CCXXXVII. LIVER GLYCOGEN.

II. ACYL DERIVATIVES AND "REGENERATED GLYCOGENS".

BY DAVID JAMES BELL AND HANS KOSTERLITZ.

*From the Physiological Laboratory, Marischal College,
The University of Aberdeen.*

(Received July 5th, 1935.)

THIS paper extends the observations of Bell and Young [1934] to the preparation and properties of acetyl and benzoyl derivatives of glycogens, the examination of the polysaccharides regenerated from these acyl derivatives and the occurrence of phosphorus in both the original and regenerated materials.

Acyl derivatives.

The triacetates and tribenzoates of pure glycogens, isolated from rabbit and fish (*Gadidae*) livers as described by Bell and Young, were obtained in good yield. A comparison of the properties of these substances revealed no difference between derivatives from the two sources (Table I). It was noted that glycogen extracted by water (no KOH treatment) displayed resistance to acetylation. Preliminary heating with 30% KOH solution, however, was found to promote complete acylation.

Table I.

Source of glycogen	Acetate		$[\alpha]_D$	Benzoate		$[\alpha]_D$
	Yield %	Acetyl %		Yield %	Benzoyl %	
Rabbit (water-extracted)	50	43.9	+172°	—	—	—
„ (KOH water-extracted)	84	44.0	+171°	80.0	65.0	+113°
„ (water-extracted, after KOH)	84	44.0	+171°	—	—	—
Fish (water-extracted)	Trace	—	—	84.7	66.4	+114°
„ (water-extracted, after KOH)	87	44.4	+170°	—	—	—

$[\alpha]_D$ was determined in chloroform solution.

The regenerated polysaccharides.

De-acylation of the acetates and benzoates yielded theoretical amounts of materials which, at present, cannot but be considered as chemically identical with the parent substances. The results are summarised in Table II.

The rates of hydrolysis with 2.4% HCl at 100° (*vide* Bell and Young) were in all cases the same and were unchanged from those of the parent substances. Regeneration likewise did not affect the coloration with iodine, deep red-brown with rabbit glycogen and yellow-brown with the material from fish.

DISCUSSION.

Phosphorus content. This is extremely small and diminishes after acylation. As no precautions were taken to employ phosphorus-free reagents, it is probable that the presence of this element in the highly purified materials is adventitious

Table II.

	I (a) signifies H ₂ O-extracted.		
	(b) „ KOH-extracted.		
II	„ regenerated from acetate.		
III	„ regenerated from benzoate.		
	Source	Rabbit	Fish
[α] _D in water <i>c</i> = 0.5 % <i>l</i> = 2 dm.	I (a)	+ 197.8°	+ 196.6°
	(b)	+ 196.9°	—
	II	+ 199.0°	+ 202.5°
	III	+ 198.5°	+ 200.0°
Reduction (Hagedorn-Jensen) (Glucose = 100)	I (a)	0.09	0.11
	(b)	0.09	—
	II	0.09	0.09
	III	0.10	0.12
Reduction (Macleod-Robison) (Glucose = 100)	I (a)	1.5	1.7
	(b)	1.6	—
	II	1.7	1.3
	III	1.2	2.0*
Inorganic P (%)	I (a)	Nil	Nil
	(b)	0.003	—
	II	0.001	0.002
	III	Nil	0.003
Organic P (%)	I (a)	0.009	0.027
	(b)	0.016	—
	II	0.004	0.005
	III	0.001	0.008

* High value due to nitrogenous impurity.

(*cf.* Somogyi [1934] who has recently prepared phosphorus-free glycogen). There are, therefore, no grounds for believing that phosphorus is an integral constituent of glycogen.

The regenerated glycogens. The only observed differences between the regenerated and parent substances were (a) a slightly increased rotatory power and (b) an appreciable diminution in the opacity of the solutions, indicating a smaller colloidal particle after regeneration. It is very probable that the enhanced rotation arises from this condition, particularly as the post-regeneration polarimetric observations were made with much less difficulty.

The most significant fact arising out of this work is that glycogen does not appear to undergo degradative changes on acylation by the method here employed using pyridine and acetic anhydride. This has an important bearing on the experiments of Haworth and Percival [1932] who postulate a "chain-length" of 12 glucose units for glycogen, basing their assumption on the amount of tetramethylglucose obtainable from methylated glycogen. One of us [Bell, 1932] and Irvine [1932] have criticised Haworth's analogous deductions in the cases of cellulose and starch respectively, when the initial acetylation was carried out in an acid system; indeed Irvine and Caldwell (unpublished work) find that the reducing power of starch (Hanes's method) increases steadily as a result of acylation, even under mild conditions, and that the amount of tetramethylglucose obtainable from methylated starch varies within wide limits, according to the method of acetylation adopted. In the present instance, however, we believe that our findings support Haworth's conclusions regarding glycogen.

The values of the reduction of alkaline hypiodite, which Haworth and Percival [1932] found for their specimen of glycogen are in close agreement with ours; we, also, are unable to correlate reducing power with chain-length, as will be shown in a further communication from this laboratory.

EXPERIMENTAL.

Glycogen triacetates. These were prepared according to the procedure of Haworth and Percival [1932]. $[\alpha]_D$ was determined in chloroform, $l=1$, $c=1$.

