This was manifested by a high mortality rate, inhibition of growth, and the occurrence of developmental abnormalities. The toxic effects of guanazolo were inhibited by the simultaneous injection of guanine HCl.

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Administration of the Hyperglycemic-Glycogenolytic Factor of the Pancreas to Non-Anesthetized and Anesthetized Subjects. (18131)

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That a hyperglycemic property may be present in pancreatic extracts and in commercial preparations of insulin has been recognized for some time. The recent preparation of a relatively purified hyperglycemic-glycogenolytic factor (HGF) from the pancreas and from gastric mucosa by Sutherland and DeDuve(1) and the demonstration of consistently reproducible *in vitro* and *in vivo* effects, have led to speculations concerning the possible role of such a factor in the regulation of carbohydrate metabolism in man, and particularly in the genesis of human diabetes. Nevertheless, Weisberg, Friedman and Levine(2) after summarizing the pertinent literature and from their own observations concluded that "as of the present, there has been no clear cut demonstration to prove that HGF is a mormone and/or that it is secreted by the alpha cell or the argentophil cells."

In view of the frequent presence of HGF in commercial preparations of insulin utilized in the treatment of diabetes mellitus in man, it was deemed advisable to assay the effects of the administration of a preparation rich in HGF to man. Since anesthesia has been shown to enhance the effect of the administration of HGF to rabbits(2), the influence of this factor in men, with and without anesthesia was determined. It was deemed pertinent also to compare the response in man with that of other species.

Method. A preparation of insulin which was relatively rich in hyperglycemic factor was prepared by one of us (E.D.C.). It is estimated that this preparation contained that amount of HGF as would be present in an equal weight of commercial insulin. Large numbers of assays with cats and other animals revealed that as little as 0.05 mg per kg body weight produced a marked rise in blood sugar. The effectiveness of this preparation was not impaired by filtration through an ultra-fine fritted Pyrex disc bacterial filter. Before each experiment, a fresh solution containing 0.5 mg HGF per ml acid water (pH 2.5) was prepared and sterilized by fritted-glass filtration. After a preliminary period of 20 to 30 minutes during which blood samples were drawn, 0.1 ml of the HGF solution per kg body weight was injected intravenously. Subsequent specimens of venous blood were withdrawn at five-minute intervals for thirty minutes. In some instances simultaneous arterial samples were drawn. The majority of the subjects were ambulatory patients on the psychiatric wards and varied from 17 to 53 years in age. In addition, 2 patients with diabetes of mild severity were tested. In 8 patients, the experiment was performed both without anesthesia and with anesthesia. Altogether, 21 experiments were

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1. Sutherland, E. W., and DeDuve, C., Origin and distribution of the hyperglycemic-glycogenolytic factor of the pancreas, *J. Biol. Chem.*, 1948, v175, 663.

2. Weisberg, H. F., Caren, R., Huddlestun, B., and Levine, R., *Am. J. Physiol.*, 1949, v159, 98.

performed on 11 subjects. The patients were all partaking well of the regular hospital diet, and with one exception, were allowed to eat breakfast between 7:30 and 8:00 a.m. on the day of the procedure. Lunch was withheld, and the experiments started between 1:00 and 2:00 p.m. In those instances where anesthesia was induced, it was accomplished by the intravenous injection of a 2.5% sodium pentothal solution in sufficient quantity to produce a light to moderately deep anesthesia before the first blood sample was obtained. Additional injections of pentothal were administered as necessary in order to maintain the anesthesia. In the 8 patients in whom the experiment was performed both with and without anesthesia, the 2 tests were performed in from 24 to 72 hours apart, and the order was varied, so that 3 patients were anesthetized during the first of the 2 tests performed on them and 5 were anesthetized during the second test. The blood was collected into tubes containing heparin and fluoride and the Nelson modification of the Somogyi method(3) was used for the determination of the glucose concentration.

Results. The intravenous injection of as little as 0.05 mg of extract containing HGF per kg body weight resulted in a rapid rise in the blood sugar in various species as illustrated in Fig. 1 and 2. The increase in venous blood sugar of the unanesthetized subjects varied from 6 mg % to 35 mg % with a mean of 19 mg %. Increasing the amount of extract administered did not increase the hyperglycemic effect in one patient on whom 3 experiments were performed without anesthesia nor in the many cats given from 0.05 to 0.5 mg per kg body weight.

