glucose nor insulin had been added. Thus insulin cannot inhibit glycogenolysis.

Mention must be made of series 6 and 7 in which animals starved for 2 days were used. Such control experiments were necessary, since results obtained with adrenalectomized rats might be ascribed to the fact that they were starving. The glycogen content of the muscles in these animals was very low, about 100 mg./100 g. The glycogen content after addition of glucose alone was 226 mg./100 g., with glucose and insulin (0·5 unit/100 ml.) 422 mg./100 g. The insulin effect is 196 mg./100 g. (difference: 322 − 126 = 196) and is equal to that of animals on a normal diet (see series 2 and 4, difference: 229 − 46 = 183).

3. *Action of deoxycorticosterone*

We tested next the action of DOC with and without glucose and with and without insulin. The results are shown in series 11–18 (Table 1). In series 11, 12 and 13 the action of 10, 5 and 1 mg. DOC/100 ml. of solution (or g. of muscle) in the presence of glucose is shown. In all experiments no glycogen was produced from glucose. In series 11 there was even a breakdown of 85 mg./100 g. as compared with the initial glycogen content. If we compare series 2, in which a glycogen production of 46 mg./100 g. from glucose was demonstrated, the real decrease in glycogen in series 11 is 131 mg./100 g. In series 12 and 13 with 5 and 1 mg. DOC/100 ml. only the glucose effect was abolished by DOC. In series 14 no glucose but 5 mg.DOC/100 ml. were added and a decrease of glycogen of 58 mg./100 g. resulted, which is very similar to the decrease without any addition (series 1).

After addition of DOC the Ringer solution contains 0·25–0·50 % ethanol, as pointed out in the Methods section. Series 15 and 16 show that ethanol without DOC has no action whatever on the glycogen formation with glucose and with glucose + insulin. The results are the same as in series 2 and 5.

Experiments with ethanol (1 %), glucose (100 mg./100 ml.) and insulin (1 unit/100 ml.) showed a diminished glycogen production (by about 30 mg./100 g.) in comparison with glucose and insulin alone; 0·5 % ethanol, the concentration used in our previous experiments with DOC, had no action. Nevertheless we now tested whether DOC has an inhibitory action on glycogen production in aqueous solution, i.e. without ethanol. It is possible to dissolve about 0·01 % DOC in water (Miescher & Meystre, 1943). We took 100 mg. of finely pulverized crystalline DOC and shook it for 3 hr. in 500 ml. of phosphate buffer Ringer solution, which thus became saturated with DOC. After addition of 100 mg. glucose/100 ml. we dropped the diaphragms into 10 ml. of this solution and performed the experiment in the usual manner. The results are shown in series 17. With glucose and without insulin, DOC caused a glycogen breakdown of 32 mg./100 g., or 78 mg./100 g. when compared with series 2. The inhibition of glycogen production and the increase of glycogen breakdown by DOC is of the same order as that caused by DOC with 0·5 % ethanol (series 11 and 19). Thus ethanol in the concentrations used by us does not influence the inhibitory action of DOC on glycogen production.

All these experiments show that DOC does not increase glycogen formation in muscle, but rather inhibits glycogen production from glucose.

4. *Combined action of deoxycorticosterone and insulin*

Since the preliminary experiments of sections 1–3 have shown the smallest effective doses of insulin on glycogen formation, and of DOC on glycogen breakdown, we can describe our main experiment, i.e. the action of DOC in the presence of insulin.

In Table 1 these results are shown as mean values in series 19–26*b*. From series 19 it is obvious that, in the presence of 100 mg. glucose/100 ml. and 1 unit insulin/100 ml. with 10 mg. DOC/100 ml., no glycogen is formed but as much is broken down as if no glucose and no insulin were present. In series 20 with 5 mg. DOC/100 ml., less action is seen; there is no production of glycogen but also no decrease. In series 21 with 1 mg. DOC/100 ml., there is an increase of glycogen content, i.e. the action of insulin is not antagonized. Smaller DOC concentrations of 0·5–0·01 mg./100 ml. in the series 22–25 are equally without antagonistic action. In these the results are the same as with insulin alone; about 100–150 mg. glycogen/100 g. are formed. In all the series a corresponding amount of ethanol was added to the controls without DOC. In all cases ethanol in concentrations of 0·25 and 0·50 % was quite without influence on glycogen synthesis and breakdown.

