homotransplantation. 2) Neither properdin nor complement was rapidly metabolized or inactivated by these cells in tissue culture. 3) Addition of properdin to tissue culture medium (in presence or absence of cells) caused a fall in C'1 titer and accelerated the fall of C'2 and C'4.

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Comparison in Rabbits of Hypoglycemia and B Cell Degranulation After Various Sulfonylureas.* (25145)

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It has been shown unequivocally that sulfonylureas produce degranulation of the pancreatic B cells of rats, rabbits and dogs when administered over prolonged periods(1-3). This degranulation has been interpreted(2) as indicative of suppression of insulinogenesis similar to that observed during chronic hypoglycemia induced by insulin administration. However, we demonstrated previously(4) that in rabbits insulin induced hypoglycemia did not produce degranulation, whereas hypoglycemia of equivalent depths and duration produced by tolbutamide caused varying degrees and frequently complete degranulation of the B cells. We undertook to determine whether the hypoglycemic sulfonylureas would produce pancreatic B cell degranulation even though their hypoglycemic action was prevented by simultaneous infusion of glucose. Furthermore, since the hypoglycemic effectiveness of these drugs apparently depends on their ability to increase insulin output from B cells and since this increased insulin output will be reflected in the extent of B cell degranulation, we attempted to correlate their blood sugar lowering efficacy with degree of B cell degranulation.

Material and methods. We used 72 New Zealand white rabbits of either sex, weighing 2.5 to 4 kg divided into 3 main groups. 1) *Single injections.* Eighteen normal rabbits, after overnight fast, each received intravenously 125 mg/kg of tolbutamide or chlorpropamide, or metahexamide. Furthermore, 6 animals received 1 cc/kg of saline intravenously and served as controls. Blood was withdrawn from marginal ear vein immediately before and at hourly intervals for 5 consecutive hours after administration of each test dose. 2) *Multiple injections.* Eighteen

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rabbits received intravenously twice daily 125 mg/kg of tolbutamide, chlorpropamide, or metahexamide for 28 consecutive days. Blood was withdrawn for glucose determination twice daily at 10 a.m. and 1:30 p.m. 3) *Infusion experiments.* Six rabbits were infused through marginal ear vein for 7 hours on 1, 2 or 3 successive days with approximately 300 cc of 5% glucose in saline. Additional 24 animals received a similar perfusate to which 625 mg/kg of either tolbutamide, chlorpropamide, or metahexamide had been added. An attempt was made to infuse a third group of animals with sulfonylureas in saline. This could not be accomplished since rabbits invariably expired during the first night. All animals were kept in individual metabolic cages and received Purina Rabbit Chow, carrots and water *ad lib.* Animals treated for prolonged periods received 200,000 units of Procaine Penicillin G and 0.25 g of dihydrostreptomycin sulfate (Combiotic Pfizer) twice weekly. Blood sugars were determined by Nelson-Somogyi micromethod(5). Rabbits were sacrificed by overdosage with barbiturates and the tail of the pancreas was immediately placed into Zenker-Formol (20%) solution for microscopic studies.

Results. A single injection of 125 mg/kg of tolbutamide caused a decline of blood sugar level which varied from −26 to −43 mg % (mean: −31 mg %). The nadir was obtained at 2 to 3 hours after which time blood sugar levels tended to rise. However, blood sugars did not regain the fasting level within 5 hours. Both chlorpropamide and metahexamide caused a more pronounced hypoglycemia. The maximum decline obtained with metahexamide varied from −33 to −57 mg % with a mean of −42 mg % while chlorpropamide decreased the blood sugar level which ranged from −46 to −66 mg % (mean: −57 mg %). In control experiments blood sugar concentration showed no significant fluctuations above and below the initial value (Fig. 1).

Multiple injections of 3 sulfonylureas produced a significant decline of blood sugars after each injection. However, the morning (non-fasting) levels were always within normal limits. No animals died in hypoglycemic shock.

