Hyperglycemic Properties of Crude Pancreatic Proteins.* (17792)

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The presence of a factor in crude insulin that produces a transitory hyperglycemia when injected intravenously has long been known(1). Its presence was reported in amorphous insulin and certain commercial preparations of crystalline zinc insulin(2,3), and in the supernatant of the first isoelectric precipitation of insulin(4). These observations prompted us to test pancreatic fractions that were obtained in the course of another investigation(5) from the supernatant of the 15% sodium chloride precipitate of the crude insulin salt-cake prepared by a slight modification of the procedure of Romans, Scott and Fisher(6). To those engaged in the isolation of this factor, its presence in the 15% sodium chloride supernatant and observations on the intravenous and subcutaneous administration of these fractions may be of interest.

Experimental. The procedure employed for the preparation of the pancreatic fractions from the 15% NaCl supernatant has been described in another publication(5), and is briefly outlined in flow sheet form in Table I. All preparations were dialyzed thoroughly against running tap water for at least 48 hours, filtered through clarifying pads (Republic K-3) and then lyophilized. The alkaline treatment described by Heard and coworkers(4) was employed to inactivate insulin.

Blood glucose was determined by the official U.S.P. XIII method(7). As a rule, each preparation was tested on a group of 5 or 6 rabbits (2 kg), and the results for each bleeding were averaged. To eliminate the variations in absolute blood sugar levels, the average blood sugars for each bleeding period were expressed in terms of percentage of the average preinjection blood sugar value.

Results and discussion. Hyperglycemic activity of pancreatic fractions. With the exception of No. 3 all preparations tested produced a mild hyperglycemia of short duration, followed by a marked lowering of blood sugar which in a few animals resulted in mild convulsions (Fig. 1). The nature of this hypoglycemic response indicated the apparent presence of about 1 unit of insulin. Only the trichloracetic acid insoluble fraction (No. 3) apparently was free of insulin.

It was evident that in preparations containing insulin, the full effects of the hyperglycemic factor were masked. Inactivation of the insulin in these fractions† with alkali(4) yielded preparations which showed no evidence of insulin (Fig. 1), though it is possible that the effects of small amounts of it might have been overshadowed by the hyperglycemic factor.

The alkali-treated preparations and the "non-treated" preparation, No. 3, were all essentially equally active in raising blood sugar levels. Similar results were obtained with two other series of fractions (tested at dosage levels between 1 and 10 mg/kg), one of which was treated with alkali before fractionation. The presence of the hyperglycemic factor in almost all fractions indicated lack

* Presented in part at the American Chemical Society meetings Jan. 20, 1949, Philadelphia, Pa.

1. For early literature confer Jensen, H. F., *Insulin*, p. 162 (Oxford University Press, 1938); for more recent literature see Sutherland and de Duve, *J. Biol. Chem.*, 1948, v175, 663.

2. Olsen, N. S., and Klein, J. R., PROC. SOC. EXP. BIOL. AND MED., 1947, v66, 86.

3. Sutherland, E. W., and Gori, C. F., *J. Biol. Chem.*, 1948, v172, 737.

4. Heard, R. H. D., Lozinski, E., Stewart, L., and Steward, R. D., *J. Biol. Chem.*, 1948, v172, 857.

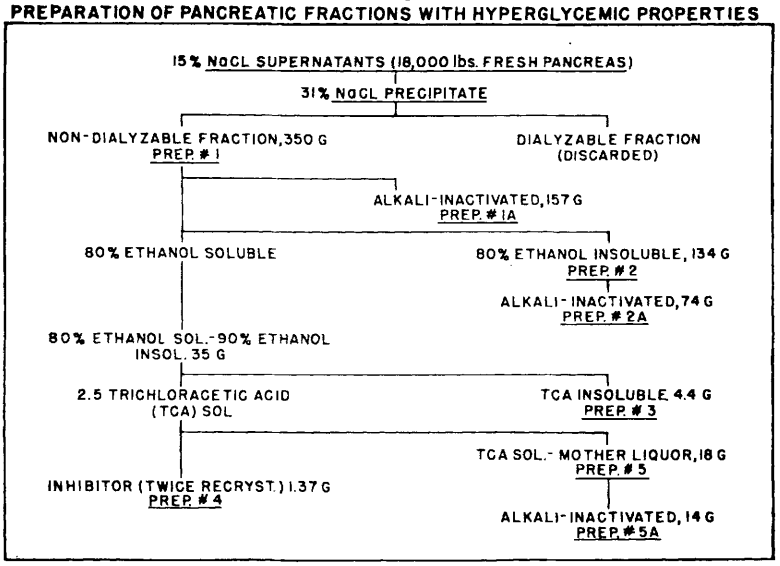
5. Kazal, L. A., Spicer, D. S., and Brahinsky, R. A., *J. Am. Chem. Soc.*, 1948, v70, 3034.

6. Romans, R. G., Scott, D. A., and Fisher, A. M., *Ind. Eng. Chem.*, 1940, v32, 908.

7. U.S.P. XIII, *Insulin Monograph* 265-268.

† Due to lack of an adequate supply of the crystalline inhibitor (No.4), this preparation was not treated with alkali.

