of 18 months. Reproducible results have been obtained by 3 different research groups in this laboratory, yielding a maximum patency of 4 weeks.

Summary. A technique is described for chronic catheterization of the coronary sinus in dogs, permitting a constant rate of sampling coronary sinus blood in states of normalcy and stress. This method, in combination with arterial blood samples and left coronary inflow, permits quantitative measurement of changes in left ventricular metabolism in chronic unanesthetized dogs.

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Submaxillary Gland Hypertrophy in Rats Fed Proteolytic Enzymes.* (28518)

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Previous studies(1) indicate that raw pancreas contains an orally active, heat-labile factor whose ingestion resulted in a marked hypertrophy of the submaxillary glands of rats. In this report data are presented indicating that a number of proteolytic enzymes of both animal and vegetable origin can induce a similar effect.

Procedure and results. Ninety male rats of the Long-Evans strain and 42 male rats of the Holtzman strain ranging between 50 and 62 g in body weight were divided into comparable groups of 6 animals each. One group of each strain was fed a basal natural food stock ration.† The remaining groups were fed the stock ration plus the various supplements indicated in Table I. Supplements were added in place of an equal amount of stock ration. Rats were placed in metal cages with raised screen bottoms (3 animals per cage) and were provided the various test diets and water *ad libitum*. Animals were sacrificed after 12 days of feeding and the submaxillary glands were removed and weighed. The latter were placed immediately thereafter in 10% neutral formalin solution, prepared for paraffin embedding in the routine manner, sectioned at 7 μ in thickness and stained with the periodic acid-Schiff technique and with hematoxylin and triosin. Data on body and submaxillary gland weights are summarized in Table I. In agreement with previous findings(1) desiccated and defatted raw pancreas at levels of 5% and 10% of the diet resulted in a highly significant increment in submaxillary gland weight. Papain and Protease P-6, enzyme preparations obtained from *Carica papaya* were also active in this regard, the former at levels of 5% and 10% of the diet and the latter at levels of 2½% and 5% of the ration. Crystalline chymotrypsin at levels of 0.2% and 0.5% of the diet and crystalline trypsin at levels of 0.1% and 0.2% of the ration also induced a marked increment in submaxillary gland weights. The parotid glands of rats fed the above supplements, however, did not differ significantly in weight from those of animals fed the unsupplemented stock ration. In contrast to findings with the proteolytic enzymes indicated above, pepsin when incorporated at levels of 2½% and 5% in the diet was without significant effect on submaxillary gland weight. In general supplements which induced a hypertrophy of the submaxillary glands also resulted in a reduction in body weight, degree of

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† Staley's Rockland Rat Diet in Meal Form, A. E. Staley Manufacturing Co., Decatur, Ill.

TABLE I. Comparative Effects of Desiccated and Defatted Raw Pancreas and Various Proteolytic Enzymes on Weight Increment and Submaxillary Gland Weight of Immature Male Rats (6 animals per group).

Dietary group	Body wt (g) Initial	Final	Submaxillary wt (mg) Avg	Range	Submaxillary wt/100 g body wt (mg)
Long Evans rats					
Basal	57	129	321	224-375	248
Basal + following supplements:					
5% desiccated and defatted raw pancreas*	57	112	566	484-657	505
10% " " " " " *	57	86 (5)	629	566-703	729
5% papain†	57	103	406	303-512	394
10% " †	56	84	553	459-701	658
5% pepsin	57	140	329	297-383	234
2½% "	57	134	340	283-423	254
5% Protease P-6‡	57	73 (3)	808	738-864	1107
2½% " " ‡	57	96	564	444-784	586
.5 % alpha chymotrypsin§	56	58 (2)	638	622-654	1100
.25% " " §	56	126	444	416-496	352
.2 % trypsin, salt free‖	56	116	695	672-707	602
.1 % " " " ‖	57	119	465	434-504	390
.2 % " 50% $MgSO_4$¶	57	121	413	382-450	342
.1 % " " " ¶	57	125	382	355-407	305
Holtzman rats					
Basal	55	116	318	297-338	274
Basal + following supplements:					
5% desiccated and defatted raw pancreas*	56	97	829	698-978	857
10% " " " " " *	56	80 (4)	817	765-829	1022
.2% trypsin**	56	73 (4)	672	542-757	920
.1% " **	55	106	736	614-922	693
.5% chymotrypsin††	55	88	908	818-1046	1035
.2% " ††	55	113	768	687-893	682

The values in parentheses indicate the number of animals which survived and on which averages were based when this number was less than the original number per group.

* Viokase Powder 4 N.F. Pancreatin, VioBin Corp., Monticello, Ill.

† Papain Powder Purified Standard, Hathaway Allied Products, Los Angeles, Calif.

