ment with ethereal hydrogen chloride. The fine, white needles (recrystallized from ethanol) melted at 239–240° with decomposition.

Anal. Calcd. for $C_{20}H_{19}ONCl_2$: C, 66.67; H, 5.32; N, 3.89; Cl, 19.68. Found: C, 66.97; H, 5.55; N, 4.35; Cl, 18.95.

dl-α- and *dl*-β-1-phenyl-1-*p*-tolyl-2-aminopropanol.—These compounds were prepared by the methods described by Tiffeneau, *et al.*,[6] and Behr.[11] The α-racemate melted after repeated recrystallization from ethanol at 76–77°, while Tiffeneau[6] reported a melting point of 65°. The compound gave the correct analysis for a hemihydrate.

Anal. Calcd. for $C_{16}H_{19}ON \cdot {}^1/_2H_2O$: C, 76.77; H, 8.05; N, 5.61. Found: C, 76.33; H, 7.87; N, 5.65.

The hemihydrate could be dehydrated by prolonged drying over phosphorus pentoxide at 56°. The resulting α-1-phenyl-1-*p*-tolyl-2-aminopropanol melted at 73–75° after repeated recrystallization from benzene or ligroin.

Anal. Calcd. for $C_{16}H_{19}ON$: C, 79.63; H, 7.94; N, 5.81. Found: C, 79.43; H, 7.95; N, 6.22.

Both the hemihydrate and the aminoalcohol yielded a hydrochloride, m.p. 252° with decomposition.[6]

Deamination of Diastereoisomeric Aminoalcohols (Table I).—The deamination of the racemic α- and β-1,2-diphenyl-1-*p*-chlorophenyl-2-aminoethanols and of the racemic α- and β-1-phenyl-1-*p*-tolyl-2-aminopropanols was carried out by a procedure described previously.[3] The deamination products obtained from the two diastereoisomeric aminopropanols were purified by chromatographic adsorption on activated alumina. The oils were dissolved in pentane and after deposition on the column eluted with redistilled benzene.

The *dl*-α-1,2-diphenyl-1-*p*-chlorophenyl-2-aminoethanol yielded 1.57 g. (60%) of *p*-chlorophenyl benzhydryl ketone, m.p. (recrystallized from ethanol) 108–109°.

Anal. Calcd. for $C_{20}H_{15}OCl$: C, 78.30; H, 4.93; Cl, 11.56. Found: C, 78.06; H, 4.73; Cl, 11.05.

(11) Behr, *Ber.*, **30**, 1521 (1897).

The β-racemate of this aminoalcohol yielded 1.43 g. (65%) of α-*p*-chlorophenyldesoxybenzoin, m.p. (recrystallized from ethanol) 102–103°. A mixed m.p. with *p*-chlorophenyl benzhydryl ketone (m.p. 108–109°) was 85–86°.

Anal. Calcd. for $C_{20}H_{15}OCl$: C, 78.30; H, 4.93; Cl, 11.56. Found: C, 78.29; H, 4.89; Cl, 11.77.

The deamination of the α-racemate of 1-phenyl-1-*p*-tolyl-2-aminopropanol afforded 1.139 g. (42%) of 4-methyl-α-phenylpropiophenone, m.p. (recrystallized from pentane) 44–45°.[6,12] A semicarbazone, m.p. 144–145°,[6,12] and an oxime, m.p. 123–124°,[12] were prepared from the ketone.

The *dl*-β-1-phenyl-1-*p*-tolyl-2-aminopropanol yielded upon deamination 0.820 g. (30%) of α-*p*-tolylpropiophenone, m.p. (recrystallized from pentane) 43–44°. A mixed melting point of this material with 4-methyl-α-phenylpropiophenone (m.p. 44–45°) was 2–5°.

Anal. Calcd. for $C_{16}H_{16}O$: C, 85.67; H, 7.19. Found: C, 85.75; H, 7.01.

An oxime of α-*p*-tolylpropiophenone, m.p. (recrystallized from ethanol) 153–155°, was prepared.

