A NOTE ON SOME PROTEIN CONSTITUENTS OF NORMAL TOBACCO AND TOMATO LEAVES.

F. C. BAWDEN AND N. W. PIRIE.

From the Rothamsted Experimental Station, Harpenden, and the Biochemical Laboratory, Cambridge.

Received for publication July 7th, 1938.

IN the course of work on the isolation of a number of viruses from infected tobacco (var. White Burley) and tomato (var. Kondine Red) plants we have gained some information on the behaviour of the proteins of normal plants. Some heat-stable proteins have already been described (Bawden and Pirie, 1937), and this note deals only with unstable proteins, some with molecular weights of the same order as the viruses, which must be removed during the isolation of the viruses. The proteins characteristic of the viruses already isolated differ so definitely from these normal proteins that they cannot easily be confused with them, and their purification presents few difficulties. Others, however, especially the relatively unstable insect-transmitted viruses such as potato virus "Y", cucumber virus 1, and *Hyoscyamus* virus 3, have properties more nearly resembling those of the normal plant proteins, and their isolation is therefore more difficult.

Different samples of sap from healthy plants differ so greatly in their protein contents and in the properties of the protein that it is impossible to make confident statements, but some material with the properties to be described is always found in both healthy and virus-infected plants. Also, the proteins are so readily changed irreversibly that it would be unwise to assume that precipitation with salts or sedimentation by high-speed centrifugation does not alter them, and we have no evidence that they have the same properties in the cell or in untouched sap.

The pH of expressed tobacco or tomato sap is usually just below 6 and is a little lower with sap from older plants. It can be cleared by centrifuging at 3500 r.p.m., but it clears more rapidly if disodium hydrogen phosphate is added. The addition of NaOH to bring the pH to 8 has no apparent effect, but the addition of the phosphate causes a precipitate of calcium compounds and proteins to separate, amounting to about 3 gm. from a litre of sap. The addition of ammonium sulphate to the clarified sap causes a bulky precipitate at one-quarter saturation. The precipitate dissolves readily in water at neutrality to give a darkly coloured viscous solution. These solutions become opalescent when one-seventh saturated with ammonium sulphate, and after a

few hours a precipitate separates, and can be removed by centrifugation. The addition of a little more ammonium sulphate to the supernatant causes a further precipitate, and a series of fractions is obtained differing in the amount of salt required to precipitate them, but not differing appreciably in any of their other properties studied. All the fractions dissolve readily in water at pH 7 and are precipitated when the pH is lowered to about 5. The precise value depends on the acid used; in general phosphoric acid precipitates the proteins more easily than other weak acids such as phthalic and acetic. The precipitation is irreversible, for 90 per cent. of the material remains insoluble at pH 7, although it can still be dissolved by the addition of excess acid.

It seems that the molecules of some of these proteins are large, for they are readily sedimented from neutral solution by high-speed centrifugation. From one-tenth to one-third of the protein usually sediments when a 0·5 per cent. solution is centrifuged for 2 hours at 12,500 r.p.m. in a rotor of 8 cm. radius. The sedimented material gives brown jellies with a similar appearance to those obtained by centrifuging crude virus preparations. The jellies are isotropic, and are easily dissolved or dispersed in water. The protein can again be sedimented and the process repeated, until the protein from a redissolved pellet sediments at the same rate as that in the supernatant poured off from it. It seems probable that these apparently homogeneous proteins with high molecular weights are normal constituents of a large number of plant species, and their existence throws some doubt on the purity of virus preparations made solely by the high-speed centrifugation of infective sap. We have found them in both healthy and virus-infected tobacco and tomato plants, and the materials centrifuged from cucumbers by Price and Wyckoff (1938) and from peas and beans by Loring, Osborn and Wyckoff (1938) are presumably proteins of a similar nature. Wyckoff, Biscoe and Stanley (1937) found no such proteins in tobacco, and they state that healthy tobacco sap contains no molecules with a weight greater than 30,000, but it is possible their treatment of the leaves before expressing the sap may account for this.

At room temperature these proteins denature and precipitate in a few days, but if kept cold and neutral they are reasonably stable. They are sensitive to freezing, which converts them irreversibly into an insoluble material. As the protein is not apparently affected by exposure to high salt concentrations for short periods, the freezing must either be done at very low temperatures or in the absence of salts, for otherwise much of the protein will be concentrated with the salt in unfrozen pockets of fluid. This effect may explain the somewhat variable results obtained when leaves are frozen before being minced for the extraction of sap. As these normal plant proteins and Bushy stunt virus (Bawden and Pirie, 1938) are denatured by freezing, it seems that freezing the tissues may be a dangerous preliminary to work on the labile protein constituents of plants. On the other hand, in isolating such viruses as tobacco mosaic virus and potato virus " X " which are apparently unaffected by freezing and thawing the treatment may provide a useful fractionation.

These normal plant proteins are denatured by a few minutes' heating at 55° C., and are readily destroyed by incubation with commercial trypsin preparations. In the presence of salts they are also sensitive to alcohol. The

addition of alcohol to salt-free solutions of the proteins causes an opalescence, but no precipitate separates until several volumes are added. In the presence of salts, especially of calcium salts, a precipitate separates at from 25 to 35 per cent. alcohol, and the precipitated material is insoluble in water.

