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Origin of Dog Serum Cholesterol Esterase.* (20064)

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Recent reports(1-4) have presented evidence for the existence of esterifying and hydrolytic cholesterol esterase systems in pancreas, dog serum, and rat intestinal mucosa. The enzymes occurring in these tissues appeared to be identical in that they required bile salts for activity, were inactivated by heating at 65°C for 15 minutes and had the same optimum pH for the hydrolytic and esterifying systems. The identity of the enzyme in rat intestinal mucosa was further verified by the demonstration that 95% depancreatized rats showed a marked lowering of cholesterol esterase activity.

In studies by Sperry and coworkers(5,6) on the cholesterol esterase of serum, it was reported that the esterification of free cholesterol occurred when human or dog serum was incubated alone. The esterification reaction in these sera was inhibited by bile salts, but in dog serum as the bile salt concentration was increased hydrolysis of the cholesterol esters of serum took place. In a previous study(3), the serum of 5 species were examined for cholesterol esterase activity. In dog serum, but not in human, rat, rabbit or guinea pig, serum highly active cholesterol esterase systems possessing the properties of the pancreatic type enzyme could be demonstrated. The occurrence of this enzyme in the serum of the dog is unique. Since a considerable amount of controversy exists regarding the occurrence and nature of serum cholesterol esterase, it was felt that a study should be conducted to ascertain more conclusively the origin of dog serum cholesterol esterase. Depancreatized dogs were prepared and the esterifying cholesterol esterase activity was followed before and after the operation with and without the addition of raw pancreas to the diet.

Procedure. Three normal healthy mongrel dogs were used. The animals were depancreatized according to the technic of Markowitz(7). Following the operation the dogs were maintained on 10 units of insulin, ½ lb raw lean beef, 10 g sucrose and 50 g raw pancreas given twice a day. The dogs were allowed to recuperate for 2 weeks. The raw pancreas was withdrawn from the diet after this period and given again after 10 days. Blood samples were obtained before the operation and then at regular intervals. Cholesterol esterase activity was measured according to the technic previously described(2) using cholesterol-oleic acid as the substrate.

Results. The results of the experiment are shown in Table I. Prior to the operation all dogs showed the presence of an active choles-

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TABLE I. Cholesterol Esterase Activity in Serum of 3 Dogs Before and After Pancreatectomy. Enzyme digests were prepared as previously described(2). Substrate for esterification, cholesterol, 25 mg, oleic acid, 54.8 mg. pH of digests, 6.2. Bile salt solution, 1 cc 10% sodium taurocholate. Enzyme, 2 cc of dog serum.

No. of dog	Days after operation	Raw pancreas in diet	Esterification* 24 hr, %
1	Pre	—	39.1
	14	—	.9
	16	—	.7
	20	—	.0
	24	+	1.0
	28	+	1.0
2	Pre	—	17.2
	14	—	.7
	16	—	.0
	20	—	.5
	24	+	.3
	28	+	2.0
3	Pre	—	
	14	+	40.0
	16	+	1.0
	20	—	.8
	24	+	2.0
	28	+	.5

* The values obtained after the operation can be attributed to the limit of error of the method(2).

terol esterase in serum. Upon the removal of the pancreas, no activity could be detected. The feeding of raw pancreas did not produce any change, possibly because gastric acidity inactivated the enzyme(8).

Summary. Pancreas appears to be the sole source of dog serum cholesterol esterase.

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Toxoplasmosis. II. Intra-Uterine Infection in Dogs, Premature Birth and Presence of Organisms in Milk.* (20065)

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Toxoplasmosis is known to be a congenital disease in the human infant. The disease has been studied in mice by Eichenwald(1), who described *in utero* infection and milk transmission. Our previous study of 2 toxoplasmosis epizootics in dogs in which pups were stillborn, born prematurely or died shortly after birth prompted this investigation. This report describes experimental congenital toxoplasmosis in dogs associated with premature birth and the presence of toxoplasma in the milk.

Materials and methods. Five healthy virgin bitches 1.5 to 2 years of age and a 3-year-old male dog were isolated for 3 weeks during which time they were treated for internal and external parasites and immunized against distemper. Diet consisted of commercially prepared cereal dog food supplemented by horse meat in a ratio of 3:1. Two preinoculation serological studies conducted prior to estrus according to the method of Sabin and Feldman(2) revealed no toxoplasma antibodies. Toxoplasma organisms of canine and human origin were used in the serological studies. The male and female were allowed to copulate daily throughout the course of the estrous cycle. Sterile caps, masks, gowns, gloves and boots were worn by all who entered the room.

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