

while the second part is strongly Raman active giving an intense second order spectrum. The character of the spectrum is also totally different in the two ranges of frequency. While the spectrum of the elastic vibrations is necessarily a continuous one, the discrete nature of the atomic vibration spectrum in the upper ranges of frequency is clearly manifested in the second order Raman effect; over-tones and summations of the primary vibration frequencies appear under adequate instrumental power clearly resolved into numerous closely spaced sharp lines. These differences are fundamental and will compel anyone to recognise that the two parts of the vibration spectrum are physically different. In the

lower ranges of frequency, we are concerned with elastic waves traversing the crystal from end to end and forming stationary wave-patterns, while in the upper ranges of frequency, we are concerned with the vibrations of the atoms in the individual cells of the crystal lattice. The spectroscopic facts thus give a decisive answer to the theoretical issues stated at the end of the second paragraph. They show that the assumptions on which the Debye and Born-Karman theories are based are unjustified and that the conclusions regarding the nature of the atomic vibration spectra to which those theories lead are altogether untenable.

PREPARATION OF ADENOSINE TRIPHOSPHATE FROM BULL FROGS

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IT has been known for some time that the phosphorylation of glucose by means of adenosine triphosphate (A.T.P.)—or adenylyl pyrophosphate, as it is sometimes called—under the influence of the so-called "Hexokinase", an enzyme found in yeast by Meyerhof,¹ and the occurrence of which has also been recently demonstrated in animal tissues,^{2,3} plays an all-important role in the carbohydrate metabolism of animals. The reaction has assumed added importance and interest in the light of the new theory of Dixon and Needham⁴ of the action of vesicant poison gases and of the observations made recently by Price, Cori and Colowick⁵ and by Colowick, Cori and Slein⁶ that the activity of animal hexokinase is inhibited *in vitro* by certain extracts of the anterior pituitary glands, and that this inhibition is counteracted again by the pancreatic hormone, Insulin. Fresh light appears thus to be thrown on the mechanism of the long known antagonism between certain hormones, that in the present case being correlated with the activities of a particular enzyme system in the body.

The adenosine triphosphate required for some of the preliminary investigations carried out in this laboratory was prepared from the thigh and hind leg muscles of frogs by a slight modification of the original method due to Lohmann.⁷ The process would appear to be both simple and efficient when compared with that described recently by Dounce, *et al.*,⁸ using rabbit muscles. There is no reflex action causing twitching of the frog muscles and the consequent fear of possible loss of A.T.P., when the brains of the animals are pithed under the conditions described below, thus dispensing with the necessity for the use of anaesthetics like Nembutal. The complicated procedure involved in the removal of stable organic phosphates which appear to be present only in traces in frog muscles as compared with the rather large amounts found in rabbits, and of inorganic phosphate, by means of alternate precipitations with mercuric nitrate in addition to barium acetate, is also found unnecessary. The preparation can be conveniently completed in 3-4 hours as compared

to 2-3 days' intermittent work, described by Dounce, *et al.*⁸ The experimental details under Indian conditions, are recorded here as they might be of interest to other workers in the field.

The bull frogs obtained locally weighed on the average 150 grams each. They were kept under ice and salt for about five minutes and when benumbed, taken out of the bath, stretched on a board and their brains pithed or smashed with a light blow from a hammer. The legs were then held by an assistant and the muscles rapidly excised using scalpels and scissors, the whole operation with a single frog lasting 1 to 1½ minutes. The muscles were placed immediately in a weighed flask immersed in a freezing mixture, the muscles quickly weighed and then passed through a "Latapie" mincer which had been cooled previously in ice. Approximately 100 grams of minced muscles were obtained from three bull frogs. The mince was allowed to fall directly into ice-cold 10 per cent. trichloroacetic acid (100 ml.) in an Erlenmeyer flask, shaken up repeatedly by taking out of the freezing bath for a few seconds at a time, and filtered through cloth at the pump into an ice-cooled receiver, after a few minutes. The residue was extracted once again with 4 per cent. trichloroacetic acid (100 ml.) in the same way and filtered. The total extract (225 ml. approx.) was centrifuged in the cold to free from precipitated proteins, the clear liquid treated with ice-cold N NaOH until only just acidic to Congo Red (50 ml. approx.) and then an equal volume of ice-cold alcohol (approx. 95%) was added. A slight precipitate, which separated after standing for a short time and which consisted mainly of glycogen,⁷ was centrifuged off. To the clear ice-cold alcoholic solution (50 ml. approx.) which still tested acidic to Congo Red, was added slowly from a burette a cold 2½% solution of barium acetate until the solution ceased to be acidic to Congo Red (8 ml. approx.). The precipitate, which is the di-barium salt of A.T.P. together with some inorganic barium phosphate, was separated at the centrifuge and then shaken up with ice-cold water (75 ml. approx.) and