Anal. Calcd. for $C_{16}H_{17}ON$: C, 80.30; H, 7.16; N, 5.85. Found: C, 80.38; H, 7.14; N, 6.32.

A semicarbazone of this ketone could not be isolated.

Alkaline Cleavage of Deamination Products (Table III).—Cleavage of *p*-chlorophenyl benzhydryl ketone and the isomeric α-*p*-chlorophenyldesoxybenzoin was carried out by a procedure discussed elsewhere.[3] 4-Methyl-α-phenylpropiophenone and α-*p*-tolylpropiophenone were cleaved by heating with about fifty times the amount of 50% aqueous potassium hydroxide at 150–160° for a period of 45 hours in a copper test-tube. The hydrocarbon cleavage fragments were purified by deposition on activated alumina from a pentane solution and eluted with freshly distilled ligroin.

Structure of Diastereoisomeric Aminoalcohols (Table II).—The oxidative degradations were carried out by a procedure discussed previously.[3]

(12) Bruzeau, *Ann. chim.*, [11] **1**, 257 (1934).

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[CONTRIBUTION FROM THE DIVISION OF AGRICULTURAL BIOCHEMISTRY, UNIVERSITY OF MINNESOTA]

The Repeating Unit of Glycogen[1]

BY M. ABDEL-AKHER AND F. SMITH

Thirty-seven different samples of glycogen have been prepared. All were found to have a repeating unit containing approximately twelve anhydro-glucose residues. Glycogen with a repeating unit of 18 glucose residues was not encountered.

Investigations by several groups of workers into the methyl derivatives of specimens of glycogen obtained from a variety of sources have led to the conclusion that two types of glycogen occur in nature. The one most frequently obtained consists of repeating units containing about twelve glucose residues,[2a,2b,3,4] while the other, encountered much less frequently and apparently fortuitously, is said to contain approximately eighteen glucose residues in each repeating unit.[5–8]

Although it might not have been surprising to find that specimens of glycogen, obtained from the livers of animals fed a special carbohydrate diet such as D-galactose[7,8] would differ from the normal type, it seemed very curious, indeed, almost unbelievable, that this variation from the 12 to the 18 unit type should be encountered from time to time in commercial samples of liver glycogen prepared presumably from a number of normal rabbits.[5,8]

Since the evidence in favor of the 18 unit type of glycogen was derived from methylation studies which involved the isolation of the cleavage fragments of the methylated polysaccharide, it seemed conceivable that the high values were due to a loss of either the relatively volatile methyl-2,3,4,6-tetramethyl-D-glucoside or, less likely, to a loss of the corresponding tetramethyl sugar. This possibility, however, seemed to be ruled out by the fact that periodate oxidation also gave high values for the repeating unit when applied to the same samples of glycogen which had given a figure of 18 by the methylation technique.[8]

In a preliminary study of this apparent anomaly

(1) Presented in part before the Division of Sugar Chemistry and Technology at the Detroit Meeting of the American Chemical Society, April, 1950. Paper No. 2265, Scientific Journal Series, Minnesota Agricultural Experiment Station. This paper will form part of a thesis to be submitted by M. Abdel-Akher to the University of Minnesota in partial fulfillment for the degree of Ph.D.

(2) (a) Haworth and Percival, *J. Chem. Soc.*, 2277 (1932); (b) Bell, *Biochem. J.*, **29**, 2031 (1935); **31**, 1683 (1937).

(3) Haworth, Hirst and Smith, *J. Chem. Soc.*, 1914 (1939).

(4) Meyer and Fuld, *Helv. Chim. Acta*, **24**, 375 (1941).

(5) Haworth, Hirst and Isherwood, *J. Chem. Soc.*, 577 (1937).

(6) Bell, *Biochem. J.*, **30**, 1612, 2144 (1936).

(7) Bacon, Baldwin and Bell, *ibid.*, **38**, 198 (1944).

(8) Halsall, Hirst and Jones, *J. Chem. Soc.*, 1399 (1947).