In series 18 an aqueous solution of DOC without ethanol was used, parallel to series 17 mentioned above. Glycogen was broken down at the same rate as in series 19.

Series 19–21 contain the main results of this paper. We give therefore 10 consecutive single experiments from series 19 in Table 2, as an example of each series with its controls.

From these single experiments the same follows as from the mean values of Table 1. In the last column the total breakdown of glycogen is calculated by comparing the two glycogen contents after 1·5 hr., those with glucose and insulin alone and those with additional DOC. With 10 mg. DOC/100 ml. the effect on glycogenolysis was 174 mg./100 g. as mean value of these 10 experiments. In Table 1 comparing series 5 and 19 we calculate a DOC effect on glycogen breakdown of 199 mg./100 g. The single differences in each of the columns 2–1 and 3–1 vary considerably. Nevertheless except with the second experiment all differences in column 3–2 are very similar.

The effect of DOC on glycogen breakdown is not a direct antagonistic action against added insulin, as is shown by a comparison with series 11 where glycogen is broken down with DOC without insulin, as well as with DOC and insulin in series 19.

The series 26*a* and 26*b* prove the same. There is no correlation between the doses of insulin and DOC, because 1 unit insulin/100 ml. as well as 100 units/100 ml. is inhibited by 10 mg. DOC/100 ml.

5. *Action of insulin and DOC on the diaphragm of adrenalectomized adynamic rats*

The action of DOC was next studied with muscle from adrenalectomized animals.

Male rats, of 100–150 g. body weight, of the Institute's own breed, were adrenalectomized. They recovered from the operation in 3 days and increased in weight. The animals were killed between the 5th and 10th day if a decrease in weight and adynamia had appeared.

Series 27*a*–28*b* (Table 1) summarize the experiments by showing mean values. Series 27*b* and 28*a* confirm what was already known from Gemmill's experiments (Koepf *et al.* 1941), that the diaphragm of adrenalectomized rats *in vitro* produces glycogen in the same way as that of normal animals. If glucose only was present (series 27*a*), 63 mg. glycogen/100 g. were formed. With 0·5 unit insulin/100 ml. (series 27*b*) 182 mg./100 g. and with 1 unit/100 ml. (series 28*a*) 118 mg. glycogen/100 g. were produced.

Table 2. *Typical experiments demonstrating the action of deoxycorticosterone* (10 *mg./100 ml.*) *on glycogen formation by diaphragms from glucose and insulin*

Initial glycogen content (mg./100 g.)	Glycogen content after 1·5 hr. (mg./100 g.)		Differences in glycogen content (mg./100 g.)		
	With glucose and insulin	With glucose, insulin and DOC			
1	2	3	2–1	3–1	3–2
160	240	40	+ 80	−120	−200
140	40	40	−100	−100	0
80	230	40	+150	− 40	−190
370	370	160	0	−210	−210
180	360	110	+180	− 70	−250
40	250	70	+210	+ 30	−180
160	290	80	+130	− 80	−210
180	360	110	+180	− 70	−250
90	200	80	+110	− 10	−120
160	290	160	+130	0	−130
Means: **156**	**263**	**89**	**+107**	**− 67**	**−174**

If, however, in series 28*b* to 1 unit insulin/100 ml. 10 mg. DOC/100 g. were added, the glycogen content decreased by 58 mg./100 g. Thus the results were exactly the same as in normal animals in the series 2, 4, 5, 18 and 19.

