for guinea-pigs under the conditions described. This was attempted, using the figures obtained in all the various assays performed, but the resulting values for the slope of the best straight line (*b*) were so divergent as to render further calculation useless. It is perhaps surprising, in these circumstances, that the method should give results which are apparently more consistent with other properties of the preparations used. We have been unable to arrive at any satisfactory explanation of the difference between the tests, and can only offer a tentative suggestion that, for the biological assay of D.F., the guinea-pig is a more satisfactory animal than the rabbit.

SUMMARY

A method is described for assaying the activity of diffusing-factor preparations in the skin of guinea-pigs, whereby twofold differences in activity can be distinguished. This method, which is based on the determination of the least amount required to produce a 20% increase in bleb area, gives reasonable agreement with assays of the hyaluronidase content of the same preparations, when this is estimated by the reduction of the viscosity of hyaluronic acid.

I wish to acknowledge the assistance of Mr C. W. Hale who suggested the investigation of this method of test and collaborated throughout.

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A Method for the Estimation of Glucose-1-phosphate and Galactose-1-phosphate in the Liver

By H. W. KOSTERLITZ AND CAROLINE M. RITCHIE (Carnegie Research Scholar),
 From the Physiology Department, Marischal College, University of Aberdeen

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The estimation of glucose-1-phosphate and galactose-1-phosphate in solutions containing only small quantities of inorganic phosphate and reducing substances does not offer any particular difficulties. Both esters are readily hydrolysed by acid and the liberated inorganic P and hexose can easily be determined [Cori & Cori, 1937; Kosterlitz, 1938]. In the liver, however, relatively large quantities of inorganic P (about 30 mg. P/100 g. liver) and considerable quantities of reducing substances (corresponding to 300 mg. glucose/100 g. liver) make it difficult to determine such small quantities of hexose-1-phosphates as may be expected to be present. The acid-soluble organic P fraction obtained from rabbits' livers during galactose assimilation contained only 5–12 mg. galactose or 0.9–2 mg. P/100 g. liver as galactose-1-phosphate and less than 1 mg. glucose or 0.17 mg. P/100 g. liver as glucose-1-phosphate [Kosterlitz, 1939b]. Furthermore, the glycogen present in the liver yields glucose on acid hydrolysis and also interferes with the fractionation of the soluble and insoluble Ba salts. For these reasons, a preliminary step in the estimation of the 1-esters in trichloroacetic acid extracts of liver is

the removal of the interfering substances, viz. inorganic P, reducing substances, and glycogen.

A description of the method as it has been used for the determination of the hexose-1-phosphates present in the livers of rats fed with glucose or galactose will be followed by a critical examination of the various steps involved in the determination.

METHODS

The excised liver is rapidly washed free from adherent blood, dried between filter paper, and cut into 4–5 pieces which are immediately immersed in liquid oxygen. When frozen, 5 g. or more are weighed and transferred to an ice-cold mortar containing 20 ml. 5% ice-cold CCl_3COOH and some acid-washed sand. Throughout the procedure, whenever the reaction is acid, all glass vessels and reagents are kept as near 0° as possible. After the liver has been thoroughly ground, additional 5% CCl_3COOH is added in quantity such that each 10 ml. of the final extract represent 1 g. of wet liver. In these experiments the water content of the liver has been assumed to be 80% of the liver weight. Therefore, a total of 9.2 ml. 5% CCl_3COOH /1 g.

