was moderately toxic although apparently less toxic than many of the other antihistaminics in mice as judged from published lethal doses in the literature(8). In guinea pigs the ratio of toxic to therapeutic doses indicated a high margin of safety. In rabbits this compound had the same toxic dosage level as other antihistaminics. 3. Rats fed diets containing as high as 0.1% of the drug in the diet for 4 weeks gained weight comparable to the weight of control rats fed normal ration. Higher concentrations impaired growth but produced no visceral damage as shown by hematological and pathological studies. 4. Dogs given large daily doses of 'Pyronil' for 4 weeks, aside from the presence of occasional red cells in the collecting tubules of the kidney, showed no significant pathological changes attributable to the drug.

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Some Factors Involved in the Fibrogenesis of Collagen *in vitro*.* (19657)

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In solutions of collagen (ichthyocol) obtained by filtration of dilute acetic acid extracts of carp swim bladder tunic, the collagen is dispersed as extremely thin filaments, or highly asymmetric particles, less than 50 A in width, rather than as cross-striated fibrils characteristic of native collagen(1). By suitable adjustment of ionic strength (usually with NaCl, though many other salts are effective) and of pH, cross-striated fibrils typical of native collagen as seen in the electron microscope, are precipitated(1-5). The authors previously reported(2) that reconstitution of collagen-type fibrils could be effected by addition to the acid filtrate of serum acid glycoprotein(6,7) in a concentration of about 10^{-9} M (0.001%) followed by dialysis against water. If the concentration of glycoprotein (GP) is higher (ca 10^{-7} M, or 0.05%), the reconstituted fibrils have the long-spacing (LS) pattern, with an axial period of about 2100 A, previously observed in the "procollagen" obtained by dialysis of acid citrate extracts of connective tissue(8). Because GP is so effective in reconstitution, it was suggested(2) that GP, or a component thereof, may be involved in the fibrogenesis of collagen. This effectiveness in reconstituting collagen-type fibrils in the absence of inorganic ions does not, in itself, constitute evidence that GP plays any role in the natural process of collagen fibrogenesis. However, analytical evidence, too scanty to warrant description at the time, supported the view that certain of the components of glycoproteins and polysaccharides are present in the precipitated cross-striated fibrils, whether of the collagen- or LS-type.

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Further experimentation proceeded along 2 lines: 1) extensive chemical analysis of collagenous tissue sufficiently purified to remove the ground substance and other materials not firmly bound within the native fibrils; 2) extensive testing of the uniqueness of GP in its ability to reconstitute collagen- and LS-type fibrils from acid solutions of ichthyocol. This paper concerns the second aspect. The results support the view that the potentiality for reconstitution resides in the extracted fibrous material itself; various substances, including some which have no obvious relationship to glycoproteins or mucopolysaccharides, were found capable of inducing reconstitution.

Experimental. Ichthyocol solutions are prepared by allowing 0.6-0.8 g of scissor-minced, water-washed carp swim bladder tunics to stand in 100 ml of 0.05% acetic acid, pH 3.5, for 24 hours at 0°C, with occasional stirring, avoiding extensive comminution of the tunics and continuous stirring during extraction. The viscous suspension is passed, consecutively, through bolting cloth, coarse, medium and fine sintered glass filters. The final filtrate is clear and viscous, containing about 0.1% protein. It may be stored in the cold for several months. All filtrates are examined carefully with the electron microscope for intact fibrils. A rare striated fibril which slips through the filters may be tolerated. One ml of solution containing the substance to be tested (dissolved in acetic acid, pH 3.5), in graded concentrations ranging from 0.002% to 5%, was mixed with one ml of ichthyocol filtrate. Any precipitate which appeared within 30 minutes was examined in the electron microscope. All preparations were then dialyzed against distilled water at room temperature without removing any precipitates which may have formed. After dialyzing 12 to 18 hours, the preparations were reexamined.

