

the gingivae, preservation of the odontoblasts to a metabolic state whereby normal dentin was produced, and cessation of bone resorption. In most cases the jaws of guinea pigs receiving the 10 mg dosage of 3-MC were indistinguishable from those animals receiving the L-ascorbic acid supplement.

Chemical analysis of L-ascorbic acid in various tissues from Groups 1, 5 and 6 indicated that 3-MC does not alter retention or synthesis of L-ascorbic acid. Experiments utilizing D-galactose-1-C¹⁴ virtually exclude the possibility of 3-MC induced L-ascorbic acid synthesis in scorbutic guinea pigs at least through known pathways(11).

The mechanism by which 3-MC reverses some of the signs of scurvy is not known. Other studies have shown that 3-MC exerts a stimulatory effect on protein synthesis and on the activity of certain enzyme systems(12, 13, 14, 15, 16). It is possible that some of the pathoses in scurvy result from impaired synthesis of certain proteins and/or a lessened activity of certain enzymes which are corrected in part by 3-MC.

Summary. 1. Some of the pathoses of scurvy in guinea pigs were reversed by administration of 3-methylcholanthrene. These included a marked reduction of incidence of hemorrhage, reduction of bone resorption, preservation of dentinogenesis and prevention of death of odontoblasts. 2. The effect 3-methylcholanthrene exerts upon scurvy does not appear to be mediated by induction of L-ascorbic acid synthesis nor by increased retention of the vitamin.

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Localization of Proelastase in the Goose-Fish (*Lophius piscatorius*).*

(26270)

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It has been suggested(1,2) that elastase (3) is localized in the pancreatic islets of Langerhans. These observations were made

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before the existence of proelastase(4) had been recognized. As a consequence very little activity could be measured. In view of the possible role of elastase in the etiology of atherosclerosis(5) it was thought of interest

TABLE I. Proelastase Assay of a Mesenteric Acinar Tissue Extract.

Elastin, mg	Extract, ml	Trypsin, ml	Buffer, ml	O.D.
50	.5	1	1.5	.604
50	.5	—	1.5	.127
—	.5	—	1.5	.137
50	—	1	2.0	.055
—	—	—	3.0	Blank

to determine the location of the proenzyme in the pancreas.

The Goose-fish, *Lophius piscatorius*, is a choice animal for such studies because the exocrine and endocrine portions of the gland are anatomically separated(6,7,8). The acinar portion is mainly located as bands of tissue in the mesentery, usually along the branches of the portal vein, while the islet portion is localized in major part as a single mass of tissue (principal islet of Rennie) near the origin of the cystic duct. In the capsule of the principal islet, however, several lobules of acinar tissue can be found. These are easily dissected by removal of the capsule which is attached to the islet only by loose connective tissue.

Approximately 500 Goose-fish were collected and dissected upon arrival at the laboratory. Three different fractions were separated and stored frozen for subsequent activity determinations: mesenteric acinar tissue, capsule of the principal islet and the central endocrine portion of the islet.

Determination of proelastase depends upon its activation by trypsin(4). The hydrolytic activity of the elastase produced is then measured, using a standardized preparation of elastin as substrate(9). The first step of the reaction is so rapid (~1 min) that it is carried out simultaneously with the second step.

Table I gives a typical example of a proelastolytic assay carried out on mesenteric acinar tissue. The capsules were ground in a hand homogenizer with Tris buffer 0.06 M, pH 9.15. The homogenate was lightly centrifuged to remove the strands of connective tissue and the supernatant used as the source of proenzyme. Six flasks were prepared as shown in the table. Trypsin was used at a concentration of 0.5 mg/ml dissolved in the

standard Tris buffer. It has been shown(9) that amount of elastase produced is proportional to concentration of trypsin until all the proelastase is activated. The concentration of trypsin used in these assays is about twice that necessary completely to activate 0.5 ml of a raw extract of acinar pancreas containing approximately 50 mg protein. Since Goose-fish elastase has an optimum pH in the range 9.1-9.5 similar to that of rat and hog elastase(9), all assays were performed at pH 9.15. After 20 minutes incubation at 37° the reaction was stopped by 2 ml of phosphate buffer, 0.7 M, pH 4.5 and 5 ml of water was added to each flask. The unhydrolyzed elastin was centrifuged out and a Lowry protein determination(10) was performed on 0.2 ml of the supernatant. The resulting optical density was read at 750 m μ .

