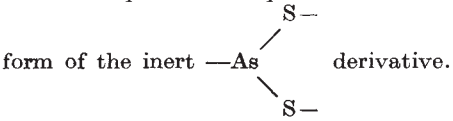
The following extract from this report gives our conclusions based on investigations, carried out in the Cardiff City Mental Hospital during 1938, 1939 and early 1940, on the effects of organic trivalent and pentavalent arsenic compounds on proteins and enzymes.

"(1) Reversible equilibria exist between pentavalent arsenic compounds (such as tryparsamide) or trivalent arsenic compounds (such as arsphenamine and derivatives of phenylarsenoxide) and purified tissue proteins. The affinity of organic arsenoxides to the proteins is far greater than that of pentavalent arsenic compounds. Affinity constants have been measured and new methods for analysis of As^{III} and As^{V} in presence of proteins elaborated. Compounds of the type *R*.As.O combine reversibly with —SH groups of the protein and with other as yet unknown groupings.

"(2) Certain compounds of the type *R*.As.O (e.g., meta-amino-*p*-hydroxyphenylarsenoxide, also known on the market as 'mapharside') combine reversibly with —SH enzymes. They can be regarded as specific inhibitors of this class of enzymes, which includes urease, succinic dehydrogenase and choline esterase. It is proved for the first time by this means that choline dehydrogenase is an —SH enzyme. Enzymes known not to possess —SH groups as part of their active centres, e.g., invertase, catalase, lactic dehydrogenase, cytochrome oxidase are unaffected by *R*.As.O. The toxicity of *R*.As.O to enzymes is neutralised by the addition of —SH compounds, e.g., cysteine, in large excess. These condense with *R*.As.O to form easily dissociated compounds, and excess —SH is required to keep most of the —AsO in the



"Compounds of the type *R*.As.O and *R*.As.Cl_2 also inhibit vigorously the respiration of intact cells in presence of glucose and sodium pyruvate. Such cells vary from intact brain tissue to bacterial suspensions. The oxidation of pyruvate and possibly other ketonic acids is greatly inhibited, and ketonic acids accumulate. The addition of —SH compounds in excess neutralizes *R*.As.O inhibition of the metabolism of intact cells.

"Compounds of the type *R*.As.O and *R*.As.Cl_2 are also highly bactericidal but the addition of —SH neutralises the lethal action.

"(3) Pentavalent arsenic compounds, e.g., tryparsamide and atoxyl, are reduced by —SH compounds (e.g., cysteine, glutathione) under biological conditions to the highly toxic trivalent —AsO derivatives. This may be demonstrated by the effects of the products of reduction on —SH enzymes (e.g., urease) and on intact cells. The reduction of tryparsamide in the body is doubtless accomplished by the —SH bodies (e.g., glutathione) present."

This work was undertaken as part of a programme to elucidate the mechanism of action of arsenicals in the treatment of syphilis of the central nervous system, and part of it was carried out with the assistance of Mr. W. J. Clarke.

The publication of these results was not allowed during the War for security reasons. Owing to war work we became separated, and only recently have we had the opportunity of reconsidering our early work for the purpose of publication.

The experimental results upon which the above conclusions have been reached will, it is hoped, be published shortly. We are much indebted to the secretary of the Medical Research Council for permission to publish the extract of the report in question.

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Splitting of Phosphocreatine

It is generally assumed that when phosphocreatine is hydrolysed either enzymatically or spontaneously, the end products are creatine and phosphoric acid. This, however, is not always found to be the case. When phosphocreatine is hydrolysed at 38° in saturated picric acid, about 10 per cent of the substance is converted into creatinine (Fig. 1). Under these conditions, creatine is not transformed into creatinine. Obviously phosphocreatine has a certain spontaneous tendency to ring formation. If heated to 100° either in picric acid or hydrochloric acid, there is a rapid formation of creatinine, followed by a linear increase due to the reaction creatine – creatinine (Fig. 2). If the two curves are subtracted, a curve of much the same appearance as Fig. 1 results. Whether picric or hydrochloric acid is used for hydrolysis is of no importance.

