SUMMARY

1. Minced muscle and liver of normal rats produce glycogen from glucose-1-phosphate *in vitro*.

2. If, after a starvation period, the glycogen content falls below 200 mg./100 g., minced liver and muscle do not produce glycogen from glucose-1-phosphate. However, if glycogen is added as well,

additional glycogen is produced, just as with pure enzyme solutions.

3. In adrenalectomized, adynamic rats the glycogen content is decreased in liver and muscle, and only if glycogen is added can production of new glycogen by minced muscle and liver be observed to occur at the same rate as with normal animals. Thus adrenalectomy does not affect the mechanism responsible for the synthesis of glycogen from glucose-1-phosphate.

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The Action of Steroids on Glycogen Breakdown in Surviving Muscle

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In a previous paper (Verzár & Wenner, 1948) the effect of deoxycorticosterone (DOC) on glycogen synthesis from glucose with or without insulin was studied in the surviving diaphragm of the rat. DOC in concentrations of 5 mg./100 ml. of suspending fluid counteracts the effect of insulin and increases glycogen breakdown. In order to establish whether this effect of DOC is specific, several other steroids,

sex hormones and similar biologically active and inactive substances have been tested in the same way.

METHODS

The technique used was that of Gemmill (1940, 1941) and of Gemmill & Hamman (1941) with modifications as described in our previous paper (Verzár & Wenner, 1948). The diaphragms of rats were incubated in oxygenated,

phosphate buffer Ringer solution containing 100 mg. glucose and 1 unit insulin/100 ml. of solution. The steroid was added in a 2% (w/v) solution in ethanol or in a 1% solution in 50% (v/v) ethanol, if this was possible without crystallization occurring. Thus 0.05 or 0.1 ml. had to be added to 10 ml. solution to give a concentration of 5 or 10 mg. steroid/100 ml. solution.

Surface tension was measured with the Traube stalagmometer (Traube, 1887; Abderhalden, 1930). For these experiments the 50% ethanolic solutions of the steroids were diluted with distilled water to a concentration of 5 or 10 mg./100 ml. The surface tension is expressed in dynes/cm. (Landolt-Börnstein, 1923).

RESULTS

Table 1 shows the effect of various steroids on glycogen breakdown. Each line contains the mean values of a series of at least 4, but mostly of 8 or more single experiments. Steroids 1-14 contain either free keto or hydroxyl groups or both, whilst steroids 15-17 contain either an ether or an ester linkage. Substances 18 and 19 are not related to steroids. The effect on glycogen breakdown is seen from a comparison of columns 2 and 3. The dose of 5 mg./100 ml. was chosen to show the different effects of the steroids. DOC in a concentration of 5 mg./100 ml. of solution inhibits glycogen production in the presence of 100 mg. sugar and 1 unit insulin/100 ml. solution. Progesterone (no. 2) and pregnenediolone (no. 4) show even a larger effect. With 5 mg. progesterone the glycogen content is decreased even below the initial value.

Pregnanediolone, corticosterone, hydroxycorticosterone, testosterone, androstanediol (nos. 5, 6, 7, 8, 9) have a distinctly smaller effect. 11-Dehydrocorticosterone (no. 17) was also active; it could only be tested as acetate. Pregnanediolone, testosterone and androstanediol were, in the higher concentration (10 mg./100 ml.), as active as DOC; but oestradiol (no. 10) and bisdehydrodoisynolic acid (no. 11) had the same activity at a concentration of 10 mg./100 ml. as the former group (nos. 5-9) had at a concentration of 5 mg./100 ml. Hydroxyaetiocolenic acid (no. 12), *allopregnanepentol* (no. 13), and androstenediol (no. 14) had no action even in the higher concentration of 10 mg./100 ml. Stilboestrol (no. 18), an oestrogen, was more active than cestradiol and its activity was equal to that of progesterone or DOC.

The glucoside of DOC (no. 15) had no activity, while the monoacetate of pregnenediolone (no. 16) had about the same activity as the non-esterified substance (no. 4). The acetate of 11-dehydrocorticosterone was also very active. Oleic acid (no. 19), with a molecular weight similar to those of the steroids tested, was without activity.

DISCUSSION

The inhibition of glycogen production and increase of glycogen breakdown is not present in substances

lacking a hormone-like action such as hydroxyaetiocolenic acid and oleic acid. On the other hand high activity was found in stilboestrol and, to a lesser extent, in bisdehydrodoisynolic acid, substances which are chemically different and only related biologically to the steroid hormones.