TABLE I

AVERAGE NUMBER OF ANHYDRO GLUCOSE RESIDUES IN THE REPEATING UNIT OF GLYCOGEN

Source of glycogen	Wt., mg.	Formic acid, mg.	No. of units	$[\alpha]^{H_2O}D$
Rabbit liver (normal)	498.2	11.6	12	+195
Rabbit liver[a,i]	514.0	12.0	12	+190
Rabbit liver[a]	520.0	12.9	11	+198
Rabbit liver[a]	498.5	11.6	12	+185
Rabbit liver[a]	525.9	12.3	12	+194
Rabbit liver[a]	163.4	3.5	13	+190
Rabbit liver[b]	457.4	11.0	12	+195
Rabbit liver (animal fed alloxan)	284.5	6.6	12	+194
Rabbit liver (animal injected with alloxan)	536.0	12.8	12	+190
Wild rabbit liver	315.6	7.1	13	...
Horse liver (normal)	476.0	12.1	11	+190
Horse liver (animal fasted and fed D-galactose)[c]	563.0	14.7	11	+192
Guinea pig liver (normal)	536.5	15.0	10	+190
Guinea pig liver (animals fasted and fed D-galactose)[d]	354.4	9.3	11	+193
Ox liver (normal)[e]	463.5	10.4	13	+192
Ox liver (tissue kept 14 hours after death)	492.2	12.8	11	+190
Human liver	506.5	12.8	11	+195
Dog liver	521.0	12.3	12	+193
Rat liver	499.6	13.2	11	+193
Domestic fowl liver	545.0	11.0	14	+194
Dogfish (*Squalus sp.*) muscle	593.0	13.5	13	+195
Northern pike (*Esox estor*) liver[f]	435.0	10.3	12	+194
Bullhead (*Ameiurus melas*) liver	516.0	12.0	12	+190
Crappie (*Pomoxis annularis*) liver	520.0	12.4	12	+194
Carp (*Cyprinus carpio*) liver	480.2	11.5	12	+198
Bass (*Micropterus dolamieu*) liver	564.6	11.1	14	+191
Garfish (*Lepisosteus osseus*) liver	240.9	5.6	12	+195
Walleyed pike (*Stizostedion vitreum*) liver	475.2	10.5	13	+197
Frog (*Rana temporaria*) spawn	204.6	4.7	12	+190
Frog (*Rana catesbeana*) liver	506.7	11.1	13	+191
Mussels (*Mytilus edulis*)[i]	76.7	1.8	12	+192
Sheep tapeworms (*Moniezi expansa*)[g]	466.0	10.9	12	+194
Hog round worms (*Ascaris lumbricoides*)	418.4	9.7	12	+194
Polysaccharide from *Neisseria perflava*	129.0	3.3	11	+196
Drone larvae	553.6	13.7	12	+191
Commercial glycogen (Merck and Co.)	460.8	9.9	13	+190
Sweet corn (Mature Golden Bantam)[h] (phytoglycogen)	604.9	18.1	10	+195

[a] Samples 2 to 6 were obtained from full grown rabbits which had been fasted 48 hours and each fed an aqueous solution (15–20 cc.) containing D-galactose (10 g.). [b] Sample 7 was obtained from rabbits treated as in (*a*) except that they were fed D-glucose instead of D-galactose. [c] The horse was fasted 27 hours and fed by mouth a solution of D-galactose (605 g.) in water (1000 cc.) and killed 4.5 hours after feeding. [d] The guinea pigs were fasted for 24 hours and each injected interperitoneally with an aqueous solution of galactose (2 g.) and killed 3 hours after injection. The livers from similarly starved control guinea pigs contained no glycogen. [e,f,g] These samples gave values of 12, 13 and 12, respectively, for the repeating unit by the potassium periodate method.[8] [h] *Cf.* Hassid and McCready, THIS JOURNAL, **63**, 1632 (1941); Meyer and Fuld, *Helv. Chim. Acta*, **32**, 757 (1949). [i] A value of 12 was obtained for the repeating unit of specimens 2 and 31 by methylation studies.[10]

glycogen was prepared from the livers of rabbits which had been fasted and then fed D-galactose[6,7]; glycogen was also extracted from edible mussels (*Mytilus edulis*).[9] Both these sources had been reported to provide the 18 unit type of glycogen. Experiments carried out prior to the war using the methylation technique showed that these two specimens possessed a chain length of 12 and not of 18.[10]

