fluid, and Tables 3 and 6 that the glycolysis of suspensions is unaffected by the compound. A small stimulation only was found in previous experiments with the same organism (McIlwain, 1944) in which, in contrast with the present work, the course of glycolysis was measured. Pantothenate is always present in small quantities in the organisms; nevertheless, if free pantothenate is present, it is metabolized. As the association of the usage of added pantothenate with glycolysis is facultative, it is understandable that pantooyltaurine should be able, as is found, to depress pantothenate-usage without much effect upon glycolysis. The organism in presence of pantothenate and pantooyltaurine is then reverting to a condition similar to that obtaining when neither is present. This condition may be adequate for glycolysis but is not adequate for growth. The association of pantothenate-usage with growth is obligatory: growth is strictly limited by the quantity of pantothenate added when that is below a given concentration (McIlwain, 1944), and the limiting quantities are completely removed from the media. Pantooyltaurine in such cases prevents both the pantothenate metabolism and growth.

(5) To explain inhibition of growth by a process which preserves the growth-essential, pantothenate, it can be supposed that, in its functioning, pantothenate undergoes a cycle of changes, which is interrupted by pantooyltaurine, and of which at least one phase is relatively unstable in the presence of the reaction mixture.

SUMMARY

1. Pantothenate disappeared from streptococcal cultures and from certain reaction mixtures containing pantothenate through a process for which the organisms were necessary but which was independent of their growth and viability.

2. This metabolism of pantothenate was associated with the presence of glucose and a casein hydrolysate (or related materials) and perhaps of magnesium, but was independent of oxygen supply.

3. When glycolysis was prevented by omitting or adding various substances, or by treating the organisms, pantothenate metabolism usually also ceased.

4. The presence of pantooyltaurine is exceptional among the circumstances investigated, in that it causes inhibition of pantothenate metabolism without a corresponding inhibition of glycolysis.

5. Streptococci normally contain pantothenate and are capable of glycolysis in the absence of added pantothenate, but if it be assumed that both glycolysis and the presence of excess pantothenate are needed for the formation of a substance essential to streptococcal growth, and that such formation is inhibited by pantooyltaurine, then it is understandable that, as observed, (i) absence of added pantothenate inhibits growth, (ii) inhibition of glycolysis stops pantothenate metabolism, (iii) pantooyltaurine stops pantothenate metabolism and growth, but not glycolysis.

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A Quantitative Micro-method for the Separation of Inorganic Arsenite from Arsenate in Blood and Urine

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Studies on the metabolism of inorganic arsenic compounds in the animal body have been greatly hampered by the lack of a suitable quantitative method for the separation of trivalent from penta-

valent inorganic arsenic in the body fluids. Precipitation methods for the separation of arsenite from arsenate in urine are unsatisfactory, as shown by Joachimoglu (1916), whose method was based on

the observation that, on electrolysis in strongly alkaline solution, arsine is evolved from arsenite but not from arsenate. However, as Joachimoglu recognized, the reduction of arsenite to arsine is not quantitative.

Klein & Vorhes (1939) described a method based on earlier work by Tarugi & Sorbini (1912), for the determination of total arsenic in biological materials, which involved the reduction of arsenate to arsenite and subsequent extraction of the latter from acid solution with sodium ethyl xanthate dissolved in carbon tetrachloride. Although the authors did not actually state that arsenic acid does not react with the xanthate reagent to form a carbon tetrachloride-soluble product, they implied that this is the case. In the present work it has been found possible to achieve a quantitative separation of arsenite from arsenate in blood and urine, freed of interfering substances, by extraction with the xanthate reagent, thereby allowing them to be separately estimated by any of the usual methods for total arsenic. This procedure seems to be more satisfactory than any that have hitherto been used.

EXPERIMENTAL

Preparation of the xanthate reagent (Klein & Vorhes, 1939). NaOH pellets were added to pure dry ethanol in the proportion of 1 g. to 10 ml. The mixture was warmed and shaken vigorously for about 15 min., centrifuged and the supernatant liquid decanted off. The alkali content was determined by titration of a sample, and adjusted to a concentration of 7% (w/v). To 9 vol. of this solution, 1 vol. of redistilled carbon disulphide was added slowly with cooling, and, after mixing and filtering, the product was stored in the ice-box. A fresh solution was made fortnightly. The

xanthate reagent was prepared as required by diluting 50 ml. of the alcoholic sodium ethyl xanthate to 1 l. with dry, redistilled carbon tetrachloride.

