collagen molecule are separated and each chain adopts a randomly coiled configuration[2,3]. As the content of the pyrrolidine residues increases, presumably the number of pyrrolidine—pyrrolidine links between the glycine residues (which in the latest collagen models appear at every third residue position along each polypeptide chain for stereochemical reasons) also increases, and there is an increasing tendency, at least along short lengths of the polypeptide chains, for a poly-L-proline II type configuration[8] to be maintained. In the solid state the poly-L-proline II helix is known[8] to be left-handed with an exact three-fold screw axis in the chain direction; recent polarimetric studies have shown that this configuration is largely maintained in solution. Aqueous solutions of poly-L-proline II are characterized by very high lævo-rotatory power ($[\alpha]_D^{25} \sim -500°$).

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Some Improvements in Colorimetric Determination of Elastin

A METHOD was previously developed[1] for the direct colorimetric estimation of elastin by treating a sample of tissue with orcein. All free stain is removed and elastin hydrolyzed with elastase (pancreatin). The stain which is brought in this way into solution is then photometrically estimated.

The method is reported here with some further improvements that made it possible to determine age variations in the elastin content of human lung[2] and aorta[3].

The following reagents are used: (1) 95 per cent ethanol containing 0·1 ml. concentrated hydrochloric acid per 100 ml.; (2) orcein stock solution: orcein 1 gm., anhydrous ethanol 100 ml., concentrated hydrochloric acid, 1 ml.; (3) before use, dilute 1 ml. stock solution with 9 ml. reagent 1); (4) glycocoll-NaCl-NaOH solution (Sörensen buffer), *p*H 9·5 at 38°C.; (5) 4 per cent pancreatin solution, prepared before use with Sörensen buffer 4). Filter until thoroughly clear.

Defatting and drying the tissues. The tissues (about 1·5 gm. fresh weight) are finely minced to 2-mm. particles and washed with water to remove blood. The tissues are then extracted at 40°C. for 8 hr. with 3 changes of 100 ml. acetone and dried at 70°C. for 12 hr.

Maceration. The dry matter is finely triturated in a mortar and a sample 30–50 mgm. is macerated in a 25-ml. glass-stoppered tube with 10 ml. 0·1 *N* sodium hydroxide at 25°C. for 8 hr. with frequent mixing.

Staining and washing. The tube is centrifuged and the residue suspended twice in 10 ml. of acid ethanol (reagent 1) at 38°C. for 30 min., centrifuged and treated with 20 ml. orcein solution (reagent 3). Staining is carried out at 25°C. for 24 hr. with occasional stirring, then the mixture is centrifuged and the residue repeatedly washed with 20 ml. acid ethanol (reagent 1) at 38°C. until the free colour is completely removed. A first washing of 2 hr., a second of 12 and a third of 5 are generally sufficient. Ethanol is removed by centrifuging and washing the residue with 10 ml. of buffer (reagent 4) at 38°C. for 1 hr. The buffer is then replaced by exactly 5 ml. of fresh buffer.

Enzymatic digestion and colorimetric estimation. Digestion is carried out by adding to the tube 5 ml. of 4 per cent pancreatin solution (reagent 5) and incubating with frequent stirring at 38°C. for 1 hr.

All undigested materials are centrifuged. A slight turbidity due to the pancreatin solution which may persist has no appreciable effect. Optical density is read at 590 mμ. As a blank in the colorimetry, a control tube with 5 ml. buffer and 5 ml. pancreatin solution is treated in the same way. Colour is completely stable.

The percentage of elastin in the sample may be obtained from the following formula (previously calculated by a calibration curve with pure elastin[1]):

$$\text{Percentage elastin} = \frac{D \times 1540}{P}$$

where D is optical density and P the dry weight of the sample in mgm.

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Biosynthesis of Corynomycolic Acid from Two Molecules of Palmitic Acid

CORYNOMYCOLIC acid, $C_{32}H_{64}O_3$, m.p. 70°, $[\alpha]_D = +7·5°$ has been isolated from the lipids of *Corynebacterium diphtheriæ*[1] and *C. ovis*[2]. Its structure (III, R = H) has been established by degradation[3] and confirmed by synthesis: the condensation of two molecules of methyl palmitate (I, $R = CH_3$) in presence of NaH gives the β-ketoester (II, $R = CH_3$) which is reduced by $NaBH_4$ to a mixture of two diastereoisomers (III, $R = CH_3$)[4]; one of these has been shown to be racemic methyl corynomycolate[5].

The presence of free palmitic acid and of palmitone (IV)[6] in the lipids of *C. diphtheriæ* as well as previous work on the mycolic acids of Mycobacteriæ[7] suggested that corynomycolic acid (III, R = H) is formed *in vivo* by the condensation of two molecules of palmitic acid (probably as palmitoyl-coenzyme *A*) and reduction of the intermediate β-ketoester (II). Palmitone (IV) could be formed through saponification and decarboxylation of the β-ketoester (II)[8,9].

The following experiments seem to prove that corynomycolic acid is, in fact, synthesized *in vivo* from two molecules of palmitic acid.

C. diphtheriæ (strain Parke–Williams 8) was grown in 500 ml. of Loiseau–Philippe medium containing 10 μc (total activity $14·4 \pm 2·0 \times 10^5$ counts/minute) of the potassium salt of $1-^{14}C$ palmitic acid (specific activity $43·1 \pm 6·0 \times 10^7$ counts/min./m. mole). After 12 days at 37°, the cells were centrifuged, washed and extracted with alcohol-ether (1 : 1).

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