TABLE I
PREPARATION OF PANCREATIC FRACTIONS WITH HYPERGLYCEMIC PROPERTIES



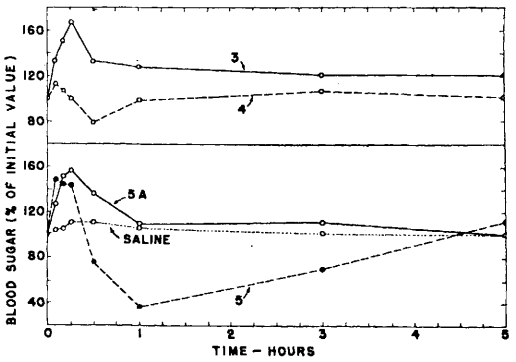


FIG. 1.

Effect on blood sugar levels of rabbits receiving pancreatic fractions before and after alkaline inactivation of insulin. Curves marked "A" were obtained with alkali-treated preparations. The numbers refer to preparations in Table I. Each curve is the average of 5 or 6 rabbits. The preparations were injected intravenously 20 mg/kg except No. 4 (10 mg/kg) and saline (0.5 ml/kg). Preparations No. 1 and No. 2 produced a biphasic glycemic effect similar to curve 5 except that the hyperglycemia was less marked and of shorter duration. Preparations No. 1A and No. 2A produced a hyperglycemic response almost exactly identical with that of preparation No. 5A.

of adequate separation of this factor from contaminating substances and suggested that it possibly was being readily adsorbed by the protein(s) in these fractions. The presence of the factor in crystalline zinc insulin and amorphous insulin(2,3), in the supernatant of first iso-electric precipitate(4) and now in the 15% NaCl supernatant and the various fractions derived from it support this possibility. Except for the separation of the factor from insulin by 2.5% trichloracetic acid (preparation No. 3), it is doubtful whether any degree of purification was effected.

Quantitative aspects. The wide variation in response among individual rabbits to the injection of the hyperglycemic factor, especially evident at lower dosages, suggested that the test employed was not suitable for quantitative studies. An analysis of variance on the data obtained (Table II) indicated the log-dose-response curve to be linear over the range covered by the 4 lowest doses. However, the slope of the regression line was too low for statistically significant results without employing an impractically large number of animals at each dose level. Therefore it was concluded that the hyperglycemic effects produced under the specified conditions could be interpreted only qualitatively.

Subcutaneous administration. When the hyperglycemic factor was injected subcutaneously in single dosages of 100 and 200 mg/kg it produced blood sugar levels as high as 225% of the initial value in some rabbits. Maximum levels were reached in approximately 30 minutes.

TABLE II.
Dose-response Effect of Hyperglycemic Factor (Prep. No. 1A) Administered I.V. in Rabbits (Groups of 6 Animals).

Blood sample (min.)	Dose (γ/kg)							
	0*	5	20	80	100	300	900	2700
10	95†	98	105	110	140	130	130	145
20	100	103	112	114	137	120	123	142

* Distilled water—0.5 ml/kg.
† Blood sugar—% of initial value.

In some of the animals multiple subcutaneous injections (Fig. 2) raised the blood sugar level above 300% of the initial value. The administration of 0.75 units of crystalline Zn insulin per kg with the initial dose did not significantly change this response. This amount of insulin is sufficient to cause a 50% or greater reduction in blood sugar level within 2 hours, and to produce convulsions in the more sensitive animals. When insulin and the hyperglycemic factor were administered together, neither hypoglycemia nor convulsions were observed during this period. However, continued administration of the hyperglycemic factor, with or without the initial dose of insulin, produced a convulsive and fatal level of blood sugar after 7 hours. Heard *et al.*(4) and Burger and Kramer(9) have eliminated the possibility that this factor produces its effect through a non-specific action or adrenal or anterior pituitary intervention. Therefore the continued administration of large amounts of this factor probably produced a depletion of the glycogen reserves of the liver.

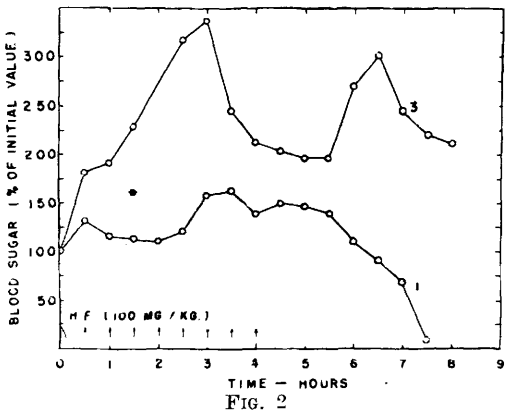


FIG. 2

Effect on blood sugar levels of rabbits receiving multiple subcutaneous injections of 100 mg/kg of Preparation No. 1A at half-hourly intervals. Each curve represents a single animal.

