The Reconstitution of Elastin from a Soluble Protein derived from *ligamentum nuchae*

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The object of this investigation has been to study the conversion of soluble elastin from ox *ligamentum nuchae* into a form resembling native elastin. Study of the precipitation of collagen fibrils from collagen solutions has provided an insight into the formation of collagen *in vivo*, but the possibility of reconstituting elastin from elastin solutions has been neglected.

Purified elastin from ox *ligamentum nuchae* dissolves slowly in 0.25M-oxalic acid at 100° to give a water-soluble polydisperse product whose number-average molecular weight is about 60 000 (Adair, Davis & Partridge, 1951; Partridge, Davis & Adair, 1955). This can be divided into an α -fraction (mol.wt. 60 000–84 000) which forms a precipitate on heating, and a β -fraction (mol.wt. 5500) which does not. The precipitate is composed of liquid droplets, rich in protein (i.e. a coacervate), which disappear when the solution is cooled again. Reversible coacervation is also a property of solutions of elastin solubilized by boiling with 40% (w/v) urea solution (Bowen, 1953). The lowest temperature at which coacervation of elastin solubilized with oxalic acid can be observed depends on pH and ionic strength, showing a minimum near the isoelectric point (pH 4.8 at ionic strength 0.01, pH 3.9 at ionic strength 0.1; Partridge *et al.* 1955).

The present investigation shows that the reversal of coacervation on cooling may be arrested under suitable conditions and the coacervate converted into a gel whose properties are similar to those of the original elastin.

MATERIALS AND METHODS

Preparation of soluble elastin. Fresh ox *ligamentum nuchae* was freed from adhering tissue, cut into 5 mm. cubes and minced in a power mincer, solid CO₂ being used to chill the material (Hall, 1955). Preparation of purified elastin from the minced ligament followed the procedure of Partridge *et al.* (1955) except that, instead of using a stainless-steel hammer mill to powder the material, it was gently rubbed through a series of standard sieves. At the end of the preparation 80% of the elastin passed through a sieve (150 mesh/in.) and this fraction was used. It appeared under the microscope as a mixture of short fibres and small bundles of fibres, and the remaining 20% consisted of larger bundles of fibres.

Samples of purified elastin were dissolved by the following methods: (i) Heating with 0.25M-oxalic acid as described by Partridge *et al.* (1955) in their preparation of the 'primary extraction product'. Soluble elastin prepared by this method is referred to below as O₀.

(ii) Serial extraction with 0.25M-oxalic acid at 100° as described by Partridge *et al.* (1955). Six fractions were obtained and the proteins isolated from them are referred to below as O₁–O₆. O₁ consists mainly of β -elastin and successive fractions of this series consist of mixtures of β -elastin and α -elastin with gradually increasing proportion of α -elastin (Partridge *et al.* 1955).

In each method the resulting solutions were dialysed against distilled water and freeze-dried. The carbohydrate contents (as glucose) of the products O₁–O₆ determined by the anthrone method of Seifter, Dayton, Norrie & Muntwyler (1950) were 0.8, 0.2, 0.1, 0.0, 0.1 and 0.1% respectively.

Special chemicals. The sample of elastase was the proteolytic elastase fraction E₄ prepared by Dr D. A. Hall (Hall, 1957a). Crystalline trypsin was supplied by L. Light and Co. Ltd.

Methods. The temperature of coacervation was determined as described by Partridge *et al.* (1955).

Protein concentrations were determined either by the micro-Kjeldahl nitrogen estimation, a nitrogen content of 16.3% being assumed, or, for comparative purposes, by determining the u.v.-light absorption at a wavelength of 275 m μ of solutions in 0.1N-NaOH with a Unicam SP. 500 spectrophotometer.

Load-extension curves were determined on strips of tissue or gel 1–2 cm. long and about 0.02 cm.² in cross-sectional area. A strip was mounted between glass hooks and suspended in a glass tube (length 15 cm., diam. 1 cm.) containing water whose temperature was kept constant by circulating through a jacket water from a thermostat. When tissue was studied the upper end of the strip was fixed. Load was applied by adding weights to a pan connected to the lower end of the strip by a length of fine stainless-steel wire which passed through a glass capillary in the bottom of the tube. With strips of gel the loads involved were much lower than with tissue and the procedure was therefore modified. The lower end of the strip was kept fixed, relative to the tube, and the upper end attached to the beam of a dial-reading torsion balance (0–500 mg.). Load was applied by rotating the balance arm. The balance beam and upper end of the strip were then restored to their zero position by lowering the tube. In both methods the length of the strip was measured with a cathetometer.