liver, has been added. The liver and the acid are once more well mixed and allowed to stand for 3–5 min. Then, the pulped tissue and sand are centrifuged down at 3000 r.p.m. for 3 min. To as large a measured sample of the clear supernatant fluid as possible are added one drop of 0.5% alcoholic thymolphthalein solution, a measured quantity of 40% NaOH, and finally of *N*/2 NaOH, drop by drop, until the solution just turns blue. Then for each ml. of the original $\text{CCl}_3\text{.COOH}$ extract, 0.1 ml. of freshly prepared and filtered 10% mercuric acetate solution is added. After 20 min. standing at 0°, the Hg precipitate is centrifuged off. If the supernatant fluid is not absolutely clear, it is filtered through an acid-washed paper (Whatman no. 541). The excess of mercuric acetate is removed from a measured sample by means of H_2S . The HgS , which has adsorbed the glycogen, is filtered off and washed 4 times with 2 ml. H_2O . The combined filtrate and washings are aerated for 20 min. One drop of 0.5% thymolphthalein solution is added and then finely powdered Ba(OH)_2 until the solution just turns blue (about 0.32 g.). The pH is adjusted, if necessary, with a drop of 5% $\text{CCl}_3\text{.COOH}$ or *N*/2 NaOH. 3 vol. redistilled ethanol are added, and the flask stoppered and allowed to stand at 0° for 2 hr. The precipitate is then quantitatively collected in a 50 ml. cup by repeated centrifuging and the flask finally washed out 4 times with 5–10 ml. of 75% ethanol. The precipitate in the centrifuge tube is dried for 15 min. *in vacuo* over CaCl_2 , and after addition of 2.5 ml. H_2O is well broken up with a fine glass rod; the rod is washed with 0.5 ml. H_2O , and the tube centrifuged for 3 min. at 3000 r.p.m. The supernatant fluid is transferred to another 50 ml. centrifuge tube. This extraction is repeated twice, the supernatant fluids being added to the fluid from the first extraction. 3 vol. ethanol (27 ml.) are then added to the combined extracts, and the centrifuge tube is stoppered and allowed to stand overnight at 0°. After centrifuging, the precipitate is dried *in vacuo* over CaCl_2 for 40 min. The precipitate is then dissolved in 5.0 ml. 0.1 *N* HCl; 5.0 ml. 0.4 *N* H_2SO_4 are added and the BaSO_4 centrifuged off. The supernatant fluid is transferred to a Pyrex test tube. Inorganic P is estimated in 1 ml. and the reducing power before hydrolysis in 2 ml. 3.75 ml. are transferred to another Pyrex tube, which is stoppered, and hydrolysed at 50° for 30 min. The remainder of the fluid in the original tube, which is fitted with an air condenser, is hydrolysed at 100° for 3 min. Of the hydrolysed solutions 2 ml. were used to estimate increase in reducing power, as previously described [Kosterlitz, 1939*a*], and 1 ml. to estimate increase in inorganic P by a modified Fiske & Subbarow [1925] procedure. The volume of the reaction mixture was 1.17 ml. with P contents from 1 to 12 μg .

Examples:

(1) Liver from a rat fasted for 24 hr.

Liver weight:	7.36 g.
Sample of $\text{CCl}_3\text{.COOH}$ extract	63.0 ml.
NaOH	1.52 ml.
10% mercuric acetate	6.3 ml.
Total volume	70.82 ml.

Sample used after centrifuging = 65.0 ml. = 5.79 g. liver. The final precipitate of the organic P was dissolved in 10 ml. 0.25 *N* acid.

1 ml. ($\equiv$ 0.579 g. liver) contained:

Before hydrol.	2.01 μg . inorg. P
After 3 min. hydrol. at 100°	2.70 μg . inorg. P, i.e. an increase of 0.69 μg .
After 30 min. hydrol. at 50°	2.22 μg . inorg. P, i.e. an increase of 0.21 μg .
The increases in P calculated/100 g. liver were	0.119 mg.
and	0.036 mg. (= 30.3%) respectively

The reducing power, calculated as glucose, of 2 ml. ($\equiv$ 1.158 g. liver) was:

Before hydrol.	41.5 μg .
After 3 min. hydrol. at 100°	58.0 μg ., i.e. an increase of 16.5 μg .
After 30 min. hydrol. at 50°	47.5 μg ., i.e. an increase of 6 μg .

