Fatty Metabolism and Ketogenesis after Liver Denervation or Bilateral Thoracolumbar Sympathectomy in Pancreatectomized Dogs

Bernardo A. Houssay, M.D., Ciro T. Rietti, Ph.D., Edmundo Ashkar, M.D., Enrique J. Del Castillo, M.D., María Elisa Galli, M.D., Alicia Roldán, Ph.D., and Enrique J. Urgoiti, M.D., Buenos Aires

SUMMARY

Bilateral extirpation of sympathetic chains from T1 to L6, including stellate ganglia and splanchnic nerves in totally pancreatectomized dogs, induces in blood: no changes in the diabetic hyperglycemia or cholesterolemia, but decrease of fatty acid mobilization from adipose tissue (lower values of FFA and lipemia), and a striking diminution of the diabetic ketonemia. In the liver, as in pancreatectomized dogs, there is less increase of total lipids (and triglycerides) but high values of cholesterol.

Acute hepatic denervation in pancreatectomized dogs does not change the mobilization of fatty acids from adipose tissue (the high levels of hyperlipemia and FFA are similar to those of pancreatectomized controls), but the increase of the ketonemic level is markedly depressed. Glycemia and cholesterolemia are increased as in the pancreatectomized dogs. In the denervated liver there is less increase of total lipids, triglycerides and cholesterol as compared with the pancreatectomized controls.

In the pancreatectomized dogs with chronic hepatic denervation the blood changes are similar to those of the pancreatectomized dogs. There is no attenuation of ketonemia. In the liver the cholesterol content is strikingly diminished as compared with the pancreatectomized controls. The diabetic hyperglycemia is high after pancreatectomy either after sympathectomy or hepatic denervation. DIABETES *16*:259-63, April, 1967.

In preceding studies[1-3] the hormonal aspects of severe ketonemia of totally depancreatectomized dogs were studied. Hyperketonemia was found to be dependent on three endocrine glands through either: (1) absence of endocrine pancreas (lack of insulin) and (2) simultaneous presence of pituitary and adrenals or their hormones so that the surgical ablation of either one of these two glands markedly diminishes diabetic hyperketonemia. Administration of the deficient hormone restores hyperketonemia.

In the present work, we have studied the role of bilateral sympathectomy or hepatic denervation on fatty metabolism and ketogenesis of pancreatectomized dogs.

MATERIAL AND METHODS

Five groups of animals were studied according to the following design: (1) normal dogs; (2) pancreatomized dogs; (3) pancreatectomized dogs with acutely denervated livers; (4) pancreatectomized dogs with chronic hepatic denervation; (5) pancreatectomized bilaterally sympathectomized dogs.

Mongrel dogs weighing 8 to 12 kg. were operated under intravenous Nembutal anesthesia (33 mg. per kg. or less). They were fed biscuits containing: total protein, 25 per cent; lipid, 5.5 per cent; fiber, 3 per cent; minerals, 3 per cent; and carbohydrates, 49 per cent. Raw pancreas was added to the diet of pancreatectomized dogs (40 gm. per day).

In Group 5, bilateral extirpation of the sympathetic chains from T1 to L6, including the stellate ganglia and the splanchnic nerves, was immediately followed by total pancreatectomy. The hepatic denervation was performed according to the following technic: section of nerves and connective sheath of the hepatic artery and of all tissue of the hepatic pedicle, except the portal vein; surgical cleaning of the bile duct, and the pancreatic and pancreatic-duodenal arteries. The liver denervation was followed immediately by total pancreatectomy in Group 3 (acute hepatic denervation) or twenty days later in Group 4 (chronic hepatic denervation).

From the Instituto de Biología y Medicina Experimental, Buenos Aires, Argentina.

TABLE 1

Blood changes in dogs pancreatectomized (Pp); pancreatectomized with acute liver denervation (Pp.A.L.D.); pancreatectomized with chronic liver denervation (Pp.C.L.D.); pancreatectomized with bilateral removal of sympathetic chains (Pp.S—x). Between brackets number of dogs. Five days of observation after pancreatectomy.

