XCIII. OBSERVATIONS ON THE CHEMISTRY OF MUSCLE GLYCOGEN

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SHORTLY after Claude Bernard had isolated glycogen from liver tissue [1857] Sanson [1857] announced that skeletal muscle contained a substance identical with that obtained by Bernard from the liver. No details of the properties of the substance isolated from muscle tissue were given, however. Despite this lack of adequate demonstration that specimens prepared from the two sources were similar in every respect, there has apparently been no doubt since the time of Sanson that muscle and liver glycogens are indeed chemically identical. Claude Bernard's views on the relationship of starch to glycogen from liver or muscle are not at all clear [Young, 1937]. In *Leçons sur le diabète* [1877, 1] he distinguishes the colours given by starch and glycogen with iodine, describing that given by glycogen as "une coloration violette qui est intermédiaire entre le bleu de l'amidon et le rouge de la dextrine obtenue par l'acide sulfurique" (p. 304), but later writes [1877, 2] "Nous avons vu non seulement le glycogène être identique à l'amidon...." According to present teaching the colour given by glycogen (generally obtained from liver) with iodine is brownish red rather than violet [cf. Bell & Young, 1934]. Bernard also states that the glycogen accumulating in resting rabbit muscle previously stimulated to exhaustion gives, with iodine, a blue coloration similar to that given by starch [1877, 1, p. 553]. The object of the present investigation was to compare the properties of specimens of glycogen prepared from different muscle tissues with those of liver glycogen, using the methods developed by Bell & Young [1934]. It also seemed of interest to check Bernard's statement about the peculiar property of glycogen accumulating in resting, previously fatigued, muscle.

METHODS

"Alkali-extracted glycogen" was prepared from muscle by the method described by Bell & Young [1934] for liver tissue. In many cases further purification was effected by precipitation with 80% acetic acid as described by these authors. The material obtained by this method is described as "acetic acid-purified glycogen". Attempts to prepare "aqueous-extracted glycogen" from muscle tissue by the method of Bell & Young and by that of Carruthers [1931] were unsuccessful. Aqueous extracts of muscle contain much nitrogenous material not precipitated by 4% trichloroacetic acid but insoluble in 66% alcohol. Moreover, only a small proportion of the glycogen present in muscle could be freed from nitrogenous matter unless the tissue had first been digested with strong alkali. In many cases a full 3 hr. preliminary heating in 30% potash was necessary. Much of the glycogen in muscle tissue probably exists in a combined form [Willstätter & Rohdewald, 1934; Przylecki & Majmin, 1934;

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Mystkowski, 1935], a form which Willstätter & Rohdewald describe as "desmoglycogen". The small amount of glycogen that could be extracted from muscle tissue by boiling water was freed from protein matter by treatment with picric acid, the picric acid being removed by thorough washing with alcohol, followed by dialysis. Material thus obtained is described as "aqueous-extracted glycogen".

During the preparation of muscle glycogen, considerable loss of material occurred in the process of dialysis. The dialysing membranes were of the type used previously for the preparation of liver glycogen, when no significant loss occurred during dialysis. These membranes appear to retain particles of molecular weight of 20,000 or more. The presence of diffusible glycogen-like substances in muscle tissue was described by Pavy [1894], although his results were not generally accepted. The present investigation is concerned only with a "non-diffusible" muscle-polysaccharide (glycogen), which was shown by Oakley & Young [1936] to possess a mean particle weight of about 2×10^6.

Results

Aqueous-extracted muscle glycogen. The specific rotation and reducing properties of aqueous-extracted glycogen were considerably altered by precipitation with acetic acid or treatment with 30 % potash, as shown in Table I.

Table I

Specimen	$[\alpha]_D$	Reduction (glucose = 100)	Iodine coloration
1. Rabbit. Aqueous-extracted	+166·5°	1·55	Reddish violet
After acetic acid treatment	188	0·25	,,
After KOH treatment	187	0·20	,,
2. Rabbit. Aqueous-extracted	160	1·28	,,
3. Rat. Aqueous-extracted	106	2·80	Brownish red
After acetic acid treatment	134	0·96	,,
After KOH treatment	186	0·26	,,

The aqueous extraction method apparently yields a product which is far from pure and is not easily further purified. The colour given with iodine by any given preparation was constant and not altered by subsequent purification, although different preparations gave very different iodine colours.