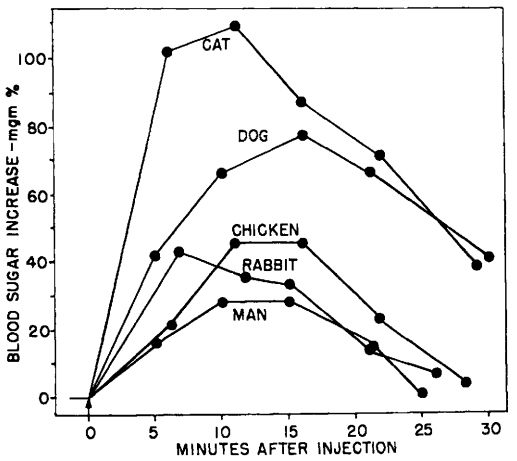

FIG. 1.

Effect of HGF in various species. 0.05 mg HGF was administered intravenously after induction of anesthesia.

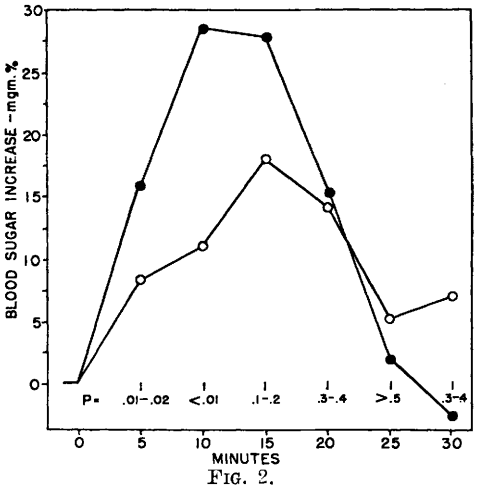

FIG. 2.

Effect of anesthesia on action of HGF in man. ○ = unanesthetized; ● anesthetized.

In all 8 patients in whom the effect of administration of HGF was studied with and without the production of anesthesia, the increase in the blood sugar during anesthesia exceeded that observed in the same subjects without anesthesia, Fig. 2. Statistical analysis of the data revealed a significant difference between the effect of HGF only during the first ten minutes after the administration of the extract irrespective of the presence or absence of anesthesia. Thereafter there was no significant difference between the 2 groups.

In the 2 subjects with mild diabetes, one of whom had been fasted overnight after a high carbohydrate meal at 10 p.m. and who received an injection of 0.2 mg of the HGF per kg, the rise in blood sugar was 8 and 12 mg % respectively.

None of the patients demonstrated any untoward reaction which could be attributed to the preparation which was administered. In most instances the pulse rate was measured before, and at frequent intervals during the

3. Nelson, N., *J. Biol. Chem.*, 1944, v152, 375.

procedure and no significant changes were observed.

Discussion. The results described, demonstrate that a consistent hyperglycemic effect, variable in degree, can be produced in man by the intravenous injection of a potent preparation of hyperglycemic factor. This hyperglycemic effect is apparently increased by anesthesia as has been reported in animals by others(2).

The nature of the influence of anesthesia on the hyperglycemic effectiveness of this factor is not understood. An adequate explanation may lie in the well-established effect of anesthesia on increasing the rate of the peripheral circulation(4). Such an increase in the peripheral blood flow can be expected to reduce the arterio-venous difference in blood sugar. If that be the case, it may be anticipated that the rise in arterial blood sugar produced by the injection of HGF will be unaffected by anesthesia while the rise in the venous blood sugar will approximate that of the arterial blood sugar. In accord is the experiment in which simultaneous arterial and venous blood sugars were determined. Fig. 3 illustrates the data obtained in an anesthetized cat following the intravenous injection of HGF. Whereas the

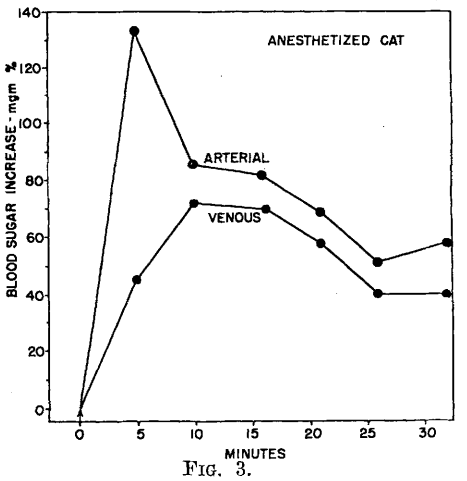

FIG. 3.

Effect of HGF on arterial and venous blood of the anesthetized cat.

4. Abramson, D. I., Vascular responses in the extremities of man in health and disease, Univ. of Chicago Press, Chicago, Ill., 1944.

