Glycogen Production from Glucose-1-phosphate by Liver and Muscle of Normal and Adrenalectomized Animals

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It has been shown by Schumann (1940), Verzár & Montigel (1941, 1942, 1943*a*, 1943*b*) and by Kutscher & Wüst (1941, 1942) that, after adrenalectomy, phosphorylation of glycogen by muscle and liver (Doetsch, 1945) is decreased. Since some authors were not able to confirm this observation (Riesser, 1943–5; Smits, 1943–5; Helve, 1940) the experiments were repeated in this laboratory by Staehelin & Voegtli (unpublished) and the earlier results were fully confirmed. The first step in the breakdown of glycogen is the formation of glucose-1-phosphate and, as shown by Cori and his co-workers (Cori, Cori & Hegnauer, 1937; Cori, Colowick & Cori, 1937; Cori, Cori & Schmidt, 1939; Cori, Schmidt & Cori, 1939; Cori & Cori, 1939; Cori, Green & Cori, 1942), this reaction is reversible. The equilibrium mixture at pH 7 consists of 77 % glycogen and 23 % glucose-1-phosphate (Colowick & Sutherland, 1942). Glycogen has been produced *in vitro* with minced tissues (Kiessling, 1936, 1937; Ostern, Herbert & Holmes, 1939; Ostern & Holmes, 1939), tissue extracts (Cori, Cori & Schmidt, 1939; Cori & Cori, 1939; Mirski & Wertheimer, 1942) and with purified or even crystalline enzymes (Cori *et al.* 1937; Cori, Colowick & Cori, 1938; Cori *et al.* 1942; Kiessling, 1939*a*, 1939*b*; Colowick & Sutherland, 1942; Schäffner & Specht, 1938, 1939).

In our former experiments only the breakdown of glycogen was studied. It was therefore of interest to see whether the reverse reaction, the production of glycogen from glucose-1-phosphate, was influenced by adrenalectomy. New experiments of Koepf, Horn, Gemmill & Thorn (1941) and of Verzár & Wenner (1948) had shown that whole muscle of adrenalectomized animals is able to produce glycogen. We have therefore studied the problem as to whether muscle and liver of adrenalectomized animals can produce glycogen from glucose-1-phosphate.

METHODS

White rats of 150–300 g. of the laboratory stock were used. Adrenalectomy was carried out on males only. The animals appeared to be well again 2–3 days after the operation and became adynamic after 5–20, days when they were used for the experiments. Our test for adynamia was as follows: the animal was put on an almost vertical wire netting and its movements were observed. Normal animals always climb upwards, whilst adynamic animals cling on to the netting and fall down after a few minutes. We generally used animals which were able to keep up for at least 0·5 min.

The animals were pithed and then bled. The liver was immediately removed and pressed through a sieve. Muscles of the abdominal wall were cut up in a Latapie mincer. The methods of Ostern *et al.* (1939) and of Ostern & Holmes (1939) were used for the estimation of glycogen production in muscle and liver. Quantities of about 0·5 g. of tissue were quickly weighed on a torsion balance and then put in either (*a*) 1 ml. of 0·8 % NaCl containing 0·005 M-NaF, or (*b*) 1 ml. of a solution containing 0·005 M-NaF and glucose-1-phosphate of a concentration which was equivalent to a glucose content of 10 mg./ml. In certain cases 5 mg. of glycogen (Hofmann-La Roche), dissolved in 0·2 ml. of water, were added to the mixture (i.e. 1 g./100 g. muscle or liver). The muscle or liver mixture was kept at 20° and the glycogen content was measured after 0, 7, 15 and 30 min. Glycogen production usually reached its maximum after 15–30 min. After 1 hr. the newly formed glycogen had disappeared again (Ostern *et al.* 1939; Ostern & Holmes, 1939).