All solutions were freshly prepared before use and the work was done under normal conditions of laboratory cleanliness. In view, however, of the long periods of

incubation in some of the experiments these were repeated with toluene as preservative. The results did not differ significantly from those obtained without toluene.

EXPERIMENTAL AND RESULTS

Precipitation of elastin by heat

Solutions of O_0 and O_3 - O_6 (1%, w/v, in acetate buffer, pH 5.0, ionic strength 0.2) heated to 50° for 5 min. showed coacervation, which disappeared on cooling. When heating was continued for 16 hr. before cooling, the coacervate, which had then collected at the bottom of the vessel as a viscous layer, did not redissolve completely but when stirred coagulated into an elastic mass. The amount of elastin which precipitated and the solubility of the precipitate in buffer depended on temperature and time of heating, pH and ionic strength of the solution and type of soluble elastin, as shown below.

Temperature. A solution of O_0 (1%, w/v, in acetate buffer, pH 5.0, ionic strength 0.15) was kept at 30° for 16 hr. After cooling, the supernatant was poured off and kept at 35° for 16 hr. The procedure was repeated at higher temperatures. The precipitates obtained at the temperatures above 30° were dissolved in buffer, dialysed, freeze-dried and weighed. The precipitate at 30° did not dissolve in the buffer but did dissolve in 7M-urea. This solution, too, was dialysed, freeze-dried and weighed. The results (Table 1) show that most precipitation occurred in the range 30–35°, but only the small amount which precipitated in the lowest temperature range was insoluble in buffer.

Table 1. *Extent of precipitation of elastin at different temperatures*

Elastin O_0 , 1% (w/v) in acetate buffer, pH 5.0; ionic strength 0.15.

Temperature range	Elastin precipitated (% original wt.)
18–30°	11
30–35	15
35–40	9
40–50	14
50–60	4

Time of heating, pH and type of elastin. Samples (2 ml.) of solutions (1%, w/v) of O_3 , O_4 , O_5 and O_6 in buffers of pH 3.2, 5.1 and 7.0 and ionic strength 0.2 were heated to 40° in stoppered tubes for different times, cooled to room temperature (18°) for 1 hr., stirred briefly to disperse the precipitate, centrifuged for 5 min. and 1 ml. of the supernatant was diluted to 10 ml. with 0.1N-NaOH. The extent of precipitation was calculated from the u.v.-light absorption of these dilutions and of similar dilutions of the original solutions. The results (Table 2) show, as was to be expected from the work of Partridge *et al.* (1955), that only a small amount of elastin precipitated at pH 7.0. Larger amounts precipitated at lower pH but there was no significant difference between the amounts formed at pH 3.2 and 5.1. In each case precipitation was near its maximum extent at 5 hr. and little further change resulted from heating for 16 hr. The data indicate that for the same pH and time of heating the extent of precipitation increased as the fraction number increased. This is consistent with Partridge's observation that as the fraction number increases the proportion of the coacervate-forming α -fraction of elastin increases. All further experiments on heat precipitation were therefore done on the fraction O_6 , which is rich in α -elastin.

Ionic strength and protein concentration. The extent of precipitation of O_6 from solution in acetate buffer (pH 5.0) at 40° was the same at ionic strengths 0.15 and 0.07 and at protein concentrations 1.0 and 0.5%.

Solubility of precipitates. The precipitates dissolved slowly if kept in buffer at 4° for a long time. Precipitates were prepared by heating samples (3 ml.) of 1% (w/v) solutions of O_6 in acetate buffer, pH 5.0, at 40° for various times. The extent of precipitation was determined and the precipitates were left in contact with 3 ml. of fresh buffer for 4 days at 4° with occasional stirring. The amount of precipitate which had redissolved was measured. The results (Table 3) indicate that precipitates prepared by heating for 23 hr. dissolved much more slowly than those prepared by heating for shorter periods, and also that elastin precipitated at ionic strength 0.075 was more soluble than that precipitated at ionic strength 0.15.