Stability in blood; hyperglycemic property of incubated blood. Sutherland and de Duve (8) were unable to identify with certainty the hyperglycemic factor in citrated plasma collected from the pancreatic duodenal vein, and found that when the factor was added to blood it was destroyed at an appreciable rate. Our factor apparently was not destroyed when incubated at 37°C for 1 hour with heparinized whole blood since the injection of the blood produced a hyperglycemic effect like that produced by the factor alone. No significant increase in blood sugar was observed in animals injected with blood incubated under the same conditions, but without the factor. In our experiments, perhaps the presence of heparin accounted for the stability of the hyperglycemic factor.

A secondary rise in blood sugar did occur in 1 to 3 hours in control animals injected with blood held for one hour at 37°C. This was confirmed in other experiments (Fig. 3) in which heparinized blood injected immediately after collection from donor rabbits did not produce any increase in blood sugar, but incubated blood did. Hence incubation of such blood apparently released a substance which had hyperglycemic properties or which acted through the adrenal or anterior pituitary glands or in some other way. Obviously these experiments afford no explanation of mechanism of action.

Protection of mice from insulin shock. The observation(9) that the hyperglycemic factor

8. Sutherland, E. W., and de Duve, C., *J. Biol. Chem.*, 1948, v175, 663.

9. Zimmermann, B., and Donovan, T. J., *Am. J. Physiol.*, 1948, v153, 197.

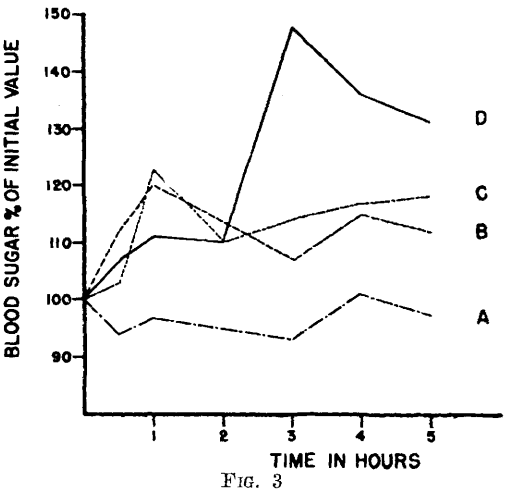


FIG. 3

Effect on blood sugar levels of rabbits receiving 10 ml injections of heparinized blood. Each curve represents average of 3 animals except C (2 animals). Curves A, B, and C were obtained by injecting pooled heparinized blood taken from several donor rabbits: A—blood injected immediately (within 10 min) after collection, B—after incubation at room temp for 1 hr, and C—after incubation 37°C for 1 hr. Curve D by reinjecting the animals' own heparinized blood after incubation 37°C for 1 hr.

does not protect mice against insulin-induced shock was confirmed by thoroughly controlled experiments with groups of 30 and 40 mice receiving 100 mg/kg (single and multiple doses) of the hyperglycemic factor at various intervals before and/or after the injection of insulin (0.016 unit/mouse). Failure of relatively large doses to protect mice against insulin-induced convulsions seems to be at variance with the results obtained in rabbits, in which the subcutaneous injection of the hyperglycemic factor in large doses successfully reversed the hypoglycemic effect of the insulin. The absence of data on the actual blood sugar levels in the mice makes interpretation of the results difficult.

Summary. The hyperglycemic activity of certain fractions derived from the 15% sodium chloride supernatant, a by-product of insulin manufacture, was determined before and after alkaline inactivation of the insulin present in these preparations. Hyperglycemic activity was studied in the rabbits but the results were interpreted only qualitatively, since a satisfactory log dose-response was not obtained. The factor was active whether intravenously or subcutaneously administered. Repeated subcutaneous administration produced hypoglycemia and convulsions in some animals. The factor appeared to be stable in heparinized blood at 37°C for 1 hour; incubated blood itself produced a delayed hyperglycemic effect when injected intravenously. The factor did not protect mice against insulin-induced convulsions, but did reverse the hypoglycemic effect of insulin in the rabbit.

Received February 8, 1950. P.S.E.B.M., 1950, v74.

Stimulatory Effect of Aureomycin on the Growth of Chicks. (17793)

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Several reports in the literature suggest that the growth-promoting activity of "animal protein factor" concentrates for chicks is not due solely to the vitamin B_{12} content of such supplements(1-3). Stokstad *et al.*(4) in studying the distribution of this additional factor(s) found that fermentation liquors of *Streptomyces aureofaciens* were capable of producing growth increments in chicks greater

1. Menge, H., Combs, G. F., and Shorb, M. S., *Poultry Sc.*, 1949, v28, 775.

2. Carlson, C. W., Miller, R. F., Peeler, H. T., Norris, L. C., Hauser, G. F., *Poultry Sc.*, 1949, v28, 750.

3. Hill, F. C., *Poultry Sc.*, 1948, v27, 536.

4. Stokstad, E. L. R., Jukes, T. H., Pierce, J., Page, A. C., and Franklin, A. C., *J. Biol. Chem.*, 1949, v180, 647.