may be necessary to alter the nutrient medium, depth of fluid, gaseous environment, pH, or temperature of incubation in order to get a particular type of cell to grow. The nutrient medium may be pre-conditioned by previous growth of living cells or an irradiated feeder layer(2) may be used.

In attempting to establish clones from primary isolates of tissue, simply plating cells out involves an initial selection process in that only those types of cells that can grow under the conditions used will do so. However, with this technic, a partciular type of cell may be selected and placed under a variety of different cultural conditions in the hope of finding one that will permit the growth of the desired type of cell. Attempts are currently under way to isolate single cells directly from bone marrow and to develop from them clone cultures which can be maintained in serial passage.

Summary. A simple method for isolating single tissue culture cells has been developed. The cells are isolated on small glass squares which can be transferred to any desired culture vessel or testing apparatus. This procedure has been used for the preparation of clone cultures.

1. Sanford, K. K., Earle, W. R., Likely, G. D., *J. Nat. Cancer Inst.*, 1948, v9, 229.

2. Puck, T. T., Marcus, P. I., Cieciura, S. J., *J. Exp. Med.*, 1956, v103, 273.

Received May 28, 1958. P.S.E.B.M., 1958, v99.

Immediate Effects of Intravenous Injections of Tolbutamide and Insulin on Blood Glucose and Amino Acids.* (24236)

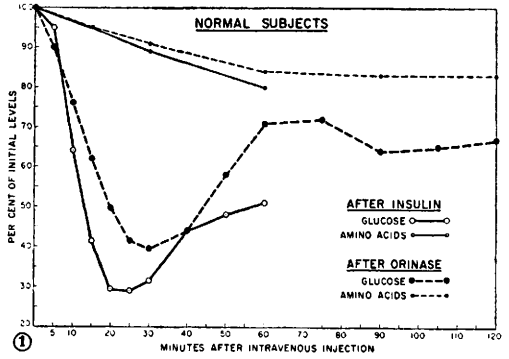
ROGER C. DEMEUTTER, AVEDIS K. KHACHADURIAN AND ALEXANDER MARBLE
Cancer Research Institute and Baker Clinic Research Laboratory, N. E. Deaconess Hospital, Boston, Mass.

After several years of study there is no general agreement regarding the details of the mechanism of action of the hypoglycemic sulfonylureas and more studies are needed to clarify this problem(1,2). The present study was undertaken with two purposes in mind: (1) To determine the time of onset of hypoglycemic action of tolbutamide following its intravenous administration and to compare, in normal and diabetic individuals, the blood glucose curve thus obtained with that produced by insulin; (2) To determine if, like insulin, tolbutamide causes a fall in the serum amino acids.

Materials and methods. Three groups of individuals were studied: (1) Five normal male volunteers, age 28 to 35, body weight 70 to 80 kg. (2) Seven patients with stable diabetes, age 42 to 73, weight 70 to 88 kg, classified as suitable for tolbutamide therapy according to commonly accepted criteria(3). (3) Two patients with unstable diabetes requiring more than 50 units of insulin daily. Prior to study, all subjects were fasted 12 hours. Patients on long-acting insulin were taken off this preparation for more than 48 hours and, when necessary, crystalline insulin was given for control of diabetes. However, no insulin was given later than 15 hours prior to test. The tests were performed on recumbent, rested subjects; no smoking was allowed. Blood samples were drawn through a needle fitted with stylet and kept in place in a brachial vein during the whole test. Stasis was avoided and no tourniquet used whenever possible. After a short control period of saline infusion, glucagon-free insulin† was injected intravenously at fixed dose of 0.1 unit/kg actual net body weight. One or several days later the same procedure was used and sodium

* This study was supported by U. S. Atomic Energy Com. contract No. A.T. (30-1)-901 with New England Deaconess Hospital; the Upjohn Co.; and Diabetes Foundation.

† Kindly furnished by Eli Lilly and Co., Indianapolis, Ind.



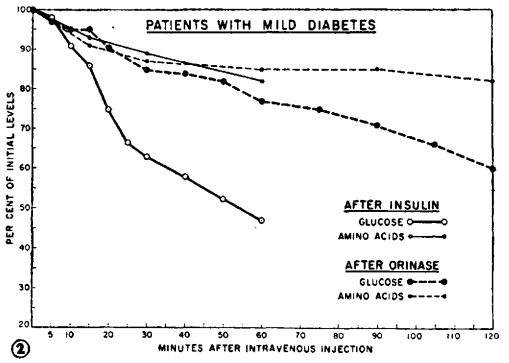


FIG. 1 (top). Response of blood glucose and serum total alpha amino acids following intrav. inj. of glucagon-free insulin (0.1 unit/kg body wt) and sodium tolbutamide (Orinase) (40 mg/kg) to 5 normal male human subjects. Points shown in the curves represent % of levels prior to injections.

FIG. 2 (bottom). Response of blood glucose and serum total alpha amino acids following intrav. inj. of glucogan-free insulin and sodium tolbutamide (Orinase) in 7 patients with stable diabetes. Doses of insulin and of sodium tolbutamide were as given in Fig. 1. Points shown in the curves represent % of levels prior to injections.

tolbutamide in the same dosage of 40 mg/kg was injected intravenously in the form of 10% solution in saline with pH 9.3. During its rapid injection (3 minutes) most subjects experienced a slight burning along the vein. Blood glucose determinations were made according to the Somogyi-Nelson method(4). Serum total alpha-amino acids were determined by the ninhydrin colorimetric method (5) using tungstic acid as the deproteinizing agent.