The optical densities given in rows 2 and 3 (Table I) are similar. Without trypsin no elastolytic activity exists in the extract. These optical densities are a measure of protein concentration in the extract. The difference between optical densities in row 1 and those in rows 2 and 4 gives the optical density due to the elastin which has been hydrolyzed under the influence of the activated proelastase. This value can be converted to milligrams of elastin hydrolyzed by means of the relation established previously (9): O.D. = $0.0248 \times (\text{mg elastin hydrolyzed})$.

Concentrations of proelastase in the 3 ana-

TABLE II. Proelastase Activity in Tissue Extracts of Different Tissues.

Extracts	mg elastin dissolved in 20 min./100 mg dry tissue
Rat pancreas	19.7
Goose-fish islet	
Acinar tissue	83
Goose-fish mesenteric	
Acinar tissue	196-394
Goose-fish islet tissue (stored frozen)	0
Goose-fish islet tissue (fresh)	0
Cellular islets granules	
(a) whole	0
(b) treated with detergent	0
(c) " " trypsin	0

tomical fractions of the pancreas of the Goose-fish are reported in Table II. All activity is concentrated in the acinar tissue and is greater, on a specific basis, than that found in rat pancreas. The mesenteric fraction seems to be more active than the capsular fraction, probably because of the larger amount of connective tissue present in the capsule. The islet tissue was tested under a variety of conditions: fresh tissue, repeatedly frozen and thawed tissue, granular and supernatant fractions obtained by light centrifuging of the raw extract, as well as granular fractions disrupted by detergent or trypsin. In all cases no proelastolytic activity could be detected.

Since one of the earlier observations(1) describing elastase activity in the endocrine portion of the islet was made by examining elastin fibers under the microscope after 24 hours incubation with the extract, we have performed an assay under similar conditions. We found that 3.5 mg of elastin had been digested, an amount corresponding to 0.2 mg of elastin digested in 20 minutes per 100 mg of tissue. This negligible amount may be due either to a slight contamination by the

capsular proelastase or to bacterial contamination.

We conclude that in the Goose-fish, elastase and its precursor proelastase are produced in the exocrine pancreas, a location typical of a proteolytic enzyme.

Summary. A proelastase resembling that of the pig and the rat has been found in the pancreas of the Goose-fish. It is localized in the acinar portion of the pancreas, both in the mesenteric bands and in the capsule of the principal islet. However it is absent from the endocrine portion of this organ.

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Pasteur Reaction and Glycogen Content of Rat Brain After Noble-Collip Drum Shock.* (26271)

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Shock produced by any method results in systemic hypoxia. Several investigators have based their work on the influence of shock upon intracellular processes on this fact. They excised tissue from normal animals and subjected it to anoxic conditions. *e.g.*, Greig(1) and Furchgott et al.(2). They interpreted then the changes observed under anoxia *in vitro* as indicative of what ought to have happened in shock.

This report describes tests on the Pasteur

reaction with brain tissue of rats, obtained during shock.

Methods. The shock procedure has already been described(3). Only "taped" rats were drummed. Their weights ranged from 150 to 250 g. The brains were excised and homogenized in Potter-Elvehjem homogenizers(4) with Teflon pestles under ice cooling in Krebs-Ringer or bicarbonate media(5) for aerobic and anaerobic tests, respectively. Each gram of fresh brain was made up with 10 ml of medium. The nitrogen content of the homogenates was determined with a mi-

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