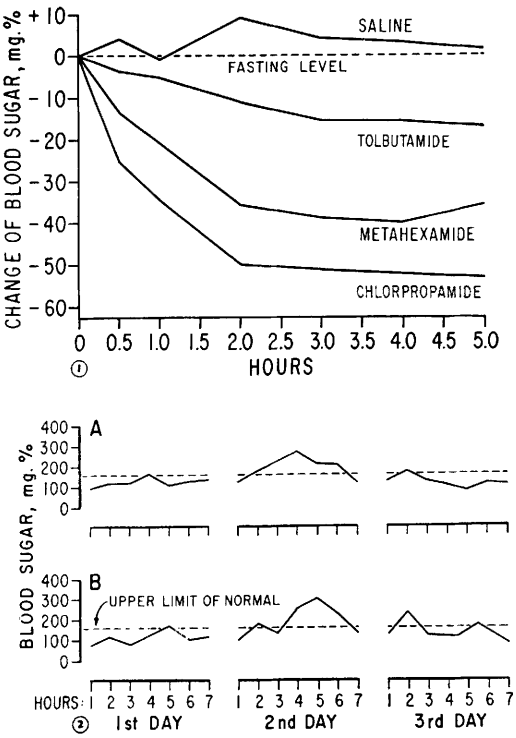

FIG. 1. Comparison of mean decline in blood sugar of rabbits after a single inj. of tolbutamide, metahexamide or chlorpropamide.

FIG. 2. Representative blood sugar curves of 2 rabbits: (A) infused through marginal ear vein for 7 hr on 3 successive days with 300 cc of 5% glucose in saline alone; (B) with a similar amount of glucose in saline to which 625 mg/kg (2 g) of metahexamide had been added.

Infusion of 300 cc of 5% glucose solution in saline for 7 hours produced the expected hyperglycemia with maximal blood sugar elevations obtained at 1 or 2 hours varying from 210 to 400 mg %. In animals in which the various sulfonylureas were administered simultaneously with glucose, degree of hyperglycemia was not significantly altered. Peak values ranged from 220 to 375 mg % (Fig. 2). In no instance was a significant decline of blood sugars below fasting levels observed.

A single injection of any sulfonylurea caused no alteration in pancreatic morphology. After chronic administration for 28 days there was usually similar extensive B cell degranulation with all 3 drugs.

Infusion of glucose alone for 3 days caused no detectable alteration in islet morphology. When sulfonylureas were added to the glucose perfusate varying degrees of degranulation

were obtained. Administration of chlorpropamide and metahexamide caused extensive degranulation after 2 days, which usually became almost complete after 3 days. On the other hand, perfusion of tolbutamide caused less pronounced degranulation after 2 days and only rarely complete loss of B cell granules even after administration of the drug for 3 successive days.

Discussion. It has been demonstrated that while daily infusion of glucose for 3 days causes no morphologic alteration of B cells, addition of various sulfonylureas to the perfusate produces distinct and usually complete B cell degranulation, despite the fact that glucose contained in the infusion is adequate to prevent reduction of blood sugar level. These findings are in keeping with our previous interpretations(4,6) that B cell degranulation is due to a primary action of the drug. They are also in accord with much clinical and experimental evidence which indicates that functioning B cells are a prerequisite for blood sugar lowering action of these drugs(1, 7-9). In further support of this interpretation is the present observation that the relative hypoglycemic effectiveness of equal dosages of these drugs is paralleled by the extent of B cell degranulation induced.

That glucose alone administered intravenously for 3 days caused no morphologic change in rabbit B cells is not in accord with the general impression based on studies in rats and guinea pigs(10,11) that glucose administration causes B cell degranulation. However, this finding is in keeping with previous demonstration that in rabbits made diabetic by administration of cortisone B cell degranulation is frequently not noted until 2 days after starting hormonal treatment(12). It is quite probable that administration of glucose immediately increases insulin output (13). This initial increase in insulin output is, however, not reflected morphologically in changes of pancreatic granulation. There is apparently a need for a sufficient extent and duration of hyperglycemia for B cell degranulation to become morphologically visible. This may be in accord with the previously expressed view(14) that the initial stage of B cell degranulation is a diminution in size of granules rather than a disappearance of granules and that morphologically visible degranulation only occurs when they have diminished very markedly in size.

