Fielder and Mr. Charles Casmer, for preparation and interpretation of brain sections; and to Mrs. Kathleen Barkley for excellent technical assistance in these studies.

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Received February 14, 1966. P.S.E.B.M., 1966, v122.

Oxytetracycline and Hypoglycemia with Convulsions in Pancreatectomized Dogs.* (31169)

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During an investigation into the source of serum amylase, dogs with vagotomy, pyloroplasty and staged removal of the pancreas and small intestine were prepared. The animals were maintained post-operatively by intravenous infusion of a solution of half normal saline containing 50 g of glucose, 15 units of regular insulin and 200 mg of oxytetracycline per liter. It was found that after several days, the dogs developed hypoglycemia with staggering and convulsions. In these animals the liver glycogen was usually normal and occasionally elevated. It was subsequently determined that neither the enterectomy nor the vagotomy and pyloroplasty was needed for the development of this condition. It was found that pancreatectomy was the only necessary operative procedure if the animal was maintained post-operatively by intravenous infusion of fluid containing oxytetracycline.

This report describes a condition of hypoglycemia with convulsions that can be induced in pancreatectomized dogs when oxytetracycline is added to intravenous fluid used to maintain them post-operatively. The report also describes the response of the blood glucose of these animals to intravenous injection of glucose and of glycogenolytic agents.

Method. Inasmuch as the operative procedures other than pancreatectomy had no influence on the experimental results, the operated animals were pooled for analysis of the influence of oxytetracycline.

Fourteen dogs of both sexes, weighing be-

* Supported by Grant AM 3672-05A1 USPHS, Nat. Inst. Health.

tween 10 and 13 kg, were used. The dogs were anesthetized by intravenous injection of 25 mg of sodium pentobarbital per kilo body weight. The abdomen was then opened and a biopsy of the liver obtained and quickly dissolved in boiling potassium hydroxide for determination of liver glycogen. The operative procedure, which included removal of the pancreas, was then carried out.

The 10 experimental animals were maintained post-operatively by intravenous infusion of 5% glucose in half normal saline with 20 meq of potassium chloride, 2 ml of water soluble vitamins, 200 mg of choline chloride, 15 units of regular insulin, and 200 mg of oxytetracycline per liter. The 4 control dogs were maintained post-operatively on the same fluid, minus the oxytetracycline. Each dog received about 750 ml of solution daily through an indwelling catheter in an external jugular vein by a slow, almost continuous drip.

In the experimental animals determination of the responses of the blood glucose to intravenous injections of glucose, glucagon and adrenalin was made before the onset of hypoglycemia. The tests were done 2 hours after discontinuing the intravenous infusion in order to permit inactivation of the circulating insulin.

With the onset of hypoglycemia with staggering and convulsions a specimen of blood was obtained for determination of serum bilirubin, SGOT and SGPT; and the intravenous infusion was discontinued for one to two hours. The interval varied inversely with the severity of the convulsions. At the end of this period the response of the blood glucose to successive intravenous injections of glucose, adrenalin and glucagon was determined. These tests were done in 5 dogs before they were anesthetized for a liver biopsy for glycogen determination; in 3 dogs after they were anesthetized and the liver biopsy obtained. Two convulsing dogs died before any tests were done or liver biopsy obtained. The 4 control dogs were anesthetized for liver biopsy 3-5 weeks after the pancreatectomy.

Blood glucose was determined by the glucostat method(1), urine glucose with Clinitest tablets, glycogen by the method of Good *et al*(2), serum bilirubin, SGOT and SGPT in the hospital clinical laboratory. Pancreatectomy was done by the avulsion technique (3).

Results. Post-operatively there was a period during which the animals treated with oxytetracycline had the moderate hyperglycemia and slight glycosuria usually observed in post-pancreatectomized dogs treated with insulin and glucose in the proportions described. During this time the response of the blood glucose to intravenous injection of glucose (glucose tolerance test) was a sharp, sustained rise. The response to intravenous injection of adrenalin was a sharp rise followed by a slower one. The response to intravenous injection of glucagon was a more uniform rise followed by a gradual fall (Tables I, III, IV).