In the DOC-21-glucoside (no. 15) the new linkage on the 21-hydroxyl group abolished the whole activity. It is possible that this substance cannot be split by intact muscle, but the acetate of pregnenediolone (no. 16) was as active as pregnenediolone itself. The acetate of 11-dehydrocorticosterone (no. 17) was also active. Thus, if the above explanation is correct, muscle must be able to split these two esters. It should be remembered that in previous experiments on glycogen phosphorylation (Montigel & Verzář, 1943) it was found that steroids esterified in the 21-position were less active.

It is much more difficult to explain the differences between the activities of the various other substances on glycogen breakdown. It might appear that the 11-hydroxysteroids which are more soluble in water have a smaller activity. Thus the most active substances progesterone (no. 2), pregnenediolone (no. 4) and stilboestrol (no. 18), which are active even in a concentration of 1 mg./100 ml., have a smaller solubility in water than corticosterone (no. 6) and *allopregnanepentol* (no. 13), which are less active. On the other hand oestradiol (no. 10) and androstenediol (no. 14) are less soluble in water than progesterone (no. 2), but have a smaller activity than corticosterone (no. 6). Thus differences in water solubility do not explain the greater activity of certain steroids on the inhibition of glycogen formation.

Another explanation might be found in the surface activity of steroids (Sobotka, 1938). The steroids, after adsorption on the cell surface might inhibit the diffusion of glucose or of oxygen into the cells. The substances most active in glycogen breakdown, such as DOC and pregnenediolone, lower the surface tension of water to 68 and 68.5 (dynes/cm.) respectively; the inactive oestradiol had in aqueous solution a surface tension of 70; and the fairly active corticosterone 58.5. Thus there was no correlation between inhibition of glycogen formation and surface tension.

There is a marked difference in inhibitory action on glycogen production between DOC and progesterone, both of which are very active, and corticosterone and 17-hydroxycorticosterone which show very little activity. The second group also has a distinct action on glycogen production in the whole animal and this is actually used in some assays of hormones of the adrenal cortex, such as the work test of Ingle (1941) and the glycogen deposition test (Reinecke & Kendall, 1942; Dorfman, Ross & Shipley, 1946; Eggleston, Johnston & Dobriner,

1946). Many authors therefore believe that 11-hydroxysteroids have a specific effect on carbohydrate metabolism, whilst DOC has none. This point has been discussed by us (Verzár, Bucher, Somogyi & Wirz, 1941), and we consider that there is a difference in the velocity but not in the nature of the action of the two groups of steroids. It is remarkable that the results described in this paper show differences between these two groups also with respect to glycogen metabolism *in vitro*.

It has been known for a long time that the sex hormones show effects on carbohydrate metabolism which are comparable with those of the adrenal cortical hormones. Montigel & Verzár (1943) have shown that progesterone, testosterone and to a smaller degree oestradiol influence phosphorylation of glycogen by muscle. The activity is of the same order as that observed in the present experiments. Ingle (1940, 1943, 1944) and Janes & Nelson (1940) have demonstrated that the naturally occurring sex

Table 1. *The effects of various steroids and related substances on the glycogen content of rat's diaphragm in the presence of glucose and insulin*