Table 2. *Extent of precipitation of elastin in different buffers for different times of heating*

Buffers: pH 3.2, glycine hydrochloride; pH 5.1, sodium acetate; pH 7.0, sodium phosphate. Ionic strength 0.2. Temperature 40°.

Elastin fraction ...			O_3			O_4			O_5			O_6		
Time of heating (hr.)			1	5	16	1	5	16	1	5	16	1	5	16
Elastin pptd. (% original wt.)	{ pH 3.2		2	13	14	23	—	32	27	47	31	18	38	36
	{ pH 5.1		4	16	15	9	25	25	19	49	50	33	41	32
	{ pH 7.0		5	9	3	2	10	6	6	12	6	5	1	0

Table 3. *Solubility of precipitated elastin*Elastin 0₆, 1% (w/v) in acetate buffer, pH 5.0. Temperature 40°.

Ionic strength	Time of heating (hr.)	Elastin precipitated (% of original amount in solution)	Elastin redissolved in fresh buffer after 4 days at 4°	
			(% of the precipitated elastin)	(% of original amount in solution)
0.15	3	20	86	17
0.15	23	49	8	4
0.075	6	39	60	23
0.075	23	51	29	15

Precipitates prepared by heating solutions of 0₆ at pH 5.0 for 16 hr. and ionic strength 0.15 were soluble in formic acid (98–100%), in dichloroacetic acid and in *m*-cresol. If, however, they were washed with water and then with ethanol and the ethanol was removed *in vacuo*, they became insoluble in these media although they swelled markedly.

Precipitation by other methods

The soluble elastins dissolved readily in formic acid (98–100%), and more slowly in dichloroacetic acid, to give viscous solutions. When the solutions in formic acid were diluted with 3 vol. of water at room temperature coacervates formed which settled to the bottom of the flask to form viscous layers after 16 hr. They coagulated on stirring to give an elastic mass. When solutions in dichloroacetic acid were diluted in the same way a fibrous friable precipitate formed, which did not coagulate on stirring.

Gel-like precipitates could also be prepared by acidifying a solution of 0₆ in 0.2 M-NaCl with N-HCl to pH 1.0 at room temperature and allowing to stand for 16 hr. The amount of elastin precipitated by this procedure was increased by the presence of up to 20% of ethanol, but when there was more than 60% of ethanol present no precipitate formed.

The precipitates formed by these methods were insoluble in water even after prolonged washing but dissolved readily in formic acid. In view, however, of difficulty experienced in removing acid completely from precipitates prepared by these methods further study was restricted to heat-precipitated elastin.

Some properties of heat-precipitated elastin

Samples of heat-precipitated 0₆ were prepared for physical measurements as follows: A solution (80 ml.) of 0₆ (0.6%, w/v, in acetate buffer, pH 5.0, ionic strength 0.15) was heated to 55° in a stainless-steel tube, internal diam. 3 cm., whose lower end was closed by a removable polystyrene disk. After 16 hr. most of the coacervate had collected on the disk and after cooling was removed from it with a scalpel as a yellow translucent gel. The gel was

Fig. 1. First, second, third and sixth cycles of a series of load-extension cycles for a strip of heat-precipitated elastin of cross-sectional area about 0.02 cm.² immersed in water at 18°. Each increment of load and measurement of length took about 1 min. Thus the time taken for the sixth cycle was approx. 12 min.

carefully washed with water and then with aqueous ethanol, the ethanol concentration gradually being increased to 100%. The gel was finally dried *in vacuo* to give an opaque, brittle disk about 1 mm. thick. When immersed in ethanol or water it swelled and became elastic.

Elastic properties. A disk of precipitate was soaked in ethanol, cut into strips and washed thoroughly with water, and load-extension curves were determined. Fig. 1 shows a series of load-extension cycles in which the maximum extension was gradually increased. There was slight hysteresis, but even after 50% extension the strip returned almost to its original length. The Young's modulus of the strip was about 5×10^4 dynes/cm.² and its extension at break was about 120%. The thermo-elasticity of precipitated elastin was studied by determining load-extension curves of a single strip at different temperatures. The variation of tension with temperature at constant length calculated from these data is shown in Fig. 2. The strip had previously been stretched 30% and allowed to retract in order to eliminate the small irreversible extension shown in Fig. 1.

Fig. 2. Load-temperature curves at constant length derived by interpolation from load-length curves for a single strip of heat-precipitated elastin immersed in water at four different temperatures. Approximate cross-sectional area 0.02 cm^2 . The length in cm. is shown against each curve.

