Table 7. *Recovery of arsenate from urine*

(Extraction procedure on aqueous dialysates)

Total arsenic ($\mu\text{g. As}$)	Arsenite found ($\mu\text{g. As}$)	Arsenate found ($\mu\text{g. As}$)
41.0	Nil	40.9
	Nil	38.7
	0.6	39.7
42.2	0.9	40.3
	Nil	41.9
	Nil	40.5

portions were used for xanthate extraction, and two 20 ml. samples for total arsenic determination (Table 7).

Separation of arsenite from arsenate in urine. Known amounts of arsenite and arsenate were added to rabbit urine and duplicate 5 ml. samples dialyzed against 60 ml. of distilled water in the ice-box for 46 hr. Table 8 gives the arsenite and arsenate values obtained by extracting the individual dialysates. For calculation of these results the small amount of arsenic remaining in the sacs—less than 10% of the total—was neglected (Table 8).

Table 8. *Separation of arsenite from arsenate in urine*

(Extraction procedure on aqueous dialysates)

Arsenite			Arsenate		
Added ($\mu\text{g. As}$)	Found ($\mu\text{g. As}$)	Recovery (%)	Added ($\mu\text{g. As}$)	Found ($\mu\text{g. As}$)	Recovery (%)
20.0	19.2	96	20.1	22.4	112
20.0	22.3	112	20.1	20.5	102

DISCUSSION

By the method described above, it is possible to separate added inorganic arsenite from arsenate in simple solution, in blood haemolysates, and in urine with a reasonable degree of accuracy. They may be estimated in the separated fractions by any suitable arsenic method, the error involved in the xanthate extraction being probably less than that associated with the subsequent determination of arsenic. The method appears to be independent of the presence of those substances which would interfere with titrimetric, colorimetric or polarographic techniques.

When the method is applied to biological fluids, it must always be borne in mind that arsenic-containing compounds other than arsenite and arsenate may be present. Any such compound soluble in carbon tetrachloride would appear in the arsenite fraction, irrespective of the valency of the arsenic. This difficulty may be overcome by carrying out preliminary extractions with carbon tetrachloride before extracting with the reagent.

When studying problems of metabolism by this method, it is necessary to assume that any arsenic compound which is water-soluble and dialyzable, or non-precipitable by trichloroacetic acid, and which is not extractable from acid solution by carbon tetrachloride, is either inorganic arsenite or arsenate. While this assumption is probably valid in studies with inorganic compounds of arsenic, it is obviously not justifiable in the case of administration of organic arsenicals.

SUMMARY

1. A method is described for the quantitative separation of inorganic arsenite from arsenate in simple solution, in blood and in urine.

2. The arsenite is separated from arsenate by extraction with a carbon tetrachloride solution of sodium ethyl xanthate. Arsenate may be present in large excess.

3. The limitations of the method are discussed.

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Glycogen

9. THE MAGNITUDE OF THE 'UNIT CHAINS' OF LIVER GLYCOGEN OF RABBITS SUPPLIED WITH GLUCOSE, FRUCTOSE AND SUCROSE

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End-group assay of glycogens from different sources has revealed that the 'unit chains' may be composed of either 12 or 18 glucose radicals. In the case of

rabbit liver glycogen 18-radical specimens have been described by Bell (1936) after administration of galactose by mouth, and by Haworth, Hirst &

Isherwood (1937) for samples of commercial origin. The present paper describes the end-group assay (Haworth & Machemer, 1932; Hirst & Young, 1938) of glycogens isolated from livers of previously fasted rabbits subsequent to the administration of massive doses of glucose, fructose and sucrose. Fructose was given both by stomach-tube and by continuous intravenous injection; glucose was intravenously injected and sucrose given by stomach-tube alone. Investigation of a specimen of glycogen isolated from rabbit livers following continuous injection of glucose has already been reported (Bell, 1935, 1936); in this case a unit chain-length of 12 was reported.

RESULTS

The results of our experiments, carried out on groups of animals, were clear-cut. Glycogen with 12 radicals was formed after the feeding of both glucose and fructose, and after the injection of fructose. Sucrose feeding, on the other hand, resulted in the formation of glycogen having a unit chain composed of 18 glucose radicals. This result should be contrasted with previous findings (Bell, 1935, 1936) on animals allowed to feed naturally on a diet of carrots augmented with sucrose. It seems possible in the light of our results that the 18-radical glycogen of commercial origin described by Haworth *et al.* (1937) may have been isolated from rabbits fasted and then fed massive doses of sucrose, this being a well-known method of stocking the liver with glycogen.