Summary. 1) A single injection of same dose of 3 sulfonylureas, tolbutamide, chlorpropamide and metahexamide showed that tolbutamide caused a lesser hypoglycemia, whereas the 2 other drugs caused a more severe lowering of blood sugar level, most marked after administration of chlorpropamide. In no case was any B cell degranulation observed. 2) Infusion of glucose alone or simultaneously with various drugs for 7 hours each day on 3 successive days caused an identical hyperglycemic response in all animals. However, infusion of glucose alone produced no B cell degranulation; when sulfonylureas were added to the perfusate, degranulation of B cells was found. This was most pronounced after administration of chlorpropamide and metahexamide. That absence of hypoglycemia did not prevent B cell degranulation is further proof that degranulation of B cells after sulfonylureas is a primary action of these drugs on B cells to increase insulin output. Furthermore, relative B cell degranulating efficacy of the 3 compounds, in accord with their hypoglycemic effectiveness, supports the conclusion that they act directly on the B cell.

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Effect of Parathyroid Extract on *in vitro* Uptake of Ca^{45} by Voluntary Muscle.* (25146)

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For many years the majority of studies investigating mode of action of parathyroid hormone was undertaken to prove that it acted solely on bone or solely on kidney, with the result that a good deal of evidence has accumulated suggesting it acts on both(1). More recently, it has been suggested that parathyroid glands might also regulate transfer of phosphorus between extracellular and intracellular fluid(2,3). The thesis that parathyroid hormone can modify the passage of electrolytes across a membrane is implicit in earlier work of Clark(4), who, by addition of parathyroid extract, could almost completely inhibit *in vitro* uptake of calcium by the lens. The present experiment indicates that parathyroid extract (PTE) also inhibits *in vitro* uptake of calcium by muscle.

Materials and methods. Rats weighing approximately 150 g were used. They were sacrificed by blow on head, and abdominal wall musculature was excised and cut into equal segments. Incubating medium was prepared by adding to one liter of distilled water 9 g NaCl, 0.42 g KCl, 0.2 g $MgCl_2$, 1 g $NaHCO_3$, 0.05 g NaH_2PO_4, and 1 g glucose. $Ca^{45}Cl_2$ was added to give an activity level of 2000 cts/min/cc of incubating medium. The final concentration of calcium was 0.1 mg/liter. Twenty-five units (0.25 cc) of parathyroid extract (Eli Lilly Co.) was added to experimental flasks, and an equal volume of saline to control flasks. Each flask contained 6 cc of buffer at pH 7.4, and incubated in water bath at 37°C. Flasks were incubated unstoppered for 30 minutes and shaken manually every 5 minutes. Then the incubating medium was removed from both sets of flasks. Three 1 cc aliquots from each flask were pipetted into planchets, dried, and counted in Geiger-Müller counter. Values were expressed as amount of radioactivity remaining after incu-

TABLE I. Inhibition of Ca^{45} Uptake by Parathyroid Extract.

	Ca^{45} activity remaining after incubation (cts/cc/min.)*	
Flask No.	Control	PTE added
1	1366	1644
2	1451	1611
3	1421	1621
Mean	1410	1623
1	1269	1574
2	1328	1544
3	1346	1498
Mean	1312	1536
1	1343	1693
2	1372	1676
3	1368	1685
4	1368	1641
Mean	1361	1672
1	1328	1446
2	1396	1568
3	1240	1598
4		1524
Mean	1319	1532
1	1423	1599
2	1487	1624
3	1470	1595
4	1270	1590
Mean	1411	1600
$t = 10.96$	$df = 4$	$p < .001$

* Initial conc. 2000 cts/cc/min.

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