Ketone bodies (mg./100 ml. of blood plasma)

Dogs	Basal	1 day	2 days	3 days	4 days	5 days
Pp. (12)	3.1±0.8	8.3±1.6	11.9±2.3	13.1±3	18.0±3	24.1±2.8
Pp.A.L.D. (9)	1.5±0.6	5.2±1.6	6.9±0.9	7.5±0.9	11.2±2.5	14.4±2.7
Pp.C.L.D. (5)	3.5±0.8	5.1±1.4	11.7±4	10.5±2	15.2±3.4	24.6±5.5
Pp.S–x (12)	3.2±0.6	4.3±0.6	5.8±0.9	7.9±1.5	11.4±1.7	10.9±2.3

Total lipids (mg./100 ml. of blood plasma)

Dogs	Basal	1 day	2 days	3 days	4 days	5 days
Pp. (12)	486±58	985±235	1,057±139	1,029±111	920±182	854±182
Pp.A.L.D. (9)	468±45	803±93	1,268±245	1,117±408	907±204	701±119
Pp.C.L.D. (5)	464±15	950±103	1,370±335	1,019±311	922±152	707±123
Pp.S–x (12)	617±65	748±88	857±107	895±90	789±72	717±59

Free fatty acids (μEq./L. of blood plasma)

Dogs	Basal	1 day	2 days	3 days	4 days	5 days
Pp. (12)	474±74	1,533±145	1,719±175	1,752±142	1,910±111	1,880±152
Pp.A.L.D. (9)	565±60	1,738±151	1,653±132	1,757±169	1,484±83	1,481±168
Pp.C.L.D. (5)	423±70	1,671±142	1,470±192	1,709±155	1,819±232	1,740±226
Pp.S–x (12)	442±60	948±115	1,259±93	1,371±85	1,427±138	1,299±105

Glycemia (mg./100 ml.; total blood)

Dogs	Basal	1 day	2 days	3 days	4 days	5 days
Pp. (12)	78±3	227±12	266±24	263±16	280±26	275±10
Pp.A.L.D. (9)	80±3	250±17	301±22	263±99	368±81	352±160
Pp.C.L.D. (5)	83±3	406±28	417±22	430±54	382±33	339±14
Pp.S–x (12)	79±3	236±23	297±29	317±45	291±23	266±17

Cholesterol (mg./100 ml. of blood plasma)

Dogs	Basal	1 day	2 days	3 days	4 days	5 days
Pp. (11)	114±8	145±10	153±8	200±9	158±13	156±11
Pp.A.L.D. (9)	112±9	127±8	160±13	151±12	161±25	144±8
Pp.C.L.D. (5)	125±8	118±3	154±12	140±12	142±7	128±19
Pp.S–x (12)	119±12	136±11	158±14	162±11	170±10	169±11

TABLE 2

Changes of liver composition in dogs: Normal (N.); pancreatectomized (Pp.); pancreatectomized with acute liver denervation (Pp.A.L.D.); pancreatectomized with chronic liver denervation (Pp.C.L.D.); and pancreatectomized with bilateral removal of sympathetic chains (Pp.S–x)

Dogs	FFA (μEq. per gm.)	Total lipids (gm. per 100 gm.)	Triglycerides (gm. per 100 gm.)	Phospholipids (gm. per 100 gm.)	Cholesterol (gm. per 100 gm.)
N. (6)	10.5±2.2	5.4±0.7	4.0±0.8	2.5±0.4	271±43
Pp. (12)	7.9±0.3	38.9±5.3	34.5±5.7	3.1±0.3	755±60
Pp.A.L.D. (4)	12.3±1.4	27.0±3.6	23.4±3.4	2.6±0.2	610±73
Pp.C.L.D. (5)	13.4±3.5	34.2±6.1	30.1±2.8	3.6±0.4	313±38
Pp.S–x (4)	9.3±1.4	13.9±2.0	10.2±2.0	4.1±0.4	815±32

Heparinized blood samples were obtained from the forearm vein after sixteen hours of fasting and the following determinations were made on the plasma of each sample: glycemia (on total blood) (Somogyi[4]); total lipids (Bragdon[5]); free fatty acids (Dole and Meinertz[6]); ketone bodies (Bessman and Anderson[7]); phospholipids (Dryer et al.[8]); total cholesterol (Searcy and Bergquist[10]). The operated dogs were maintained on the previous diet for five days and they were killed under anesthesia on the sixth day after pancreatectomy.

