lation between tumor incidence and tumor size. The occurrence of multiple tumors is sufficiently rare in all groups so as not to permit any conclusions as to the presence of differences.

The foregoing data strongly suggest that the nature of the dietary fat may have a significant bearing on the development of the C_3H mouse hepatoma. The effect observed here is similar to the one reported by Shear and Leiter(9), who fractionated lard by filtration at 38°C and found that the liquid fraction when used as a carrier for benzpyrene gave rise to more tumors than the solid fraction.

It still remains to be established however, whether the differences in the effects are due to qualitative peculiarities of the different fractions or to differences in the actual amounts of fat absorbed. No simple correlation between weight and tumor incidence is apparent from a comparison of the weight curves given in Fig. 1 and the data in Table II. This would suggest a qualitative, rather than a quantitative, difference, but the question as to the nature of the responsible factor remains open for the present.

Summary. Five fractions of hydrogenated vegetable fat (commercial crisco) have been prepared by crystallization from ether at +15, +5, —5, and —15°C. When incorporated in the standard diet at equivalent levels, fractions of low melting point, presumably containing largely unsaturated and shorter-chain fatty acids as well as unsaponifiable matter, are found to give rise to a high proportion of hepatomas in C_3H male mice, while fractions of high melting point give rise to but a few. The magnitude of the observed difference in incidence ($p<.001$) is believed sufficient to justify further studies on the nature of the responsible factors.

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Received October 28, 1955. P.S.E.B.M., 1955, v90.

Collagenase Activity of Dog Pancreatic Juice. (22124)

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Pancreatic juice obtained from pancreatic fistulae artificially created in dogs produces striking digestion of collagen. This is not due to trypsin, chymotrypsin, or carboxypeptidase. It is the purpose of the present work to describe these studies.

Methods and materials. Adult mongrel dogs of both sexes were used. Under aseptic technic the abdomen of the anesthetized animal was opened through a midline incision and the pancreas exposed. The accessory pancreatic duct was dissected free, ligated, and cut, following which the main duct was identified. After placing a ligature close to the point of its entrance into the duodenum, the duct was cannulated with a polyethylene tube whose lumen measured 1.14 mm in diameter; this was tied securely in place. The tube was then brought out through a separate stab incision, following which a Witzel jejun-

ostomy was performed using a number 8 French rubber catheter which was brought through another stab incision. The wound was then closed in layers and a sterile condom was tied to the polyethylene tube. The jejunostomy tube was then securely clamped and a dressing applied. Following recovery from the anesthetic, secretions started flowing in 12-24 hours from the polyethylene tube reaching a maximum of 20 ml per hour in 36-48 hours. The animals could be kept alive for prolonged periods if the pancreatic secretions were returned through the jejunostomy tube. Otherwise they became dehydrated, anorexic and lethargic and died within 7 to 8 days. The secretions tested were collected within the first 24-48 hours in each instance and were also tested for sterility by routine bacteriologic methods. These were free of bacterial growth. Collagen was prepared by the method of Buechler(1) using fresh beef achilles tendon instead of steer hide. 100 mg of prepared tendon was suspended in 2.0 ml of secretion from a pancreatic fistula and incubated at 37°C for 5 hours. The suspension was then filtered and the precipitate washed. The precipitate was then dissolved in 9.0 ml of 0.5 N HCl and autoclaved for 30 minutes at 15 lb. pressure. The solution was then made up to 10.0 ml volume. Collagenase activity was measured by the quantitative assay of the hydroxyproline in the residue as described by Neuman and Logan(2). Since hydroxyproline forms a constant fraction of collagen, this method is an accurate assessment of the amount of undigested collagen, permitting ready calculation of the amount of collagen digested. Commercially available preparations* of trypsin, chymotrypsin, and carboxypeptidase were used. 0.1 M phosphate buffers at pH 7.0 and 8.0 were used. 0.5 mg of each of the commercial enzymes were dissolved in 2.0 ml of buffer solution for testing the activity. Changes in collagen apparently occur at pH 4.5 and below and will permit digestion by trypsin and vegetable cathepsins(3). These findings have been confirmed by us and are not included in this report.

TABLE I. Percent Digestion of Collagen and Casein by Pancreatic Juice and Trypsin.

