The linoleic acid content of the diets containing 10 per cent linseed fatty acids or 2 per cent ethyl linoleate is about the same (1.7 per cent and 1.5 per cent respectively). Nevertheless, the percentage of linoleic in the liver of chickens fed the linseed fatty acids was approximately twice the level observed in the livers of the birds fed 2 per cent linoleate. The arachidonic acid was significantly ($P < 0.05$ by the t test) lower in the birds consuming linseed oil fatty acids. Since arachidonic acid is synthesized from linoleic acid³ our observations suggest that the linolenic acid in the linseed fatty acids is inhibiting the conversion of linoleic acid to arachidonic acid.

This hypothesis, however, should be confirmed by the feeding of pure linolenic acid and preferably by the use of carbon-14 labelled linoleic acid.

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Elastin a Sialoprotein

THE aorta consists of elastin, collagen and a number of other proteins; it also contains about 2 per cent of carbohydrate¹. Elastin is usually isolated from aorta or ligamentum nuchae by treatment with acids and alkalis at elevated temperatures; however, a small amount of ill-defined carbohydrate appears to be held strongly and cannot be easily removed². It is possible that this sugar moiety is an integral part of elastin.

The object of the work reported here was to isolate a fragment containing the elastin-sugar linkage using very mild conditions, and to investigate its nature.

A bovine aorta was cleaned, minced in the cold and treated in turn with pepsin at pH 2.5, trypsin at pH 7.5, and collagenase at pH 7.0. The residue was washed well after each treatment, which lasted 30–40 hr. at 37°. The tryptin digest contained most of the acid mucopolysaccharide, as judged by the content of hexosamine and hexuronic acid. The final residue, which accounted for about 50 per cent of dry aorta, was then treated exhaustively with pure elastase at pH 8.5 (preparation E₂I, Hall and Czerkawski³), which resulted in more than 90 per cent solubilization. Dialysis of the solubilized material gave approximately equal amounts of diffusate and retentate.

Although both fractions of elastolysate had similar amino-acid composition, the diffusate was devoid of carbohydrate. The retentate, which is practically resistant to further action by elastase, contains about 0.5 per cent of a substance giving the usual reactions for sugars. It contains no glucosamine or galactosamine, but approximately equal amounts of sialic and hexuronic acids. The presence of the former was first indicated by considerable humin formation during hydrolysis in strong mineral acid, and finally confirmed by the positive Bial and direct Ehrlich reactions. The actual amount of sialic acid was estimated after a mild acid hydrolysis (1 hr. at 80° in 0.1 N sulphuric acid) by the thiobarbiturate and alkali-Ehrlich methods of Aminoff⁴. Such hydrolysis did no damage to the protein and certainly did not remove the hexuronic acid (Table 1, col. 3), and although it made the sialic acid reactive to thio-

barbiturate, probably due to hydrolysis of the glycosidic bond on C2, it did not produce a complete cleavage, because the sugar could not be separated from the residual protein by dialysis. When the products of mild hydrolysis were subjected to dialysis, no sialic acid could be detected in either the diffusate or retentate. The reactivity of sialic acid with the thiobarbiturate reagent could be restored when the retentate was again hydrolysed for 1 hr. at 80° in 0.1 N acid. This can be explained by supposing that the mild acid hydrolysis breaks only one of two linkages joining sialic acid to elastin, and that subsequent dialysis results in the reformation of this bond (Table 1, col. 2).

Table 1. ANALYSIS OF SUGAR COMPONENTS AND REACTIVITY TO ELASTASE OF THE RETENTATE FRACTION

Treatment	Apparent content of sialic acid (per cent)	Content of hexuronic acid (per cent)	Elastolytic activity (arbitrary units)
None	0.02	0.23	7.0
Mild acid hydrolysis	0.29	0.23	19.8
Dialysis (retentate)	0.02	0.19	12.0
Another hydrolysis (retentate)	0.23	—	—

The sialic acid was determined by Aminoff's method (ref. 4), and the hexuronic acid was determined directly by the method of Bitter and Ewins (ref. 5). The elastolytic activity was determined by the trichloroacetic acid precipitation method as described by Hall and Czerkawski (ref. 6).

The ability to react with thiobarbiturate is paralleled by the susceptibility of the retentate fraction to further attack by elastase. The mild acid hydrolysis makes the protein susceptible to further proteolytic attack, but this newly acquired susceptibility is greatly reduced after dialysis (Table 1, col. 4).

It is too early to venture any firm conclusions at this stage; but there is every indication that sialic acid is doubly linked to elastin and so is a cross-linking agent, probably in association with the hexuronic acid. It is possible, that it occupies a strategic position, because, although present in such small amount (about 1 mole/10⁵ gm.), it plays an important part in the reaction with elastase. This last fact also makes it unlikely that the sialic and hexuronic acids are part of a large polysaccharide polymer; on the contrary, they are probably uniformly distributed throughout the solid elastin.

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m-Hydroxyphenylpropionic Acid, a Major Urinary Metabolite of (+)-Catechin in the Rat

ALTHOUGH substances of the flavonoid type are present in many foodstuffs of plant origin the metabolic fate of these compounds in mammalian metabolism was until recently largely unknown. The compounds on which recent investigation has been centred, however, are of either the flavonol^{1–5}, the flavanone or the flavone type⁶. It appeared to be of interest, therefore, to study the fate of a compound of the related catechin type, in which the keto group at C4 is absent.