The increases in reducing power calculated as glucose/100 g. liver were 1.43 and 0.52 mg. (= 36.4%) respectively. The reducing power before hydrolysis ('initial reduction')/100 g. liver was equivalent to 3.59 mg. 'glucose'.

(2) Liver from a rat fed 1.5 ml. 60% galactose solution/100 g. body weight after a 24 hr. fast, and killed 2 hr. afterwards.

Liver weight:	5.40 g.
Sample of $\text{CCl}_3\text{.COOH}$ extract	45.0 ml.
NaOH	1.15 ml.
10% mercuric acetate	4.5 ml.
Total volume	50.65 ml.

Sample used after centrifuging = 45 ml. = 4.00 g. liver. The final precipitate of the organic phosphates was dissolved in 10 ml. 0.25 *N* acid.

1 ml. ($\equiv$ 0.400 g. liver) contained:

Before hydrol.	2.70 μg . inorg. P
After 3 min. hydrol. at 100°	5.36 μg . inorg. P, i.e. an increase of 2.66 μg .
After 30 min. hydrol. at 50°	4.57 μg . inorg. P, i.e. an increase of 1.87 μg .
The increases in P calculated/100 g. liver were	0.665 mg.
and	0.463 mg. (= 70.3%) respectively

The reducing power, calculated as glucose, of 2 ml. ($\equiv$ 0.800 g. liver) was:

Before hydrol.	90.5 μg .
After 3 min. hydrol. at 100°	125.5 μg ., i.e. an increase of 35 μg .
After 30 min. hydrol. at 50°	114.0 μg ., i.e. an increase of 23.5 μg .

The increases in reducing power calculated as glucose/100 g. liver were 4.38 and 2.94 mg. (= 67.2%) respectively. The initial reduction was 11.3 mg. 'glucose'/100 g. liver.

Certain corrections have to be introduced: for these see the recovery experiments.

The significance of the various values obtained will be discussed later: here the following points may suffice.

The 'initial reduction' is an approximate measure of the quantity of equilibrium ester (hexose-6-phosphate) present. Both glucose-1-phosphate and galactose-1-phosphate are completely hydrolysed by a mixture of 0.05N HCl and 0.2N H₂SO₄ at 100° in 3 min., while hydrolysis at 50° for 30 min. splits 28.6% of the glucose-1-phosphate present and 77.5% of the galactose-1-phosphate present.

EXAMINATION OF THE METHOD

Acid hydrolysis of glucose-1-phosphate and of galactose-1-phosphate

I. At 100°.

(1) Glucose-1-phosphate, prepared according to Hanes [1940] is hydrolysed:

(a) in 0.1N H₂SO₄ at 100° to the following extents: after 2 min. 81.8%, after 3 min. 94.7%, after 5 min. 99.3%;

(b) in a mixture of 0.05N HCl and 0.2N H₂SO₄ at 100°: after 2 min. 98.5%, after 3 min. 100%.

(2) Galactose-1-phosphate is completely hydrolysed after 2 min. in 0.09N H₂SO₄ at 100° [Kosterlitz, 1939a].

For the estimation of the sum of the two esters, hydrolysis in a mixture of 0.05N HCl and 0.2N H₂SO₄ at 100° for 3 min. has been adopted. The HCl is necessary to ensure solution of the Ba salts before the Ba is precipitated with H₂SO₄.

Table 1. *Hydrolysis at 50°*

(1) Hydrolysis of glucose-1-phosphoric acid.
Concentration of ester 0.75 m.-molar

Time min.	Glucose formed %	$k \times 10^3$	Inorg. P formed %	$k \times 10^3$
15	15.6	4.91	15.5	4.88
30	29.0	5.01	28.9	5.00
45	40.1	4.92	40.6	5.21
60	49.3	4.83	48.6	4.19
	Average	4.92	—	4.82

Percentage of hydrolysis after 30 min., calculated for $k = 4.87 \times 10^{-3}$: 28.55%.

