Effect of Prolonged Administration of Tolbutamide in Depancreatized Dogs

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In a preliminary communication[1] we have reported that depancreatized dogs treated with tolbutamide for prolonged periods showed derangements of liver function. The present paper gives a detailed account of our studies on twelve depancreatized dogs treated orally with various doses of the drug. When given relatively large doses, the animals required appreciably smaller amounts of exogenous insulin to maintain a standard degree of control.

MATERIALS AND METHODS

The series represents a total of sixteen dogs and consists of the following groups of animals:

1. Depancreatized adult dogs

(a) Two dogs maintained on 150 mg. per kilogram body weight of tolbutamide; (b) three dogs on 100 mg. per kilogram body weight; (c) three dogs on 30 mg. per kilogram body weight; (d) two controls.

2. Depancreatized pups

(a) Three pups maintained on 70 mg. per kilogram body weight; (b) one control.

3. Partially depancreatized adult dogs (approximately 50 per cent of pancreatic tissue removed from the splenic end):

(a) One dog maintained on 30 mg. per kilogram body weight; (b) one control.

The adult animals weighed about 10 kg. and the pups, which at the time of pancreatectomy were approximately six months old, about 5 kg. The diabetic dogs were fed measured amounts of a commercial ration supplemented with raw pancreas. They were kept in metabolism cages and the urine was collected daily. The total amount of sugar was determined by Clinitest* tablets. Regular and Protamine Zinc insulin were administered daily, prior to feeding, in two separate subcutaneous injections in amounts required to keep blood sugar levels on the average below 200 mg. per 100 cc. and the glycosuria very slight. Because of considerable fluctuation from day to day and week to week, it was found more convenient to express the effect of tolbutamide upon blood sugar in terms of the amount of insulin spared. The diabetic dogs were kept under these standard conditions for at least two weeks and in some cases much longer before treatment was commenced.

Blood samples were obtained frequently before and during the test period. Blood sugar was determined by the micro-micromethod described by King.[2] Bromsulphalein clearance tests were performed according to Gornall and Bardawill.[3] Serum bilirubin was determined according to Malloy and Evelyn.[4] Prothrombin clotting time was determined in whole plasma and in 25 and 50 per cent saline dilutions by the one-stage method of Quick.[5] Total cholesterol was determined by a modification of the method of Bloor, Pelkan and Allen.[6] Total serum protein was determined by the method of Weichselbaum.[7] Serum albumin was determined by salt precipitation with 26 per cent sodium sulphate[8,9] and the precipitated globulins were separated by centrifugation under ether according to Kingsley.[10] Serum alkaline phosphatase was estimated by the amount of phenol liberated from a phenolphosphate substrate and expressed in King-Armstrong units per 100 ml. of serum.[11] Serum glutamic-pyruvic and glutamic-oxalacetic transaminases were determined by a procedure in which the oxalacetate and pyruvate formed an intensively brown-colored hydrazone; the serum levels were expressed in Sigma-Frankel units per 1 ml. of serum.[12] Total serum protein-bound hexose was determined with the orcinol reagent according to Lustig and Langer[13] using Winzler's modification.[14]

RESULTS

The first change in liver function noted at any dose level was usually an elevation of the serum alkaline phosphatase. Figures 1-a,b,c show the behavior of the alkaline phosphatase in the first two groups of animals. The same elevation was observed in the partially depancreatized dog. A fall in total serum proteins was also encountered which was due to a decrease in serum albumin. Figures 2-a,b,c present the results obtained in the same groups of animals. Again, the partially depancreatized animal showed the same change. The glutamic-oxalacetic and glutamic-pyruvic transaminase levels became elevated and the highest values as well as the normal ones are recorded in table 1. The serum cholesterol levels were not followed closely, but in several dogs a profound fall was encountered in the terminal stages. The lowest figures as well as the control values for each animal are included in table 1. Total protein-bound hexose was determined on a number of blood samples and an elevation from the average normal of 106 mg. per 100 cc. up to 153 mg. per 100 cc. was found in all our dogs treated with tolbutamide.

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* Ames Company, Inc.

