

by the borate. The reaction is obviously not as satisfactory as other methods which depend on the isolation of the amino sugar or on the identification of a derivative, for it may prove to give misleading results if other amino sugars are present. For qualitative and rough quantitative guidance it has however proved very useful.

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Inosine phosphates in muscle*

Rabbits were killed by decapitation, quickly skinned, eviscerated, and packed in ice for ten minutes; then their muscles were removed, minced in a precooled meat mincer, and suspended on 50% methanol, 100 ml being used for 100 g. After putting the suspension through the colloid mill 250 ml absolute methanol were added, and the suspension was pressed out through muslin and clarified in the Sharples centrifuge. On addition of Zn a precipitate was formed which, illuminated by near UV, showed a brilliant blue fluorescence. The substance responsible for this fluorescence could also be precipitated as a strongly fluorescent Ca-complex. In the absence of salts no fluorescence was observed.

The extracts showed a strong pH-independent absorption at 250 $m\mu$ but none at 260, indicating the presence of inosine and absence of ATP. From the absorption for inosine-nucleotide (E 10,000), the muscle was calculated to contain 1 mg per gram of fresh tissue.

If the alcohol-extracted muscle residue was eluted with water, an extract was obtained which showed a strong pH-independent absorption at 260 $m\mu$ and none at 250 $m\mu$. Calculated for ATP the absorption corresponded to 2 mg of ATP per gram of fresh tissue.

The substance responsible for the 250 $m\mu$ absorption was crystallized and found to contain phosphate and ribose. It contained one labile and one stable phosphate pro mole. The former split off spontaneously in a few weeks time on storage, indicating its great lability. On addition of Ca salts and alcohol the IMP also was fluorescent, suggesting that the non-localized electronic structure revealed by the fluorescence was probably due to a complex formation between the inosine and the metal. Since ATP does not form fluorescent metal complexes, it seems likely that the OH group at position 6 was involved in the formation of a metal chelate. It seems not unlikely that in IDP or ITP also the phosphate-end of the molecule complexes with the metal to form a bidentate chelate, two valencies of the metal being left free to complex with myosin.

These results suggest that inosine phosphates play a major role in muscle. Possibly a chelate formation between Ca, the inosine phosphates and myosin is involved in the detachment of the terminal phosphate and the energy transmission from the nucleotide to the protein. Since ITP, prepared by the deamination of ATP by nitrous acid, does not show fluorescence under identical conditions, it seems probable that the IDP isolated from muscle is an isomer of the IDP obtained *in vitro*.

Technical details and possible biological bearings will be discussed in more detail on a later occasion.

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