

somes. This result agrees very closely with that observed histologically by Sulkin<sup>1</sup> and McKellar<sup>2</sup>.

From the results it may be concluded that the high values which have been obtained for the mean amount of deoxynucleic acid per nucleus in the liver of the rat have been due to the presence of nuclei containing the tetraploid and octoploid number of chromosomes. The striking agreement between the values for the degree of polyploidy obtained by histological measurements and by calculation from chemical analyses suggests that a doubling in the number of chromosomes is accompanied by a doubling in the amount of deoxynucleic acid per nucleus. Therefore, it would appear that for the rat there is a direct relationship between the amount of deoxynucleic acid per nucleus and the number of chromosomes. The significance of these findings in the calculation of the mass of the liver cell will be discussed in a later paper.

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### Coenzyme of Kidney Phosphatase

It has been deduced from dialysis at different pH that kidney phosphatase can be separated according to the scheme:

holophosphatase  $\rightleftharpoons$  cophosphatase + apophosphatase<sup>1</sup>.

By mixing the dialysis liquid with the solution of inactivated phosphatase and adding magnesium, reactivations up to 100 per cent can be reached: by adding to the apoenzyme boiling solutions of the enzyme, in which the coenzyme remains unaltered, partial reactivations can also be obtained. Albers thinks that the coenzyme cannot be magnesium, but a labile organic substance which has not been identified.

These results have been ascribed alternatively to the presence of small amounts of zinc or cobalt ions, which are able to form a dissociable complex<sup>2</sup>. On the same lines, kidney phosphatase has been considered as an enzyme with dissociable metal (magnesium and also zinc) which may not contain a dissociable coenzyme; so the enzymatic activity would be connected with co-ordination in a metal complex of various fragments of the apoenzyme<sup>3</sup>.

It has also been suggested that the dialysable prosthetic group could be a combination of a metal and a substance containing amino-acids; this has been inferred from experiments of reactivation of phosphatase with amino-acids in the presence of metallic ions<sup>4</sup>. But the active entity of boiled solutions cannot be replaced by amino-acids, according to another series of experiments<sup>5</sup>.

Following our research on organic models of phosphatase<sup>6</sup> and in order to get a better knowledge of the composition of the enzyme, we have carried out experiments on the dialysis of kidney phosphatase. This was obtained by the Albers method, and we have verified previous results on inactivation, going further in the study of the dialysed liquid.

The liquid of acid dialysis, on evaporation *in vacuo*, gives a residue having 5.2 per cent nitrogen. The spectral analysis of the ash gives magnesium, zinc, nickel, calcium, iron, sodium and potassium—the same metals present in the phosphatase before dialysis<sup>7</sup>, but which are not contained in the apoenzyme.

The same residue, treated with alcohol, deposits the mineral part—an insoluble white solid containing the metals referred to before, except magnesium. It also contains phosphorus (24.1 per cent of phosphate) corresponding to a formula  $\text{PO}_4\text{HM}^{\text{II}}$  with an average atomic weight of 30 for  $\text{M}^{\text{II}}$ .



From the filtrated solution a very hygroscopic organic residue (N = 10.7–11 per cent) containing magnesium can be crystallized (see accompanying illustration); it melts at 110–120° (with softening), is precipitated by ammonium molybdate and gives yellow needles, m.p. 180° (decomp.), with picric acid.

By treatment with hot, concentrated hydrochloric acid, another portion containing  $\text{PO}_4^{3-}$  is separated, a pentose is identified and another nitrogen compound (N = 23.5 per cent), which is not precipitated with ammonium molybdate and picric acid, is isolated. The spectrum of this substance is now under study.

The results so far obtained suggest that kidney cophosphatase is a metallic derivative of a diphosphonucleoside, probably derived from adenosine, as can be deduced from analytical data and spectral analysis.

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