(2) Hydrolysis of galactose-1-phosphoric acid.
Concentration of ester 0.965 m.-molar

Time min.	Galactose formed %	$k \times 10^3$	Inorg. P formed %	$k \times 10^3$
15	51.2	20.8	49.8	20.0
30	78.2	23.3	76.8	22.3
45	89.4	20.9	89.2	22.1
	Average	21.7	—	21.5

Percentage of hydrolysis after 30 min., calculated for $k = 21.6 \times 10^{-3}$: 77.5%.

II. At 50°.

The acid used consists of a mixture of 0.2N H₂SO₄ and 0.05N HCl (Table 1).

After 30 min. the ratio hydrolysed galactose-1-phosphoric acid/hydrolysed glucose-1-phosphoric acid is 2.72/1. If the values for P and for reducing power for the total hydrolysis (3 min. at 100°) and for the partial hydrolysis (30 min. at 50°) are known, the quantities of the two esters can be calculated (Table 2).

Table 2. *Ratios of hydrolysis percentages at 50° and 100° of mixture of glucose-1-phosphate and galactose-1-phosphate*

Mixture of		Ester hyd. at 50° Ester hyd. at 100°	50° value 100° value calculated from uncorrected P values	50° value 100° value calculated from reducing power expressed as 'glucose'
Glucose-1-phosphate %	Galactose-1-phosphate %			
100	0	0.286	0.286	0.286
80	20	0.384	0.378	0.364
60	40	0.482	0.472	0.451
40	60	0.579	0.568	0.547
20	80	0.677	0.665	0.654
0	100	0.775	0.765	0.775

Note. The values in column 4 are necessitated by the fact that 4.5% of the galactose-1-phosphate present is hydrolysed during the process of analysis (see note to Table 3), and the values in column 5 by the fact that galactose has only 76.3% of the reducing power of glucose.

Removal of glycogen. A small proportion of the glycogen comes down with the precipitate due to the addition of mercuric acetate but most is precipitated along with the HgS after passing H₂S through the filtrate. Up to 2% liver glycogen is removed in this way. HgS prepared from HgCl₂ and H₂S has been used by Flock, Bollman & Mann [1936] for the removal of glycogen.

Effect of mercuric acetate. Mercuric acetate, when added to the neutralized CCl₃.COOH extract, precipitates, amongst other compounds, phosphoric esters containing nitrogenous bases, and consequently the removal of the Hg-precipitate has the following effects: (1) It decreases the 'initial reduction' of purified phosphates by about 75%, e.g. from 61.3 mg./100 g. liver to 14.7 mg. (2) It decreases the labile P (3 min. 100°) considerably, from 0.55 mg. % P (6 exps.) to 0.09 mg. % (10 exps.). (3) It has no influence on the increase in reduction after 3 min. hydrolysis at 100°.

Mercuric acetate also precipitates bile pigments so that a colourless filtrate is obtained.

Removal of inorganic phosphate. This can be done either (1) by precipitating it at pH 9.5* as BaHPO₄

* pH 8.4 is frequently used for the separation as Ba salt of inorganic P from organic phosphates; we found this to be insufficiently alkaline, and therefore used thymolphthalein as indicator which just turns blue at pH 9.5.

from the filtrate, after aerating to remove H_2S ; removing it by centrifuging and adding 3 vol. ethanol to precipitate the organic phosphates, or (2) as described above, by precipitating the organic and inorganic phosphates together with 3 vol. ethanol, drying the precipitate, and then extracting the organic phosphates. The initial reduction is lower with method (2) than (1), and the increase in reduction in preparations from livers of fasted rats after acid hydrolysis with method (2) is only one-third to one-half of that with method (1). Recovery of added galactose-1-phosphate is the same with the two methods, and since smaller blank values are obtained with method (2) than with method (1) the former has been adopted.