Except for their high molecular weights these normal plant proteins have little in common with the plant viruses that have yet been isolated. They contain from 14 to 16 per cent. of nitrogen but less than 0·02 per cent. of phosphorus and, if repeatedly sedimented by high-speed centrifugation, less than 1 per cent. of carbohydrate, whereas all the viruses we have obtained in crystalline or liquid crystalline form have been nucleoproteins. Best (1937) and Loring (1938) have confirmed the fact that tobacco mosaic virus is a nucleoprotein. The proteins have no serological relationship with any of the viruses we have studied. They also differ from the viruses in being relatively poor producers of precipitating antibodies when injected intravenously into rabbits. No evidence has yet been obtained suggesting a possible mechanism for the multiplication of viruses, but the suggestion that they are autocatalysts produced from virus-precursors is frequently made. We feel that it would be extremely premature to look upon these normal plant proteins as in any sense virus precursors merely because of a similarity in molecular weight, especially as they differ so definitely from the viruses in their chemical, physical and serological properties. Tobacco mosaic virus has been added to concentrated solutions of these normal plant proteins, but so far from there being any evidence of virus increase, the dilution of the virus with the protein caused a greater loss in infectivity than a similar dilution in water or buffer solutions. Muller (1922) suggested that viruses might be "free genes", and since it has been found that certain viruses are nucleoproteins, the suggestion that viruses might be portions of nuclear material or the naked nuclei of hypothetical small bacteria has frequently been made. However, in this connection it should be noted that the nucleic acids characteristic of the nuclei of plant and animal cells contain desoxy pentose, whereas all the plant viruses that have been isolated have contained a nucleic acid of the ribose type.

In describing the isolation of tobacco mosaic virus (Bawden and Pirie, 1937) we commented on the larger amount of protein in clarified sap from infected plants than in that from healthy plants. The sap was usually prepared from frozen leaves and was often acidified slightly to facilitate clearing. It had therefore lost at least a part of the normal proteins described in this paper. Even when sap from unfrozen leaves is used and clarified by centrifuging there is a real difference, for the yield of unstable proteins in normal or infective sap does not exceed 2 gm. per litre, and this amount of virus can be isolated from a litre of infective sap. The soluble protein in the sap will of course represent only a portion of the total protein of the leaf, and Martin, Balls and McKinney (1938) have shown that there is no change in the total protein content of infected leaves at all comparable with the change in the content of soluble protein. This suggests that infection causes a change in the ratio of soluble to insoluble protein, and it is possible that a detailed study of the normal insoluble nucleoproteins of the cell might give some information on the mechanism whereby the abnormal nucleoproteins are formed in the infected plants.

REFERENCES.

BAWDEN, F. C., AND PIRIE, N. W.—(1937) *Proc. Roy. Soc.*, B, **123**, 274.—(1938) *Brit. J. exp. Path.*, **19**, 251.
BEST, R. J.—(1937) *Nature*, **140**, 547.
LORING, H. S.—(1938) *J. Biol. Chem.*, **123**, lxxvi.
Idem, OSBORN, H. J., AND WYCKOFF, R. W. G.—(1938) *Proc. Soc. Exp. Biol. Med.*, **38**, 239.
MARTIN, L. F., BALLS, A. K., AND MCKINNEY, H. H.—(1938) *Science*, **87**, 329.
MULLER, H. J.—(1922) *Amer. Nat.*, **56**, 32.
PRICE, W. C., AND WYCKOFF, R. W. G.—(1938) *Nature*, **141**, 685.
WYCKOFF, R. W. G., BISCOE, J., AND STANLEY, W. M.—(1937) *J. Biol. Chem.*, **117**, 57.

THE INDOPHENOL-REDUCING CAPACITY OF GUINEA-PIG LEUCOCYTES.

A. E. KELLIE AND S. S. ZILVA.*

From the Division of Nutrition, Lister Institute, London, S.W. 1.

Received for publication July 18th, 1938.

IMPRESSED by the unusually high ascorbic acid content of the whole blood in patients suffering from leukæmia, as determined by indophenol titration, Stephens and Hawley (1936) followed up this observation by investigating the partition of ascorbic acid in the blood of a number of normal subjects and of patients suffering from a variety of diseases. Samples of blood from thirty individuals were examined in this connection. They found that, although the values varied widely, the ascorbic acid content of the leucocytes was consistently and significantly higher than that of the other fractions of the blood examined. The high titration values observed in patients with leukæmia were, therefore, ascribed to the preponderance of white cells in the blood.

It is mostly assumed that the rapid reduction of indophenol in acid solution by extracts of animal tissues is due entirely to the presence of ascorbic acid. This assumption is often but not invariably justified. Thus Johnson (1936) found that whilst in the humor of the eyes of the horse, ox, sheep, pig and guinea-pig the capacity for reducing indophenol was entirely due to ascorbic acid, the eye lens contained a substance or substances, other than ascorbic acid, which reduced indophenol. We, therefore, performed a series of experiments on guinea-pigs with the object of ascertaining whether in the case of leucocytes the reduction of indophenol was due entirely to ascorbic acid, and a few typical experiments will be described.

* Member of the Scientific Staff, Medical Research Council.