To summarize results up to the present, rabbit liver glycogen having 18 radicals composing the unit chain has now been found when both galactose and sucrose are fed to fasted animals. Feeding or intravenous injection of glucose and fructose, likewise to fasted animals, leads to formation of 12-radical unit chains. Feeding rabbits on a diet rich in sucrose therefore appears to have an effect different from that obtained when sucrose is given in concentrated solution by stomach-tube to fasted animals.

Interpretation of our results does not seem possible at the moment in view of our lack of knowledge of the mechanisms that underlie the formation of glycogen in the liver from substances other than glucose. Even with glucose our knowledge is incomplete, since the action of liver phosphorylase appears to produce a long, unbranched chain of glucose radicals analogous to amylose, whereas natural glycogen is composed of much shorter chains, aggregated in the manner Haworth and his colleagues have suggested and termed the 'laminated structure'.

During the course of this and similar investigations in this laboratory it became apparent that no rapid method was available for evaluating the properties of 2:3:4:6-tetra-methyl- and 2:3:6-trimethyl-methylglucosides present in the mixed fractions encountered during end-group assays.

One of us (J.S.D.B.) has devised a graphical method of analysis based on the following arguments:

Hirst & Young (1938) showed that, if the refractive indices of mixtures of the α - and β -isomers of certain methylated glucosides are plotted against their optical rotations, the straight lines shown in Fig. 1 are obtained.

Fig. 1. Relationships between $[\alpha]_D$ (water) and n_D^{16} for mixtures of α - and β -methylglucosides of 2:3:6-tri- and 2:3:4:6-tetra-methylglucosides. For explanation see text.

AB represents all possible mixtures of 2:3:4:6-tetramethyl- α -methylglucoside with its β -isomer: *CD* represents the values for the two 2:3:6-trimethyl-methylglucosides. It will be seen that the two lines are almost parallel, and that both are finite. If it be assumed that a fraction obtained by distillation of the mixed glucosides (produced by hydrolysis of methylated glycogen, and believed to contain nothing but the four substances represented by the points *A*, *B*, *C* and *D*) has the properties represented by the point *P*, it is possible to make certain deductions as to its probable composition.

If any straight line *EPF* be drawn through *P*, then the fraction *P* can be expressed as a mixture of the two α - β -mixtures represented by *E* and *F*. In order to determine exactly the composition of a given fraction, the line *EPF* must be drawn in the right place.

It can be seen, however, that since *AB* and *CD* are almost parallel and since they are also finite, the limits of variation of the ratio *EP/PF* are narrow. If *AB* and *CD* were quite parallel the ratio would be constant. But *EP/PF* represents the ratio of tri- to tetra-methylglucosides in the fraction *P*, and is all that is required to be known. Consequently, if the variation possible in this ratio is sufficiently small, a suitably approximate answer to the problem of the composition can be obtained by graphical means.

The validity of this argument has been confirmed by algebraic methods.

EXPERIMENTAL

Preparation of the glycogens

(a) The sugars injected were administered by the method of Bridge & Noltie (1935). A 25% solution of glucose or fructose in normal saline was injected into the ear veins of

Table 1. *Summary of feeding and injection experiments*

No. of animals in group	Body-weight (kg.)		Sugar administered				Weight of liver tissue		Liver glycogen isolated	
	Total	Average	Injected	Fed	(g.)	(g./kg.)	(g.)	(g./kg.)	(g.)	(g./kg.)
5	8.5	1.7	Glucose	—	156	18.3	415	4.88	26	6.25
8	16.0	2.0	Fructose	—	294	18.3	505	3.16	22	4.35
4	6.0	1.5	—	Fructose	130	21.6	325	5.4	21	6.46
4	5.6	1.4	—	Sucrose	130	23.2	280	5.0	20	7.15

Table 2. *Properties of the glycogen specimens and of their derivatives*

Origin of specimen	$[\alpha]_D$ in water	Triacetyl glycogen		Trimethyl glycogen		
		$[\alpha]_D$ in chloroform	Acetyl (%)	No. of treatments	$[\alpha]_D$ in chloroform	Methoxyl (%)
Glucose injection	+195°	+169°	45	20	+208°	45.2
Fructose injection	+199°	+167°	44	8	+210°	45.2
Fructose feeding	+193°	+167°	44	12	+202°	45.3
Sucrose feeding	+197°	+163°	47	8	+200°	43.4