For glycogen estimation (Pflüger, 1909; Simonovits, 1933; Good, Kramer & Somogyi, 1933; Bomskov & Kaulla, 1942; Kiessling, 1939*a*; Cori & Cori, 1939, 1940) 2 ml. of 40 % (w/v) NaOH were added and the mixture was hydrolyzed on a boiling water bath for 15–30 min. The solution was made up with water to 5 ml. and 5 ml. of 96 % (v/v) ethanol were added. The mixture was kept at 0° for 30 min. and the precipitated glycogen was then centrifuged (3000 r.p.m.) for 5 min. Remaining glucose-1-phosphate was extracted by dissolving the precipitate in 1 % NaCl (2 ml.), reprecipitating the glycogen with ethanol (2 ml.) at 0° and centrifuging. The glycogen was finally dried by adding 96 % ethanol (2 ml.), centrifuging and evaporating the ethanol on a water bath. The glycogen was hydrolyzed with 2 N-H_2SO_4 for 2 hr. on a boiling water bath, the solution was transferred to a 50 ml. flask, made alkaline by addition of 2 N-Na_2CO_3 and diluted with water. The glucose was determined by the Hagedorn-Jensen method.

Glucose-1-phosphate was synthesized from glucose according to Cori *et al.* (1937). The preparation contained 80 % of the Ba salt. For the conversion to the Na salt, Ba was precipitated by Na_2SO_4 from a solution acidified with H_2SO_4 and the solution neutralized with 2 N-Na_2CO_3 (litmus).

RESULTS

The glycogen content of minced liver decreases after the death of the animal. To allow for this glycogenolysis, a control in which no glucose-1-phosphate was added was carried out for each experiment. Uncut

Table 1. *Glycogen production by minced normal liver*

Exp. no.	Wt. of rat (g.)	Time (min.)	Glycogen content (mg./100 g.) Without additions	With addition of glucose-1-phosphate	Glycogen produced (mg./100 g.)	Remarks
4	230 ♂	0	4350	—	—	Concentration of glucose-1-phosphate 5%
		15	4300	5000	700	
		30	3890	4750	860	
5	215 ♀	0	6500	—	—	—
		15	6000	6700	700	
		30	5800	6900	1100	
13	250 ♂	0	6190	—	—	—
		7	6010	6440	430	
		15	5870	6470	600	
		30	5730	6020	290	
14	280 ♂	0	3510	—	—	—
		7	3300	3910	610	
		15	3000	3950	950	
		30	3070	4050	980	
15	220 ♀	0	2420	—	—	New glucose-1-phosphate used
		15	770	1870	1100	
		30	520	1880	1360	
	Means	0	4590	—	—	
		15	3990	4800	810 (±90)*	
		30	3800	4720	920 (±180)*	

* Standard error in brackets.

Table 2. *Glycogen production by minced normal muscle*

Exp. no.	Wt. of rat (g.)	Time (min.)	Glycogen content (mg./100 g.) Without additions	With addition of glucose-1-phosphate	Glycogen produced (mg./100 g.)
4	230 ♂	0	690	—	—
		15	480	1060	580
		30	430	780	350
5	215 ♀	0	790	—	—
		15	600	610	10
		30	520	620	100
12	180 ♀	0	510	—	—
		30	350	590	240
13	250 ♂	0	260	—	—
		7	240	390	150
		15	160	280	120
		30	60	140	80
14	280 ♂	0	440	—	—
		7	340	530	190
		15	200	470	270
		30	230	430	200
	Means	0	540	—	—
		15	360	600	240 (±130)*
		30	400	510	110 (±80)*

* Standard error in brackets.

muscle shows only a little glycogenolysis in the first 30 min. after death, but in minced muscle, diluted with 0·8 % NaCl, the glycogen content decreases rapidly. Identical glycogen values were obtained with whole muscle, hydrolyzed directly, or with muscle which was first minced and then immediately hydrolyzed by hot NaOH. The glycogen content of different muscles varies widely (Noll & Becker, 1936). We therefore used the muscles of the abdominal wall, because they present a relatively large quantity of

muscle of uniform glycogen content. It was found that the error of glycogen estimation for amounts under 100 mg./100 g. was about 20%, between 100–500 mg./100 g. about 10–20%, and between 500–5000 mg./100 g. it was under 10%.