Subsequently each of the oxytetracycline-treated dogs developed hypoglycemia with staggering and, in 2-4 hours, convulsions. The time of onset was 5-9 days in 8 of the dogs. One began on the second day and one on the twelfth day. With the onset of staggering and convulsions, discontinuance of the intravenous infusion for 1-2 hours resulted in no change in clinical condition or blood glucose level. Now, the responses of the blood glucose to the intravenous injections of glucose and of adrenalin were unlike those previously described; the response to glucagon was unchanged. Intravenous injection of glucose resulted in a brisk rise of the blood glucose, followed by a swift fall to the pre-injection level (Table I). A rather similar sequence was observed in 5 dogs tested with 10 times the dose (1.5 g/kilo) following the standard glucose tolerance test (Table II).

TABLE I. Glucose Tolerance*
Pancreatectomy and Oxytetracycline.

	Blood glucose	
Time (min)	Before hypoglycemia and convulsions	During hypoglycemia and convulsions
0	215 (186–244)	37 (10– 60)
5	265 (250–281)	82 (47–112)
30	255 (231–280)	72 (18–115)
60	265 (231–295)	40 (0– 95)
90	265 (236–294)	34 (8– 57)

* I.V. injection of 0.15 g glucose per kilo body weight.

TABLE II. Glucose Tolerance (5 Dogs)* Pancreatectomy and Oxytetracycline.

Time (min)	Blood glucose, during hypoglycemia and convulsions
0	51 (32– 77)
5	263 (213–339)
30	164 (129–194)
90	106 (60–155)

* I.V. injection of 1.5 g glucose per kilo body wt.

TABLE III. Response to Adrenalin* Pancreatectomy and Oxytetracycline.

Time (min)	Blood glucose: Before hypoglycemia and convulsions	Blood glucose: During hypoglycemia and convulsions
0	221 (187–256)	75 (60– 95)
5	315 (310–321)	96 (88–107)
30	305 (250–359)	84 (73–100)
60	342 (300–385)	75 (63– 93)
90	377 (344–410)	78 (69– 92)

* I.V. injection of 10 gamma of adrenalin per kilo body weight.

The injection of adrenalin was followed by a small rise of blood glucose and a quick fall to pre-injection level (Table III). The intravenous injection of glucagon was followed by the same fairly uniform rise and gradual fall of the blood glucose observed in the animals before the onset of hypoglycemia with staggering and convulsions (Table IV).

In 9 of the experimental animals, the hypoglycemia was associated with essentially normal (unchanged) or even elevated levels of liver glycogen. Two of the three animals whose livers were biopsied before administration of glycogenolytic substances showed significant elevations of the liver glycogen (68 and 97%). In only one dog, dead when the liver was biopsied, did the liver glycogen fall significantly (48%) (Table V).

Three of the animals given oxytetracycline showed a small elevation of the serum bilirubin (1.4 to 1.8 mg%). Elevated serum bilirubin occurred only in animals with enterectomy. In these dogs, swelling of the suture line near the duodenal papilla could make for a moderate degree of obstructive jaundice. There was no evidence of liver dysfunction as measured by the serum bilirubin, SGOT and SGPT in any of the other dogs given intravenous oxytetracycline: on microscopic examination, sections of the livers showed no inflammation or liver cell degeneration (Fig. 1).

In most of the animals, the convulsions could not be terminated by intravenous injection of glucose and proved fatal. Only one animal was restored to "normal," by intravenous injection of 50 ml of 50% glucose over a 5-hour period. This animal, subsequently kept on the parenteral maintenance fluid with only 3.5 units of insulin per liter, developed hypoglycemia with irreversible convulsions in 48 hours.