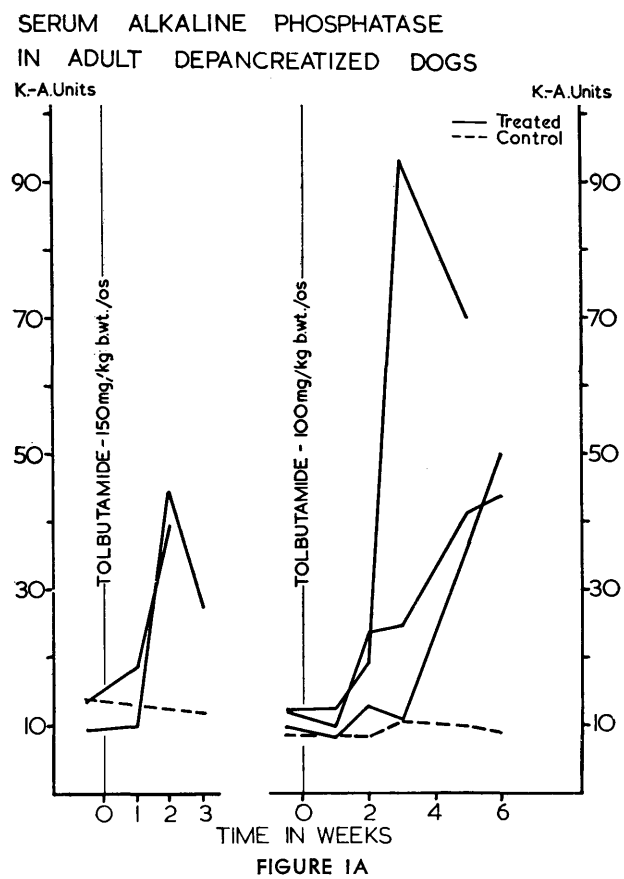

FIGURE 1A

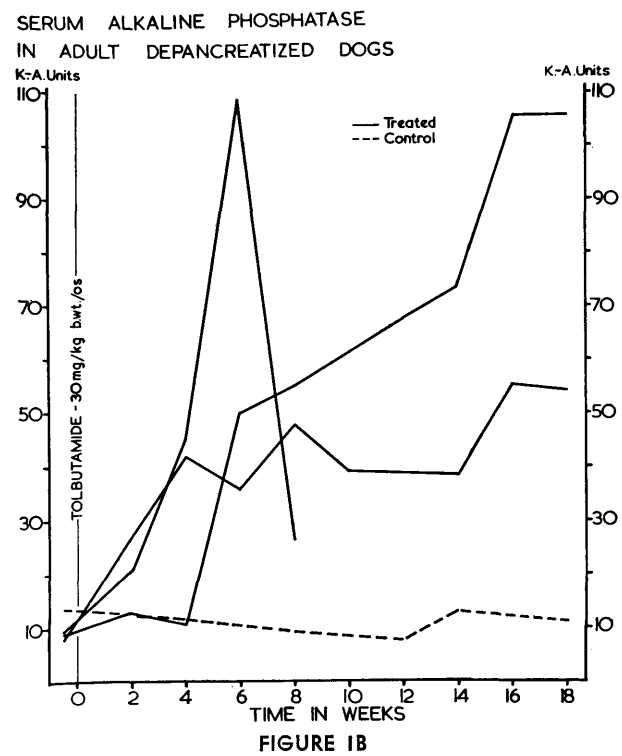

FIGURE 1B

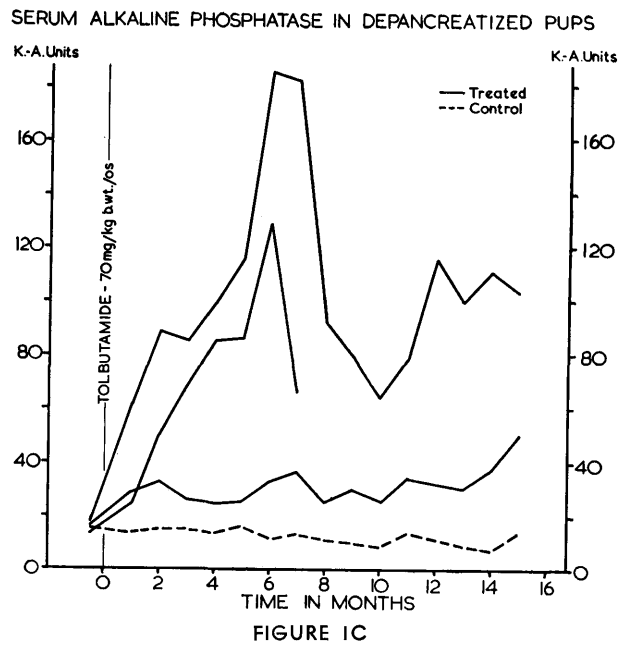

FIGURE 1C

TABLE 1

Serum transaminase and cholesterol values before and during tolbutamide treatment