The test substances may be grouped in 3 categories depending upon the type of response evoked.

1. *Reconstitution of collagen- and LS-type fibrils.* In this group are included: Serum acid glycoprotein(6,7), thrombin (Lilly, Seegers), papain, *Cl. histolyticum* proteinase (MacLennan), and ACTH (Armour, Parke-Davis). Upon mixing with ichthyocol filtrate no precipitate was produced. After dialysis against water, heavy precipitates appeared which, with the exception of those from the ACTH mixture, contained well-formed collagen- and LS-type fibrils. Only collagen-type fibrils occurred in the ACTH precipitates. Seegers'(9) data suggest that his thrombin preparations may contain a constituent having electrophoretic properties similar to those of GP. Solutions of the above substances may be heated at 95°C for 30 minutes, followed by treatment with 20% trichloracetic acid, without altering the capacity to reconstitute striated fibrils; if anything, the perfection of structure thus produced was greater than with the untreated substances. In the above experiments, when precipitation occurred on treatment with trichloracetic acid, the solutions were clarified by centrifugation, the supernates dialyzed and aliquots used for the experiment. GP is not precipitated by boiling or subsequent treatment with 20% trichloracetic acid.

2. *Reconstitution of unstriated and LS-type fibrils.* In this group are included: Sodium and potassium hyaluronates, calcium and potassium chondroitin sulfates (K. Meyer, R. W. Jeanloz), and heparin (Lilly). Immediate heavy, clumped, fibrous precipitates appeared on addition of the acid mucopolysaccharide solutions to the collagen filtrate in the absence of salt. Electron microscope examination revealed fibrils of a variety of sizes, anastomosing and forming sheets, but exhibiting no cross-striations. Dialysis against water did not alter this picture. One preparation of calcium chondroitin sulfate from cartilage (K. Meyer) produced some typical LS fibrils on addition to filtrate, but no collagen. Although these polysaccharides reconstitute chiefly unstriated fibrils, as reported previously(2), it was found that LS- but not collagen-type fibrils were produced when the reaction was carried out in the presence of salt (NaCl, $\mu = 0.17$). Under these conditions, pronounced opalescence with some fibrous precipitate appeared immediately. Examination revealed large masses of unstriated fibrils plus smaller clumps of typical LS forms. No collagen-type fibrils were seen. Subsequent

dialysis of the preparation against water produced no apparent change. These structures were observed over the entire range of concentrations of mucopolysaccharides tested.

3. *No reconstitution.* In this group are included: Crystallized bovine serum albumin, fibrinogen (Seegers), gliadin, and protamine sulfate. No precipitation or opalescence appeared upon mixing solutions of these substances with ichthyocol filtrates or after dialysis against water. Electron microscope examination revealed no fibrils.

As controls for all of the above experiments, ichthyocol filtrates were dialyzed against water. In all cases clear gels were produced in which very thin, unstriated fibrils and filaments were observed.

It is important to note that salt-reconstituted ichthyocol contains all the materials necessary for the production of LS- as well as collagen-type fibrils. This was demonstrated as follows: An ichthyocol solution was reconstituted by dialysis against 1% NaCl, pH 6-7. The saline-washed precipitate was extracted with citrate buffer ($\mu = 0.2$, pH 3.8) and the extract dialyzed against water. A fibrous precipitate formed which contained both collagen- and LS-type fibrils. Apparently the salt-reconstituted collagen contains something which is required for the production of LS-type fibrils. The fact that hexosamine and tyrosine have been demonstrated to be present in the salt precipitate may be significant in this connection.

Discussion. The above experimental results suggest that, in seeking to understand the mechanism of *in vitro* collagen fibrogenesis, it will be profitable to focus attention on the fibrous system extracted from the connective tissue rather than upon the substances which, under various conditions, may be able to induce the reconstitution of collagen- or LS-type fibrils. From the present results it is obvious that a variety of inorganic and organic compounds, under specified conditions of ionic strength and pH, may be able to cause the fibrous material to aggregate into fibrils having specific structural patterns. Such behavior requires the assumption that the test substances interact with one or another aspect of the structural organization inherently present in the extracted fibrous material.