The control pancreatectomized animals, those maintained by parenteral fluid without oxytetracycline, manifested no staggering or convulsions in periods of from 3 to 5 weeks. At the time the experiment was terminated in these animals, the liver glycogen was unchanged from that of pre-operative level.

Discussion. It appears from these experiments that the inclusion of oxytetracycline in

TABLE IV. Response to Glucagon* Pancreatectomy and Oxytetracycline.

Time (min)	Blood glucose: Before hypoglycemia and convulsions	Blood glucose: During hypoglycemia and convulsions
0	260 (210–325)	60 (24– 72)
5	305 (246–367)	106 (94–132)
30	342 (275–405)	179 (164–193)
60	375 (302–430)	286 (233–355)
120	386 (315–445)	280 (166–380)
180	370 (310–435)	274 (134–406)
240	324 (275–380)	261 (103–437)

* I.V. injection of 2 mg of glucagon.

TABLE V. Liver Glycogen.*

Pre-pancreatectomy	During hypoglycemia and convulsions, Pancreatectomy and Oxytetracycline: Before administration glycogenolytic substances	During hypoglycemia and convulsions, Pancreatectomy and Oxytetracycline: After administration glycogenolytic substances
7.3	12.3	
6.9	13.6	
7.1	6.9	
8.8		8.1
7.2		6.5
8.6		4.4
8.0		7.4
6.8		7.0
6.8		7.7
6.3		8.4

* g per 100 g liver wt.

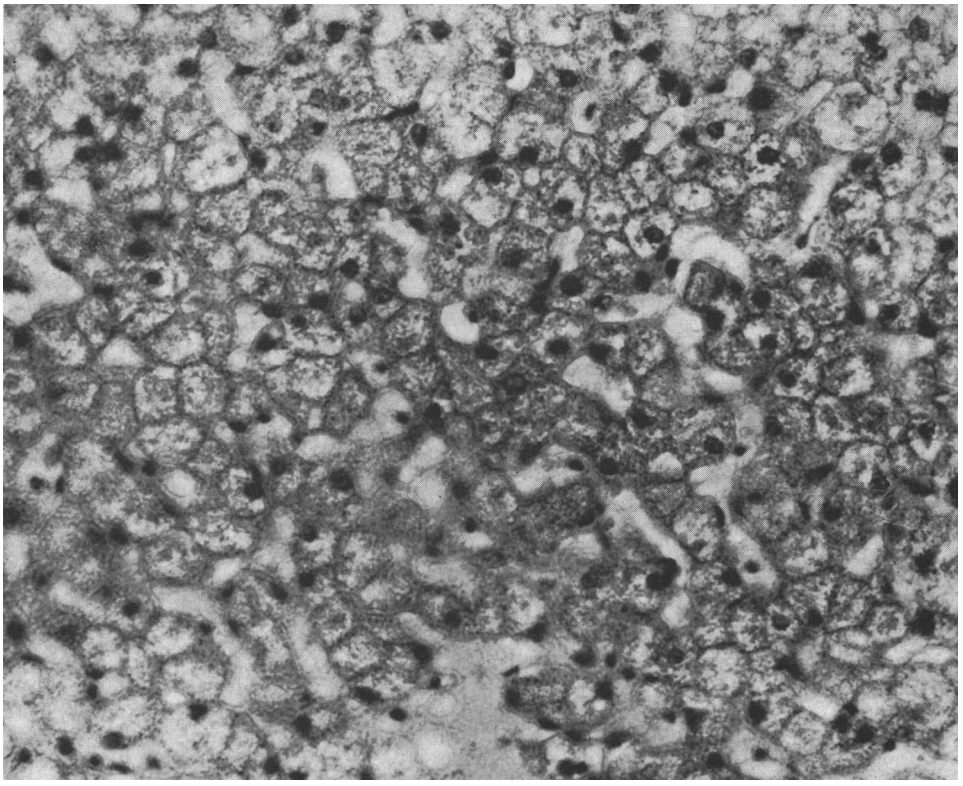

FIG. 1. Liver of Hypoglycemic Dog. (H and E × 400).

the appropriate intravenous fluid used to maintain pancreatectomized dogs induces a state of hypoglycemia and convulsions that does not usually respond to the intravenous injection of glucose. This state is associated with a normal or even elevated content of glycogen in the liver.