In all experiments, unless otherwise stated, the amounts of glucose-1-phosphate and of glycogen added were equivalent to a concentration of 3 g. and 1 g./100 g. minced tissue respectively.

1. *Normal fed animals*

Table 1 shows that in all 5 animals minced liver produced glycogen from glucose-1-phosphate. In Exps. 4, 5, 14 and 15 the maximum was reached after 30 min. and in Exp. 13 after 15 min. The greatest glycogen production was seen in Exp. 15 (1360 mg./100 g.). In 0·8% NaCl the glycogen content decreased during the same period from 2420 to 520 mg./100 g.

Whilst, as shown in Table 1, the glycogen content of liver was between 2·4 and 6·5%, the glycogen content of abdominal wall muscle was only 0·26–0·79% (Table 2).

In the five experiments of Table 2, some of which were done on the same animals as in Table 1, addition of glucose-1-phosphate caused the greatest glycogen production in Exp. 4 (580 mg./100 g.) and this was reached after 15 min. Without glucose-1-phosphate the glycogen content decreased from 690 to 480 mg./100 g.

2. *Normal starved animals*

The following experiments were made in order to study the glycogen production by muscle and liver tissue from rats whose glycogen content had been decreased by fasting. Such experiments were required, since in adrenalectomized animals the glycogen content of both liver and muscle is very much decreased, and it appeared possible that this factor might influence glycogen production.

In rats, fasting for 24 hr. reduces the glycogen content of the liver to very low values (FitzGerald, 1938; FitzGerald, Laszt & Verzár, 1938; Verzár, 1939; Kreienberg & Wiesenhüter, 1943), while in muscles the decrease is less (Kreienberg & Wiesenhüter, 1943).

Table 3. *Glycogen production by minced liver from starved animals*

Exp. no.	Wt. of rat (g.)	Time (min.)	Glycogen content (mg./100 g.) Without additions	With addition of glucose-1-phosphate	With addition of glycogen	With addition of glycogen and glucose-1-phosphate	Glycogen produced from glucose-1-phosphate: Without addition of glycogen	With addition of glycogen	Period of starvation (hr.)
6	230 ♂	0	100	—	—	1310	—	—	24
		15	60	80	960	1350	20	390	
		30	80	—	—	1360	—	—	
7	260 ♂	0	70	—	1070	—	—	—	24
		15	20	5	900	970	−15	70	
		30	10	5	880	880	− 5	0	
8	200 ♂	0	—	—	1420	—	—	—	24
		15	—	—	990	1700	—	710	
		30	60	210	810	1350	150	540	
10	200 ♂	0	730	—	—	1600	—	—	24
		15	560	890	1280	1850	330	570	
		30	380	—	1200	1260	—	60	
22	180 ♀	0	40	—	1040	—	—	—	48
		15	20	30	800	1130	10	330	
23	170 ♀	0	30	—	1050	—	—	—	48
		15	20	30	800	1000	10	200	
24	245 ♂	0	160	—	1140	—	—	—	48
		15	90	700	910	1520	610	610	
26	240 ♂	0	180	—	1160	—	—	—	72
		15	50	670	680	1360	620	680	
27	265 ♂	0	30	—	1030	—	—	—	48
		15	20	120	1070	1390	100	320	
25	260 ♂	0	50	—	1070	—	—	—	48
		15	20	610	770	1240	590	470	
28	325 ♂	0	80	—	1130	—	—	—	72
		15	30	310	880	1430	280	550	
	Means	0	150	—	1120	—	—	—	
		15	110	340	920	1360	230 (±80)*	440 (±60)*	

* Standard error in brackets.

