

agreement with the results of Smillie and Neurath<sup>7</sup> and Kozo Narita<sup>8</sup>.

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<sup>1</sup> Josefsson, L., Edman, P., *Biochim. et Biophys. Acta*, **25**, 614 (1957).  
<sup>2</sup> Synthesis will be published in *Rec. trav. chim.*

<sup>3</sup> Hestrin, S., *J. Biol. Chem.*, **180**, 249 (1949).

<sup>4</sup> Fevold, H. L., *Adv. in Protein Chem.*, **6**, 222 (1951).

<sup>5</sup> Thompson, H. W., Torkington, P., *J. Chem. Soc.*, 640 (1945).

<sup>6</sup> Bellamy, L. J., 'The Infra-red Spectra of Complex Molecules' (Wiley, 1954).

<sup>7</sup> Smillie, L. B., and Neurath, H., *J. Biol. Chem.*, **234**, 355 (1959).

<sup>8</sup> Kozo Narita, *J. Amer. Chem. Soc.*, **81**, 1751 (1959).

### Determination of Tissue Mucoproteins

STUDIES in blood serum mucoproteins have stressed the importance of the alterations of this fraction in various pathological and physiological conditions<sup>1</sup> as well as in the administration of certain drugs<sup>2</sup>. However, only very scant information on the biosynthesis and function of seromucoid is at present available<sup>1</sup>. It seemed to us that a method for the determination of histomucoid<sup>3</sup> would be a practical tool for the study of seromucoid biosynthesis.

Studies were made in male Wistar rats weighing 185–225 gm. The animals were killed by decapitation and the blood was left to flow off. Immediately, the liver and the kidney were perfused with isotonic saline, excised and weighed after dissection of capsular and vascular connexions. The tissues were ground in a mortar and 20 vol. of cold acetone added. After centrifugation, the precipitates were washed with 20 vol. of cold acetone, centrifuged and dried. One per cent suspensions of the dried acetone powders were made in 0.14 M sodium chloride, pH 6.6, and the mucoproteins extracted with shaking for 30 min. according to the method used by Weimer *et al.*<sup>3</sup> The supernatants were filtered through Whatman No. 42 filter paper and 5.0 ml. aliquots of the filtrates were used for mucoprotein determinations by the same method employed for seromucoid<sup>1</sup>. Results were expressed as galactose-mannose per 100 gm. of acetone powders. A summary of the results obtained is presented in Table 1.

Table 1. MUCOPROTEINS IN ACETONE POWDERS OF RAT LIVER AND KIDNEY

	No. of rats	Histomucoid	
		(mgm. hexose/100 gm. acetone powder)	Mean $\pm$ S.D.
Liver	10	Range 96–220	162 $\pm$ 40
Kidney	7	70–130	98 $\pm$ 22

The method gave significant means with 10 and 7 animals for liver ( $t = 4.050$ ,  $P < 0.01$ ) and kidney ( $t = 4.454$ ,  $P < 0.01$ ) respectively. The difference between the two organs was highly significant ( $t = 4.230$ ,  $P < 0.001$ ). Higher levels of liver mucoproteins are probably related to the synthesis of seromucoid, since it is a well-known observation that this fraction is lowered in parenchymatous liver disease<sup>1</sup>.

Acetone powders of rat sera were prepared by the same technique and used in recuperation experiments. Table 2 presents the results of typical experiments.

Further work on histomucoid in other tissues and in various experimental conditions are now in progress.

Table 2. RECUPERATION OF SEROMUCOID IN ACETONE POWDER OF

Acetone Powders (mgm.)	RAT SERUM		Recuperation Per cent
	Found $\mu$ gm. hexose	Calculated $\mu$ gm. hexose	
Serum 50	96*	—	—
Kidney 50	41	—	—
Serum 50 + Kidney 50	140	137	102
Serum 42.8 + Kidney 150	206	205	100
Liver 100	117	—	—
Serum 50 + Liver 50	167	155	108

\* This value is comparable of that obtained by Weimer *et al.*<sup>3</sup> for lyophilized serum.

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### Paper Chromatography of Cell Fragments

THE observation and analysis of isolated cell fragments gives much information on the way in which the more complex structures are put together. The usual method used for separating cell fragments is differential centrifugation, in which the difference in size and particle density is the determining factor. Albertsson<sup>1</sup> has studied the separation of cells and cell fragments using column chromatography. This is a method of interest since it separates the cell fragments on the basis of the physical and chemical properties of the surface of the particles. This communication points out that the well-known technique of paper chromatography can be extended from the separation of molecules to the separation of cell fragments of very large molecular weight.

To illustrate this phenomenon an experiment is described: to 0.15 ml. of spinach chloroplasts were added 0.1 ml. of water and 0.2 ml. of 'Teepol' and the whole mixed. (The chloroplasts were prepared by the method of Arnon *et al.*<sup>2</sup>, except that 0.5 M sucrose was used in place of 0.35 M sodium chloride. 'Teepol' is a detergent manufactured by Shell Oil Co. It is a 21 per cent solution of sodium sec. alkyl (C<sub>10</sub>–C<sub>13</sub>) sulphonate.) Five  $\mu$ l. of this was placed on the origin of a paper chromatogram and run while still moist, using water as solvent. A green spot ran with an  $R_F$  of 0.5. Untreated chloroplasts do not run but remain on the origin. That this is not simple chromatography of water-soluble material, can be demonstrated by elution of the spot with water and centrifugation at 30,000  $g$  for 1 hr. at 4° C. This treatment removes all the green colour to the bottom of the centrifuge tube. That cell fragments can be separated by paper chromatography can be demonstrated by running the above material on a two-dimensional (water-water) chromatogram. Under an ultra-violet lamp the green spot can be seen to be in reality two spots—one which appears black, followed by one which fluoresces red. Both these spots have been eluted separately and centrifuged at 30,000  $g$  for 1 hr. at 4° C., which removes the colour to the bottom of the centrifuge tube.

The mechanism of the above phenomenon is somewhat puzzling. The spots do not tail but run as nice round spots, even exhibiting (when run in two dimensions) the 'swiggles' so familiar in normal paper