Separation technique. The arsenic-containing solution (80 ml. or less) was measured into a 150 ml. separating funnel and acidified with 10 ml. conc. H_2SO_4 diluted with 2-3 vol. of water. The solution was extracted by vigorous shaking for 2 min. with one 40 ml. and two 20 ml. portions of the xanthate reagent, 5 ml. of carbon tetrachloride being run through the funnels to wash down the stems after each extraction. The combined extracts were washed with one 10 ml. portion of 2N- H_2SO_4 (this washing being added to the aqueous phase), and evaporated down after addition of water. Total arsenic was determined (Levy, 1943) on the evaporated extract (arsenite fraction), and on the aqueous phase (arsenate fraction).

To avoid the extremely stable emulsions, which form with the xanthate reagent and blood or urine, the extractions were carried out on dialysates, or, in the case of blood, on trichloroacetic acid filtrates.

'Blank' corrections. Concurrently with each experiment, 'blank' solutions were extracted under identical experimental conditions. The corrections so obtained were applied to all results.

Separation of arsenite from arsenate in simple solution. Various amounts of arsenite and arsenate (as standard solutions of their sodium salts) were pipetted into volumetric flasks, and the solutions diluted to 100 ml. Total arsenic was determined on 40 ml. of each solution, and the remaining 60 ml. submitted to the xanthate procedure (Table 1).

Separation of arsenite from arsenate added to blood dialysates. 40 ml. of a haemolysate prepared from washed red blood cells were dialyzed in 'Visking' sausage casing against 80 ml. distilled water for 24 hr. in the ice-box. To this dialysate in a volumetric flask, known quantities of arsenite and arsenate were added, and the volume made up to 100 ml. Samples were analyzed for total arsenic, arsenite and arsenate, the results of four separate experiments being shown in Table 2.

Table 1. *Separation of arsenite from arsenate in simple solution*

Total arsenic			Arsenite			Arsenate		
Added (μ g. As)	Found (μ g. As)	Recovery (%)	Added (μ g. As)	Found (μ g. As)	Recovery (%)	Added (μ g. As)	Found (μ g. As)	Recovery (%)
105.0	113.7	108	20.0	16.3	82	85.0	68.0	80
112.0	107.3	96	52.0	48.3	93	60.0	57.7	96
113.0	99.0	88	88.0	81.0	92	25.0	23.2	93
120.5	120.0	100	50.0	47.3	95	70.5	73.2	104
119.4	117.8	99	64.0	64.3	100	55.4	57.6	104
119.2	117.8	99	84.0	81.7	97	35.2	32.5	92
55.0	54.8	100	30.0	31.3	104	25.0	26.5	106
104.6	112.0	107	24.0	28.0	117	80.6	82.5	102
49.0	54.3	111	16.0	14.7	92	33.0	34.2	104
184.0	195.8	106	24.0	27.0	113	160.0	158.7	99

Table 2. *Separation of arsenite from arsenate added to blood dialysates*

Total arsenic			Arsenite			Arsenate		
Added (μ g. As)	Found (μ g. As)	Recovery (%)	Added (μ g. As)	Found (μ g. As)	Recovery (%)	Added (μ g. As)	Found (μ g. As)	Recovery (%)
210.2	205.5	98	180.0	165.0	92	30.2	35.0	116
180.8	179.5	99	60.0	63.0	105	120.8	111.3	92
70.3	66.5	95	30.0	30.7	102	40.3	42.0	104
185.1	185.5	100	24.0	23.7	99	161.1	163.3	101

Table 3. *Separation of arsenite from arsenate added to blood haemolysates*

(Extraction procedure on aqueous dialysates)

