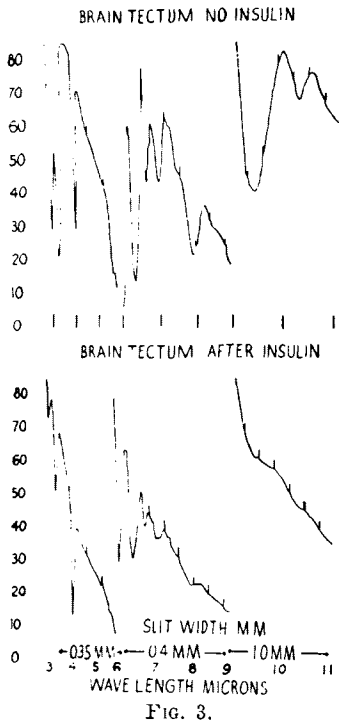

FIG. 3.

ences. In 4 other brain sections of rabbits in insulin shock, the K_1 and K_2 figures were considerably lower than those found in any brain section of control animals. A shift in the relation of K_1 to K_2 occurring in other brain sections from animals in insulin shock is also indicated in the graph and needs further evaluation. In 3 animals free of shock symptoms after insulin injection, sections through different parts of the brain gave about the same K figures as found in control rabbits.

Summary. 1. A method for recording of infra red spectra from frozen tissue section of 25-50 μ thickness and from areas as small as 3 x 3 mm is described. 2. The infra red spectra of tissues show a fingerprint region which is so characteristic of the individual organ that it was frequently possible to identify the tissue from its infra red spectrum. 3. The assignment of tissue spectra to chemical structures as nucleic acid derivatives has been discussed. 4. A method for quantitative infra red spectroscopy in brain tissue has been described. 5. Significant spectroscopic changes in brain tissue of animals in insulin shock have been demonstrated.

Received December 18, 1950. P.S.E.B.M., 1951, v76.

Effect of Proteolytic Enzymes and Fixation on Metachromasia of Skin Collagen. (18459)

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In the course of some histochemical observations on generalized and circumscribed myxedema we(1) have had occasion to treat sections of fixed tissue with certain proteolytic enzymes. We were surprised to find that incubation of sections with pepsin and trypsin led to the appearance of metachromatic staining quality in collagen which ordinarily does not display this property(2). This observation has led us to pursue the subject further in order to compare the effects of these two enzymes on unfixed and fixed preparations.

Methods. Human skin, obtained at autopsy or operation, has been used throughout. Frozen sections were made of unfixed material. Some were treated with crystalline pepsin* or trypsin* in the unfixed state; others were fixed in either neutral formalin or 80% ethanol, after which they were washed and treated with the same enzymes. The enzymes were used in a concentration of 5%; the pepsin was dissolved in acetate buffer, pH 5.0, the trypsin in veronal buffer at pH 7.4.

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TABLE I. Effect of Incubation With and Without Enzymes on Unfixed and Fixed Collagen.

Treatment	pH	Unfixed	Fixation in 80% ethanol	Fixation in formalin
Pepsin	5.0	Meta.*	Meta.	Meta.
Buffer	5.0	Ortho.†	Ortho.	Ortho.
Trypsin	7.4	Ortho.	Meta.	Meta.
Buffer	7.4	Ortho.	Ortho.	Ortho.

* Meta. = Metachromatic.
† Ortho. = Orthochromatic.

Control sections were incubated in buffer without enzyme. The time of incubation was 2-6 hours. All sections were stained for similar periods with weak toluidine blue.

Results. The results are shown in Table I. When unfixed skin is stained with toluidine blue the collagen takes up very little stain in contrast to the purplish color of the epidermis and appendages. With the concentrations utilized here there was only the slightest blue tint to the connective tissue bundles. In contrast, in unfixed sections which had been digested with pepsin the collagenous fibers exhibited intense metachromasia while those treated in a similar fashion with trypsin did not. On the other hand when frozen sections were fixed in either alcohol or formalin before incubation with the enzymes metachromasia was present after enzymatic digestion with both pepsin and trypsin.

Discussion. We fully realize that metachromatic staining is not a specific histochemical test for the demonstration of mucopolysaccharides in tissue. However, metachromatic dyes such as toluidine blue are useful tools with which to reveal alterations in the chemical nature of certain materials. The physiochemical basis of metachromasia is poorly understood. According to Michaelis (3) certain substrates have the ability to promote polymerization of the dye molecules so that the wave length of the peak absorption of light is shifted. Mucopolysaccharides are among the materials which are capable of doing this.

It is well known that skin contains chrondroitin sulfuric acid, hyaluronic acid and doubtless other mucopolysaccharides(4,5). Despite this, fresh skin collagen is not metachromatic. On the other hand fresh tendon, cartilage and umbilical cord all of which contain these materials are metachromatic. On purely chemical grounds Meyer(6,7) has called attention to the ease with which chrondroitin sulfuric acid may be removed from cartilage, *i.e.* by extraction with $CaCl_2$; from this he has assumed that this mucopolysaccharide is loosely bound to protein. On the other hand much more drastic measures, *i.e.* strong alkali, are necessary to separate this material from skin collagen. One would therefore infer that this mucopolysaccharide is more strongly bound to the protein in skin than that in cartilage.

The unmasking of metachromasia in undenatured skin by pepsin but not by trypsin is of interest because of the well known differences in action of these two enzymes on collagen(8,9). The former is able to digest collagen while the latter is not. It would seem highly suggestive then that pepsin may attack the protein component so as to free groups of the mucopolysaccharide which might then combine with the dye. On the other hand, since trypsin is not able to split the protein, no groups would be available to bind the dye. This is, of course, purely conjectural but may furnish a possible way to study chemically the combination of mucopolysaccharide and protein in collagen.

Summary. When unfixed skin collagen, which ordinarily is only lightly orthochromatic, is treated with pepsin it becomes metachromatic. Trypsin does not have this effect. Collagen denatured by alcohol or formalin fixation is rendered metachromatic by both pepsin and trypsin.

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Received December 18, 1950. P.S.E.B.M., 1951, v76.