Alkali-extracted muscle glycogen. The properties of alkali-extracted muscle glycogen specimens prepared from different sources are given in Table II. The

Table II

Source	$[\alpha]_D$	Reduction (glucose = 100)	Ash %	Iodine coloration
1. Rabbit	+189°	0·12	0·64	Reddish violet
2. Rabbit	184·5	0·17	—	Brownish red
3. Rat	184	0·07	—	,,
4. Ox	189	0·35	—	,,
5. Guinea-pig	193	0·20	0·66	,,
6. Dog-heart	187	0·20	—	Faint brown
7. Dog-heart	186	0·09	1·96	,,
8. *Mytilus edulis*	188·5	0·04	—	Brown-yellow
9. *Mytilus edulis*	192	0·06	0·51	,,
Average	188·0°	0·14		
Liver glycogen (Bell & Young)	193·5°	1·14		

average figures for alkali-extracted rabbit liver glycogen [Bell & Young] are given for comparison. Owing to individual variations the differences between the average values for the specific rotation and reducing power of muscle and liver glycogens cannot be considered as significant. This may also be said about the figure for acetic acid-purified preparations given in Table III. Here the

Table III. *Properties of acetic acid-purified muscle glycogen*

Source	$[\alpha]_D$	Reduction (glucose = 100)	Ash %	Total phosphorus (as P_2O_5) %	Iodine coloration
1. Rabbit	+197°	0·20	—	—	Reddish violet
2. Rabbit	193	0·12	—	—	,,
3. Rabbit	192·5	0·05	—	0·02	Brownish red
4. Guinea-pig	191	0·09	0·02	0·015	,,
5. Guinea-pig	191	0·03	0·49	—	,,
6. Dog heart	187	0·08	0·21	0·12	Faint brown
7. Dog heart	190	0·05	0·95	0·12	,,
8. *Mytilus edulis*	192	0·03	0·11	0·047	Brown-yellow
9. *Mytilus edulis*	196	0·10	0·43	—	,,
Average	192·0°	0·084	0·368	0·088	
Liver glycogen	196·0°	0·090	0·170	0·093	

difference is smaller, but it is somewhat surprising that the specific rotation of muscle glycogen specimens so frequently falls below that for liver glycogen. The lower values for muscle glycogen can hardly be explained by the slightly higher ash contents.

In some cases, though not in all, the purified muscle glycogen preparations dissolved in water more rapidly than similar liver glycogen specimens, the former giving aqueous solutions which were much less opalescent than those of the latter. The colour given by dog-heart glycogen with iodine was much less intense than that given by other specimens; that given by glycogen from the muscles of *Mytilus edulis* was rather more yellow than the tint obtained with muscle glycogen from other sources, being reminiscent of that given by fish liver glycogen [Bell & Young, 1934].

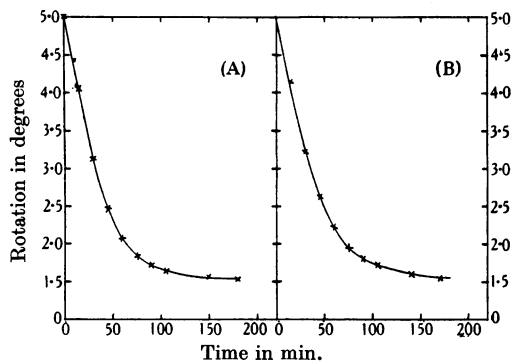

Fig. 1. Hydrolysis curves of acetic acid-purified glycogen (*a*) from rabbit liver, (*b*) from rabbit muscle. Concentration equals 1·3% and theoretical end-point equals 1·51° in both cases.

Hydrolysis by acid. The hydrolysis curves published by Bell & Young were obtained by heating the flask containing the acid glycogen solutions on the rings of a boiling water-bath. The temperature of the hydrolysing liquid, although

constant, was not 100° but somewhat below this temperature. In the present investigation hydrolysis has been carried out by immersing the flask containing the experimental solution in a vigorously boiling water-bath. Determinations of the temperature of the hydrolysing liquid have been made from time to time and have not varied significantly from 100°. Apart from this modification the technique of polarimetric determination of the rate of hydrolysis of glycogen by 2·4% HCl was similar to that described in the earlier paper. The typical curves in Fig. 1 show that the rate of hydrolysis of purified muscle glycogen by 2·4% HCl at 100° is not significantly different from that of liver glycogen.