Dose/kg. b.wt.	TGO—units control	TGO—units highest	TGP—units control	TGP—units highest	Cholesterol mg. per cent control	Cholesterol mg. per cent lowest
150	31	1,010	20	230	306.2	65.9
	21	660	21	200	400.5	67.6
100	—	25	—	28	—	229.6
	—	14	—	18	—	156.6
	—	380	—	200	—	—
70	16	71	18	72	194.0	156.6
	14	78	24	240	288.4	131.0
	11	98	11	120	201.1	34.6
30	17	194	19	800	314.0	120.0
	23	155	21	670	251.0	90.8
	15	130	3	120	261.7	184.2
	* —	1,102	—	960	—	27.3

* Partially depancreatized.

Bromsulphalein clearance tests were performed on all animals frequently and the results were found to be within normal limits, i.e., less than 10 per cent retention fifteen minutes after injecting 10 mg. per kilogram body weight.

The insulin requirements are recorded in figure 3. It can be seen that those dogs which were maintained on 100 mg. or 150 mg. per kilogram body weight of tolbutamide required, after a certain time, considerably less insulin. The insulin requirements of the puppies which have been maintained on 70 mg. of tolbutamide per kilogram body weight for almost sixteen months, (and thus are now adult dogs) and the insulin requirements of the dogs maintained on 30 mg. per kilogram body weight have not appreciably changed. These figures

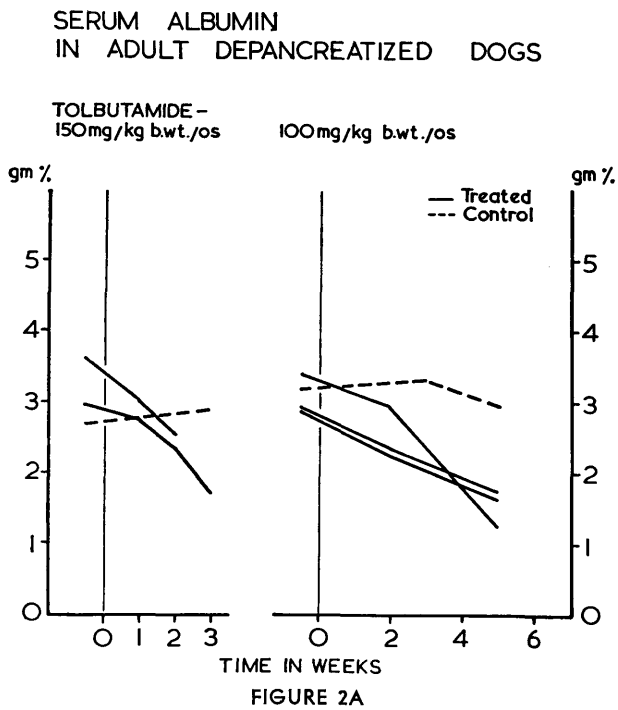

FIGURE 2A

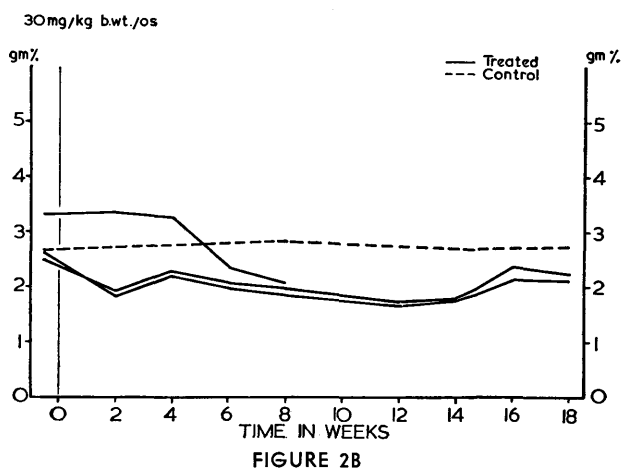

FIGURE 2B

are omitted from the graph. The partially depancreatized dog did not require exogenous insulin at any time and showed a moderate but substantial fall in blood sugar directly after the first dose of tolbutamide. In the totally depancreatized dogs which responded to the drug with a fall in blood sugar, there was no immediate drop after the first dose, but the blood sugars gradually fluctuated at lower levels and thus enabled us to decrease the daily amount of injected insulin.