	Total arsenic			Arsenite			Arsenate		
	Added ($\mu\text{g. As}$)	Found ($\mu\text{g. As}$)	Recovery (%)	Added ($\mu\text{g. As}$)	Found ($\mu\text{g. As}$)	Recovery (%)	Added ($\mu\text{g. As}$)	Found ($\mu\text{g. As}$)	Recovery (%)
1	90.7	85.5	94	45.2	37.8	84	45.5	47.5	104
	90.7	94.5	104	45.2	39.2	87	45.5	41.5	91
2	91.2	93.0	102	46.1	37.5	81	45.1	39.0	87
	91.2	—	—	46.1	39.8	86	45.1	39.0	87

Separation of arsenite from arsenate added to blood haemolysates (extraction procedure on aqueous dialysate). Two experiments were performed as follows: To 150 ml. of a haemolysate were added measured amounts of arsenite and

or arsenate were added. Three 5 ml. samples were removed for total arsenic determinations, and 55 ml. were run slowly into 2 vol. of 20% trichloroacetic acid, made up to 250 ml. with distilled water and filtered. 20 ml. samples of the filtrate were used for determination of total arsenic, and 50 ml. samples for arsenite and arsenate separation. The results of these experiments are shown in Table 4, which records average figures for triplicate determinations.

Table 4. *Recovery of arsenite and of arsenate added to blood haemolysates*

(Extraction procedure on trichloroacetic acid filtrates)

	Arsenite ($\mu\text{g. As}$)	Arsenate ($\mu\text{g. As}$)
Total arsenic in laked blood	226	262
Total arsenic in trichloroacetic acid filtrate	236	289
Arsenite in trichloroacetic acid filtrate	226	3
Arsenate in trichloroacetic acid filtrate	16	250

arsenate. Two 40 ml. samples were each dialyzed against 80 ml. of water in the ice-box for 24 hr., and the dialysates diluted to 100 ml. with distilled water; 40 ml. were used

Separation of arsenite from arsenate added to urine dialysates. Known amounts of arsenite and arsenate were added to 50 ml. portions of a dialysate of rabbit urine (1 vol. of urine against 10 vol. of distilled water), and the resulting solutions submitted to the xanthate procedure (Table 5).

Recovery of arsenite from urine (extraction procedure on aqueous dialysate). 15 ml. samples of rabbit urine, containing 30 $\mu\text{g. As}$ as arsenite, were dialyzed against 60 ml. of water in the ice-box for 40 hr., and extracted as previously described. To obtain the results (Table 6), the experimentally determined values were multiplied by 5/4, it being assumed that there was an even distribution of arsenite and arsenate on both sides of the dialysis sac.

Table 5. *Separation of arsenite from arsenate added to urine 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	19.6	98	20.1	22.2	110
20.0	18.2	91	20.1	22.3	111
40.0	38.3	96	40.3	39.6	98
40.0	34.2	86	40.3	43.2	107
120.0	111.7	93	80.6	83.3	103
240.0	241.2	100	120.8	125.8	104
80.0	82.8	104	282.0	282.1	100

for total arsenic determination and the remaining 60 ml. for the xanthate extraction. For calculation of the results (Table 3), the assumption was made that the arsenite and arsenate were evenly distributed on either side of the dialysis membrane, and accordingly, the amounts of total arsenic, arsenite and arsenate found in the dialysate were multiplied by 3/2 to obtain the corresponding amounts in the original 40 ml. of haemolysate. Although this assumption may introduce some error in the actual figures for total arsenic, arsenite and arsenate, the ratio of the two latter should not be affected.

Recovery of arsenite and of arsenate added to blood haemolysates (extraction procedure on trichloroacetic acid filtrates). To a haemolysate prepared by diluting 15 ml. of blood with 65 ml. of distilled water, about 250 $\mu\text{g. As}$ as arsenite

Recovery of arsenate from urine. Approximately 450 $\mu\text{g. As}$ (as arsenate) were added to 45 ml. of rabbit urine, which

Table 6. *Recovery of arsenite from urine*

(Extraction procedure on aqueous dialysates)

Arsenite added ($\mu\text{g. As}$)	Arsenite found ($\mu\text{g. As}$)	Arsenate found ($\mu\text{g. As}$)
30.0	26.0	2.9
30.0	25.0	0.8
30.0	27.5	1.1
30.0	29.4	3.8

was dialyzed against 180 ml. of distilled water in the ice-box. After dilution of the dialysate to 200 ml., six 20 ml.

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.

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 &