Iodine colour given by glycogen accumulating in resting, previously fatigued, muscle. Rabbits were decerebrated or anaesthetized with amytal and the muscles of one hind limb tetanized by faradic stimulation. The limb was then allowed to rest and alkali-extracted glycogen prepared from the muscles of both hind limbs. Control experiments in which glycogen estimations were carried out showed that a considerable increase in glycogen content occurred in the exhausted muscles during the period of rest. In ten experiments no difference was found between the iodine colour given by the glycogen of the unstimulated muscles and of those which had been exhausted and allowed to recover. The iodine colours varied greatly in the different experiments, ranging from liver-glycogen reddish brown to a frankly violet tint, but the colour given by the muscle glycogen of any given animal was unaffected by the experimental procedure.

Discussion

It has been known since the days of Claude Bernard that although the physiological degradation of glycogen in the liver yields mainly glucose, lactic acid results from the breakdown of glycogen in the muscles. In spite of this difference in metabolism the glycogen in liver appears, from the results of the present investigation, to differ remarkably little from that in muscle tissue. The differences, by no means constant, in iodine colour and in opalescence in solution between muscle and liver glycogens might be considered as due to a physical, rather than a chemical, difference. Nevertheless Oakley & Young [1936] found that solutions of muscle glycogen differing from those of liver glycogen in these respects gave similar mean particle weights determined osmometrically. The difference in specific rotation of purified liver and muscle glycogens amounts, on the average, to only 2% and cannot be considered as significant, although a slight difference may, in fact, exist. The ash content of muscle glycogen was somewhat higher than that of liver glycogen, and in general muscle glycogen seemed more difficult to purify than glycogen from the liver. Otherwise muscle and liver glycogens do not differ appreciably in the properties examined.

Bell has recently applied Haworth's "end-group assay" method of investigation to glycogen from the livers of rabbits fed on galactose and from *Mytilus edulis*. In both instances [Bell, 1936] he has found evidence for a chain length of 18 glucose units for the glycogen molecule, compared with 12 units for glycogen from the livers of normally fed rabbits. The results of the application of this method to mammalian muscle glycogen will be of great interest.

Summary

1. Application of the methods of Bell & Young [1934] to muscle glycogen showed that liver and muscle glycogens exhibit no very great differences in the properties examined.

2. The mean $[\alpha]_D$ for purified muscle glycogen of $+192{\cdot}0°$ cannot be considered as significantly different from that for liver glycogen ($+196{\cdot}0°$).

3. Some specimens of muscle glycogen gave solutions which differed from those of liver glycogen in opalescence and in coloration with iodine. These differences are not, however, associated with a significant difference in mean particle weight [Oakley & Young, 1936].

4. Attempts to confirm Claude Bernard's statement that the glycogen accumulating in resting, previously fatigued rabbit muscle "donne par l'iode une coloration tout à fait bleue comme de l'amidon" were not successful.

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REFERENCES

Bell (1936). *Biochem. J.* **30**, 1612 and 2144.
—— & Young (1934). *Biochem. J.* **28**, 2144.
Bernard (1857). *C.R. Acad. Sci., Paris*, **44**, 578.
—— (1877, 1). Leçons sur le diabète. (Baillière, Paris.)
—— (1877, 2). *C.R. Acad. Sci., Paris*, **85**, 519.
Carruthers (1931). *Chin. J. Physiol.* **5**, 85.
Mystkowski (1935). *Biochem. Z.* **278**, 240.
Oakley & Young (1936). *Biochem. J.* **30**, 868.
Pavy (1894). The Physiology of the Carbohydrates. (Churchill, London.)
Przylecki & Majmin (1934). *Biochem. Z.* **273**, 262, 272.
Sanson (1857). *C.R. Acad. Sci., Paris*, **44**, 1159, 1323.
Willstätter & Rohdewald (1934). *Hoppe-Seyl. Z.* **225**, 103.
Young (1937). *Ann. Sci.* **2**, 47.