Samples of hepatic tissue for lipid determinations in the four groups were taken immediately after the animals were killed. According to the following technic (Folch et al. modified by Chernick[9]), an aliquot of hepatic tissue was transferred to chloroform methanol 2/1-V/V mixture for total lipid extraction. On the chloroform extracts, determinations of total lipids, free fatty acids and total phosphorus were run. An aliquot of the original chloroform was allowed to hydrolyze with alcohol solution of KOH (1 ml. of saturated solution of KOH with 10 ml. of 50 per cent alcohol), and then ether extraction of the nonsaponifiable fraction (cholesterol) was performed.

Another aliquot of the original chloroform extract was transferred to 100 mesh silicic acid columns in order to separate triglycerides from phospholipids with chloroform and methanol elutions. These two fractions were evaluated as total lipids according to Bragdon's method. Phosphorus determinations were run on the phospholipidic fractions.

RESULTS

1. *Blood determinations*

Glycemia. The four groups of pancreatectomized dogs developed typical diabetic hyperglycemia twenty-four hours after pancreatectomy. During the whole period no significant difference was observed among groups 2, 3 and 5. In the pancreatectomized chronic-liver-denervated dogs (Group 4) the glycemia was higher, especially on the first, second and third day. It is clear that neither bilateral sympathectomy nor acute liver denervation decreased the hyperglycemia as compared with the pancreatectomized controls.

Cholesterolemia. A continuous and moderate increase of total blood cholesterol was observed after pancreatectomy in all the groups studied.

Lipemia. The pancreatectomized control dogs as well as the pancreatectomized dogs with hepatic denervation exhibited a clear-cut increase of lipemia from the second day. The values were high to the end of the experiments, even when a slight decrease was observed on the fourth and fifth days. The pancreatectomized dogs with bilateral sympathectomy had lower values of lipemia than the other three pancreatectomized groups of dogs.

Plasma free fatty acids (FFA). The pancreatectomized control dogs showed a continuous and marked increase of plasma FFA from the second day to the end of the experiments. The pancreatectomized dogs with bilateral sympathectomy showed an increase of FFA only from the second to the fourth days with a decrease on the fifth day to values lower than those of the pancreatectomized controls. The pancreatectomized dogs with liver denervation exhibited an intense increase of FFA from the beginning up to the third day and a slight diminution on the fourth and fifth days after pancreatectomy. The values were similar to those found in pancreatectomized controls.

Ketonemia. The pancreatectomized control dogs had a clear-cut and progressive hyperketonemia from the

first day until the end of the experiments (around eight times the initial values). On the other hand, the hyperketonemia of the pancreatectomized dogs with bilateral sympathectomy or with acute hepatic denervation followed a curve of similar slope but with lower absolute values (40 to 50 per cent less) than the pancreatectomized controls, during the whole period of observation. This attenuation of ketonemia was not observed in the pancreatectomized dogs with chronic hepatic denervation; therefore, the attenuation was not maintained in dogs with twenty days of liver denervation (Group 4).

2. *Liver determinations*

(a) *Fatty liver infiltration.* The classical macroscopic picture of diabetic fatty liver infiltration was observed in every group of pancreatectomized dogs, with the exception of the pancreatectomized-sympathectomized dogs where it was not so intense.

(b) *Liver lipid content.* Pancreatectomy, as well as pancreatectomy and chronic hepatic denervation, induced a sharp augmentation of the liver total lipids equivalent to seven times the normal values.

This increase was slightly lower in the pancreatectomized, acute-liver-denervated dogs (five times the normal values) and clearly and significantly depressed in the pancreatectomized-sympathectomized dogs (only three times the normal values).

