The observation of Dr. Banga that thermally contracted tendon collagen is dissolved under the action of elastase is obviously of great importance. There is considerable evidence that elastin preparations from aorta and from ox ligament, even after the presumed removal of collagen, do not present single substrates to the action of elastase[1]. In the course of a search for a purer substrate for elastase, we have also observed the dissolution of thermally contracted collagen both from Achilles tendon and from rat tail tendon. In addition we have found that elastase, acting at *p*H 8·7, causes the dissolution of the water-insoluble protein in bovine crystalline lens and of the lens capsule. The dissolution of lens capsule is particularly interesting, since the material shows some similarity in amino-acid composition to collagen, its X-ray diffraction pattern is like that of a partially oriented collagen, it contains much polysaccharide and has a definite marked shrinkage temperature between 60° and 70° [2]. We have thus shown that elastase will attack a collagenous material even when it has not been thermally contracted.

The complex structure of aorta and the close association of collagen and elastin fibres in this material makes it difficult for us to accept Dr. Banga's interpretation of the work of Schwarz and Dettmer[3]. With reference to their observations that the elastin fibrils are not dissolved under the action of elastase and are similar to collagen fibres in certain respects, we would point out that the particular set of conditions they employ, namely, incubation for ten hours at 37°, and *p*H 7, are not optimum for the action of elastase[4], and, moreover, they do not seem to have taken any special steps to remove collagen. The possibility pointed out by Dr. Banga, however, that the fibrillar structure of elastin may be altered in some way during the heat treatment employed for the removal of associated proteins is of fundamental importance, and one which must be constantly borne in mind.

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'Multiple Dipping' Procedures in Paper Chromatography: a Specific Test for Hydroxy-proline

Much industry has been devoted in recent years to methods for locating the position of components separated on paper chromatograms, and claims have been made for many reagents of greater or less specificity. We wish to report our experiences in locating compounds on paper chromatograms by the successive application of several reagents with different specificities.

We have used mainly the better-known amino-acid and amine reagents, and find that several of them can be used one over the other if applied in an appropriate order. The use of Pauly reagent to confirm the identity of histidine after location with ninhydrin was advocated by Dent[1], and the use of two superimposed reagents, usually with the second applied to selected areas of the chromatogram only, has been mentioned by others[2]. Application to selected areas only is to be avoided if possible, since this is likely to miss the presence of other interesting reactors, as well as ending with a blotched chromatogram. We have used only the 'dipping' technique[3] for application of the reagents used; and we obtain a very uniform background even after four reagents have been superimposed.

Ninhydrin, isatin and Ehrlich reagents are prepared and used in the manner already reported[3]. Our dip reagent for the Sakaguchi reaction is a 0·1 per cent solution of 8-hydroxy-quinoline (oxine)[4] in acetone; after drying in air, the paper is then dipped into a solution of 0·2 ml. bromine in 100 ml. *N*/2 sodium hydroxide; arginine and other guanidines give brilliant orange-red areas, fading slowly. The modified diazo reagent formed from amyl nitrite and *p*-anisidine in ethanol[5] gives excellent results as a dip reagent for histidine and other iminazoles, but poor results with tyrosine. The nitroso-β-naphthol/nitric acid reagent of Acher[2c], specific for tyrosine and certain of its derivatives, can be applied as acetone solutions.

After applying one reagent, the chromatogram is heated or left for full colour development to proceed. Spots and colours are then marked with lead pencil, the next reagent applied to the whole chromatogram, and so on. We term this a 'multiple dipping' procedure.

Maximum information is given by sequences such as: ninhydrin or isatin, followed by Ehrlich reagent, followed by Sakaguchi reagent or diazo reagent. Ninhydrin can be applied over isatin. With the notable exception of hydroxy-proline (see below), the colours given by amino-acids with ninhydrin and/or isatin are rapidly 'rubbed out' by the strongly acidic Ehrlich reagent, which must therefore be applied after these two. It can, however, be followed by Sakaguchi reagent, or by the diazo reagent if 2*N* sodium hydroxide is used as dip solution for the coupling reaction. The nitroso-β-naphthol reagent is applicable after ninhydrin or isatin, but its sensitivity is considerably diminished. Other sequences of these reagents are incompatible or of little value.

This multiple dipping technique is useful for confirming, by specific reagents, the nature and position of components first located by a general reagent; for revealing the composite nature of a single spot; and for revealing two or more sets of components which do not each respond to the same general reagent (for example, urinary indolic compounds in the presence of amino-acids). A single one-way chromatogram treated with four separate and specific reagents can often give more information than a two-way chromatogram, with consequent saving of time and labour. We are at present utilizing the technique in the photometric scanning of chromatographic strips, the application of the various reagents yielding records which are directly comparable.

In general, the usual colour responses and sensitivities of amino-acids to the reagents mentioned are almost unaltered by previous treatment. One important exception constitutes a highly sensitive and specific test for *hydroxy-proline*. After heating with isatin, the duck-egg blue colour due to hydroxy-proline is replaced by an intense purplish-red (cerise) colour within a few seconds of applying Ehrlich reagent over it. The effect is not seen when either acid or dimethylamino-benzaldehyde is applied alone. This colour test will detect hydroxy-proline at a concentration on paper chromatograms of 0·1 μgm./sq. cm.—considerably beyond the sensitivity