TABLE I. Excretion of C-14 Morphine, Counts/Hr.

Hr	I		II		III		IV	
	Morphine	Morphine and Nalline	Morphine	Morphine and Nalline	Morphine	Morphine and Nalline	Morphine	Morphine and Nalline
1	0	26	19	7	26	9	0	0
2	47	1580	11	766	11	1336	10	1036
3	28	0	375	43	13	0	0	28
4	1347	0	50	98	6	0	0	656
5	77	13	41	27	49	0	0	56
6	134	0	228	14	39	187	1189	164

TABLE II. Percentage of Dose Excreted.

Hr	I		II		III		IV	
	Morphine	Morphine and Nalline	Morphine	Morphine and Nalline	Morphine	Morphine and Nalline	Morphine	Morphine and Nalline
1-2	.022	76.5	.014	36.8	.017	64.0	.004	49.3
3-4	65.5	0	20.2	6.7	.9	0	0	32.6
5-6	10.0	.619	12.8	1.95	4.19	8.9	56.6	10.4

data obtained during this period will be presented here. The results of Table I and II were obtained in this study.

The data in Table I and II indicate that in each instance in which N-allyl normorphine was administered, a large portion of the injected dose appeared in the urine before the release of morphine by the control animal. These findings suggest that N-allyl normorphine produces in the morphinized animal effects in addition to the central stimulation for which it is well known. Such *secondary* effects could include(1) antagonism of antidiuretic hormone formation by the posterior pituitary or (2) facilitation of excretion by direct effect on the renal threshold. The antagonism by Nalline of morphine antidiuresis has been tested(5) and it has been found that Nalline significantly increases the volume of urine excreted by a group of morphine-treated animals as compared to morphinized controls. Other compounds have been found to antagonize the anti-diuretic action of morphine(5), and the mechanism of action of both these possible *secondary* effects is being studied further.

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Received November 2, 1953. P.S.E.B.M., 1953, v84.

Presence of Elastase in Teleost Islet Tissue.* (20753)

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Balo and Banga(1) recently described an enzyme, elastase, which solubilizes elastic tissue and is exclusively extractable from pancreas. Elastase, which is apparently specific for elastic tissue, acts upon the substrate without the release of peptides or amino acids. It appears to effect a conversion of this insoluble fibrous protein to a soluble globular form. Elastase has been effective in unravelling the chemical morphology of elastic fibers(2). Partially solubilized elastic fibers of horse ligamentum nuchae have been shown to contain fine, longitudinally oriented fibrils bound together by a matrix material of the same or

* This investigation was aided by a grant from the USPHS.

similar composition. Morphologically, such partially solubilized elastic fibers closely resemble the frayed and fragmented elastic fibers generally found in arteriosclerotic vessels. Balo and Banga have reported that a markedly lower amount of elastase is present in the pancreases of arteriosclerotic than in normal individuals(3). Examination of their data indicates, however, that the fall in elastase activity is associated at least as much with age as with arteriosclerosis.

There is some reason to question the assumption that elastase is a component of the acinar portion of the pancreas. The yield of elastase on extraction of whole pancreas is small compared to known acinar enzymes. Beef pancreas is a good source of elastase. Being a herbivore, it is difficult to picture a digestive function for elastase in this animal. Further, analysis of human pancreatic juice has not indicated the presence of elastase activity(4) and it is well known that elastic fibers pass through the alimentary tract with no apparent digestion. These, and other questions, led us to explore the possibility that elastase is a component of the islet tissue of the pancreas.

Methods. The teleost fish, *Lophius piscatorius,* is particularly suited for analysis of islet and acinar tissue of the pancreas since these two components are anatomically separate [Rennie(5); Macleod(6); McCormick (7)]. The islet tissue in Lophius exists as discrete nodules as much as 1 cm in diameter containing both alpha and beta cells(8). A distinct capsule is present with small islands of acinar tissue sandwiched between its layers of connective tissue. This capsule is readily removed by blunt dissection. Two large or principal islets and several smaller ones are present in the individual fish and are readily located in the mesenteries. Approximately 700 *Lophius piscatorius* (goose, monk, or angler fish) were collected at sea by a commercial dragger, placed on ice, and brought to the laboratory for removal of the islets. To check on a possible postmortem loss of elastase activity a limited number of newly caught fish were dissected at sea (by AIL) and the islets were stored in acetone for later fractionation. The bulk of the fish used varied in weight from 10 to 35 lb but a small number of small fish (1 to 10 lb) were used. No fewer than the two principal islets (1 cm in diameter) were collected and usually 2 to 4 of the smaller islets were large enough to be removed intact. After dehydration in acetone the connective tissue capsule was removed and stored separately. This capsule, as already indicated, includes small amounts of acinar tissue.