Fig. 3. Analysis of load (f)-extension curves of elastin into contributions (f_E and f_s) due to changes of internal energy and entropy. Data are shown for heat-precipitated elastin at 30° ($\circ$), calculated from data given in Fig. 2, and for collagen-free elastin at 30° ($\square$), calculated from similar unpublished data. In order to facilitate comparison, the values of f , f_E and f_s for collagen-free elastin have been multiplied by 5.5×10^{-3} so that its load-extension curve is coincident with that of heat-precipitated elastin.

Thermodynamic analysis of elasticity. When any material is stretched the equilibrium tension (f) may be expressed in the following form (see, for example, Treloar, 1949):

$$f = (\partial E / \partial L)_{T, v} - T(\partial S / \partial L)_{T, v} = f_E + f_s,$$

where f_E and f_s are the contributions to f due respectively to change of internal energy (E) and

entropy (S) with length (L) at constant temperature (T) and volume (V). Since

$$(\partial S / \partial L)_{T, v} = -(\partial f / \partial T)_{L, v}$$

knowledge of f , T and $(\partial f / \partial T)_{L, v}$ enables one to calculate f_s and f_E . Application of these equations to the data shown in Fig. 2 leads to the values of f_s and f_E shown in Fig. 3. The figure includes data derived in a similar way from experiments on a strip of ox *ligamentum nuchae* from which the collagen had been removed by treatment with boiling 1% (v/v) acetic acid.

X-ray diffraction. Dr W. G. Horton of Bradford Technical College examined the dried gel by wide-angle X-ray diffraction with the beam perpendicular to the plane of the gel. A pattern characteristic of an amorphous structure with diffuse rings at 4.4 and $9 \text{ \AA}$ was obtained.

Solubility. Like the other dried elastin precipitates the dried elastin gel was insoluble in aqueous buffer solutions, formic acid (98–100%), dichloroacetic acid and *m*-cresol at room temperature. To test the solubility of the dried elastin gel in hot water or hot 1% (w/v) acetic acid, in which solvents native elastin is insoluble, samples (100 mg.) were boiled under reflux with 5 ml. of solvent. At 6 hr. intervals undissolved material was removed by centrifuging and treated with a further 5 ml. of solvent, and the protein content of the extract determined. After six extractions water had dissolved only 5% of the material. Acetic acid dissolved 50% in the first extraction and after six extractions over 90% had dissolved. Similar experiments with purified elastin showed that six extractions with either solvent dissolved less than 5% of the material.

Susceptibility to enzymes. Samples of precipitated elastin (50 mg.) were immersed in 3 ml. of (i) a solution of the proteolytic elastase fraction E_2 (Hall, 1957a) (0.03 mg./ml. in borate buffer, pH 8.4), or (ii) a solution of trypsin (0.03 mg./ml. in phosphate buffer, pH 7.1). Elastase dissolved the material completely in 1 hr. at room temperature (18°), whereas a similar weight of purified elastin dissolved only after several hours at 37° . Trypsin dissolved precipitated elastin completely within 16 hr. at 37° but under the same conditions dissolved only 4% of a sample of purified elastin. Neither buffer on its own dissolved precipitated elastin.

Staining properties. Precipitated elastin appeared globular under the microscope. When stained with the Weigert stain some areas stained red like the whole of the elastin fibres of native *ligamentum nuchae*, but there was a greater proportion of dark-staining areas. Dark-staining areas were also observed in purified elastin but to a smaller extent.

DISCUSSION

Heat-precipitated elastin resembles purified elastin in the following properties: (i) both show long-range elasticity, the same shape of load-extension curve and approximately the same extension at break (Astbury, 1940; Burton, 1954; Wood, 1954); (ii) analysis of their thermo-elastic behaviour shows that the elasticity of both materials is of a type found in only a few fibrous proteins (Meyer & Ferri, 1936; Wöhlisch, Weitnauer, Grüning & Rohrback, 1943; Lloyd & Garrod, 1946). The tension is composed of a high, positive, entropy contribution and a high, negative, internal-energy contribution; (iii) both materials give similar X-ray-diffraction patterns (Astbury, 1940); (iv) both are insoluble in all the solvents considered except hot 1% (w/v) acetic acid, which dissolves precipitated elastin; (v) both are dissolved by elastase (Balo & Banga, 1950; Hall, 1957*a*) but precipitated elastin is dissolved more rapidly; (vi) both stain in a similar manner with the Weigert stain.