Table 4. *Glycogen production by minced muscle from starved animals*

Exp. no.	Wt. of rat (g.)	Time (min.)	Glycogen content (mg./100 g.) Without additions	With addition of glucose-1-phosphate	With addition of glycogen	With addition of glycogen and glucose-1-phosphate	Glycogen produced from glucose-1-phosphate: Without addition of glycogen	With addition of glycogen	Period of starvation (hr.)
8	200 ♂	0	130	—	1140	1110	—	−30	24
		15	—	—	1080	1430	—	350	
		30	60	50	910	1520	−10	610	
9	210 ♂	0	170	110	—	—	−60	—	24
		15	160	180	1280	1460	20	180	
		30	100	—	1200	1450	—	250	
10	200 ♂	0	120	50	1000	1050	−70	50	24
		15	40	90	760	1610	50	850	
		30	50	—	490	1250	—	760	
22	180 ♀	0	40	—	1070	—	—	—	48
		20	10	20	850	1280	10	430	
23	170 ♀	0	30	—	1020	—	—	—	48
		15	20	30	870	1410	10	540	
24	245 ♂	0	60	—	1120	—	—	—	48
		20	80	110	970	1230	30	260	
25	260 ♂	0	40	—	1020	—	—	—	48
		20	10	10	760	1230	0	470	
26	240 ♂	0	40	—	1040	—	—	—	72
		20	30	130	910	1350	100	440	
27	265 ♂	0	30	—	1040	—	—	—	48
		20	30	60	780	1100	30	320	
28	325 ♂	0	30	—	1020	—	—	—	72
		20	20	30	870	1060	10	190	
	Means	0	70	—	1050	—	—	—	
		15	40	70	910	1320	30 (±10)*	410 (±20)*	

* Standard error in brackets.

From Tables 3 and 4 it can be seen that if the glycogen content of liver or muscle is less than 100 mg./100 g., no or almost no production of glycogen from glucose-1-phosphate is observed *in vitro*. Only in Exps. 25 and 28 (Table 3) is glycogen produced in normal quantities by liver, in spite of a low initial glycogen content. Cori & Cori (1940) have shown that the addition of some glycogen to tissue extracts of very low initial glycogen content initiates glycogen formation from glucose-1-phosphate. We therefore added 1 g. glycogen/100 g. liver or muscle in addition to glucose-1-phosphate. This addition of glycogen caused normal quantities of glycogen to be formed, thus confirming Cori's observation.

3. *Adrenalectomized animals*

The animals were killed in severe adynamia. In Tables 5 and 6 + + + + means a state in which the animals would have died in a few hours, while + + + indicates that the rats were still moving but unable to climb, and were falling from a vertical wire netting in 0·5–2 min.

Table 5 shows that the glycogen content of the liver was extremely low and in Exps. 2, 3, 8, 9, 10 and 11 no glycogen was produced by addition of glucose-1-phosphate alone. However, addition of 1 g. glycogen/100 g. liver produced in the same experiments just as much new glycogen as with tissue from normal animals. This glycogen production varied between 150 and 570 mg./100 g. in 15 min. Only in rat no. 7 was the glycogen content of the liver as high as in normal animals, in spite of a distinct adynamia. Without addition of glycogen 470 mg./100 g. of glycogen were produced from glucose-1-phosphate, whilst with addition of glycogen 890 mg./100 g. new glycogen were produced from the phosphate ester. Rat no. 6, in spite of an extremely low glycogen content, produced without addition of glycogen 200 mg./100 g., and with addition of glycogen 400 mg./100 g., of new glycogen from glucose-1-phosphate.