Since BaHPO_4 is sparingly soluble in water, small quantities of inorganic P are always found; the average of 13 expts. was 0.55 mg. P/100 g. liver, ranging from 0.35 to 1.03 mg. P. If more water is used for the extraction or the precipitate extracted more than 3 times, the values for inorganic P increase considerably. Extraction with 3 ml. H_2O , repeated twice, is sufficient. Actually the recovery of added galactose-1-phosphate (10.06 mg. P to 30 ml.) from the filtrate after HgS precipitation was 88.5 and 100% if the precipitate was extracted 5 times with 5 ml. H_2O , and 99% when successively extracted with 3.5, 2.5 and 1.5 ml. H_2O .

Significance of 'initial reduction'. Although it is likely that the 'initial reduction' is a measure of the amount of equilibrium ester (hexose-6-phosphates) present, there is so far no direct proof of this assumption. Relatively large amounts of glycerol phosphates are present in liver which make it

difficult to estimate the P corresponding to the 'initial reduction'. The findings of Cori & Cori [1931-2] suggest that, under the conditions of our experiments, the recovery of hexose-6-phosphates may not be quantitative: the pH may be too alkaline and extraction may not dissolve all the 6-phosphates present.

Recovery of galactose-1-phosphate and glucose-1-phosphate added to trichloroacetic acid extracts of liver

Example 1. 2 ml. galactose-1-phosphate solution containing 0.114 mg. P were added to 40 ml. extract (test solution). 1 ml. NaOH was needed for neutralization and 4 ml. 10% mercuric acetate solution were then added. After centrifuging, a sample of 40 ml. was used. The final volume was 10 ml.; 2 ml. ($\equiv 112\mu\text{g.}$ galactose) were used for the estimation of reduction and 1 ml. ($\equiv 9.7\mu\text{g.}$ P) for that of P. Another 40 ml. were treated similarly with the exception that 2 ml. H_2O were added instead of the galactose-1-phosphate solution (blank solution) (Table 3).

Example 2. 1 ml. galactose-1-phosphate solution containing 0.0527 mg. P and 1 ml. glucose-1-phosphate solution containing 0.0524 mg. P were added to 40 ml. extract. This mixture was treated in the same way as the galactose-1-phosphate solution of example 1 (Table 4).

The results of several recovery experiments are summarized in Tables 5 and 6. The standard errors of the means of the recoveries and of the ratios 50° value/100° value were calculated according to the directions given by Burn [1937].

Table 3. *Recovery of galactose-1-phosphate*

	Blank solution 'glucose' or P $\mu\text{g.}$	Test solution 'glucose' or P $\mu\text{g.}$	Increases in test after deduction of blank 'glucose' or P $\mu\text{g.}$	$\frac{50^\circ \text{ value}}{100^\circ \text{ value}}$	Recovery %
(1) Reduction (in 2 ml. $\equiv 112\mu\text{g.}$ galactose $\equiv 85.5\mu\text{g.}$ 'glucose')					
Before hydrol.	28.5	28			
After 3 min. hydrol. at 100°	38.5	105			
Increase	+10	+77	+67		78.5
After 30 min. hydrol. at 50°	33	83		0.755	
Increase	+4.5	+55	+50.5	(calc. from Table 2: 0.775)	
(2) Phosphorus (1 ml. $\equiv 9.7\mu\text{g.}$)					
Before hydrol.	2.86	4.77			
After 3 min. hydrol. at 100°	2.86	11.81			
Increase	0	+7.04	+7.04		After correction* with $f=1.05$ 76.2
After 30 min. hydrol. at 50°	2.43	10.08		0.755	
Increase	-0.43†	+5.31	+5.31	(calc. from Table 2: 0.765)	

* This correction is necessitated by the great sensitivity of galactose-1-phosphate to acid. Under the conditions of these experiments, 4.5% of the ester present is hydrolysed by the analytical procedure alone, therefore the factor for correction is $1.00/0.955=1.05$. The factor for the 50° value is $0.775/0.73=1.06$. The amount of glucose-1-phosphate hydrolysed during the analysis is so small as to make a correction unnecessary in the present experiments.

