

juice 4%) is prepared in salt solution lacking both Ca and P, it contains approximately one half the usual levels of these elements. Though this solution delays the darkening of the central portion of transplants, there is no deficit in calcium compounds, and the central transplants eventually become opaque to transmitted light. This solution is superior to the usual formula for prolonged maintenance of cells in a plasma base.

Discussion. The demonstration of a 5-fold increase of Ca ions in a plasma base following serial renewals of a supernatant fluid, and of the necessity of hydrogen ions for its replacement, indicates that the plasma substrate in cell cultures is the site of an ionic exchange. A more complete analysis could be expected to show that not only Ca but also other preferentially adsorbed ions tend to replace less tightly bound but physiologically important ions. It may be inferred that cells maintained in an original or lightly patched plasma base spend a considerable portion of their existence in an inorganic environment quite different from what is intended and that the inorganic composition of renewal super-

nates might be improved by altering radically the ionic balance now normally present in fluids prepared from complex nutrients. It is also evident that existing data on the role of different ions in plasma cultures may require re-evaluation by culture methods which expose the cells directly to the fluids being studied.

The present data would appear to confirm the observations of Simms and Stillman² on the nature of at least one of the inhibitors which occur in plasma and adult tissues and is capable of being removed by tryptic digestion.

Summary. The adsorption of calcium phosphate complexes to plasma substrates, and the conditions required for their removal, indicate that the plasma gel is the site of an ionic exchange in which preferentially adsorbed ions may greatly alter the desired ionic balance. This process is one of the factors which causes plasma to become growth inhibitory during prolonged maintenance of cells in this type of substrate.

Received May 18, 1949. P.S.E.B.M., 1949, 71.

17176. Tolerance of Normal and Partially Depancreatized Rats for Insulin.

DWIGHT J. INGLE AND JAMES E. NEZAMIS.

Research Laboratories, The Upjohn Company, Kalamazoo, Mich.

It was shown by this study that the average lethal dose of insulin is smaller in force-fed depancreatized rats than in normal rats.

Methods. Male rats of the Sprague-Dawley strain were maintained on Archer Dog Pellets prior to the beginning of the experiments. At a weight of 310-320 g, some of the animals were subjected to a single-stage pancreatectomy¹ in which all but tiny remnants lying between the duodenum and bile duct were removed. After the rats recovered from the operation, they were placed in metabolism

cages and were adapted to the force-feeding² of a medium carbohydrate diet (Table I) in amounts of 13 cc each morning and late afternoon. The urines were collected at 8:00 to 8:30 A.M. each day and were preserved by thymol. Urinary glucose was determined by the method of Benedict.³

Experiments and results. Severely diabetic rats were selected for these experiments. The average amount of glucose excreted by the 18 depancreatized rats used in this study was 6096 mg per day. The amount of glucose es-

¹ Ingle, D. J., and Griffiths, J. Q., Jr., Chapter 16, *The rat in laboratory investigation*. J. B. Lippincott Co., Philadelphia, 1942.

² Reinecke, R. M., Ball, H. A., and Samuels, L. T., *Proc. Soc. Exp. Biol. and Med.*, 1939, **41**, 44.

³ Benedict, S. R., *J.A.M.A.*, 1911, **57**, 1193.

TABLE I.
Medium Carbohydrate Diet.

Constituent	g
Cellu flour (Chicago Dietetic Supply)	120
Osborne & Mendel salt mixture	40
Dried yeast (Pabst)	100
Wheat germ oil	10
Cod liver oil	10
Vit. K (2-methyl-1,4-naphthoquinone)	100 mg
Mazola oil	200
Casein (Labeo)	160
Starch	200
Dextrin	190
Sucrose	200
Water to make total of	2000 cc

TABLE II.
Lethal Dose of Regular Insulin Expressed as Units
for Rat per Day. Means and standard deviations
of the means and differences.

	No.	Mean	Difference
Normal	20	68.5 $\pm$ 1.77	
Depancreatized	18	50.5 $\pm$ 4.27	18 $\pm$ 4.68

timated to be available from the carbohydrate, glycerol and protein (assuming 60% conversion of protein to glucose) of the diet is approximately 6500 mg per rat per day. These severely diabetic rats were then compared to normal force-fed rats in respect to the amount of insulin required to kill the animal. Regular insulin (Lilly) was administered by subcutaneous injection immediately following each feeding. The initial dose was 5 units given twice each day. The dosage was increased by 5 units per injection on every second day until the animal died.

The data are summarized in Table II. Normal rats tolerated greater average amounts of insulin than did the diabetic rats. The variability among individual animals was greater among the depancreatized rats than among the normal animals. The difference between the means for the two groups is highly significant from the statistical standpoint.