The phenomenon described may possibly be developed into a specific method for the estimation of phosphocreatine in blood and other tissues. Generally this substance is estimated as the difference ('direct determinable' P — 'true' inorganic P), thus neglecting other labile phosphorus compounds which may be present.

These experiments may perhaps throw some light on the creatinine formation in living organisms. It is well established that the daily excretion of creatinine

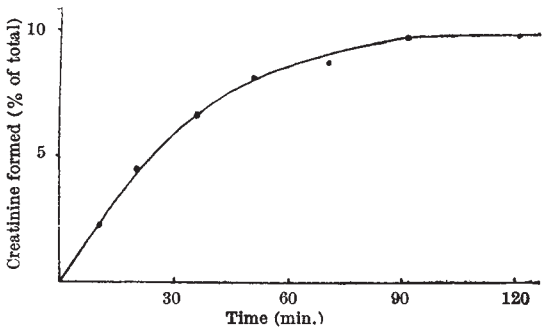

Fig. 1. Formation of creatinine from phosphocreatine in 8/9 saturated picric acid at 38°. Conc., 0·35 mM./l.

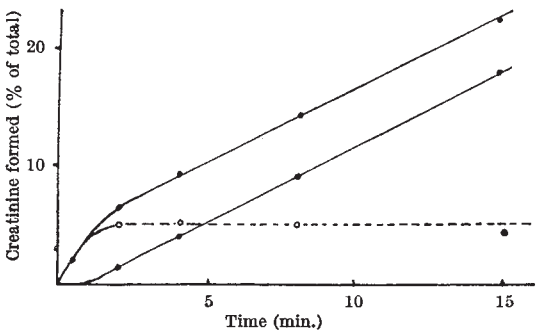

Fig. 2. Formation of creatinine from phosphocreatine (upper curve) and creatine (lower curve) at 100° in 8/9 saturated picric acid. ------, The difference between the two curves. Concentrations, 0·35 mM./l.

is rather closely proportional to the total amount of muscle tissue and independent of muscular activity. This may be explained if it is assumed that there is a spontaneous breakdown of phosphocreatine resulting in the formation of some creatinine. The phosphocreatine breakdown through specific activity yielding the energy-rich phosphate group to some acceptor can readily be imagined to give rise to no creatinine formation, thus explaining why the creatinine output is independent of muscular activity.

Another problem which may perhaps be attacked on the basis of the present observation is the active renal secretion of creatinine.

According to the hypothesis that a necessary condition of renal tubular secretion is that the substance is an acid[1], creatinine should not be secreted ; and it is in fact the only substance not found to obey this rule, at least in mammals.

Rehberg[2] has suggested that phosphocreatine, known to occur in plasma after the injection of creatinine[3], may be converted into creatinine in the tubular cells and thus be responsible for part of the urinary creatinine. This suggestion seems to find support in the observation that phosphocreatine may form creatinine spontaneously. The tubular cells are supposed to exhibit a strong non-specific phosphatase activity, thus making possible a partial conversion of phosphocreatine into creatinine. That phosphocreatine should be absorbed from the blood stream by the tubular cells is well in accord with the hypothesis mentioned[1].

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Dec. 3.

[1] Lundquist, F., *Acta Pharm. et Tox.*, **1**, 307 (1945).
[2] Rehberg, P. B., XVI Int. Physiol. Kongress, *Kongressber.*, **1**, 4 (1938).
[3] Abdon, N-O., *Kungl. Fysiograf. Sällsk. i Lund Förhandl.*, **5**, 14 (1935).

