constituents are similar to those of man. This would not necessarily be the case were other tests employed. Thus, in a number of samples in which thymol turbidity values were normal, the cephalin flocculation test was invariably positive, making the latter an invalid criterion for normal liver function in the dog. The differences may be a consequence of the dissimilar electrophoretic patterns that have been observed with canine and human serum.

The values observed for sodium and potassium in these "normal" dogs are higher than found in this laboratory for "trained" dogs that have been subjected to frequent venoclysis and venipuncture. It should be noted that whereas in the human the K^+/Na^+ ratio in erythrocytes is 220 times that in plasma, the K^+/Na^+ ratio in erythrocytes in dogs is similar to that in plasma. These considerations must be kept in mind in evaluating the importance of hemolysis in plasma samples and the significance of determinations of Na and K^+ in whole blood.

The differences in renal clearance values between dog and man and the closely associated differences in metabolism of drugs are illustrated abundantly in the literature and need not be summarized here.

Summary. Hematologic and clinical chemical data were obtained on 3 occasions in over 100 apparently healthy female mongrel dogs. Procedures are described and the normal ranges presented.

The authors wish to express their appreciation to members of the Pathology and Pharmacological Chemistry Departments, to whom should go much credit for the chemical and biological phases of this report.

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Plasminogen in Pancreatic Disease and in Pancreatic Secretion. (23128)

ANNA B. BRIDGWATER AND H. NECHELES*
Department of Gastro-Intestinal Research, Medical Research Institute of Michael Reese Hospital, Chicago, Ill.

Plasmin is a proteolytic enzyme, normally existing in blood as inactive precursor, plasminogen(1). The latter may be transformed to plasmin by streptokinase(2) or other kinases(3-5). Plasminogen has been reported to be increased in a number of pathological and physiological conditions and, in some cases, blood has been found to possess spontaneous proteolytic activity, presumably due to presence of plasmin itself(1,6-14). In one report, excessive blood proteolytic activity was found in a patient undergoing surgery for carcinoma of the pancreas(15). Because several other serum enzymes are increased in pancreatic disease, it was thought of interest to investigate serum plasminogen levels when the pancreas was in an abnormal state. For the sake of clarity, several of the terms used are defined. *Plasmin.* Spontaneous proteolytic activity of blood; such activity is ascribed generally to plasmin. However, one should be mindful that characterization of this enzyme has not been sufficiently specific to rule out the possibility that this phenome-

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non may be caused by some other proteolytic enzyme. *Plasminogen.* Proteolysis brought about through activation by streptokinase; as determined here, it also includes any plasmin activity, if present.

Methods. Pancreatic and fat necrosis was produced in 12 dogs by one of 2 methods, and blood samples were drawn before and after operation for determination of serum plasmin, plasminogen and amylase. *Bile injection.* Bile from the dog's own gall bladder (5-10 ml), was aspirated and injected under pressure into the main pancreatic duct which then was ligated. Most of these animals died with hemorrhagic necrosis of the pancreas and abdominal fat necrosis. *Pancreatic duct section.* In aseptic operations, the distal end of the main pancreatic duct was excised together with a piece of duodenal wall including mucosa, and the defect in the duodenum was closed(16). The open duct permitted pancreatic secretion to drain freely into the peritoneal cavity, and the presence of duodenal mucosa on the sectioned duct made activation of trypsinogen possible. Most of these animals developed abdominal fat necrosis and died. The animals in both series received antibiotics post-operatively. *Determination of Plasminogen.* Hemoglobin substrate†: Trypsin substrate was prepared according to Orringer *et al.*(17), except that 300 g of urea was used instead of 400 g. Streptokinase (Varidase, Lederle‡): The amount in a vial was dissolved in 0.85% saline, so that 1 ml contained approximately 5000 units of streptokinase. Plasminogen in 2.5 ml of serum was precipitated with the euglobulin fraction at pH 5.2(18), and the precipitate dissolved in 2.5 ml of veronal buffer (pH 7.4)(19). Varidase (2.5 ml) was added to this solution and the tube placed in a water bath at 37°C for 10 minutes to obtain activation of the plasminogen into plasmin (20); 2 ml of the plasmin-Varidase preparation was then added to 5 ml of hemoglobin substrate and the whole incubated at 37°C for 2 hours. Inactivation of the enzyme with 0.3 N trichloracetic acid, filtration (Whatman #50 is recommended), preparation of a "control" or blank filtrate and development of color by phenol reagent are described by Anson under estimation of trypsin(21). Intensity of color was measured in Evelyn photoelectric colorimeter, (540 mμ filter). The results, read against a standard tyrosine curve, are expressed as mg of "tyrosine" released by amount of enzyme contained in 1 ml of serum. *Plasmin*: The method is the same as for plasminogen, except that veronal buffer was substituted for Varidase. *Serum amylase* was measured by the method of Somogyi, and results are expressed according to his definition of the amylase unit(22). Phosphate buffer (pH 7.2) was used to dilute the dogs' sera; the copper-reducing action of starch filtrates and glucose content of sera were determined iodometrically, using 2 ml of filtrate (23).

Results. As seen from Table I, a postoperative increase in both plasminogen and amylase was noted in all animals except one (No. 11), in which serum amylase failed to rise appreciably, because the bile had been injected into the peripancreatic tissues and only slight fat necrosis was present. In most dogs (exceptions No. 2, 6, 11), the percentage rise in amylase was greater than that of plasminogen, and in some (No. 3, 7, 10, 12), the latter assumed a downward trend, while the amylase remained high. In view of the seeming non-specificity of the various conditions in which increased blood proteolytic activity has been observed, it is possible that any increase noted here might be the result of tissue breakdown (necrosis) or of acute state of animal immediately following operation (shock, "stress") rather than to any effect specific for the pancreas only(6,7,24,25). Thus, this reaction may well be a secondary one.