The two materials differ in the following respects: (i) the Young's modulus of precipitated elastin is only about one-hundredth of that of purified elastin (Burton, 1954); (ii) precipitated elastin is dissolved by hot 1% (w/v) acetic acid and by trypsin but purified elastin is not (Balo & Banga, 1950; Partridge & Davis, 1955); (iii) precipitated elastin is non-fibrous, unlike purified elastin.

The similarity between the X-ray diagram, insolubility and elasticity of precipitated and purified elastin, together with the fact that α -elastin has an amino acid analysis almost identical with that of purified elastin (Partridge & Davis, 1955), strongly suggests that in the two materials the molecules have essentially the same configuration. To this extent heat-precipitated elastin may be termed reconstituted elastin. The X-ray pattern indicates an amorphous structure and distortion of such a structure by stretching would be expected to give rise to the high positive-entropy tension that is in fact observed. The high negative internal-energy tension might be due to molecular repulsions between the bulky non-polar amino acid side chains, of which there are many in elastin (Partridge & Davis, 1955). In this respect elastin resembles casein (Waugh, 1954), fibres of which also show hyperentropic elasticity (Woods, 1946). Recently Ramachandran & Santhanam (1957) have suggested that elastin has a small degree of ordered structure, and Flory (1956) has shown that hyperentropic elasticity might arise from such a structure.

The differences between reconstituted and purified elastin indicate that although the arrangement

of the molecules appears to be the same in the two materials, some factor or factors which stabilize purified elastin are lacking in reconstituted elastin. The solubilization of elastin by oxalic acid is accompanied by the breaking of peptide linkages and release of aspartic and possibly glutamic acid (Adair *et al.* 1951). End-group studies (Partridge & Davis, 1955) indicate that the number-average chain length of elastin may be reduced 100-fold during solubilization. The length of the polypeptide chains in reconstituted elastin is therefore probably much lower than in purified elastin and this would be expected to result in differences of Young's modulus, solubility in the solvents studied and resistance to the solubilizing action of proteolytic enzymes such as are observed. Breaking of cross-linkages of purified elastin (Partridge & Davis, 1955) during solubilization would be expected to have a similar effect.

Those properties held in common by reconstituted and purified elastin must be characteristic of the protein of which both are composed. The results indicate therefore that in purified elastin these properties are not due to the fibrous nature of the material. In view of the way in which it is precipitated, reconstituted elastin probably does not have a fibrillar fine structure like that observed in native elastin (Lansing, Rosenthal, Alex & Dempsey, 1952; Hall, Reed & Tunbridge, 1954). Nor is it likely to consist of a protein core surrounded by a sheath of different composition, as has been suggested for native elastin (Hall, Reed & Tunbridge, 1952; Hall, 1957*b*). The common properties (elastic behaviour, insolubility, staining with the Weigert stain, X-ray-diffraction pattern) cannot therefore be due primarily to either of these two-component structures. Such structures might, however, contribute to the stability of native elastin and their importance for other properties is not excluded (see, for example, Saxl, 1957).

Consideration of the conditions of heat precipitation shows that the longer the time of heating the greater is the extent of precipitation and the lower the rate at which the precipitated elastin redissolves. This indicates that heat coacervation, which is rapid and reversible, is followed by a slower process which retards resolution. Partridge *et al.* (1955) suggested that coacervation of elastin is due to unfolding of the molecules and their subsequent aggregation. The slow process is probably increase in the extent of aggregation. Covalent linkages are probably not formed to any great extent during this process since the resulting precipitates dissolve in hydrogen-bond-breaking agents such as formic acid and dichloroacetic acid and become insoluble in these solvents only after being washed with ethanol.

The present work shows that a soluble degradation product of elastin can be converted into an insoluble form, having many of the characteristic properties of the original elastin. The way in which elastin is formed *in vivo* is not known with certainty but it is at least a possibility that it is formed from a soluble precursor by a mechanism similar to reconstitution.

SUMMARY

1. The high-molecular-weight α -fraction of soluble elastin prepared from ox *ligamentum nuchae* (Adair *et al.* 1951) becomes insoluble on prolonged heating of aqueous solutions under certain conditions.