6. *Action of insulin and DOC on glycogen formation from glucose-1-phosphate*

The experiments on glycogen formation by normal diaphragms were repeated using, instead of glucose, glucose-1-phosphate (Cori ester) as substrate. This substance, according to present knowledge, is the first product of glycogen breakdown, and possibly also the last intermediate in glycogen synthesis. Cori, Schmidt & Cori (1939) and others have shown that *in vitro* an enzyme fraction of muscle extracts, the so-called phosphorylase, can synthesize glycogen from glucose-1-phosphate.

The glucose-1-phosphate which was used for these experiments was prepared according to Cori, Colowick & Cori (1937). For these experiments the concentration used was equivalent to 100 mg. glucose/100 ml. (150 mg. glucose-1-phosphate/100 ml.). This was verified after hydrolysis by estimation of glucose by the Hagedorn-Jensen method.

Table 3. *Glycogen content of diaphragm from normal animals after incubation with glucose-1-phosphate*

Series no.	No. of rats	Mean initial glycogen content (mg./100 g. of muscle)	Additions to suspending fluid: Glucose-1-phosphate (mg./100 ml.)	Insulin (units/100 ml.)	DOC (mg./100 ml.)	Ethanol (%)	Mean glycogen content after 1·5 hr. (mg./100 g. of muscle)	Mean differences (mg./100 g. of muscle)	S.E. of mean differences
29	16	329	150	—	—	—	282	− 47	25
30	8	231	150	1	—	—	225	− 6	27
31	8	425	150	—	10	0·50	285	−140	53

The results are shown in Table 3. In series 29 (with glucose-1-phosphate alone) the glycogen decreased in the same way as in series 1 (Table 1) without glucose. Even with 1 unit insulin/100 ml. added there was no glycogen synthesis. DOC (5 mg./100 ml.) increased the glycogenolysis, as series 31 shows.

Thus glucose-1-phosphate cannot act as substrate for glycogen formation with intact muscle. Since Verzár & Wenner (1948) have found that minced muscle can synthesize glycogen from glucose-1-phosphate, the explanation seems to be that glucose-1-phosphate cannot diffuse into the intact muscle and is therefore not used. This is the more probable since it is known that glucose phosphates are only formed inside the cells and do not diffuse in or out of them.

DISCUSSION

We have thus confirmed the finding of Gemmill (Gemmill, 1940, 1941; Gemmill & Hamman, 1941) and of Soskin (Hechter *et al.* 1941) that the surviving diaphragm of rats can produce glycogen from glucose in the presence of insulin. We were also able to show that, contrary to the above authors, even without insulin glycogen can be formed in a solution containing not more than 100 mg. glucose/100 ml. Insulin increases this glycogen production three or four times. Whilst the American authors used 100–200 units insulin/100 ml., we found that even with 0·5–1 unit/100 ml. an almost maximal glycogen production was obtained. The glycogen production occurs mainly in the first half hour and slows down afterwards.

Diaphragms of adrenalectomized rats produced glycogen in the same amounts as those of normal animals. Thus the specific disturbance in adrenal cortical insufficiency cannot be an inability to produce muscle glycogen from glucose.

It was also shown that the addition of DOC with glucose alone, or with glucose and insulin, totally inhibited glycogen formation. The hormone was active in quantities of 1–10 mg./100 g. muscle, or for a diaphragm of 100 mg. in quantities of 1–10 μg. About 1 mg. DOC daily would be needed to keep alive an adrenalectomized rat of 150 g. body weight with 50 g. muscle tissue; this corresponds to about 2 mg./100 g. muscle. Thus the concentrations which were effective in these experiments *in vitro* are equivalent to the doses needed to keep an adrenalectomized animal alive.

