having only 4·5 per cent nitrogen (on free acid) and 7·7 per cent ash (3·3 per cent sodium approximately); whereas the mucin clot, after reprecipitation, had 15 per cent nitrogen and negligible ash content. In fact, hyaluronic acid, containing only 6–10 per cent protein, had been extracted at *p*H 8·0 from the mucin clot after acetone treatment.

The hyaluronic acid (potassium salt) prepared from the mucin clot by repeated application of the Sevag procedure has a high ash content, namely, 20 per cent approximately, and 3·2 per cent nitrogen[1]. While the Sevag method is useful for removing small quantities of protein, it is laborious for larger quantities and rather cumbersome when used for a large-scale preparation. This new procedure is simple and easily worked on a large scale. The product may be treated, if desired, by the Sevag method to remove the last traces of protein.

Hitherto, most workers, in preparing hyaluronic acid, have aimed at reducing the nitrogen content as much as possible. However, it is feasible that protein may be attached to hyaluronic acid by linkages other than the usually assumed salt type, and which may be ruptured by prolonged Sevag-treatment at the usual *p*H 8·0. Consequently, it is possible that the present method of preparation might yield products of interest from this point of view.

Details of this and further work will be published at a later date.

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Use of Hyaluronidase in Microtechnique

The highly viscous cement or matrix of connective tissue consists of proteins and mucopolysaccharides, the latter containing hyaluronic acid (or acids)[1]. The viscosity of the matrix can be reduced by hyaluronidase (the spreading factor)[2] almost to that of water by depolymerization and hydrolysis of hyaluronic acid. McClean[3] showed that testicular extract containing the spreading factor allowed a more extensive spread of indian ink when injected into the dermis than did Ringer's solution. The extract produced a "swelling and splitting" of collagen fibres due to increased permeability of the fibre bundles rather than to any change in the collagen itself. Robb-Smith[4] also found that testicular hyaluronidase brought about separation of the reticulin network from muscle fibres, but had no histologically demonstrable effect on other parts of the fibre or its nuclei.

We have, therefore, used commercially available hyaluronidase to facilitate the diffusion of impregnating substances in skin. Post-mortem human skin was treated for one hour at 37° C. with a solution containing 10 μgm. of active freeze-dried testicular extract per ml. For complete penetration the strips of material should not exceed 2 mm. in thickness, and be freed of subcutaneous tissue. Carey's[5] modification of Ranvier's gold chloride technique was then applied, the epidermis removed by gentle shaking and the material dissected in glycerine using glass instruments. In this way it has been possible to demonstrate, without resorting to sectioning, the nerve plexus in the dermis, and the enervation and vascular supply of the dermal papillæ, among which a variety of encapsulated sense organs are to be found.

We have also used solutions of hyaluronidase in Ringer's solution to separate cells in the liver and in the snail's foot.

The method presents great advantages over the classical macerating agents, since it is rapid in action, causes a minimum of disturbance to cytoarchitecture and does not, so far as we can tell from oxygen uptake measurements on liver, result in the death of the cell.

Hyaluronidase is active over a wide range of *p*H and has an optimum round about *p*H 5·0 in solutions of buffer salts[2]. It is inactivated by temperatures above 60° C., and its activity falls rapidly at temperatures above 40° C. Dilute solutions can be stored for about a week at 4° C., provided they are not contaminated. Inhibitors to be avoided or carefully washed out of tissues before treatment with hyaluronidase are formaldehyde, iodine, sodium nitrite, ammonium sulphate, sodium sulphate and sterols[6]. Preparations mounted from water are very susceptible to infection, so that a suitable fungicide (cresol, phenylmercurinitrate, etc.) must be added to mount media.

Our thanks are due to Bengers, Ltd., who provided the sterile freeze-dried preparation of testicular hyaluronidase used in this work.

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Enzymic Formation of a New Riboflavin Derivative

Homogenates of rats' livers, and aqueous extracts of acetone powders of rats' livers, have been found to convert riboflavin into a flavin chromatographically different from riboflavin, flavin mononucleotide, and flavin adenine dinucleotide. The amount formed increases with time, and the conversion is accelerated by rise in temperature, but does not occur after heating at 100° C. The reaction is therefore thought to be enzymic. The flavin cannot be detected in unincubated homogenates which have been concentrated many times by Crammer's[1] method for extracting flavins.

Table of *Rf* Values

Solvent:	1	2	3	4	5
Riboflavin	0·30	0·46	0·18	0·47	0·25
Flavin mononucleotide	0·09	0·17	0·00	0·06	0·5
Flavin adenine dinucleotide	0·03	0·05	0·00	0·02	0·35
New flavin	0·20	0·36	0·09	0·23	0·4
Lumiflavin	0·40		0·28		
Lumichrome	0·70				

Solvent 1. 4/1/5 *n*-butanol/acetic acid/water.
,, 2. 5/3/2 *n*-propanol/pyridine/water.
,, 3. 2/2/1 *n*-butanol/*n*-propanol/water.
,, 4. 3/1/1 benzyl alcohol/ethanol/water.
,, 5. 5 per cent aqueous solution of Na_2HPO_4, $12H_2O$ (ascending chromatogram).

This flavin is not a photodecomposition product of riboflavin. Lumiflavin can be prepared from it, using Warburg's[2] conditions for preparing lumiflavin from riboflavin; this indicates that the modification has occurred in the ribityl side-chain, not in the