We have found no observation in the literature relating oxytetracycline and hypoglycemia. However, tetracyclines can damage the liver parenchyma(4). Hypoglycemia may occur when chemical toxins cause acute and profound parenchymal disease of the liver (5). The dogs in this experiment showed no evidence of such liver disease.

It is possible that the pancreatectomized dogs given oxytetracycline post-operatively develop an increased sensitivity to insulin. This would make for hypoglycemia and convulsions—the manifestations of an overdose of insulin in a diabetic dog. Since insulin stimulates glycogen synthesis in the diabetic dog an increased sensitivity to this hormone might explain the substantial levels of liver glycogen found in some of these animals(6). The one dog that survived the state of hypoglycemia with convulsions showed an unusual sensitivity to insulin. The state recurred in 48 hours with administration of oxytetracycline with insulin and glucose in the ratio of only one unit of regular insulin to each 15 g of glucose.

The mechanism of the possible increase in sensitivity to insulin is not clear. It may involve an impaired response to adrenalin. With the onset of hypoglycemia, staggering and convulsions in the experimental animals, the intravenous injection of adrenalin does not elicit the hyperglycemia expected in diabetic dogs with adequate amounts of glycogen in the liver(6). However, the impaired response to adrenalin may be only apparent. Both adrenalin and glucagon are believed to bring about glycogenolysis by the same mechanism —activation of cyclic AMP(7). Since the response of the blood glucose to intravenous in-

jection of glucagon remains unchanged in the experimental animals, the impaired response to adrenalin requires further study with larger doses of hormone before it can be established as a consequence of treatment with oxytetracycline.

Interference with the mechanism that inactivates insulin could also make for an apparent increase in sensitivity to the hormone. Insulin is rapidly inactivated in the body; its half-life in man is about 40 minutes(8). Much of the inactivation of insulin seems due to an enzyme system in the liver(9). Interference with the activity of this enzyme by oxytetracycline would cause insulin to accumulate and possibly reach a level that precipitates hypoglycemia and convulsions. It is known that oxytetracycline concentrates in the liver and that the drug interferes with the activity of enzymes—those involved in protein synthesis by staphylococci(10).

The data are limited and do not permit final conclusions. However, the induction of hypoglycemia and irreversible convulsions by oxytetracycline treatment may be of significance in the study of hormonal control of the blood sugar.

Summary. Addition of oxytetracycline to intravenous fluid used to maintain pancreatectomized dogs brings about a state of hypoglycemia with staggering and convulsions that only infrequently responds to administration of glucose. The liver glycogen of these dogs is usually normal and occasionally elevated. During the state of hypoglycemia with staggering and convulsions the rise of the blood glucose following intravenous injection of adrenalin or glucose (glucose tolerance test) is transient; the rise of the blood glucose following intravenous injection of glucagon is large and sustained.

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Received February 14, 1966. P.S.E.B.M., 1966, v122.

The Estrous Cycle and Splenic Erythropoiesis in the Mouse.* (31170)

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The normal adult mouse displays significant splenic erythropoiesis(1). Among the many experimental manipulations that further stimulate this process are several which may have in common the evocation of an inflammatory response. These include grouping of male mice which results in the wounding of subordinate animals by dominant ones (2,3), graft *vs* host reactions(4), and injection of bacterial endotoxins(5). The periodic migration of neutrophilic leukocytes into the vagina of the mouse at termination of estrus is well known and presents an opportunity for examining the possible relationship between a "physiological inflammation" and splenic erythropoiesis. Therefore, it seemed of interest to see whether or not this type of inflammation would cause increased splenic erythropoiesis.

* Supported by USPHS Research Grant CA-03071-09.