If the protein part of the virus appears initially in the cells in the form of soluble antigens, with low molecular weight and without ribonucleic acid, the latter must be synthesised independently. One is thus led to imagine that the maturation of the virus, that is the acquisition of its infectious properties, must result from the final union of the ribonucleic acid thus synthesised with the soluble antigens to form a huge elongated particle, which is the only state of the constituents of the virus in which infectivity is displayed.

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[1] R. MARKHAM, R. MATTHEWS AND K. SMITH, *Nature*, 162 (1948) 88.
[2] R. JEENER ET P. LEMOINE, *Arch. intern. physiol.*, 60 (1952) 547.
[3] W. TAKAHASHI ET M. ISHII, *Am. J. Bot.*, 40 (1953) 85.
[4] B. COMMONER, M. YAMADA, S. RODENBERG, T. WANG AND E. BASLER, *Science*, 118 (1953) 529.
[5] R. JEENER, P. LEMOINE ET C. LAVAND'HOMME, *Biochim. Biophys. Acta*, 14 (1954) 321.
[6] R. JEENER, *Biochim. Biophys. Acta*, 13 (1954) 161.
[7] C. DELWICHE, P. NEWMARK, W. TAKAHASHI AND M. NG, *Biochim. Biophys. Acta*, 16 (1955) 127.

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A rapid colorimetric distinction between glucosamine and galactosamine

Published methods for distinguishing between glucosamine and galactosamine in small quantities depend on their separation, or the separation of substances derived from them, by paper or column chromatography[1-4]. Borate depresses colour formation in the well known colorimetric estimation of ELSON AND MORGAN[5,6]. Under the conditions to be described this depression is of the order of 50% for galactosamine and 75% for glucosamine. If therefore in the routine determination of amino sugars by the ELSON AND MORGAN method a second set of samples of double the amino sugar content is heated with borate in addition to the usual reagents the sugars can be distinguished (using double the volume of amino sugar sample with borate avoids undue spread of colour intensity). If galactosamine alone is present the colour intensity in the two sets after development will be about the same, if glucosamine alone is present the intensity in the borate set will be about half that in the first set. If mixtures are present intermediate values will be obtained.

Aliquots containing 2–8 μg amino sugar N are made to a final volume of 1 or 2 ml in tubes calibrated at 10 ml and matched for use in a photoelectric colorimeter. A second set is prepared in which the volume of each sample is double that in the first and the final volume is the same as in the first set. Two sets of standards of glucosamine and galactosamine, the second containing double the amounts in the first are also set up together with two blanks. 1 ml of borate solution (3.2 g $Na_2B_4O_7 \cdot 10H_2O$ in 100 ml water) is added to the samples and standards of doubled concentration and to a blank. 1 ml of water is added to the other set. To all tubes 1 ml of acetylacetone solution (1 vol. redistilled acetylacetone in 25 vols. 1.5 *N* Na_2CO_3) is added and the contents of the tubes well mixed. The tubes are closed with glass balls, heated for 7 min in a boiling water bath and cooled in cold water. Ethanol is added to the tubes to bring the total volume to 10 ml and is followed by the addition of 1 ml of Ehrlich reagent (1.6 g purified *p*-dimethylaminobenzaldehyde in 30 ml conc. HCl and 30 ml ethanol). The contents of the tubes are mixed by thorough shaking in an inverted position. After development (30–45 mins) the colour intensities are read in a suitable colorimeter using a filter with a maximum transmission in the region of 520–540 mμ. There should be little difference between the values for the two sets of galactosamine standards while the glucosamine standards that have been heated in the presence of borate should give readings about half of those in the control set. The effect of borate is rather variable and it is therefore advisable to include both sets of standards. In terms of colour produced/μg N the colour intensity in the presence of borate has been found to vary between 24 and 30% of the control (glucosamine) and between 50 and 65% of the control (galactosamine). The composition of mixtures of the two amino sugars can be estimated with an accuracy of about 5%. If values for the colour produced in the presence of borate as a % of that produced in its absence are calculated for known mixtures it will be found that the results, if plotted against the composition, will fall on a straight line joining the values for glucosamine and galactosamine. The method gave satisfactory results when applied to hydrolysates of known mixtures of chitin and chondroitin sulphate.

The addition of borate has little or no effect on the form of the absorption curve of the coloured complex finally produced in the reaction. The final pH of the solutions before heating is also unaffected

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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1 P. W. KENT, *Research (London)*, 3 (1950) 427.
2 E. F. ANNISON, A. T. JAMES AND W. T. J. MORGAN, *Biochem. J.*, 48 (1951) 477.
3 S. GARDELL, *Acta Chem. Scand.*, 7 (1953) 207.
4 P. J. STOFFYN AND R. W. JEANLOZ, *Arch. Biochem. and Biophys.*, 52 (1954) 373.
5 L. A. ELSON AND W. T. J. MORGAN, *Biochem. J.*, 27 (1933) 1824.
6 M. V. TRACEY, *Modern Methods of Plant Analysis*, Springer-Verlag, Heidelberg, 2 (1955), p. 264.

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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 mμ 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 mμ and none at 250 mμ. Calculated for ATP the absorption corresponded to 2 mg of ATP per gram of fresh tissue.

The substance responsible for the 250 mμ 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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