‡ Protease P-6 (a purified, standardized proteolytic preparation made from the latex of the green fruit of *Carica papaya* with 6,000 hemoglobin digestion units per g), Chas. Pfizer & Co., Inc., New York.

§ Alpha Chymotrypsin, General Biochemicals, Inc., Chagrin Falls, Ohio.

‖ Trypsin 2 × Crystalline, Salt Free, General Biochemicals, Inc., Chagrin Falls, Ohio.

¶ Trypsin, 2 × Crystalline, 50% $MgSO_4$, General Biochemicals, Inc., Chagrin Falls, Ohio.

** Trypsin, Crystallized (potency not less than 2500 Armour Units/mg), Armour Pharmaceutical Co., Kankakee, Ill.

†† Chymotrypsin, Crystallized (potency, 800-1200 Armour Units/mg), Armour Pharmaceutical Co., Kankakee, Ill.

growth retardation being proportional to the level of supplement fed. This relationship, however, was not invariable since chymotrypsin at a 0.2% level in the diet was without significant effect on body weight but did induce a marked hypertrophy of the submaxillary glands. Histologically, the submaxillary glands of rats fed the desiccated and defatted raw pancreas exhibited marked enlargement of the alveoli with pale, mucoid appearing, hypertrophied acinar cells, abundant PAS-staining cytoplasmic granules and a peripherally displaced, disfigured and hyperchromatic nucleus. Qualitatively similar changes were observed in the submaxillary glands of rats fed the papain, Protease P-6, chymotrypsin and trypsin supplements. In contrast to the above, the submaxillary glands of rats fed the unsupplemented stock ration had smaller alveoli, dense staining acinar cells and less PAS-staining cytoplasmic granules.

No data are available as to the *modus operandi* whereby trypsin and other proteolytic enzymes induced the effects noted above. It was not anticipated that significant amounts of these enzymes would have been absorbed from the intestinal tract or buccal cavity when administered as part of the diet although these possibilities have not been ex-

cluded. Further studies are indicated on the comparative effects of oral *vs* parenteral administration of proteolytic enzymes and of stomach tube feeding *vs* ingestion as a component in the diet. Such studies might indicate whether the effects of the proteolytic enzymes reported above were mediated at the buccal, intestinal or systemic level.

Summary. Immature male rats were fed a natural food stock ration supplemented with graded levels of trypsin, chymotrypsin or proteolytic enzymes derived from *Carica papaya*. A marked hypertrophy of the submaxillary glands occurred in rats fed the above diets in contrast to those of rats fed the unsupplemented stock ration.

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Activity of the Antibody-Complement System and Lysozyme Against Rough Gram Negative Organisms.* (28519)

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The killing of gram negative bacteria by serum has been attributed generally to the antibody-complement system(1). Once killed by the substances of this system, these bacteria may be lysed by the action of lysozyme (2). Recent observations that purport to modify this view, at least with rough organisms, have been reported(3,4,5). In contrast to smooth strains of the gram negative enteric bacteria, rough variants are extremely sensitive to the bactericidal action of normal serum and this property constitutes one of the distinguishing characteristics of rough variants(6). The bactericidal activity of precolostral calf serum(3) and of the serum of piglets reared under sterile conditions without maternal colostrum(4) against rough strains has been attributed to non-specific substances in the apparent absence of antibody. In another approach toward determining the serum substances responsible for bactericidal action, with which this report is directly related, the presence of antibody was considered, but the interesting suggestion was made that it played no role in bactericidal action of normal serum(5). This was based upon the finding that absorption of normal serum with cells of a rough variant, *Escherichia coli* strain Lilly, resulted in a loss of lysozyme in the serum with a concomitant loss of bacteriolytic activity that was restored by addition of lysozyme to the absorbed serum. It was postulated that the killing and lysis of that organism resulted from the combined actions of complement and lysozyme without the mediation of antibody. The loss of activity resulting from the absorption was attributed to lysozyme rather than to antibody(5). This conclusion, which is incompatible with the classic concept that complement functions in the killing and lysis of bacteria sensitized by antibody, warranted further study.

Materials and methods. Egg white lysozyme, 2× crystallized, and histone, products of Worthington Biochemical Corp., were used. The cultures of *Escherichia coli* B and *Salmonella typhosa* Mrs. S were obtained from Mr. A. Abrams, Walter Reed Army Inst. of Research, and *Escherichia coli* Lilly from Dr. A. Wardlaw, Connaught Laboratories, Univ. of Toronto. These cultures satisfy most of the criteria used in defining rough variants(6). Sera were absorbed with 3× saline washed, heat killed (60°C for 1 hr) organisms using about 10^{11} cells per ml of serum. Antisera were prepared in rabbits by 2 subcutaneous injections of about 10^9 living organisms suspended in saline after 15 hours growth on meat extract. An interval of one week was allowed between injections and the

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