

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; overtones 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-Karmann 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,<sup>1</sup> and the occurrence of which has also been recently demonstrated in animal tissues,<sup>2,3</sup> 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 Needham<sup>4</sup> of the action of vesicant poison gases and of the observations made recently by Price, Cori and Colowick<sup>5</sup> and by Colowick, Cori and Slein<sup>6</sup> 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.<sup>7</sup> The process would appear to be both simple and efficient when compared with that described recently by Dounce, *et al.*,<sup>8</sup> 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.*<sup>8</sup> 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,<sup>7</sup> 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 25% 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



the precipitate dissolved by the addition of a small amount of cold N HCl, about 6 ml. being found sufficient. A small amount of insoluble material was removed by centrifuging. To remove inorganic phosphates, the barium salt was again precipitated as described before by adding an equal volume of alcohol (75 ml. approx.) and then 25% barium acetate solution until the suspension was just alkaline to Congo Red, centrifuged and the dissolution, precipitation and centrifuging repeated two more times. Each dissolution required less HCl than the previous one, and more and more of the nucleotide salt was precipitated by alcohol each time. For the last precipitation no barium acetate or at most one drop was required to render alkaline to Congo Red. This is due to the formation of a mixture of the mono and the di-barium salts of A.T.P.,<sup>9</sup> of which the former is soluble and the latter insoluble in water. It also explains the rather high phosphorus and nitrogen percentages of the product. The product is finally centrifuged, washed with alcohol of increasing strength and then with ether and dried *in vacuo* over sulphuric acid, at room temperature. 286 milligrams of a product containing only traces of inorganic phosphate were obtained from 3 bullfrogs yielding 100 grams of muscle. The purity of the product was determined by estimating the ratio of labile or hydrolysable phosphate by hydrolysis with N HCl on boiling water-bath for 7 minutes—to stable phosphate by the method of Fiske and Subbarow,<sup>10</sup> and also the total nitrogen. The data obtained from the analyses of two of the preparations are given below:—

Prep. 1. 572 mg. Ba-salt were obtained from 202 grams of muscle derived from 6 bullfrogs.

Total phosphate	..	11.58%
Hydrolysable phosphate	..	7.83%
Hence, hydrolysable P : stable P ::		7.83 : 3.75, i.e., 2.09 : 1 (Theory, 2 : 1).
Nitrogen	..	8.91%

Prep. 2. Similar yield.

Total phosphate	..	11.36%
Hydrolysable phosphate	..	7.49%
Hence, hydrolysable P : stable P ::		1.94 : 1. (Theory, 2 : 1).
Nitrogen	..	8.77%
$C_{10}H_{14}O_{13}N_5P_3, Ba_4H_2O$		
requires N	..	9.8%
$C_{10}H_{12}O_{13}N_5P_3, Ba_24H_2O$		
requires N	..	8.2%

In all preparations, inorganic phosphate directly estimated was negligible.

The sodium salt of A.T.P. was readily prepared from the barium salt by removing the barium directly with the calculated amount of N sulphuric acid, neutralising with N NaOH to pH 7.0 and centrifuging off the precipitated barium sulphate; as little as 1 ml. of solution can thus be prepared in 15 ml. graduated centrifuge tubes. For such small volumes a loss of A.T.P. due to adsorption on the barium sulphate<sup>11</sup> is unavoidable. The A.T.P. content of the solution is determined by estimation of the total phosphate. The salt is stored as barium salt in desiccator and solutions are prepared just before use. The compound appears most stable as sodium salt, stored in solution (pH 6.8) at  $-12^{\circ}C$ .

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## NOR'WESTERS IN BENGAL

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THE arguments and the results from a study of winter rain in the U.P. are applicable to a study of nor'westers in Bengal. Bengal is a densely populated part of India where flat bottomed river craft form a chief mode of transport. The nor'westers cause damage to these river craft and sometimes cause loss of life.

Intensive meteorological observations have been taken in Bengal to study the nor'westers. Many workers have studied the various aspects of the phenomenon.<sup>2</sup> Quoting from Pramanik "Nor'westers or Kalbaisakhis (calamities of the month of Baisakh) are severe thunderstorms which occur in Bengal during the summer months March to May, some of which reach Tordanic violence and cause considerable damage to property and sometimes even loss of life. The winds in these thunderstorms come generally from some north-

westerly direction and hence they are called nor'westers. In some cases, funnel-shaped clouds characteristic of tornadoes have been noticed.....It is generally agreed that the nor'westers are not 'local heat' thunderstorms. This appears to have been recognised by Eliot as early as 1876. They also do not occur when the whole of north-east India is over-run by one air mass, i.e., during winter when west to north-west winds prevail and during the periods of strong monsoon when moist winds from the Bay prevail. They occur mostly during the transition period from the winter season to the rainy season, i.e., when two different air-masses, west to northwest winds of land origin and moist winds from the Bay co-exist over Bengal".

Eliot brought in the idea of a cold wedge. M. G. Subramanyam<sup>3</sup> used to say that though there was a southerly feed in the lower levels