Table 3. *End-group assay on the specimens of glycogen*

Fraction	Weight (g.)	n_D^{16}	$[\alpha]_D$ in water	Methoxyl (%)	Methylglucosides					
					Tetramethyl		Trimethyl		Dimethyl	
					(%)	(g.)	(%)	(g.)	(%)	(g.)
A. Product from glucose-injected animals. 24.0 g. of methylated glycogen assayed										
I	1.364	1.4428	+32.6°	60.5	(100)	1.364	—	—	—	—
II	0.353	1.4439	72.0	60.0	(100)	0.353	—	—	—	—
III	3.668	1.4557	17.0	52.5	(3)	0.11	(97)	3.558	—	—
IV	10.844	1.4580	49.0	52.5	—	—	(100)	10.844	—	—
V	3.976	1.4586	63.1	52.5	—	—	(100)	3.976	—	—
VI	0.797	1.4597	—	51.2	—	—	(91)	0.725	(9)	0.072
VII	0.473	—	—	44.2	—	—	(30)	0.142	(70)	0.331
VIII	0.550	1.4720	—	41.8	—	—	—	—	(100)	0.550

Residue (essentially derived from 2:3:6-trimethyl glucose), 0.40 g. Total pentamethyl glucose amounted to 1.872 g. With the addition of the usual 10% correction for experimental losses, a chain-length of 12 radicals is indicated.

B. Product from fructose-injected animals. 17.5 g. of methylated glycogen assayed										
I	0.641	1.4446	+76.2°	61.8	(95.6)	0.612	(4.4)	0.029	—	—
II	0.533	1.4461	95.5	60.6	(89.1)	0.474	(10.9)	0.059	—	—
III	2.844	1.4569	52.3	52.4	(7.0)	0.199	(93)	2.645	—	—
IV	5.006	1.4601	75.6	51.4	—	—	(100)	5.006	—	—
V	2.248	1.4601	122.0	50.2	—	—	(79)	1.776	(21)	0.472
VI	4.139	1.4737	—	42.0	—	—	(10)	0.414	(90)	3.725

Residue (see A above), 0.92 g. Total pentamethyl glucose amounted to 1.285 g. With the usual 10% correction, the chain-length of this material is 11–12 radicals.

C. Product from fructose-fed animals. 6.01 g. of methylated glycogen assayed										
I	0.512	1.4463	+52.0°	59.0	(76)	0.389	(24)	0.122	—	—
II	0.685	1.4572	42.9	52.2	(4)	0.072	(96)	0.658	—	—
III	1.008	1.4586	63.8	51.5	—	—	(100)	1.008	—	—
IV	1.671	—	75.5	51.6	—	—	(100)	1.671	—	—
V	1.200	—	—	44.1	—	—	(30)	0.36	(70)	0.84

Residue, 0.4 g. Total pentamethyl glucose amounted to 0.4 g. With the 10% correction the chain-length of this material is 11–12 radicals.

D. Product from sucrose-fed animals. 26.0 g. of methylated glycogen assayed										
I	0.912	1.4435	+33.6°	61.1	(100)	0.912	—	—	—	—
II	2.833	1.4543	15.6	53.0	(14)	0.400	(86)	2.433	—	—
III	2.660	1.4570	27.7	51.8	—	—	(100)	2.660	—	—
IV	10.000	1.4586	63.4	52.0	—	—	(100)	10.000	—	—
V	2.902	1.4630	—	—	—	—	—	Not analyzed	—	—
VI	4.400	1.4700	—	—	—	—	—	Not analyzed	—	—
VII	0.76	1.4758	—	40.2	—	—	—	—	(100)	0.76

Residue, 0.4 g. Total pentamethyl glucose amounted to 1.312 g. With the 10% correction the chain-length of this material is 18–19 radicals.

normal rabbits, previously fasted for 36 hr., at an average rate of 4.6 g. of sugar/kg. body weight/hr. for a period of 4 hr. The animals were then anaesthetized by injection of urethane into the ear vein, the livers removed and the glycogen isolated by the Pflüger technique.

(b) Rabbits, approximately 1.5 kg. in weight, were fasted for 36 hr., and 130 ml. of a 25% solution of fructose or sucrose were administered by stomach-tube. After 4 hr. the liver glycogen was isolated as for the injected animals.

(c) Purification of the samples was effected by the method of Bell & Young (1934). Data relating to the preparation of the specimens of glycogen obtained are summarized in Table 1. It will be seen that fructose administered by injection appears to form less glycogen than fructose given by mouth.

End-group assay

The specimens were acetylated and then subjected to simultaneous deacetylation and methylation, followed by fractional distillation of the products of methanolysis as described in earlier parts of this series. The results are given in Tables 2 and 3.

SUMMARY

1. Three sugars were administered to fasting rabbits and the unit chain-length of the liver glycogen was determined.

2. Glycogen with a chain-length of 12 glucose radicals was produced by feeding fructose and by the continuous intravenous injection of fructose or of glucose.

3. Glycogen with a chain-length of 18 radicals was produced by feeding sucrose.

4. Fructose administered by injection appears to form less glycogen than when given by mouth.

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