Preparation of Cell-free Tryptophanase

IN our earliest studies on the tryptophanase complex[1] active non-viable suspensions of *E. coli* were used. These had been sterilized by shaking with chloroform, 1 volume to 2 volumes of suspension. The mixture was shaken vigorously to 200 r.p.m. for 4 hours.

An active dry enzyme preparation was also prepared by precipitation of the cells with 66 per cent alcohol (at 0°) and subsequent washing with ice-cold absolute alcohol. Since at that time we were mainly concerned with the mechanism of the reaction, further work was not attempted ; though occasional studies on the supernatants from viable cells suggested that the enzyme complex could be obtained as a cell-free preparation. More recently we have been concerned to know more of the nature of the enzyme complex, and the following represents the preparation of such a cell-free preparation and which, moreover, can be divided into a dialysable and non-dialysable portion.

Cells grown for 22 hours at 37° on a tryptic digest of cæsin solidified with agar were harvested, washed and filtered through glass wool. Thick creamy suspensions thus obtained were cooled in ice and poured into five volumes of ice-cold acetone with constant stirring. The flocculated cells were allowed to settle and then filtered off with gentle suction on a Buchner funnel, washed successively with acetone, acetone and ether, and finally ether, and dried in a desiccator.

Tests were made with buffer solutions of *p*H 5, 7 and 9 to extract the enzyme from the powder. After overnight extraction, the suspensions were centrifuged and supernatants adjusted to *p*H 7·4. Activity of extraction was nil at *p*H 5, faint at 7 and more marked at 9.

The present technique employed is overnight extraction with borate buffer *p*H 8·6 using 20 mgm. acetone-dried powder per ml. buffer. Whereas the optimum *p*H for the reaction by the intact organism is 7·6, the cell-free enzyme has an optimal *p*H range of 8–8·5. The activity obtained from such cell-free extracts has been, at the maximum, approximately 25 per cent of that obtained from intact organisms, presumably due to destruction of some portion(s) of the tryptophanase complex by acetone drying.

Dialysis of the cell-free extract has been performed, and both dialysate and protein portion have been shown to be inactive by themselves. When added to one another activity was regained.

Work is now in hand on the elucidation of the various factors of the enzyme complex, and a full report will be published at a later date.

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[1] *Biochem. J.*, **29**, 1918 (1935).

Yeast and Rickets

IN his address to the Seventh Congress of Biological Chemistry in Liège on October 3–6, 1946, Dr. H. D. Kay summarized some experiments on feeding pigs with yeast, carried out during the War at the National Institute for Research in Dairying in Reading[1]. It was found that when the total food ration contained 8 per cent dried yeast, some of the animals developed stiffness and lameness, and were finally unable to stand. If the diet contained 20 per cent yeast, this condition developed rather rapidly in all pigs. X-ray examination of the bones indicated that the pigs were, in fact, suffering from rickets. The rachitic symptoms could be prevented by a sufficient supply of vitamin D, and could be almost completely prevented by increasing the calcium carbonate content of the diet from 1·5 to 4 per cent. When the yeast phosphorus was replaced by the corresponding amount of sodium phosphate phosphorus, no symptoms were produced.

During a recent visit to England, I discussed this problem with the Reading workers, who were now of the opinion that the rachitogenic effect of yeast might be connected with the high phytic acid content in the diet. The results of our experiments, which show that dried yeast is a strong inhibitor of the enzyme phytase, support this hypothesis.

The experiments were made on a crude enzyme solution extracted from rye bran. 25 ml. of the solution correspond to the phytase activity of about 15 gm. barley. The yeast used was dried brewers' top-yeast. The system contained 25 ml. enzyme solution and 72·0 mgm. phytate phosphorus, as pure sodium phytate ; concentration of acetate buffer, 0·2 *M.* ; total volume, 100 ml. ; *p*H, 5·20 ± 0·05 ; temp. 30° ± 0·1° C. ; time of reaction 2 hr., at the end of which 10 ml. was taken out to control the *p*H