Thus the production of glycogen by tissue from these adrenalectomized animals was not diminished as compared with that with tissue from normal, starved rats. These ten experiments do not indicate any marked effect of adrenalectomy on glycogen

Table 5. *Glycogen production by minced liver from adrenalectomized rats*

Exp. no.	Wt. of rat (g.)	Time (min.)	Glycogen content (mg./100 g.) Without additions	With addition of glucose-1-phosphate	With addition of glycogen	With addition of glycogen and glucose-1-phosphate	Glycogen produced from glucose-1-phosphate: Without addition of glycogen	With addition of glycogen	Days after adrenalectomy, and degree of adynamia
2	195 ♂	0	50	—	—	—	—	—	17
		7	20	10	—	—	-10	—	+ + + +
		15	10	10	—	—	0	—	
		30	20	20	—	—	0	—	
3	190 ♂	0	10	—	—	—	—	—	18
		15	10	5	—	—	- 5	—	+ + +
		30	5	5	—	—	0	—	
4	225 ♂	0	—	—	1220	—	—	—	19
		7	—	—	900	1470	—	570	+ + +
		15	—	—	920	1490	—	570	
5	200 ♂	0	20	—	—	—	—	—	18
		7	—	—	820	1300	—	480	+ + +
		30	—	—	680	1300	—	620	
8	130 ♂	0	30	—	1030	—	—	—	10
		15	10	40	—	1320	30	(320)	+ + +
9	150 ♂	0	20	—	1030	—	—	—	10
		15	30	40	930	1080	10	150	+ + +
10	150 ♂	0	20	—	1020	—	—	—	11
		15	20	10	970	1150	-10	180	+ + + +
11	145 ♂	0	40	—	—	—	—	—	11
		15	30	60	1010	1270	30	260	+ + +
6	117 ♂	0	20	—	1030	—	—	—	5
		15	20	220	830	1230	200	400	+ + +
7	102 ♂	0	2000	—	2900	—	—	—	5
		15	1850	2320	2220	3110	470	890	+ + +
	†Means	0	30	—	1070	—	—	—	
		15	20	60	930	1260	40 (±30)*	330 (±80)*	

* Standard error in brackets.
† Nos. 5 and 7 are not included.

formation from glucose-1-phosphate by liver tissue. Many more experiments would be necessary to reveal possible differences of velocity or slight, statistically significant, differences.

Table 6 shows that muscles of the same adrenalectomized animals too have very low initial glycogen contents. In none of these experiments was there a production of glycogen from glucose-1-phosphate, without addition of extra glycogen. If, however, 1 g. glycogen/100 g. muscle was added, new glycogen was produced in about the same quantity as with starved normal animals. Thus the muscle from adrenalectomized animals too, has not lost the capacity to produce glycogen from glucose-1-phosphate.

DISCUSSION

The above experiments show that normal minced liver and muscle produce glycogen from glucose-1-phosphate. In starved rats with very low glycogen content, however, no glycogen is produced by minced muscle or liver, unless glycogen is added to the solution. This seems to show that the glycogen-producing enzyme (phosphorylase, Cori *et al.* 1942) can only produce glycogen from glucose-1-phosphate if some glycogen is already present. Kiessling (1939*a*, 1939*b*), Cori, Cori & Schmidt (1939) and Colowick & Sutherland (1942) have obtained similar results with pure enzyme solutions. Phosphorylase thus appears to increase the size of glycogen molecules which are already present without increasing their number.

It is well known that adrenalectomized rats show a very low glycogen content in the liver and later also in the muscles. Since starvation for 24–48 hr. in rats produces similarly low glycogen values, it seemed possible that the low glycogen contents of adrenalectomized rats are caused, at least partly, by the concomitant starvation. Liver or muscle tissue from adrenalectomized animals produces no glycogen without addition of extra glycogen and thus behaves like that from starved animals. But glycogen is produced in the same quantities as by tissue from normal animals, if glycogen is added. Thus the disturbance of carbohydrate metabolism

