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Extraction of Collagen from Tissues

In the course of some estimations of deoxyribonucleic acid by Schneider's method[1], we observed incidentally that the hot trichloroacetic acid extraction of the tissue removed the collagen almost completely, judged by estimations of hydroxyproline on the hydrolysates of the solubilized and insoluble material.

We have now examined this method of solubilizing collagen in other tissues with the view of using it instead of the normal autoclaving procedure in conjunction with the method of Neuman and Logan[2] for estimation of collagen from the hydroxyproline content of an acid hydrolysate of the solubilized material. In order to estimate the completeness of the extractions, we estimated the hydroxyproline content of the extracts and of the residual insoluble material. On the basis of a preliminary investigation along these lines using various times of extraction, we decided on two half-hour extractions with 0·3 *M* trichloroacetic acid at about 90° C. followed by two washes with cold trichloroacetic acid. Elastin of dog's aorta does not appear to be dissolved by this procedure, though it is rendered soluble in *N*/10 sodium hydroxide, so that the usual method of estimating it after collagen is not applicable[2,3]. Some results with the method are given in Table 1.

Table 1

	Collagen (gm. per 100 gm. wet weight)	Collagen extracted (per cent)
Rat muscle	0·77	97·4
Rat skin (with hair)	4·52	98·3
Rat tail tendon	15·70	100·1
Rat mammary gland (lactating)	1·47	98·0
Rat liver	0·18	96·4
Ox liver (*a*) pulp	0·25	92·8
(*b*) vessels	5·1	97·5

(*a*) Material pressed through 1 mm. diameter holes in a metal plate.
(*b*) Material retained by perforated metal plate.

The completeness of extraction tends to drop slightly as the collagen content of the material diminishes, possibly owing to increased relative importance of adsorption of gelatin on precipitated proteins. The liver pulp, which gave the lowest extraction, probably contains a large proportion of reticulin which is known to be difficult to solubilize, a difficulty which occurs also with other methods of extraction. This degree of extraction would in any event be quite adequate for many purposes.

The method is quicker and simpler than the usual procedure[2]; it also has great advantages for sampling a large bulk of tissue which is difficult to homogenize. The tissue is roughly broken up and subjected to the first period of heating with trichloroacetic acid. It can then be homogenized very easily in a blender and sampled; tissue containing bone can be dealt with easily in this way.

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Inhibition of Histamine Release in Anaphylaxis

Although the effects of anaphylaxis and chemical histamine releasers are similar and mainly attributable to histamine release, the mechanism by which the release occurs in the two cases is fundamentally different. We have found that lack of oxygen and also inhibitors of oxidative and glycolytic metabolism have a powerful inhibitory action on the release of histamine in anaphylaxis but enhance its release by compound 48/80 (a condensation product of *p*-methoxyphenyl-ethyl-methylamine and formaldehyde) and octylamine.

By the use of minced guinea pig lung from animals sensitized to egg albumin, it is possible to release with the specific antigen 20–40 per cent of the total histamine contained in the tissue. Addition of 1 m*M* iodoacetate reduces this release to 5 per cent or less. Suitable concentrations of compound 48/80 (1 mgm./ml.) and octylamine (0·15 mgm./ml.) also release 20–40 per cent of the histamine content of the tissue; but in the presence of 1 m*M* iodoacetate this release is nearly doubled. Addition of the antigen in the presence of nitrogen produces similar effects, namely, an almost complete inhibition of the anaphylactic release and a potentiation of histamine release by the chemical releasers. It has previously been shown by Parrot[1] that anoxia inhibits the release of histamine in anaphylaxis. The effect of anoxia could be partially reversed by methylene blue; but that of iodoacetate was not reversed by pyruvate.

These results suggest that the release of histamine in anaphylaxis is a process which requires energy and can easily be disrupted. The action of chemical releasers, on the other hand, is enhanced by processes which interfere with normal cell metabolism. These findings may possibly explain why isolated intracellular particles containing histamine are able to release histamine when treated with chemical releasers[2], but cannot be made to release histamine in anaphylaxis[3].

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