Steroid				Glycogen content (mg./100 g.)			Mean difference (mg./100 g.) 3-2	S.E. $\pm$ (mg./100 g.)
				After 1.5 hr. with addition of glucose and insulin				
No.	Name	Concentration (mg./100 ml.)	No. of animals	Initial 1	Without steroid 2	With addition of steroid 3		
1	Deoxycorticosterone	10	21	213	309	156	-153	18
		5	30	246	380	244	-136	13
		1	20	287	422	402	-20	24
2	Progesterone	10	12	325	410	237	-173	27
		5	8	359	456	252	-204	30
		1	7	283	400	308	-92	36
3	17-Hydroxyprogesterone	10	7	209	443	241	-202	53
4	Pregn-5-ene-3(β):21-diol-20-one	10	4	275	405	212	-193	20
		5	4	290	475	305	-170	20
		1	12	287	415	368	-47	14
5	Pregnane-3(α):12(β)-diol-20-one	10	8	305	412	285	-127	22
		5	8	356	455	392	-63	21
6	Corticosterone	5	16	339	433	367	-66	16
7	17-Hydroxycorticosterone	5	8	331	485	441	-44	27
		1	8	260	365	355	-10	37
8	Testosterone	10	8	405	536	292	-244	20
		5	8	281	384	312	-72	25
		1	8	200	360	228	-132	36
9	Androstane-3(β):17-diol	10	6	423	527	395	-132	52
		5	12	257	389	320	-69	18
10	Oestradiol	10	8	201	360	278	-82	15
		5	8	190	344	334	-10	24
11	Bisdehydrodoisynolic acid	10	8	254	420	310	-110	18
		5	8	192	351	355	+4	22
12	3(β)-Hydroxyaetiochol-5-enic acid	10	8	309	366	381	+15	18
13	<i>allo</i> Pregnane-3(β):11(β):17:20:21-pentol	10	7	285	360	321	-39	33
14	Androst-5-ene-3(β):17-diol	10	8	280	428	400	-28	35
		5	8	269	421	385	-36	13
15	Deoxycorticosterone- β -D-glucoside	10*	20	252	364	336	-28	26
16	Pregn-5-ene-3(β):21-diol-20-one-21-acetate	10	7	237	351	214	-137	28
17	11-Dehydrocorticosterone-acetate	10	8	340	462	370	-92	24
18	Stilboestrol (4:4'-dihydroxy- α β -diethylstilbene)	10	6	202	302	148	-154	37
		5	8	199	348	204	-144	27
		1	10	206	310	252	-58	28
19	Oleic acid (Na salt)	10	4	438	655	790	+45	33
		5	4	358	562	594	+35	34

* Calculated as pure DOC.

hormones and stilboestrol have a marked influence on the sugar excretion of pancreatectomized rats. It has also been shown (Kochakian, 1945-6) that testosterone lowers the activity of the alkaline and enhances the activity of the acid phosphatase of the kidney. Androstenediol has an effect on phosphatases similar to that of testosterone, but androstenediol has no effect.

In our present experiments on glycogen breakdown we found that testosterone (no. 8) was very active, androstenediol (no. 9) less active, while androstenediol (no. 14) had no activity. It is probable that the effect on glycogen breakdown in our experiments, which is especially marked with progesterone and weak with oestradiol, has its parallel in the action of these substances on phosphorylation of glycogen. Thus the experimental results of the inhibition of insulin activity and stimulation of glycogen breakdown in isolated muscle, shown here with DOC and some sex hormones, has its parallel in many other facts. This is not an argument against the specificity of the action of the adrenal cortical hormones on carbohydrate metabolism. Sex hormones appear to act specifically on certain tissues of secondary sexual character, and their main metabolic function might be of the same nature, as was pointed out long ago by one of us. It might be mentioned that we observed that the insulin effect on glycogen synthesis changes throughout the year, which may be connected with varying sex-hormone production in the animals.

It has not been possible to explain how the steroid hormones act on cell metabolism (Long, 1942; Ingle, 1945). It has been suggested that they are 'velocity factors' acting on enzymic reactions of carbohydrate metabolism (Montigel & Verzár, 1943). Another possibility is that the steroids are adsorbed on the surface of the cells and inhibit diffusion of oxygen so that an anaerobic breakdown of glycogen would result. Alternatively, it is possible

that the diffusion of glucose from the solution into muscle fibres may be inhibited by the adsorption of the steroids on the cell surface. Whilst such possibilities cannot be denied, an action on intracellular enzymes active in the intermediary carbohydrate metabolism still seems the most probable, although unproven, possibility.

SUMMARY

1. The effect of deoxycorticosterone (DOC) on glycogen breakdown in the intact diaphragm of the rat *in vitro* has been compared with the effect of several other steroid hormones and related substances.

2. Progesterone, 17-hydroxyprogesterone and pregnenediolone had an equal or even stronger effect. Corticosterone, 17-hydroxycorticosterone, testosterone, androstenediol and pregnenediolone showed a smaller effect. Oestradiol, bisdehydrodoisynolic acid, 3-hydroxyaetiocholenic acid, *allo*-pregnanepentol and androstenediol had the smallest activity, whilst stilboestrol showed a very high activity.

3. The steroids with corticoid activity possessing a hydroxyl group in the 11-position were less active than DOC. The biological activities of the steroids tested did not go parallel with their water solubilities, nor did differences in surface activity explain the differing activities of these substances on glycogen breakdown and the inhibition of the effect of insulin on glycogen production. There is, however, a certain parallelism between the activities of these substances towards glycogen breakdown as shown in the present experiments and their effects on glycogen phosphorylation by minced muscle.

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