Table 6. *Glycogen production by minced muscle from adrenalectomized animals*

Exp. no.	Wt. of rat (g.)	Time (min.)	Glycogen content (mg./100 g.) Without additions	With addition of glucose-1-phosphate	With addition of glycogen	With addition of glycogen and glucose-1-phosphate	Glycogen produced from glucose-1-phosphate: Without addition of glycogen	With addition of glycogen	Days after adrenalectomy and adynamia
2	195 ♂	0	30	—	—	—	—	—	17
		7	20	—	—	—	—	—	+ + + +
		15	10	10	—	—	0	—	
		30	10	10	—	—	0	—	
3	190 ♂	0	20	—	—	—	—	—	18
		7	10	10	—	—	0	—	+ + +
		15	10	5	—	—	- 5	—	
		30	5	0	—	—	- 5	—	
5	200 ♂	0	40	—	—	—	—	—	18
		7	—	—	1090	1340	—	250	+ + +
		30	—	—	900	1300	—	400	
4	225 ♂	0	—	—	1170	—	—	—	19
		7	—	—	970	1240	—	270	+ + +
		30	—	—	930	1150	—	220	
6	117 ♂	0	30	—	1070	—	—	—	5
		20	—	40	940	1700	(10)	760	+ + +
7	102 ♂	0	80	—	—	—	—	—	5
		20	—	100	—	2240	(50)	(1000)	+ + +
8	130 ♂	0	25	—	—	—	—	—	10
		20	15	25	1000	1420	10	420	+ + +
9	150 ♂	0	110	—	1150	—	—	—	10
		20	20	10	1010	1320	-10	310	+ + +
10	150 ♂	0	30	—	—	—	—	—	11
		20	20	20	930	1350	0	420	+ + + +
11	145 ♂	0	60	—	—	—	—	—	11
		20	30	70	950	1250	40	300	+ + +
	†Means	0	50	—	—	—	—	—	
		20	20	40	950	1360	20 (±10)*	410 (60)*	

* Standard error in brackets.
† No. 7 is not included.

after adrenalectomy cannot be due to a lack of glycogen production from glucose-1-phosphate. This result is in agreement with the observation with surviving diaphragms of rats (Koepf *et al.* 1941; Verzár & Wenner, 1948) that there is no difference as regards glycogen formation from glucose between diaphragms obtained from normal and adrenalectomized animals. Thus only minced muscle seems to be unable to produce glycogen when the initial glycogen content is low, since undamaged whole muscle retains the ability to produce glycogen under these conditions. The surviving diaphragm produces glycogen from glucose, but not from glucose-1-phosphate (Verzár & Wenner, 1948). This is not incompatible with the present finding that minced liver and muscle produce glycogen from glucose-1-phosphate. The latter, unlike glucose, does not diffuse through cell membranes into the cells of the undamaged surviving diaphragm. Glucose-1-phosphate, like other phosphoric acid esters of glucose, is produced inside the cells. The production of those esters inside the cell is, as pointed out by Verzár & McDougall (1936) and Minibeck & Verzár (1940), the factor responsible for the increased diffusion rate of glucose through cell membranes. In minced liver and muscle most of the cells are damaged. The greater part of the glycogen is not produced inside the cells but by dissolved enzymes in the solution. This may explain the fact that addition of 1% glycogen to the solution increases glycogen production from glucose-1-phosphate. Moreover, it is known that with pure solutions of phosphorylase production of new glycogen takes place only when some glycogen is already present.

The result that muscle and liver tissue from adrenalectomized animals has not lost the ability to form new glycogen is in conformity with the finding that the surviving diaphragm of adrenalectomized rats produces glycogen (Koepf *et al.* 1941; Verzár & Wenner, 1948). The disturbance of carbohydrate metabolism after adrenalectomy is thus due either to an inhibition of phosphorylation, or, more probably, to a disturbance of the breakdown of glycogen and not of its synthesis.

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.