		% collagen digested	% casein digested
Pancreatic juice	Dog 1	52	0
	2	41.5	0
	3	45	0
Trypsin before heating	No. 1	16	96
	2	13	92
Trypsin after heating	No. 1	0	85
	2	0	79

Results. Unbuffered pancreatic juice was used in the tests on collagen. The usual pH range was 8.2-8.6. The digestion of collagen by dog pancreatic juice varied. Dog No. 1 digested 52.0%, No. 2 digested 41.5%, and dog No. 3, 45.0%. (Table I). Commercially prepared trypsin, both at pH 7 and 8 had slight collagen digesting ability. Preparation No. 1 digested 16.0% and preparation No. 2 digested 13.0%. As pointed out by Northrop(4) and confirmed by us, heating to 95°C for 4½ minutes, then cooling to 20°C has little effect on the proteolytic activity of trypsin, as determined by the method of Grob(5). However, such heating markedly alters its ability to digest collagen. Neither trypsin preparation digested any collagen after such heating (Table I). When pancreatic juice was heated in the same fashion all collagenase activity disappeared. The inactivated pancreatic juice had no tryptic activity. Bacterial collagenase prepared from *Cl. histolyticum* was heated to 95°C for 4½ minutes then cooled, with complete loss of collagenase activity. Chymotrypsin and carboxypeptidase did not digest collagen at pH 7.0 or 8.0. Duodenal content, extract of duodenal mucosa and bile had little effect on the collagenase activity of the pancreatic secretion. The activating substance, if any, for collagenase activity remains unidentified.

Discussion. The marked collagenase activity of crude pancreatic juice was present in each of the dogs tested. This cannot be accounted for by the action of any of the pre-

* Commercial preparations used: Trypsin preparation no. 1: Tryptar, Armour Lab.; trypsin preparation no. 2: Trypsin, Worthington Biochemical Co., 2x crystallized Chymotrypsin, Worthington Biochemical Co.; Carboxypeptidase, 3x crystallized.

viously described proteolytic enzymes of the pancreas for 3 reasons: 1. The degree of digestion is at least 3 times as great as that obtained from trypsin. 2. Heating trypsin to destroy its collagen digesting ability, but not its proteolytic properties, reduces the amount of collagen digested to zero. 3. Pancreatic juice from a dog fistula which has not been activated by intestinal juice has no tryptic activity, nevertheless, it has marked collagenase activity. The inability of trypsin to digest any significant amount of collagen has also been reported by Neuman and Tytell(6).

These findings suggest that collagenase must be considered in the pathogenesis of pancreatitis and other pancreatic diseases.

Summary. Evidence has been presented to show that pancreatic juice of the dog has the ability to digest collagen. This ability is not due to the proteolytic enzymes of the pancreas, trypsin, chymotrypsin, or carboxypeptidase.

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Received October 31, 1955. P.S.E.B.M., 1955, v90.

Effects of Thyroid Hormone and Propylthiouracil on Eruption Rate of Upper Incisor Teeth in Rats. (22125)

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Though the rat's incisor teeth continue to erupt throughout life, they are kept to a fairly constant length by the process of attrition. The rate of eruption, that is, the linear quantity by which the incisor teeth extrude into the oral cavity in a unit of time, is subject to alteration by hormonal action. Hoskins(1) found that thyroid hormone caused the incisors of newborn rats to erupt earlier than those in untreated control rats. This observation was confirmed by Karnofsky and Cronkite(2). Herzberg(3) noted that thyroid hormone caused an increase in the incisor eruption rate (I.E.R.) in adult rats; however, he offered no data to substantiate his claim. Baume, Becks and Evans(4) reported that ablation of the thyroid in adult rats produced a decrease in the I.E.R. In conformity with this view it seemed of interest therefore to extend these data by 1) quantitating the effect of thyroid hormone on the I.E.R. of adult rats and 2) testing the effect of a thyroid blocking agent, propylthiouracil (PTU) on I.E.R. The latter procedure has the advantage that it leaves the parathyroid glands in a functional state. In many of the earlier studies the parathyroids were removed indiscriminately at the time of surgical thyroidectomy.

Procedures. Twenty-three male white rats (Holtzman strain), 80 days of age, were treated as follows: 8 animals were fed ground rat chow containing 0.5% desiccated thyroid; 8 rats were fed processed chow containing 0.15% PTU and 7 animals, which served as untreated controls, were fed only ground rat chow. Four rats on PTU-feeding were shifted at the end of the fifth week to a diet of ground rat chow containing instead 0.5% desiccated thyroid for an additional 2 weeks. The I.E.R. of all animals was measured according to the method of Schour(5) once each week during the course of the experimental period. Dental roentgenograms of upper incisors of rats fed thyroid were taken after 2 weeks on the diet, and those of the animals receiving PTU were taken after 5

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