† Occasionally small negative values have been observed after 30 min. hydrolysis at 50° ; they have been neglected.

Table 4. *Recovery of galactose-1-phosphate and glucose-1-phosphate*

	Blank solution 'glucose' or P μg.	Test solution 'glucose' or P μg.	Increases in test after deduction of blank 'glucose' or P μg.	$\frac{50^\circ \text{ value}}{100^\circ \text{ value}}$	Recovery %
(1) Reduction (in 2 ml. $\equiv$ 51.7 μg. glucose + 52.1 μg. galactose $\equiv$ 91.5 μg. 'glucose')					
Before hydrol.	44.5	37.5			
After 3 min. hydrol. at 100°	55.5	121			
Increase	+11	+83.5	+72.5		79.3
After 30 min. hydrol. at 50°	53.5	80		0.462	
Increase	+9	+42.5	+33.5	(calc. from Table 2: 0.498)	
(2) Phosphorus (1 ml. $\equiv$ 8.95 μg.)					
Before hydrol.	1.99	2.26			
After 3 min. hydrol. at 100°	2.30	8.57			After correction with $f=1.02$ 68.5
Increase	+0.31	+6.31	+6.0		
After 30 min. hydrol. at 50°	1.91	5.23		0.495	
Increase	0	+2.97	+2.97	(calc. from Table 2: 0.520)	

Table 5. *Recovery of glucose-1-phosphate*

2.65-3.1 mg. ester P/100 g. liver

Amount of ester P added to extract mg./40 ml. ($\equiv$ 4 g. liver)	Glucose liberated in 3 min. at 100° % of calc.	Glucose liberated in 30 min. at 50° calc. = 28.6	$\frac{50^\circ \text{ value}}{100^\circ \text{ value}}$ calc. = 0.286	P liberated in 3 min. at 100° % of calc.	P liberated in 30 min. at 50° calc. = 28.6	$\frac{50^\circ \text{ value}}{100^\circ \text{ value}}$ calc. = 0.286
0.106	85.4	23.5	0.276	85.2	27.5	0.323
0.111	88.2	26.6	0.302	75.5	18.6	0.246
0.111	91.5	25.0	0.273	87.2	26.9	0.309
0.124	83.3	22.6	0.272	81.0	21.4	0.265
Mean	87.1 $\pm$ 1.78	24.4 $\pm$ 0.88	0.280 $\pm$ 0.0116	82.2 $\pm$ 2.59	23.6 $\pm$ 2.16	0.287 $\pm$ 0.0278
Coefficient of variation (%)	2.04	3.61	4.15	3.15	9.15	9.68

Table 6. *Recovery of galactose-1-phosphate*

(a) 2.68-2.85 mg. ester P/100 g. liver

Amount of ester P added to extract mg./ml.	Galactose liberated in 3 min. at 100° % of calc.	Galactose liberated in 30 min. at 50° calc. = 77.5	$\frac{50^\circ \text{ value}}{100^\circ \text{ value}}$ calc. = 0.775	P liberated in 3 min. at 100° corrected % of calc.	P liberated in 30 min. at 50° corrected calc. = 77.5	$\frac{50^\circ \text{ value}}{100^\circ \text{ value}}$ corrected calc. = 0.775
0.12/40 ($\equiv$ 4 g. liver)	76.5	56.6	0.74	73.8	56.9	0.77
0.12/40	84.5	60.4	0.715	83.6	61.0	0.73
0.114/40	78.5	58.7	0.75	76.2	58.3	0.765
Mean	79.8 $\pm$ 2.40	58.6 $\pm$ 1.10	0.735 $\pm$ 0.0261	77.9 $\pm$ 2.95	58.7 $\pm$ 1.20	0.755 $\pm$ 0.0325
Coefficient of variation (%)	3.01	1.88	3.55	3.79	2.04	4.30