Results. Blood glucose. Fig. 1 and 2 represent mean values for blood glucose expressed as per cent of fasting level. In normal subjects, tolbutamide produced a rapid decrease in blood glucose which began within 5 minutes in 3 of 5 subjects. The maximum fall with insulin occurred at 25 minutes and was 71% of the fasting value, while with tolbutamide it occurred at 30 minutes and was 61% of the fasting value.

In 7 patients with stable diabetes decrease in blood glucose with insulin was slower and smaller than in the normal group, averaging 56% at 60 minutes (end of experiment). However, the response to tolbutamide was even less than that due to insulin; a mean maximum fall of 40% was attained at the end of experiment at 120 minutes.

In the 2 non-responsive diabetics there was no drop in blood sugar after tolbutamide while one of them who received insulin showed a fall of 34% at 2 hours.

Serum amino acids. There was no difference in fasting levels of normal and diabetic subjects. In normal patients with stable diabetes, tolbutamide caused a decrease in serum amino acids comparable to that produced by insulin. At 60 minutes the normal subjects had a fall of 20% with insulin and 16% with tolbutamide, while the responsive diabetics had a fall of 18 and 15% respectively. Following tolbutamide there was no decrease in the amino acids of the 2 patients with unstable diabetes, while a 20% fall occurred after insulin in one of these patients. The onset of the fall in amino acids was comparable in the different experiments and a significant fall occurred in the majority of patients at 15 minutes.

Discussion. Our results with the effect on blood glucose of tolbutamide agree with those of other workers(1) and, moreover, they indicate that onset of action of tolbutamide is almost immediate since a significant fall in blood glucose occurs within 5 minutes after its injection.

If tolbutamide acts by increasing insulin output from the pancreas, it would appear to do so, at least initially, by releasing preformed insulin. The fact that the pancreas of the diabetic contains less extractable insulin than that of the normal, fits with the observation that the hypoglycemic effect of tolbutamide is less marked in the diabetic subjects. The fall observed in amino acids after tolbutamide is

consistent with the observations of Recant and Fischer(6) that pretreatment of rats with tolbutamide causes a greater incorporation of glycine into protein by their livers. Therefore, as determined by these 2 studies, tolbutamide when effective in producing hypoglycemia, has an action parallel to that of insulin on amino acid metabolism(7). This similarity of action adds support to the evidence that tolbutamide acts by increasing the output of insulin and speaks against the possibility that depression of gluconeogenesis from amino acids could be a factor in production of hypoglycemia.

Summary. 1) In normal subjects sodium tolbutamide given intravenously in dosage of 40 mg/kg of actual body weight produced hypoglycemia comparable to that produced by insulin (0.1 unit/kg body weight) intravenously. In 3 of 5 subjects the fall in blood glucose took place within 5 minutes following injection of tolbutamide. 2) In 7 patients with stable diabetes the decrease in blood glucose following tolbutamide was slower than that with insulin and no fall occurred in 2 patients with unstable diabetes. 3) In normal persons and in the 7 diabetic patients who experienced a fall in blood glucose following tolbutamide, there was likewise a decrease in serum amino acids comparable to that following insulin. No fall in serum amino acids occurred in the 2 patients with unstable diabetes.

1. Symposium on Clinical and Experimental Effects of Sulfonylureas in Diabetes Mellitus, Rachmiel Levine and Garfield Duncan, Special Editors. *Metabolism,* 1956, v5, 721-977.

2. Conference on the Effect of Sulfonylureas and Related Compounds in Experimental and Clinical Diabetes, *Ann. N. Y. Acad. Sci.,* 1957, v71, 1-292.

3. Marble, A., Camerini-Davalos, R., *ibid.,* 1957, v71, 239.

4. Nelson, N., *J. Biol. Chem.,* 1944, v153, 375.

5. Meyer, H., *Biochem. J.,* 1957, v67, 333.

6. Recant, L., Fischer, G. L., *Ann. N. Y. Acad. Sci.,* 1957, v71, 62.

7. Krahl, M. E., *J. Biol. Chem.,* 1953, v200, 99.

Received June 2, 1958. P.S.E.B.M., 1958, v99.

Pituitary and Plasma Bioassay for Trophic Hormones in the Alloxan-Diabetic Rat.* (24237)

A. M. LAWRENCE,† A. N. CONTOPOULOS AND M. E. SIMPSON

Depatrment of Anatomy and Inst. of Experimental Biology, University of California, Berkeley

Among endocrine disturbances reported in experimentally induced diabetes mellitus have been impairment of reproductive physiology and hypoactivity of the thyroid(1-6). In the present study the gonadotrophic, thyrotrophic, and growth-promoting potencies of the pituitary glands and blood plasma of diabetic rats were determined by bioassay in hypophysectomized immature female rats.

Materials and methods. Female rats of the Long-Evans strain maintained on an adequate natural food diet‡ were injected with alloxan at 50 days of age and sacrificed at 100 days of age. Pituitaries and plasma were assayed in hypophysectomized rats, 28 days old at operation, used 14 days later. Hypophysectomy was performed by the parapharyngeal approach. Completeness of ablation was checked by observation of the sella turcica at autopsy. Atrophy of target organs furnished

* Supported by USPHS Grant.

† Part of this work was done during tenure of Lederle Medical Student Summer Research Fellowship.

‡ All diabetic rats were maintained on a modified McCollum Diet I consisting of ground whole wheat 67.5%, casein 15%, skim milk powder 7.5%, sodium chloride iodized 0.75%, calcium carbonate 1.5%, melted fat 6.75%, fish oil (Vit. A and D concentrate) 1%. KI solution added to 1 μg of iodine/g of diet (450 mg KI/liter, 300 cm^3/270.5 lb of diet). Hypophysectomized rats were offered daily a wet mash of this diet. All rats received a supplement of lettuce 2-3 times a week and were maintained at 74° ± 1°F temperature.