More recently, we have resumed and extended this work on the length of the repeating unit to a larger variety of glycogen samples and in order to facilitate the search for the 18 unit type of glycogen a periodate oxidation procedure was developed to determine the average length of the repeating unit on a semimicro scale. Based on the previous findings of others,[8,11–14] the method, which is applicable to all those polysaccharides tried so far and especially to those soluble in water, consisted of treating the glycogen with an excess of 0.1 *N* sodium metaperiodate at 5–6°. The combination of a low concentration of periodate and a low temperature avoided over-oxidation even though the reaction mixture was kept for many days after acid production had become constant.[13,15,16,17] The oxidation of glycogen proceeded smoothly without shaking[8] and the maximum yield of formic acid was produced in 80 to 90 hours. At 0° the liberation of acid was completed in 160 to 180 hours.

In some experiments the formic acid was titrated with dilute barium hydroxide[8] or sodium hydroxide, in others an iodometric method was used.[18] The latter was preferred since the end-point is definite and the troublesome baryta is avoided.[15,19]

Thirty-seven different samples of glycogen have been tested so far. The results confirmed the figure of approximately 12 obtained by the methylation technique for the repeating unit of the four samples prepared by methods previously reported[6,7] to give the 18 unit type of glycogen. Moreover, a specimen of liver glycogen isolated from a horse which had been fasted and fed D-galactose *per os* and another from the livers of guinea pigs similarly fasted and fed the same sugar interperitoneally both gave values of approximately 12 and not 18 for the repeating unit. The other thirty-one samples of glycogen were also found to have a repeating unit containing about 12 glucose residues (see Table I).

(9) Bell, *Biochem. J.*, **30**, 2144 (1936).

(10) Jackson, James and Smith, to be published.

(11) Malaprade, *Bull. soc. chim.*, [4] **43**, 683 (1928); *Compt. rend.*, **186**, 382 (1928).

(12) Barry, *J. Chem. Soc.*, **578** (1942); *Nature*, **152**, 537 (1943).

(13) Potter and Hassid, THIS JOURNAL, **70**, 3488 (1948).

(14) Dillon, *Nature*, **155**, 546 (1945).

(15) Halsall, Hirst and Jones, *J. Chem. Soc.*, 1427 (1947).

(16) Huebner, Lohmar, Dimler, Moore and Link, *J. Biol. Chem.*, **159**, 503 (1945).

(17) Sprinson and Chargaff, *ibid.*, **164**, 433 (1946).

(18) Kolthoff and Sandell, "Textbook of Quantitative Inorganic Analysis," The Macmillan Company, New York, N. Y., 1943, p. 616.

(19) Jeanes and Wilham, THIS JOURNAL, **72**, 2655 (1950).

We found quite often that after specimens of glycogen, prepared from liver tissue by the method of Bell and Young,[20,21] had been dried *in vacuo* they dissolved slowly in water to give pronounced opalescent solutions. In a few cases, some of the material failed to dissolve. Some of these were found to contain protein impurity,[22] and they invariably gave high values, some even as high as 24, for the repeating unit. However, purification of such samples by retreatment with aqueous trichloroacetic acid followed by alcoholic precipitation has always given the 12 unit variety of glycogen.

It is not yet possible to say whether all the samples of glycogen listed in Table I are structurally identical or whether each sample is homogeneous,[23a,b] but it is hoped that further work now in progress will indicate whether any significance is to be attached to a variation of 2 units in the repeating unit.