The pure islet tissue obtained after ecapsulation was ground to a fine powder with a mortar and pestle followed by a ground glass tissue homogenizer. Following established procedure(2) the islet powder was extracted with phosphate buffer at pH 6.0. This crude extract was either tested directly for elastase activity or else the elastase was salted out from it with $(NH_4)_2SO_4$ at 0.4 saturation. In the latter case, the salted out elastase was dissolved for use in carbonate buffer at pH 9.0 (optimum for elastase activity). The substrate was a standardized preparation of elastin from horse ligamentum nuchae(2). The elastase activity of fish islet powder and of acinar tissue in the islet capsule was compared with elastase prepared from equal amounts of mammalian pancreatic powder (Viokase, Viobin Corp.). Solubilization of the elastin fibers was observed with the aid of the compound microscope.

Mammalian elastin was readily solubilized by the extracts of the fish islets. Strong elastolytic activity was exhibited by samples extracted from as little as 100 mg of dried, defatted islet tissue. On the basis of rate of solubilization of elastin, the fish islet extract was more active than mammalian elastase prepared from equivalent amounts of mammalian pancreatin (Viokase). There was some difference in the course of action; although mammalian elastase does not begin to act on the elastin as rapidly as does the fish islet elastase, the former carries the solubilization of elastin to completion within a few hours while fish elastase may require as much as a few days.

Extracts prepared from the capsules of islets which contain acinar cells were completely inert with respect to elastolytic activity in so far as could be determined by the method of direct observation.

Discussion. In Lophius, it seems that elas-

tase is confined to the islet tissue and that its mode of action differs somewhat from that of mammalian elastase. The latter may be due to species differences in the enzyme or the substrate, or both. The fact that elastase may be found in such divergent forms as fish and mammals indicates need for further study of the phylogenetic distribution of this enzyme and of a possible basic function for elastase. Experiments are currently being conducted to determine if elastase is also a component of the islet tissue of mammals.

Summary. Islet tissue of the teleost fish, *Lophius piscatorius,* contains elastase whereas acinar tissue seems to be free of this enzyme.

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Received November 23, 1953. P.S.E.B.M., 1953, v84.

Antigenic Differences Among Strains of Newcastle Disease Virus.* (20754)

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Newcastle disease, first recognized in the Dutch East Indies in 1926, is now known throughout the poultry raising regions of the world. Described in the Far East as a highly fatal infection of chickens, the virus evaded early recognition in many regions by appearing as a mild respiratory disease. In spite of the variations in pathogenicity and in other properties, most investigators have found that strains of the virus whether isolated in Asia, Africa or America are antigenically homogeneous(1,2). Contrary evidence is scanty and based on the relative immunogenicity of vaccines(3,4). The repository of strains of Newcastle disease virus assembled at the University of Wisconsin under the auspices of the Bureau of Animal Industry and the North Central Regional Technical Committee on Newcastle Disease provided material for an investigation of antigenic diversity among 13 strains of the virus. The cultures available had been isolated in many localities in the United States, Canada, and Mexico and from a few places in Europe and Australia over a period of 20 years.

* Published with the approval of the Director of the Wisc. Exp. Station Paper NS 127.

Methods. The relative abilities of homologous and heterologous antisera to neutralize the embryo infectivity of the virus were compared. The immune sera were obtained from chickens and rabbits about 21 days after injection of virus and used unpooled. The rabbit serum was inactivated at 56°C for 30 minutes and chicken serum was used uninactivated. Infected allantoic fluids kept at −20°C were used as the virus source. Serial ten-fold dilutions of virus which had been incubated 30 minutes at room temperature with undiluted antiserum were inoculated intrallantoically in 0.1 ml amounts into 10- or 11-day embryonating eggs. Six eggs were used for each dilution, and all eggs candled twice daily for 7 days. The number of 50% lethal doses of virus neutralized by each serum was calculated by the procedure described by Smadel(5). On the basis of replicate tests prepared simultaneously by 2 individuals using the same stock virus and same serum, a difference of greater than one log was considered significant, 0.6 to 1.0 log of possible significance and 0.5 log or less of no significance (Table I). To keep the amount of non-specific difference at a minimum, heterologous serum-virus combinations were always compared to a homolo-