2. Heat-precipitated elastin has many of the physical properties of purified elastin and it is concluded that the protein molecules have essentially the same configuration in the two materials.

3. Differences between heat-precipitated (reconstituted) elastin and purified elastin may be attributed to degradation of the latter when it is converted into soluble elastin.

4. The precipitation of elastin appears to consist of two processes, namely a rapid one which may involve configurational changes and results in coacervation and a slow one which is probably due to aggregation of the protein molecules.

5. The bearing of the results on the structure of elastin and its fibrogenesis is discussed.

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REFERENCES

- Adair, G. S., Davis, H. F. & Partridge, S. M. (1951). *Nature, Lond.*, **167**, 605.
 Astbury, W. T. (1940). *J. Soc. Leath. Tr. Chem.* **24**, 69.
 Baló, J. & Banga, I. (1950). *Biochem. J.* **46**, 384.
 Bowen, T. J. (1953). *Biochem. J.* **55**, 706.
 Burton, A. C. (1954). *Physiol. Rev.* **34**, 619.
 Flory, P. J. (1956). *J. Amer. chem. Soc.* **78**, 5222.
 Hall, D. A. (1955). *Biochem. J.* **59**, 459.
 Hall, D. A. (1957*a*). *Arch. Biochem. Biophys.* **67**, 366.
 Hall, D. A. (1957*b*). *Connective Tissue, C.I.O.M.S. Symp.*, p. 238. Ed. by Tunbridge, R. E., Keech, M. K., Delafresnaye, J. F. & Wood, G. C. Oxford: Blackwell Scientific Publications.
 Hall, D. A., Reed, R. & Tunbridge, R. E. (1952). *Nature, Lond.*, **170**, 264.
 Hall, D. A., Reed, R. & Tunbridge, R. E. (1954). *Exp. Cell Res.* **8**, 35.
 Lansing, A. I., Rosenthal, T. B., Alex, M. & Dempsey, E. W. (1952). *Anat. Rec.* **114**, 555.
 Lloyd, D. J. & Garrod, M. (1946). *Fibrous Proteins, Soc. of Dyers and Colourists Symp.*, p. 24.
 Meyer, K. H. & Ferri, C. (1936). *Arch. Physiol.* **238**, 78.
 Partridge, S. M. & Davis, H. F. (1955). *Biochem. J.* **61**, 21.
 Partridge, S. M., Davis, H. F. & Adair, G. S. (1955). *Biochem. J.* **61**, 11.
 Ramachandran, G. N. & Santhanam, M. S. (1957). *Proc. Ind. Acad. Sci. A*, **45**, 124.
 Saxl, H. (1957). *Gerontologia*, **1**, 142.
 Seifter, S., Dayton, S., Norrie, B. & Muntwyler, E. (1950). *Arch. Biochem. Biophys.* **25**, 191.
 Treloar, L. R. G. (1949). *Physics of Rubber Elasticity*, p. 19. Oxford: Clarendon Press.
 Waugh, D. F. (1954). *Advanc. Protein Chem.* **9**, 342.
 Wöhlisch, E., Weitnauer, H., Grüning, W. & Rohrback, R. (1943). *Kolloidzsch.* **104**, 14.
 Wood, G. C. (1954). *Biochim. biophys. Acta*, **15**, 311.
 Woods, H. J. (1946). *J. Colloid Sci.* **1**, 407.

The Site of Protein Synthesis in *Bacillus megaterium*

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In recent years much work has been done on the incorporation of isotopically labelled amino acids into the various fractions of mammalian cells. It has been found that the ribonucleoprotein particles associated with the microsomal fraction become labelled at a very early stage (Borsook, Deasy, Haagen-Smit, Keighley & Lowy, 1950; Keller, Zamecnik & Lofffield, 1954; Littlefield, Keller, Gross & Zamecnik, 1955; Simkin & Work, 1957;

Cohn & Butler, 1957), although evidence for a preliminary activation process in the 'soluble fraction' has been given (Hoagland, Keller & Zamecnik, 1956; Hoagland, Zamecnik & Stephenson, 1957). On the other hand, little information has been obtained about the sites of protein synthesis in the bacterial cell. It has, however, been shown that in *Torulopsis utilis* absorbed amino acids do not exist in the free state at all but are bound in such a way