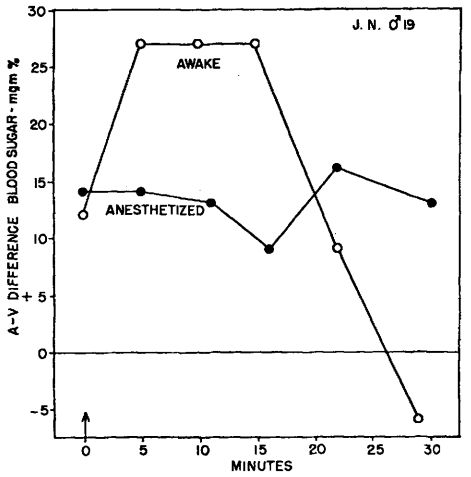

FIG. 4.

Effect of HGF on arterial-venous blood glucose difference.

arterial blood glucose was increased by 105 mg per 100 cc, within 5 minutes, the concentration of glucose in the venous blood increased by only 43 mg within the same interval of time. Thereafter the glucose concentration of both the arterial and the venous blood approached similar levels. In one patient (J.N.), simultaneous capillary and venous blood specimens were obtained in experiments performed with and without anesthesia. In the experiment without anesthesia, the rise in venous blood sugar following injection of the HGF was 29 mg % while the rise in capillary blood sugar was 44 mg %; under anesthesia, the rise in venous sugar was 30 mg and that of the capillary sugar was 27 mg %. Thus, calculation of the A-V differences reveals a marked effect of the HGF injection in the absence of anesthesia and an insignificant effect in the presence of anesthesia (Fig. 4).

The preceding suggests that anesthesia does not affect the glycogenolytic action of the hyperglycemic factor of the pancreas. Since the effect of this factor in both anesthetized and unanesthetized men is relatively small, it can be postulated that the amount present in commercial insulin will produce negligible effects when administered to patients in diabetic coma.

Summary. The intravenous injection of a purified preparation of the hyperglycemic factor of the pancreas produces an increase in

the venous blood sugar of man. This response is variable in quantity and is not increased by increased dosage. The production of anesthesia enhances the increase in venous blood sugar produced by the hyperglycemic factor. This effect of anesthesia is attributed to an increase in peripheral blood flow rather than to an augmented hyperglycemic effect.

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Experimental Histoplasmosis in the White Rat.* (18132)

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The true effectiveness of any substance of potential promise in the therapy of an infectious disease can be evaluated only in terms of its capacity to inhibit or kill the pathogenic microorganism in the tissues of an infected host. It is perhaps not unreasonable to suggest that lack of a standardized laboratory procedure for testing the *in vivo* activity of chemotherapeutic agents has been responsible for the delay in the development of an effective method of treatment of the systemic mycotic infections. With increasing recognition of the prevalence of these diseases has come an awareness of the necessity for some specific form of therapy, since the systemic mycoses, though not of common occurrence, have a high mortality rate. Extensive experience with histoplasmosis at the Vanderbilt University Hospital led us, a little more than a year ago, to undertake the development of a standardized technic for infecting animals with *Histoplasma capsulatum*. The widespread interest in histoplasmosis is reflected in the recent appearance of two articles on the experimental production of this fungous disease in animals. Campbell and Saslaw(1), and Howell, Kipkie and Bruyers(2), have been able to demonstrate consistently successful infection of mice with *H. capsulatum*. The present paper is a report and discussion of the procedures we have developed for inducing generalized histoplasmosis in the white rat.

Methods and experiments. Animals. Since it was anticipated that certain drugs to be tested might have to be administered by gavage, mice were not used because of their small size. Guinea pigs had been shown (unpublished data) to be unsuitable because of a too great lack of uniformity in reaction to infection with *H. capsulatum*. White rats, of the Sherman strain, were tried and found to be reasonably satisfactory; healthy, white females, weighing 125 ± 8 g were used throughout the experiments.

Inoculum. Preliminary experiments, over a period of a few months, with small groups of animals inoculated with equal numbers of organisms in each experiment, showed a great variation in mortality rate. The hypothesis was advanced, then proven correct, that even though the absolute numbers of organisms injected into the rats were identical, the number of *viable* organisms in the inoculum differed greatly. Accordingly, a rigid technic and schedule of transfer was instituted: under these conditions, transfer of cultures at three-day intervals produced a growth which, at the time of transfer, yielded a relatively constant percentage of viable organisms. After virulence tests on several strains of *H. capsulatum* obtained from different sources, V. U. strain No. 621,† which appeared more viru-

* This work was aided in part by grants from the Institute of Paper Chemistry, Appleton, Wisc., and the National Tuberculosis Association.

1. Campbell, C. C., and Saslaw, S., PROC. SOC. EXP. BIOL. AND MED., 1950, v73, 469.

2. Howell, A., Jr., Kipkie, G. F., and Bruyers, P. T., *U. S. Public Health Rep.*, 1950, v65, 722.