The prothrombin clotting time in plasma diluted with saline to 25 per cent was in some cases prolonged to 300 seconds, in comparison with about twenty seconds

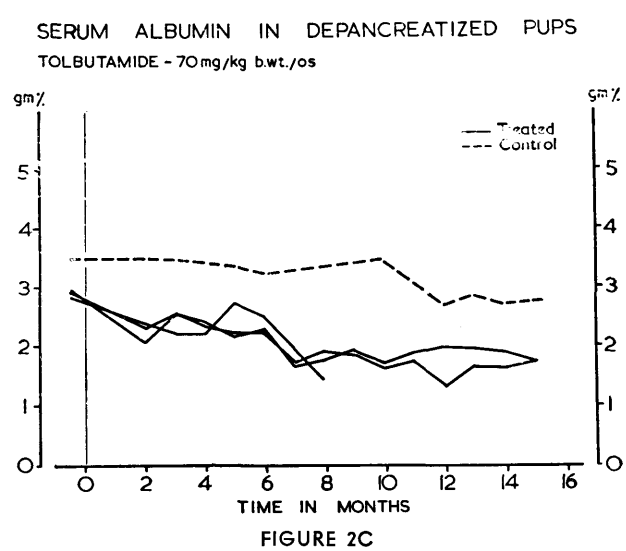

FIGURE 2C

for normal dogs. This prolongation usually could be eliminated by parenteral administration of vitamin K_1. In two animals the prothrombin clotting times were elevated in the terminal stages and here vitamin K_1 was without effect. In two other animals bleeding into various organs and into the peritoneal cavity was encountered but the prothrombin clotting time was normal. The dying animals were always jaundiced. The elevation in serum bilirubin levels was due to a rise in direct reacting bilirubin. It appears then that bleeding tendencies, prolonged prothrombin clotting times and jaundice were abnormalities which occurred independently of each other but a final conclusion on this point is not possible in this small series of animals.

All five dogs treated with 100 or 150 mg. tolbutamide per kilogram body weight deteriorated within three to six weeks and either died or had to be sacri-

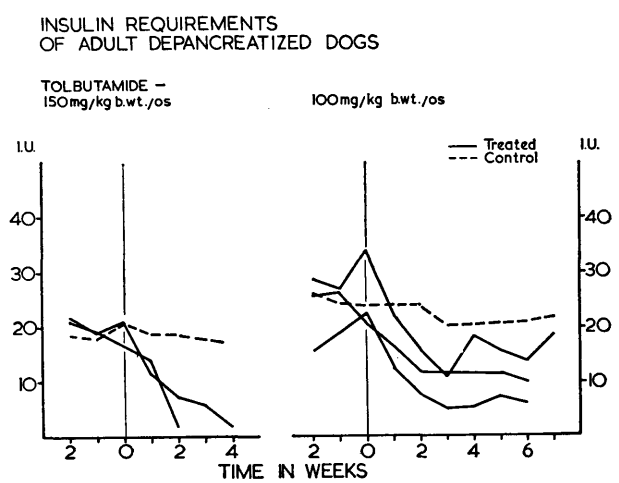

FIGURE 3

ficed. One animal in each of the other groups, i.e., those receiving less than 100 mg. tolbutamide per kilogram body weight, followed the same course as those receiving the higher doses. The gross and microscopic findings in all of our autopsied animals will be reported in a separate communication. The completeness of the pancreatectomy was verified in each case.