In 7 animals, free serum plasmin was determined also; in 3 (No. 7, 11, 12), no free plasmin was present in the controls, but relatively high values appeared when pancreatic necrosis developed; in 2 (No. 9, 10), free plasmin was found before operation, followed by increases afterwards.

† Bovine Hemoglobin Enzyme Substrate Powder, Wilson Laboratories, Chicago, Ill.

‡ Varidase was furnished through the courtesy of Lederle Laboratories, Pearl River, N. Y.

TABLE I. Plasmin, Plasminogen, and Amylase in Dog's Serum before and after Operation to Produce Pancreatic or Fat Necrosis.

Dog No.	Blood sample time (hr)		Plasmin	Plasminogen	Amylase
1	Bile injection	0		.0153	2482
		12		.0595	9263
		24		.0510	14111
2	*Idem*	0		.0085	2021
		6		.0663	7569
3	"	0		.4471	3994
		2		.6290	30017
		24		.2822	31343
4	Pancreatic	0		.0986	3206
	duct section	21		.3392	15642
5	*Idem*	0		.3978	2797
		4		.5117	3932
		21		.4608	12076
6	Bile injection	0	.0	.1547	1048
		2	.0	.2737	2028
7	*Idem*	0	.0	.0527	1586
		2	.0918	.0493	5816
		8	.0	.1445	7954
		22	.0306	.1003	11180
8	"	0	.0	.0187	1418
		3.5	.0	.0349	3879
9	"	0	.0247	.4208	1604
		7.5	.1998	.8404	6087
10	"	0	.0136	.2244	2199
		7	.0391	.2669	18623
		19	.0	.2822	18983
		22	.0196	.2499	18623
11	"	0	.0	.2686	3240
		7	.0493	.3638	4030
12	"	0	.0	.0595	2041
		3	.0442	.2091	10204
		7	.1046	.1088	11216

All animals inj. with bile (except #11) showed pancreatic and fat necrosis.

All animals with severed pancreatic ducts showed fat necrosis.

More blood samples were taken, but only the most significant are presented.

Plasminogen was determined in the serum of 7 normal adults and of 4 patients with high serum amylase, but no patient exhibited increased plasminogen when compared with normal controls.

Obviously, the question arose as to whether plasminogen is present in the pancreatic secretion. Treatment of samples of pancreatic juice from 4 dogs with streptokinase (Varidase) brought about no increase in proteolysis over that caused by spontaneous proteolytic activity of the juice itself. On the other hand, when each sample of secretion was treated with enterokinase,§ greatly increased proteolysis, over and above that shown spontaneously, resulted. These results indicate that the enzyme activated by streptokinase and that activated by enterokinase are not the same. This conclusion concurs with a previous report by Kaplan(26) in which somewhat different methods were used.

Summary. 1. Serum plasminogen and amylase levels were measured in 12 dogs in whom pancreatic necrosis and/or fat necrosis was produced. An increase of plasminogen along with the expected increase in amylase was noted under the conditions described. 2. Samples of pure pancreatic juice obtained from 4 dogs were tested for the presence of plasminogen. No proteolytic activity due to the activating effect of streptokinase was present by the method employed.

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Strain Differences in Response of Mice to Mammary Gland Stimulating Hormones.* (23129)

J. P. MIXNER† AND C. W. TURNER

Department of Dairy Husbandry, University of Missouri, Columbia.

Most species of mammals require a combination of progesterone and estrogen to obtain full development of the mammary gland while in a few species, notably the guinea pig and the monkey, estrogen alone will cause complete development(1). Possible strain differences in mammary gland growth responses within a species are less well known. In studies on mammary cancer susceptibility in mice, strain differences have been found to exist. Also Richardson(2,3) and Richardson and Cloudman(4) have reported variation in extent of rudimentary duct development in various inbred strains of male mice. Mixner and Turner(5) have used ovariectomized virgin albino mice rather extensively in experiments on factors responsible for and influencing mammary lobule-alveolar growth. In these studies a single strain of albino mice had been used. In subsequent studies a second strain of mice showed a marked difference in mammary growth responsiveness as compared to the first strain of mice. Other strains of mice were then tested in this respect.

The present paper deals with strain differences in the mammary lobule-alveolar growth responsiveness of mice to estrone in conjunction with both progesterone and pituitary mammogenic lobule-alveolar growth factor.

Procedure. Two anterior pituitary extracts and progesterone were assayed for their ability to promote mammary lobule-alveolar growth in 4 strains of mice. Our original strain of albino mice (designated "Schwing") were obtained from Edward Schwing, Harrison, Ohio, who stated that his strain of mice was started about 1906 from a pair of mice. A second strain of mice (designated "Swiss") was obtained from Rockland Farms, New City, N. Y. The third strain of mice (designated "Kansas") was obtained from Mrs. Ray Whitehouse of Hardtner, Kansas, who stated that the original pen of mice was obtained from James W. Howck & Co., Tiffin, Ohio. The last strain of mice (designated "Sutter") was obtained from Arthur Sutter, Springfield, Mo., who advertised them as "Rockland All-Purpose Strain White Mice." The criterion of response in the assay method used was the per cent of ovariectomized mice showing a minimal mammary lobule-alveolar growth response after 10 daily injections of standard anterior pituitary preparation or progesterone, each injected in conjunction with a daily dose of 0.75 μg estrone. A mouse unit of mammogenic material is that amount of material required per mouse which will cause 50 $\pm$ 10% of 10 or more mice to show this minimal stimulation of the mammary glands.

Results. Mice of the 4 strains selected

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† Present address: Rutgers University, Dairy Research Farm, Sussex, N. J.