tonin, 5-hydroxytryptophan, had no effect. (4) Closure of chromatophores by an active substance was not reversible by electrical stimulation. (5) Attention is called to the fact that constricting agents all share a common property, that of resonance, and that a number of them are capable of electron donation. (6) Results obtained are consonant with theory that chromatophore control is achieved by an amine-amine oxidase system (closure), opposed by an acetylcholine-choline esterase system (expansion).

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Effect of Tolbutamide on Glucose and Nitrogen Metabolism in Totally Depancreatized Dog. (24659)

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The manner in which sulfonylureas lower blood sugar has not been fully elucidated. There is considerable evidence that they may stimulate release of insulin from the pancreas when that organ is present and functioning (1-6), and it is well established that the drugs are ineffective when pancreas is absent(1,7,8). We and others have demonstrated, however, that the sulfonylurea, tolbutamide, diminishes blood and urinary glucose in totally depancreatized dog maintained with suboptimal doses of insulin(9-13). Clearly, then, the pancreas is not essential for the action of this compound provided small amounts of insulin from other sources are available. The question now arises, whether, under these circumstances, tolbutamide potentiates the injected insulin or whether, alternatively, its action takes place primarily in tissues where insulin plays only a permissive role. In our experiments, to answer this question, changes in urinary nitrogen excretion of the inadequately treated diabetic animal were used as criterion of insulin potentiation.

Methods. Mongrel dogs approximately 10 kg were subjected to total pancreatectomy. Completeness of operation was verified at autopsy. The animals were kept in metabolism cages and given constant diet (Rival Dog Food) in 2 daily feedings with measured amounts of pancreatin. Acidified 24-hour specimens of urine were collected daily and combined in 3-day pools for later analysis. Feces were collected quantitatively over brief periods from 2 dogs. Total nitrogen was determined in food, pancreatin and feces after acid digestion, and also in urine, using steam distillation(14a) and titration with boric acid (14b). Pooled urine was analyzed quantitatively for glucose(15). Fasting blood sugar (16) was determined several times a week. Dogs were weighed periodically. Animals were maintained on constant suboptimal amount of regular insulin (2 to 6 units twice

TABLE I. Effect of Added Insulin Compared to Added Tolbutamide on Urinary Glucose and Nitrogen of Totally Depancreatized Dogs Inadequately Treated with Insulin.

Dog	Wt, kg: Initial/terminal	Mean blood sugar, mg %			Mean urine sugar, g/day			Mean urine N, g/day		
		All control periods	+ PZI	+ Tol.	All control periods	+ PZI	+ Tol.	All control periods	+ PZI	+ Tol.
28	12.0/7.0	302	254	217	32.1	6.8	5.9	9.2	7.8	9.3
32	7.8/5.4	314	265	221	34.6	13.0	24.2	6.1	5.6	5.9
19*	10.7/8.3	379	206		43.3	0.7		9.0	8.0	
36*	9.7/6.8	364		242	38.4		11.4	6.0		5.85
37*	8.8/6.3	302		246	38.9		25.8	9.2		9.6

* Died before some of planned observations could be completed.

Data for first 3 days of each period (control, PZI and tolbutamide) were excluded from calculations.

PZI = protamine zinc insulin, 2 units once daily. Tol. = tolbutamide, 0.25 g twice daily.

a day). No studies were performed sooner than 2 weeks after laparotomy. The experimental design consisted of 5 periods of 11 to 27 days each: (1) initial period when animal received only constant small doses of regular insulin; (2) experimental period when either supplementary protamine zinc insulin 2 units once daily, or tolbutamide 0.25 g twice daily, was administered; (3) recovery period following withdrawal of supplementary drug; (4) second experimental period employing alternate supplementary drug; and (5) final recovery period.

Results. Unfortunately, some animals succumbed before all of planned observations could be completed. Premature death of several dogs seemed attributable to extensive bleeding accompanied by high prothrombin time after treatment with tolbutamide(13). Similar results have been found with carbutamide(17) and isopropylthiodiazole(18).

A significant fall in urinary nitrogen, synchronous with reduction of glucose levels, occurred when supplementary protamine zinc insulin (PZI) was given (Table I).* In contrast, tolbutamide produced no demonstrable decrease in urinary nitrogen. Differences in nitrogen loss with PZI as compared with tolbutamide were statistically significant ($p < .01$).

Nitrogen balance, determined roughly during control observations for two 3-day periods in Dog 32 and for one 3-day period in Dog 36, seemed to be at equilibrium or slightly positive (+0.89, +0.3 and −0.08 g/day respectively).

Discussion. Nitrogen sparing effect of insulin(19,20) is demonstrated best, although probably not only(21), when nitrogen balance is negative(22). In our experiments, marked weight loss of all animals (Table I), in most to the point of cachexia, renders it probable that overall nitrogen loss exceeded intake despite what seemed to be equilibrium in 2 dogs during brief periods of observation. The endogenous origin of at least some urinary nitrogen is proved by decrement that occurred when small additional amounts of insulin were given. Tolbutamide, on the other hand, under similar conditions and in doses sufficient to cause significant reduction in glucose levels, was without effect on nitrogen excretion.

These observations are incompatible with the notion that tolbutamide potentiates exogenous insulin, at least in the dog. They can best be explained by the assumption that the drug reduced glucose output from the liver, as shown by earlier work(23), under permissive action of injected insulin.

Summary. In totally depancreatized dogs maintained on a constant regimen with suboptimal amounts of insulin, additional insulin, but not tolbutamide, caused a decrease in urinary nitrogen along with the expected decline in glucose levels. The failure of tolbutamide to duplicate the action of insulin suggests that it does not potentiate the injected hormone.

* One animal with only mild glycosuria during control periods failed to show a fall in urinary nitrogen on PZI and was therefore excluded.

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Concentration of Azoproteins in Yolk Sac Placenta of the Rat.* (24660)

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The striking role of the rabbit yolk sac placenta in transmission of homologous gamma globulin from the maternal system into the embryo and the apparent non-permeability of this membrane to heterologous protein has been described by Brambell(1). Few attempts have been made to study the permeability of either the chorio-allantoic or the inverted yolk sac placentae of the rat to protein molecules although both homologous and heterologous protein compounds have been used extensively in various studies of maternal-fetal relationships in this animal. The present work is an attempt to study the fate of 2 different azoprotein molecules injected into the pregnant rat, specifically with respect to placental membranes at various stages of gestation.

Materials and methods. The azo dye, echt säure blau B, has been shown to be rapidly excreted in the bile and urine of animals injected with moderate amounts of this compound(2). It is not phagocytyzed by the reticulo-endothelial system and apparently does not combine with serum proteins *in vivo*. Echt säure blau B, an intensely blue azo dye, was diazotized and coupled to crystalline bovine serum albumin and rat serum albumin utilizing the technic described by Kruse and McMaster(2). Bovine serum albumin was obtained commercially and rat serum albumin was obtained by ammonium sulfate precipitation of pooled rat serum. After final dialysis and filtration, the bovine serum albumin-azo dye preparation (BSA-ESBB) contained 20 mg of protein and 1408 μg of dye/ml. The

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