(a) *H₂O-extracted glycogen.* A 50% yield was the best obtained in two experiments. Much unchanged glycogen was recovered; this did not react with fresh acylating agents. The product showed $[\alpha]_D^{17^\circ} + 172.1^\circ$. (Found: OAc, 43.9%. $C_6H_7O_5(OAc)_3$ requires OAc, 44.7%.)

(b) *H₂O-extracted glycogen after treatment with KOH.* 2 g. of material were heated for 2 hours at 100° with 30% KOH solution, neutralised with acetic acid and the glycogen precipitated by the addition of 2 volumes of 95% alcohol. The precipitate was washed with alcohol and ether and introduced into the acetylating mixture in which it rapidly dissolved. Yield 3 g. (84.3%); $[\alpha]_D^{17^\circ} + 170.9^\circ$; OAc, 44.0%.

(c) *KOH-extracted glycogen.* From 2 g. lots, two experiments gave the following yields:

3.00 g. (84.5%); $[\alpha]_D^{17^\circ} + 170.9^\circ$; OAc, 44.0%.
3.01 g. (84.6%); $[\alpha]_D^{17^\circ} + 171.7^\circ$; OAc, 44.1%.

(d) *Fish glycogen (H₂O-extracted).* As this material proved totally resistant to acetylation it was treated as (b). 1.90 g. yielded 2.97 g. acetate (87.4%); $[\alpha]_D^{18^\circ} + 170.2^\circ$; OAc, 44.4%.

Glycogen tribenzoates. The procedure was similar to that employed by Irvine and Caldwell (unpublished work) for the benzylation of starch, and was as follows. 1 g. glycogen was dissolved in 30 ml. 10% NaOH and shaken with 11 g. (9.3 ml.) benzoyl chloride. A white solid which separated was filtered off, washed with 10% NaOH and dried by alcohol-benzene under reduced pressure. The dry material was dissolved in 10 ml. dry pyridine and 3.5 ml. benzoyl chloride were added with cooling. After standing for 24 hours excess of alcohol was added and the semi-solid mass triturated with dilute HCl and then dilute NaOH, washed with water, then alcohol, dissolved in a mixture of 3 parts chloroform and 1 part acetone and precipitated by adding alcohol. Finally, the solid was dissolved in chloroform, shaken with norite and filtered through a Seitz filter. From the pale yellow, opalescent solution by the addition of alcohol the tribenzoate was obtained as a creamy solid, soluble in the usual organic solvents except alcohol, petroleum and ether.

(e) *Fish glycogen.* Yield 84.7%; $[\alpha]_D^{19^\circ} + 115.0^\circ$ ($l=1$, $c=0.6$); $+112.8^\circ$ ($l=2$, $c=0.5$) in $CHCl_3$. (Found: COC_6H_5 , 66.35%. $C_6H_7O_5(COC_6H_5)_3$ requires COC_6H_5 , 66.45%.)

(f) *Rabbit glycogen.* Yield 80%; $[\alpha]_D^{21^\circ} + 113.4^\circ$ ($c=1.0$, $l=2$) in $CHCl_3$. (Found: COC_6H_5 , 65.0%.)

Regeneration of the carbohydrates. The acetates were de-acetylated by allowing them to stand for 24 hours at room temperature in contact with a 50% excess of 50% aqueous methyl alcoholic 3% KOH. After acidifying with acetic acid, 2 volumes of alcohol were added and the resulting precipitate was washed with 66% alcohol and reprecipitated twice from water by 2 volumes of alcohol. The final product was examined by the procedure of Bell and Young, and in addition the hypiodite reducing values were determined by the method of Macleod and Robison [1929], and phosphorus, inorganic and organic, by the methods of Lohmann [1928] and Lohmann and Jendrassik [1926].

Owing to their insolubility in alcohol, the benzoates were dissolved, in 10% concentration, in acetone and shaken at room temperature for one hour with two-fifths volume of 20% KOH. The reaction was completed by warming at 50°

for 10 min. The addition of 2 volumes alcohol rendered the gummy precipitate solid; this was reprecipitated three times from water (after acetic acid neutralisation) with 2 volumes alcohol.

The properties of the regenerated materials appeared little altered (Table II), except with regard to the opacity in solution. This was appreciably lessened in the case of material regenerated from the acetates and considerably so in that regenerated from the benzoates. The yields were theoretical.

SUMMARY.

Rabbit and fish (*Gadidae*) liver glycogens do not appear to undergo degradation when acetylated or benzoated by the methods employed. Examination of the regenerated glycogens with respect to reducing power (ferricyanide and hypiodite), rotatory power and rate of hydrolysis with acid revealed no significant differences from the same properties of the original materials.

The organic phosphorus contents were very small and were diminished to even lower values on regeneration.

We desire to acknowledge with gratitude the award of a Senior Studentship of the Exhibition of 1851, and a grant from the Royal Society to one of us (D. J. B.). We are also indebted to Sir J. C. Irvine for his continued interest.

REFERENCES.

- Bell (1932). *Biochem. J.* **26**, 590, 598.
— and Young (1934). *Biochem. J.* **28**, 882.
Haworth and Percival (1932). *J. Chem. Soc.* 2277.
Irvine (1932). *Chem. Ind.* **51**, 263.
Lohmann (1928). *Biochem. Z.* **194**, 306.
— and Jendrassik (1926). *Biochem. Z.* **178**, 419.
Macleod and Robison (1929). *Biochem. J.* **23**, 517.
Somogyi (1934). *J. Biol. Chem.* **104**, 245.