In these experiments, therefore, DOC acts by increasing the breakdown of glycogen (glycogenolysis). This is in accordance with the fact previously announced in several papers from this laboratory (Verzár & Montigel, 1942, 1943*a*, 1943*b*; Doetsch, 1945), that after adrenalectomy the phosphorylation of glycogen by muscle (and liver) decreases. Glycogen-phosphorolysis is the first step in glycogen breakdown to form glucose-1-phosphate. As was shown by the above authors, DOC acts by accelerating this process.

Since however it is known that the same enzyme (phosphorylase) can also catalyze the synthesis of glycogen (Cori *et al.* 1939; Cori, Green & Cori, 1942; Kiessling, 1939; Ostern & Holmes, 1939), it has been thought until now that the disturbance might be on the side of glycogenesis rather than of glycogeno-

lysis. The experiments in this paper show that the disturbance in the undamaged muscle lies only on the side of glycogenolysis, which is greatly increased by DOC in normal as well as in adrenalectomized animals.

It is difficult to explain the decrease of glycogen in the organs of adrenalectomized animals. The apparent result of the administration of DOC or other adrenal cortical hormones *in vivo* is an increase of glycogen content, as we ourselves and others have shown (e.g. Verzár & Montigel, 1943*a*, 1943*b*); Koepf *et al.* (1941, 1942) have also shown that in liver slices of adrenalectomized animals which were starved for 24 hr. the so-called total carbohydrate was lower than in normals; and that normal and adrenalectomized animals produced more total carbohydrate from lactate and pyruvate if cortin was previously injected.

In spite of these difficulties, our experiments can only be explained by assuming that DOC increases the glycogenolysis and thus counteracts the effect of insulin, which increases glycogenogenesis.

Since it was shown by Verzár & Wenner (1948), that the minced muscle of adrenalectomized animals can produce glycogen from Cori ester, while the undamaged diaphragm cannot, it seems that glucose-1-phosphate cannot diffuse into the undamaged muscle fibres (Conway, 1942). On the other hand, it can be used for glycogen formation in minced muscle from adrenalectomized as well as from normal animals. Thus the disturbance which leads to a decrease of phosphorylation of glycogen after adrenalectomy is on the breakdown side of glycogen metabolism: it is the first step of glycogen breakdown (glycogenolysis), the phosphorylation of glycogen in muscle, which is decreased after adrenalectomy. This process is increased in the whole muscle by DOC in normal and adrenalectomized animals. The increase of glycogenolysis through DOC can totally antagonize the action of insulin upon glycogen synthesis in muscle.

Further experiments with other steroids which we propose to do, are intended to show whether this effect which we have found is specific for DOC.

SUMMARY

1. The surviving diaphragm of the rat, in Ringer solution saturated with oxygen and buffered with phosphate, synthesizes glycogen when 100 mg. glucose/100 ml. are added. In pure Ringer solution, it breaks down its own glycogen stores.

2. The addition of 0·5–1 unit of insulin/100 ml. to the Ringer solution containing 100 mg. glucose/100 ml., increases the formation of glycogen. A further addition of insulin, up to 100 units/100 ml., produces no further increase in glycogen formation.

3. After the addition of 5–10 mg. of DOC/100 ml. to the Ringer solution there is a breakdown of glycogen in the diaphragm. When the Ringer solution contains glucose, the synthesis of glycogen is completely inhibited by the addition of even 1 mg. DOC/100 ml. When 1 unit of insulin/100 ml. is added to the Ringer solution with glucose, then 5–10 mg. DOC/100 ml. completely prevent the synthesis of glycogen. With 1 mg. DOC/100 ml. scarcely any action could be detected. Even when 100 units of insulin/100 ml. are added, no synthesis occurs in the presence of 10 mg. DOC/100 ml.

4. The muscles of adrenalectomized animals synthesize glycogen to the same extent as those of normal animals, and in these animals also DOC favours the breakdown of glycogen and inhibits the action of insulin.

5. If glucose-1-phosphate is taken instead of glucose then no synthesis of glycogen occurs even in the presence of insulin. The explanation may be that the cell membrane is non-permeable to this ester.

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