Although the fibrous protein is doubtless responsible for the major structural properties of the extracted material as well as those of the native fibrils, it has long been known that a carbohydrate component is also present in skin collagen in a concentration of about 1% (10-12). The present authors have been able to show by analyses of purified cowhide collagen, to be described in detail elsewhere, that there is a carbohydrate-containing fraction in the collagen fibrils. This fraction includes hexoses (glucose, galactose, mannose), and hexosamine as well as tyrosine and tryptophan. Although these substances are constituents of the serum acid glycoprotein molecule, it has not yet been possible to isolate a glycoprotein from collagen fibrils demonstrably free of ground substance.

We have been able to demonstrate the presence of hexose, hexosamine and tyrosine in the fibrils reconstituted by salts from acid solutions of ichthyocol as well as in ichthyocol purified by thrice repeated cathodal electrodeposition (13) from acid solutions.

To what extent the carbohydrate-containing fraction is involved in the structural organization and fibrogenesis of collagen remains to be demonstrated.

Summary. 1. A variety of organic substances derived from animal and plant sources can induce the precipitation of collagen- and long-spacing-type fibrils from acid extracts of ichthyocol in a manner similar to that of serum glycoprotein. These fibril-precipitating agents appear to be thermostable and are not altered by subsequent exposure to 20% trichloracetic acid. 2. Hyaluronate, chondroitin sulfate and heparin produce fibrous precipitates directly on addition to ichthyocol filtrates. At low ionic strength these fibrils are unstriated. At ionic strengths of 0.1 to 0.2 (NaCl) some long-spacing-type fibrils are formed but collagen-type fibrils have not been observed. 3. The components necessary for the formation of cross-striated fibrils appear to be contained in the fibrous material extracted from the connective tissue; the action of the precipitating substances seems to be relatively non-specific under the conditions of these experiments.

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Reactions of the Vessels of the Rat Kidney after Experimental Occlusion of the Renal Artery for Various Periods. (19658)

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The kidney of the rat is well adapted to the study *in vivo* of gross vascular changes. The renal vessels of the rat appear to be especially sensitive to vasoconstrictor influences, and the vessels on the surface of the kidney are too few to interfere with color changes resulting from changes in the caliber of, and in the blood flow through, vessels of the superficial cortex. Therefore, we have used the rat in making gross observations concerning the reactivity of renal cortical vessels after varying periods of occlusion of the renal artery.

We previously observed that renal vasoconstriction appears to be limited to the vessels of the cortex(1,2). When the renal artery was suddenly completely occluded, the entire cortex of the kidney blanched severely, provided the vessels had not been manipulated previously. The cortical vessels evidently constricted briefly and squeezed the blood from the cortex. However, study of cut sections showed that the medulla retained its normal red color and its vessels remained filled with erythrocytes.

When the occlusion of the renal artery was released after only 5 to 15 seconds, the rat kidney appeared hyperemic for the next 10 to 30 seconds, as indicated by the bright pink color and the fullness of all vessels on the surface of the kidney when magnified and observed through a dissecting microscope. The kidney rapidly regained its normal color and appearance after this transient occlusion. Evidently, a brief period of hyperemia followed the brief period of ischemia.

When the occlusion of the renal artery was maintained for approximately one minute, after release of the occlusion the surface of the kidney appeared dark red or blue for 3 to 7 minutes, after which the normal color returned. If only one branch of the renal artery was occluded for a similar period, that portion of the kidney supplied by this vessel showed the congested appearance after release of the occlusion. The congested appearance of the portion of the kidney supplied by the vessel previously occluded could be contrasted with the normal appearance of the remainder of the kidney. After an intravenous injection of India ink, the region supplied by the vessel