REFERENCES

Bomskov, C. & Kaulla, K. N. (1942). *Z. ges. exp. Med.* **110**, 604.
Colowick, S. P. & Sutherland, W. (1942). *J. biol. Chem.* **144**, 424.
Cori, C. F., Colowick, S. P. & Cori, G. T. (1937). *J. biol. Chem.* **121**, 465.
Cori, G. T., Colowick, S. P. & Cori, C. F. (1938). *J. biol. Chem.* **123**, 375.
Cori, C. F. & Cori, G. T. (1939). *J. biol. Chem.* **131**, 397.
Cori, G. T. & Cori, C. F. (1940). *J. biol. Chem.* **135**, 733.
Cori, C. F., Cori, G. T. & Hegnauer, A. H. (1937). *J. biol. Chem.* **120**, 193.
Cori, G. T., Cori, C. F. & Schmidt, G. (1939). *J. biol. Chem.* **129**, 629.
Cori, C. F., Green, A. A. & Cori, G. T. (1942). *J. biol. Chem.* **142**, 447.
Cori, C. F., Schmidt, G. & Cori, G. T. (1939). *Science*, **89**, 464.
Doetsch, R. (1945). *Helv. chim. Acta*, **28**, 31.
FitzGerald, O. (1938). *Verh. Ver. Schweiz. Physiol.* **13**, 14.
FitzGerald, O., Laszt, L. & Verzár, F. (1938). *Pflüg. Arch. ges. Physiol.* **240**, 619.
Good, C. A., Kramer, H. & Somogyi, M. (1933). *J. biol. Chem.* **100**, 485.
Helve, O. E. (1940). *Biochem. Z.* **306**, 358.
Kiessling, W. (1936). *Biochem. Z.* **287**, 291.
Kiessling, W. (1937). *Biochem. Z.* **292**, 26.
Kiessling, W. (1939*a*). *Biochem. Z.* **302**, 50.
Kiessling, W. (1939*b*). *Naturwissenschaften*, **27**, 129.
Koepf, G. F., Horn, H. W., Gemmill, C. L. & Thorn, G. W. (1941). *Amer. J. Physiol.* **133**, Proc. 353.
Kreienberg, W. & Wiesenhüter, E. (1943). *Pflüg. Arch. ges. Physiol.* **247**, 22.
Kutscher, W. & Wüst, H. (1941). *Naturwissenschaften*, **29**, 314.
Kutscher, W. & Wüst, H. (1942). *Hoppe-Seyl. Z.* **273**, 235.
Minibeck, H. & Verzár, F. (1940). *Helv. med. Acta*, **7**, Suppl. v, 7.
Mirski, A. & Wertheimer, E. (1942). *Biochem. J.* **36**, 230.
Noll, A. & Becker, M. (1936). *Biochem. Z.* **287**, 88.
Ostern, P., Herbert, D. & Holmes, E. (1939). *Biochem. J.* **33**, 1858.
Ostern, P. & Holmes, E. (1939). *Nature, Lond.*, **144**, 34.
Pflüger, E. (1909). *Pflüg. Arch. ges. Physiol.* **129**, 362.
Riesser, O. (1943–5). *Enzymologia*, **11**, 323.
Schäffner, A. & Specht, H. (1938). *Naturwissenschaften*, **26**, 494.
Schäffner, A. & Specht, H. (1939). *Naturwissenschaften*, **27**, 195.
Schumann, H. (1940). *Pflüg. Arch. ges. Physiol.* **243**, 686.
Simonovits, S. (1933). *Biochem. Z.* **265**, 437.
Smits, G. (1943–5). *Enzymologia*, **11**, 334.
Verzár, F. (1939). *Die Funktion der Nebennierenrinde*, p. 62. Basel: Schwabe.
Verzár, F. & McDougall, E. J. (1936). *Absorption from the Intestine*. London: Longmans, Green and Co. Ltd.
Verzár, F. & Montigel, C. (1941). *Schweiz. med. Wschr.* **71**, 101.
Verzár, F. & Montigel, C. (1942). *Helv. chim. Acta*, **25**, 9, 22.
Verzár, F. & Montigel, C. (1943*a*). *Helv. chim. Acta*, **26**, 883.
Verzár, F. & Montigel, C. (1943*b*). *Helv. physiol. pharmacol. Acta*, **1**, 115, 137.
Verzár, F. & Wenner, V. (1948). *Biochem. J.* **42**, 35.

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,