The liver total lipid infiltration was due mainly to the triglyceride fraction, its proportion being: of triglycerides, 89 per cent of total lipid for the pancreatectomized controls; 87 per cent for the pancreatectomized, acute-liver-denervated dogs; 88 per cent for the pancreatectomized, chronic-liver-denervated dogs and 73 per cent for the pancreatectomized-sympathectomized dogs. The last condition was very close to the normal values of 74 per cent of triglycerides found in the normal control dogs.

There was no clear variation observed in the liver FFA content of the different experimental and control groups when compared each against the others.

The liver phospholipid content was about the same in every group studied but the pancreatectomized-sympathectomized dogs showed a slight increase over the normal values.

The liver cholesterol content was 2.7 times the normal observed values for the pancreatectomized control dogs; 2.2 times for the pancreatectomized, acute-liver-denervated dogs; a very low value of 1.1 times for the pancreatectomized, chronic-liver-denervated dogs and 3 times the normal values for the pancreatectomized-sympathectomized dogs.

DISCUSSION

Diabetic hyperglycemia was marked in the four groups of pancreatectomized dogs studied, higher in the chronic-denervated-liver animals. No attenuation was obtained by bilateral sympathectomy or hepatic denervation. This lack of attenuation of hyperglycemia is in accordance with previous observations of Lewis and Turcatti (1935)[11] on pancreatectomized dogs with bilateral extirpation of sympathetic chains; or with bilateral abdominal sympathectomy or bilateral vagotomy (Etcheverry 1937[12]); or on pituitary diabetes of dogs submitted to bilateral section of the splanchnic nerves (Houssay, Biasotti and Rietti[13]).

Bilateral sympathectomy performed on pancreatectomized dogs was followed by a diminution of the typical diabetic hyperlipemia, blood total lipids and FFA, as well as by a marked decrease of blood ketone bodies, and a diminished deposition of fat in the liver, in comparison with the pancreatectomized control dogs. These facts suggest that sympathectomy in the pancreatectomized dog produced a diminution of fatty acid mobilization from the peripheral tissues and a decrease of deposition of fat in the liver, less formation of ketone bodies in blood, and an increase of cholesterol in the liver.

The first experimental demonstration that adipose tissue mobilization is under nervous control is due to Wertheimer (1926).[14-16] Scission of the spinal cord in the vicinity of the first thoracic segment prevents the transport of fat from the adipose tissue to the liver and the formation of a fatty liver in a phloridzin-treated, fasting dog.

In the pancreatectomized dogs, acute hepatic denervation induced a sharp diminution of the diabetic blood levels of ketone bodies, and diminution of the hepatic total lipid content and no modifications of diabetic hyperglycemia, hypercholesterolemia, hyperlipemia and FFA as compared with the pancreatectomized control dogs.

Hepatic chronic denervation in the pancreatectomized dogs was followed by high hyperketonemia, high content of liver total lipids, and severe hyperglycemia as compared with the other groups of pancreatectomized dogs, with an almost normal amount of hepatic cholesterol in contrast with the marked increase in all the other groups of pancreatectomized dogs. It is remarkable that the attenuated ketonemia and relatively low

value of liver lipid observed in the pancreatectomized, acute-liver-denervated dogs express a transient phenomenon. They reach high values as soon as a chronic state of denervation is established, but the liver cholesterol is very much diminished at this moment.

This diminution of cholesterolemia and cholesterol content in the liver is striking in the pancreatectomized, chronic-liver-denervated dogs and deserves further study.

Sympathectomy in the pancreatectomized dog clearly lowers the amount of liver total lipids and the blood ketone bodies level, the cholesterolemia remaining at high values. Similar effects, even when attenuated, are observed after the acute denervation of the liver.

From our observations it appears that all three pancreatectomized-denervated groups of dogs exhibit a net and inverse relationship between the hyperketonemia and the hepatic cholesterol content: hyperketonemia corresponds to low hepatic cholesterol and its reciprocal. This fact is in contrast with the observed parallelism of hyperketonemia and hepatic increase of cholesterol in the pancreatectomized control dogs.

In the four groups of pancreatectomized dogs, a parallelism was observed between the amount of lipid in the liver and the level of ketonemia.

The experiments described in this paper show some influences of sympathetic nerves and hepatic inervation on lipid metabolism.

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