RAMAN SPECTRA OF CRYSTALS AND THEIR INTERPRETATION*

ITS universal applicability, simplicity of technique, precision of results obtained and ease of interpretation make the Raman effect a powerful tool for the study of the structure of matter. The information yielded by Raman spectra has been of great value in the investigations of relatively simple molecules as well as of the more complicated polyatomic ones. Its utility in the investigations of the physico-chemical problems set by the crystalline state of matter is equally great. Although numerous papers have appeared on the Raman spectra of crystals since the discovery of the effect, very little progress was made till 1943 in using Raman effect data for the elucidation of the physical properties of solids. This state of affairs may be attributed to the fact that the physics of the solid state was dominated for many years by theories which had their birth before the discovery of the Raman effect. The reference here is to the well-known theories of solid behaviour originally put forward about the same time by Debye and by Max Born.

It is a significant fact that, subject to some noteworthy qualifications and exceptions, the modes of atomic vibration appearing in the Raman spectra of crystals are represented by sharp lines irrespective of the nature of the substance, or of the frequency of the vibrations. Basing himself on this and similar optical effects observed in crystals, e.g., luminescence and absorption spectra at low temperatures, Sir C V. Raman (1943) formulated a new theory of lattice dynamics. The new theory leads among other things to the most important result, namely the vibration spectrum of a crystal consists essentially of a finite number of discrete frequencies. On the basis of the Raman theory, crystal lattice has in general $(24p-3)$ modes of normal vibration (excluding simple translations) in $(3p-3)$ of which all the units in the structure have the same phases, while in the remainder equivalent atoms in neighbouring cells along one, two or all three primitive translations vibrate with opposite phases. The experimental confirmation of this prediction became a matter of fundamental importance for the progress of crystal physics. As the available data on the Raman spectra of crystals were insufficient, it became necessary to carry out a series of investigations using an improved experimental technique to give a decisive answer to the following issues raised by the new lattice dynamics: (1) Are the vibrations in crystals which manifest themselves in the Raman spectrum, waves extending through the volume of the crystal, or are they the vibrations of the atoms in the individual cells of the lattice? (2) Is the complete vibration spectrum of the crystal in the infra-red region a continuous diffuse spectrum, or is it a discrete spectrum exhibiting a finite set of monochromatic frequencies ?

It has been the practice generally to use the λ 4046 and λ 4358 radiations of the mercury arc as exciter in the studies on the Raman spectra of crystals. The Raman lines excited by these radiations fall in the very region where the weak fluorescence exhibited by many crystals appear and where the spectrum of the mercury arc itself shows a weak continuum. Consequently feeble Raman lines may not be detected as they will appear overpowered by the continuum In view of these difficulties, the technique of using the λ 4046 and λ 4358 radiations for the study of the Raman effect in crystals appeared to have already been pushed to the limit of its utility during recent years. It became clear that some radical improvement in the technique of study had to be effected in order to get further useful information about the Raman spectra of crystals. This was achieved by the so-called ultra-violet technique described below.

As is well-known, it is essential to employ a monochromatic light source which is very intense for recording the extremely feeble Raman scattering in crystals. It is also of great importance that there should be no continuous spectrum accompanying the same. This is secured by using the λ 2533·5 mercury reasonance radiation from a water-cooled magnet-controlled quartz arc. The enormously increased scattering power of the resonance radiation arising from its exceptional intensity as compared with the other mercury radiations and from the λ^{-4} law has made it an ideal source for Raman effect studies in the case of crystals that are transparent to the ultra-violet and do not get coloured by prolonged exposure to this radiation. The λ 2533·5 radiation from the light scattered by the medium is effectively suppressed before its entry into the spectrograph by absorption in a column of mercury vapour, as otherwise the photographic plate would be fogged. This makes it possible to record faint Raman lines with small frequency shifts on a clear background.

Using the improved experimental technique for exciting the Raman spectra, many crystals have been studied by Dr. R S Krishnan during the last five years and a rich harvest of results obtained in every one of the cases investigated. The results obtained in the case of diamond, rocksalt, potassium bromide, ammonium chloride and ammonium bromide afford a direct experimental verification of the predictions of the new theory of crystal dynamics. In all these cases, the second order Raman spectra exhibit a series of sharply defined frequency shifts. The numerical evaluation of the eigen frequencies for the above crystals on the basis of the new lattice dynamics leads to results in good agreement with observational data. Their activities in light-scattering and in infra-red absorption are also in accordance with the theoretical predictions.

From the experimental facts presented herein on the Raman spectra of crystals, it is clear that the parts of the vibration spectrum in the lower and upper ranges of frequency differ radically in their behaviour. The first part is totally inactive in light scattering,

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 adenyl 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 Needham[4] of the action of vesicant poison gases and of the observations made recently by Price, Cori and Colowick[5] and by Colowick, Cori and Slein[6] 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 anæsthetics 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. trichloracetic 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. trichloracetic 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 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