DISCUSSION

The data presented above indicate that the livers of our experimental animals have been seriously affected by tolbutamide. The data also show that prolonged administration of the drug in doses of 100 mg. and 150 mg. per kilogram body weight may produce a fall in blood sugar in depancreatized dogs and diminish but not abolish their requirements for exogenous insulin. A fall in blood sugar after prolonged administration of tolbutamide to depancreatized dogs was observed by Ricketts and associates[15] and Schambye.[16] Bleeding into organs of depancreatized dogs treated with large doses of tolbutamide was reported previously by Schambye,[17] but he did not conduct any extensive study of liver function. In another study Tarding and Schambye,[18] using C^{14}-labeled glucose, were able to demonstrate a reduction in hepatic glucose output after a single injection of tolbutamide to normal dogs. Ashmore, Cahill, Earle and Zottu,[19] by cannulating the portal and hepatic veins, provided direct evidence of a diminished glucose output from the liver when normal dogs were given a single intravenous injection of tolbutamide. These results would suggest that an analogous situation may have existed in our depancreatized animals receiving 100 mg. or more per kilogram body weight of the drug for prolonged periods. The diminished insulin requirements of our animals may prove to be due to a diminished glucose output from the liver. It would also be indicated that doses such as 30 mg. or 70 mg. per kilogram body weight, while effective in producing the described changes in liver function, were perhaps insufficient to interfere with glucose output as judged by the relatively steady blood sugar levels and insulin requirements. This suggestion is strengthened by the numerous reports on experiments with liver slices. Tolbutamide had to be present in the incubation medium in rather large concentration in order to depress the activity of enzymes involved in the release of free glucose from this organ.[20-24]

It might be argued that the derangements in liver function observed in our dogs are the result of nutritional deficiencies due to malabsorption. All our depancreatized animals were kept under standard conditions for from several weeks to one year before they were given tolbutamide. None of the dogs showed any evidence of digestive or hepatic disturbance during the control period and these observations and the results of our previous experiences with depancreatized dogs on similar diets and insulin therapy tend to rule out nutritional deficiencies as a cause of the hepatic changes. In the partially depancreatized dog the pancreatic tissue was unable to protect the animal from the toxic effects of the drug. In recent experiments we have observed that even normal dogs, when treated with small amounts of tolbutamide, may show derangements in liver function similar to those described in the depancreatized animals.

The severe change in liver function found in dogs is in a striking contrast to the results of similar studies in human subjects. We have carried out the same liver function tests in a series of human diabetics treated with tolbutamide by our clinical colleague, Dr. B. Leibel, and in accord with other authors[25] have thus far seen no abnormalities. The results of the study of Mohnike and Wittenhagen[26] indicate that in the dog this drug is metabolized in a different way from that occurring in man. The possibility that the tolbutamide treated dog produces a toxic metabolite either in the form of para-toluol-sulfonamide or some other by-product is now being investigated.

SUMMARY

Tolbutamide (Orinase-Hoechst) was given in various doses orally to twelve depancreatized dogs for prolonged periods of time. All the animals suffered derangement in liver function, two died and six had to be sacrificed because they became jaundiced and were deteriorating rapidly. It was observed, in confirmation of other authors (Ricketts, Schambye), that depancreatized animals when treated with relatively large doses of tolbutamide may require appreciably smaller amounts of exogenous insulin to maintain a standard degree of control. The present findings are discussed in the light of those of other workers.

SUMMARIO IN INTERLINGUA

Effecto Del Administration Prolongate De Tolbutamido In Canes Dispancreatisate

Tolbutamido (Orinase-Hoechst) esseva administrate in varie doses per via oral a dece-duo dispancreatisate canes durante prolongate periodos de tempore. Omne le animales suffreva un disrangiamento del function hepatic. Duo moriva, e sex debeva esser sacrificate proque illos disveloppava jalnessa e se deteriorava rapidemente. Esseva observate, in confirmation de altere autores (Ricketts, Schambye) que dispancreatisate animales—quando tractate con relativemente grande doses de tolbutamido —require in certe casos appreciabilemente plus micre

quantitates de insulina exogene pro mantener un grado standard de controlo. Le presente constatationes es discutite in le lumine de illos de altere autores.

ACKNOWLEDGMENT

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The metabolic blocks thus far discussed have a direct relation to the production of genetic disease. The relation, however, may not always be so direct, and the pathological consequences of a gene may manifest themselves only in specific environmental situations. It has recently been shown, for example, that a genetic deficiency of the enzyme glucose-6-phosphate dehydrogenase may cause an alteration in glutathione metabolism, resulting in an instability of reduced glutathione.[1] As a consequence of this instability, the red blood cells are liable to hemolysis following the ingestion of certain drugs such as naphthalene, primaquinine, sulfanilamide, and nitrofurantoin. Thus an induced hemolytic anemia develops, which, though appearing to be environmentally produced, has a definite genetic basis. From a practical standpoint, an assay of blood cells for this enzyme may make it possible to detect those individuals who are drug-sensitive and would be harmed by these drugs.

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