Of all the tissues examined in this work none yielded more than a small fraction of its total glycogen upon extraction with cold water although the so-called pure glycogen is freely soluble in cold water. This phenomenon, which is, perhaps, not unexpected since glycogen is the reserve animal carbohydrate, would appear to favor the view put forward by Willstätter and Rohdewald[24] that in the living tissue the polysaccharide is combined with protein.[23,25]

Experimental

Isolation of Glycogen.—The procedure, similar to that of Bell and Young,[20] was as follows: The fresh tissue (10 g.) was boiled for 30 minutes with water (50 ml.) and then disintegrated. After 2 hours boiling, the liquid was decanted and the residue re-extracted with more boiling water (50 ml.) for 1 hour. This extraction process was repeated until the extract showed no opalescence. The extracts were filtered, combined and evaporated to 25 ml. in an open metal dish. The concentrate was treated with trichloroacetic acid (1.25 g.) (added slowly with stirring) and the solution kept for 12 hours at 5°. The protein precipitate was removed and the crude glycogen precipitated by ethanol (three volumes). The glycogen was purified by two reprecipitations from aqueous solution with ethanol. Any small amount of material found to be insoluble in cold water during the purification steps was carefully eliminated. Unless this was done, the samples of glycogen contained protein; they gave high values for the repeating unit by the periodate methods and furthermore, the solutions were highly opalescent and showed a low specific rotation.

It was found advisable to treat all the crude glycogen extracts with trichloroacetic acid whether the protein impurity was large, as in the case of animal livers, or small as in the case of fresh water fish livers, the helminths and honey bee larvae.

Fresh water fish livers are an excellent source of glycogen. For example, fresh bullhead liver contained 12.5% of glycogen.

Determination of the Average Length of the Repeating Unit.—Glycogen (100–500 mg.), dried *in vacuo* at 100°, was weighed and dissolved in water (180 ml. approx.). Sodium metaperiodate (50 ml., 0.5 *N*) was then added and the volume adjusted to 250 ml. by the addition of water. The reaction mixture was quickly cooled to 5–6° and kept at this temperature in the dark. A blank experiment was carried out at the same time under the same conditions. Aliquots were removed at suitable intervals (the first was taken after 2–3 days) from the reaction mixture and from the blank. The formic acid was determined in aliquots, measured at room temperature and freed from periodate with ethylene glycol, either by titration with 0.1 *N* barium hydroxide or 0.01 *N* sodium hydroxide using methyl red as the indicator[8] or preferably by an iodometric method,[21] as follows: To an aliquot (20 ml.) ethylene glycol (0.5 ml.) was added, followed after 10 minutes by excess potassium iodide (it is unnecessary to add iodate since this is produced during the oxidation). An excess of 0.01 *N* sodium thiosulfate (10 ml.) was then added and the excess back-titrated with 0.01 *N* iodine using starch as the indicator. A similar titration was also carried out on the blank solution (20 ml.) and its acidity, in terms of 0.01 *N* thiosulfate subtracted from that shown by the reaction mixture. At suitable intervals (12 hours) the titrations were repeated until three consecutive results agreed. At 5–6° the reaction was complete in about 80 hours (at 0° it required 180 hours). The number of anhydroglucose units required to furnish one mole of formic acid was then calculated. The results are given in Table I. The results were identical with those obtained by the periodate method of Halsall, Hirst and Jones.[8] (see footnotes *e*, *f*, *g*) which, however, necessitates shaking and requires 150 hours for completion.[26]

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(20) Bell and Young, *Biochem. J.*, **28**, 882 (1934).

(21) Meyer, *Advances in Enzymology*, **3**, 109 (1943).

(22) *Cf.* Kent and Stacey, *Biochem. et Biophys. Acta*, **3**, 641 (1949).

(23) (a) Kavalskiĭ, *Acad. Sci. U. S. S. R. Biokhimiya*, **13**, 131 (1948); *C. A.*, **42**, 7817 (1948); (b) Bloom, Schumpert and Lewis, *Federation Proc.*, **9**, 152 (1950).

(24) Willstätter and Rohdewald, *Z. physiol. Chem.*, **225**, 103 (1934).

(25) Wajzer, *Bull. chim. biol.*, **21**, 1243 (1939).

(26) This modified periodate method of oxidation gave a value of 20 for the repeating unit of a sample of defatted waxy corn starch.