Discussion. If it were assumed that the secretion of insulin is unaltered by the administration of exogenous insulin, it would be expected that a lethal level of insulin dosage would be reached more rapidly in the normal animal than in the depancreatized animal. It seems probable from these and other data⁴

⁴ Haist, R. E., *Physiol. Rev.*, 1944, **24**, 409.

that the secretion of insulin by the pancreatic islets is inhibited by the administration of an excess of exogenous insulin. Even this assumption does not explain the increased sensitivity of the depancreatized animal to insulin. It has been noted that totally depancreatized patients⁵ may show greater sensitivity to insulin than the average patient with diabetes mellitus. Young⁶ reported that pancreatectomy of a dog with pituitary diabetes lowered its insulin requirement. Dragstedt *et al.*⁷ noted that partially depancreatized dogs may have a higher insulin requirement than totally depancreatized dogs. Thorogood and Zimmerman⁸ found that dogs having alloxan diabetes showed a decrease in insulin requirement following pancreatectomy. Howard⁹ has commented upon "The almost uniform finding that an organism is highly oversensitive to a hormone that it lacks, and the corollary that the normal is relatively insensitive to doses of a hormone which would seriously injure the deficient animal. . . one wonders if the gland itself may not sometimes reverse its processes, and, in the presence of an administered excess of its hormone, actually begin to destroy it or elaborate some counteracting hormone." A substance which causes glycogenolysis and hyperglycemia has been obtained from extracts of pancreatic islets¹⁰ and has been considered as a possible antagonist of insulin but it is not known if it is a normal secretory product of the pancreas or if it has any physiological role.

An additional explanation of these results could be based upon the assumption that the

⁵ Waugh, J. M., Dixon, C. F., Clagett, O. T., Bollman, J. L., Sprague, R. G., and Comfort, M. W., *Proc. Staff. Meet. of the Mayo Clinic*, 1946, **21**, 25.

⁶ Young, F. G., *New England J. Med.*, 1939, **221**, 635.

⁷ Dragstedt, L. R., Allen, J. G., and Smith, E. M., *PROC. SOC. EXP. BIOL. AND MED.*, 1943, **54**, 292.

⁸ Thorogood, E., and Zimmerman, B., *Endocrinology*, 1945, **37**, 191.

⁹ Howard, J. E., *American Practitioner*, 1948, **2**, 674.

¹⁰ Heard, R. D. H., Lozinski, E., Stewart, L., and Stewart, R. D., *J. Biol. Chem.*, 1948, **172**, 857.

assimilation of food was incomplete due to a deficiency in the external secretion of the pancreas and that fatal hypoglycemia developed more rapidly for this reason. There was no diarrhea in these experiments, the feces were well formed and firm but were larger than normal in the depancreatized rats. The completeness of intestinal absorption was not studied. Similarly, it is possible that the depancreatized rats were debilitated by operation and by the period of uncontrolled glycosuria so that their resistance to insulin shock was lower than normal. Although the depancreatized animals appeared vigorous and healthy during the period of insulin administration, it would be unsafe to conclude that their lower than normal resistance to insulin

was due solely to the absence of the pancreatic islets.

Summary. Eighteen extensively depancreatized male rats and 20 normal rats were forced a medium carbohydrate diet. Following periods of adaptation and control, all of the animals were tested for their resistance to twice daily injections of regular insulin. The initial dose was 5 units and was increased in increments of 5 units per dose every second day until the animal died. The average lethal dose for the depancreatized rats was 50.5 units per 24 hours as compared to an average of 68.5 units for the normal animals. This difference in averages was calculated to be statistically significant.

Received June 1, 1949. P.S.E.B.M., 1949, 71.

17177. Isolation of Three Strains of Type B Influenza Virus Incompletely Related to Lee.

A. S. LAZARUS.

From the Department of Public Health and Preventive Medicine, University of Washington School of Medicine, Seattle.

During December 1948, reports of widespread distribution of influenza began to appear in the Seattle area. The majority of illnesses were of relatively short duration and were accompanied in many cases by gastrointestinal disturbances. Proper specimens of throat washings and serum were not obtainable until the return of students to the University of Washington campus after the New Year holidays.

During the first 9 weeks of 1949, there were 116 admissions for upper respiratory infection to the University of Washington Health Center. It was apparent, through random questioning of the campus population, that many others were ill at home. Since approximately 60% of the 16,000 University students live at home in or close to the City of Seattle, and there were a number of students known to be ill in living quarters on and off the campus, it seems obvious that the outbreak was much more widespread than the 116 admissions to the Health Center would

indicate. A conservative estimate of 500 cases, of varying severity, during the period from the end of December to the first of March does not seem unreasonable. Observations of classroom attendance supported this estimate.

The majority of illnesses were mild, with the average period of hospitalization being between 3 and 4 days. The mildness of the disease further supports the idea that the cases seen in the campus infirmary represented only a relatively small proportion of the total number occurring in the Seattle area.

Materials. Blood specimens and throat washings were obtained during the acute stage from patients in the infirmary whenever possible. Serum was separated promptly and stored at -2°C until used. Throat washings were obtained by having the subjects gargle 15 ml of nutrient broth (Bacto) to which had been added penicillin G in an approximate concentration of 1000 units per ml. The garglings were collected in clean beakers, trans-