(b) 0.60-1.5 mg. ester P/100 g. liver

0.06/40 ($\equiv$ 4 g. liver)	72.5	52.0	0.715	69.8	60.0	0.86
0.06/40	72.5	40.6	0.56	91.6	70.9	0.775
0.057/40	86.0	64.3	0.75	80.9	54.4	0.675
0.0306/50 ($\equiv$ 5 g. liver)	95.0	65.5	0.69	68.7	54.1	0.785
0.0301/50	70.5	70.5	1.00	75.1	58.8	0.785
0.0422/50 containing 0.2% glycogen ($\equiv$ 2% liver glycogen)	69.0	58.3	0.845	72.9	49.1	0.675
Mean	77.6 $\pm$ 4.28	58.5 $\pm$ 4.43	0.754 $\pm$ 0.0705	76.5 $\pm$ 3.50	57.9 $\pm$ 3.05	0.757 $\pm$ 0.0528
Coefficient of variation (%)	5.51	7.57	9.36	4.58	5.27	6.98

In the recovery experiments the esters were added to liver extracts in proportions similar to those found in determinations of these esters in livers of animals assimilating glucose or galactose. Approximately 80% of the added esters were recovered; there was no significant difference between the recoveries of hexose and phosphorus. The ratios 50° value/100° value were sufficiently close to the calculated values. As was to be expected, the standard errors of the means were relatively high in the experiments in which only 0.6–1.5 mg. ester P/100 g. liver was added. These facts have to be taken into consideration when experiments are designed. The limiting factors obviously are the methods used for the estimation of reducing power and of phosphorus. These difficulties are best overcome by increasing the quantities of liver used for the estimation but, unfortunately, in the rat at least, this cannot easily be done, particularly when other substances, as, for example, glycogen and hexose, have to be determined simultaneously.

SUMMARY

1. Glucose-1-phosphate and galactose-1-phosphate are determined in trichloroacetic acid extracts of rats' livers. After removal of interfering reducing substances, i.e. nitrogenous phosphoric esters, bile pigments, and glycogen, by means of mercuric acetate and HgS, the organic phosphates

are separated from inorganic P as water-soluble barium salts.

2. The reducing power of the organic phosphate fraction gives an approximate measure of the amount of hexose-6-phosphates present. The quantity of glucose-1-phosphate and galactose-1-phosphate taken together is determined by the increase in reducing power and the amount of inorganic P liberated after hydrolysis in a mixture of 0.05N HCl and 0.2N H₂SO₄ at 100° for 3 min. Incubation with 0.25N acid at 50° for 30 min. hydrolyses 28.6% of glucose-1-phosphate and 77.5% of galactose-1-phosphate. In a mixture of the two esters, the ratio 50° value/100° value is determined by the relative concentrations of the esters.

3. In experiments with addition of known quantities of ester to CCl₃.COOH extracts of liver, 84.5% of added glucose-1-phosphate (2.65–3.1 mg. P/100 g. liver), 79% of added galactose-1-phosphate (2.68–2.85 mg. P/100 g. liver), and 77% of added galactose-1-phosphate (0.6–1.5 mg. P/100 g. liver) were recovered. The ratios 50° value/100° value did not show any significant deviation from the expected values. The significance of the standard errors of the means of the results for the recovery of added material is discussed.

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An Improved Method for the Fractionation of Protein Mixtures by Electrophoresis

By H. GUTFREUND, *From the Department of Biochemistry, University of Liverpool*

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The application of the electrophoresis technique to the problem of analysing protein mixtures was greatly refined by Tiselius [1937 *a, b*], who used the method particularly for the analysis of serum proteins. Since then it has been used by a variety of workers, Kekwick & Record [1940], for instance, having applied it to the isolation of the biologically active globulins from immune horse serum. Al-

though the method is of great value, only relatively small quantities of proteins can be separated by it and at a high cost. Moreover, attempts to increase the yields have met with considerable difficulties [Philpot, 1940].

In their purification of a variety of colloids by electrodialysis between two semi-permeable membranes, Pauli, Stamberger & Schmidt [1937] ob-