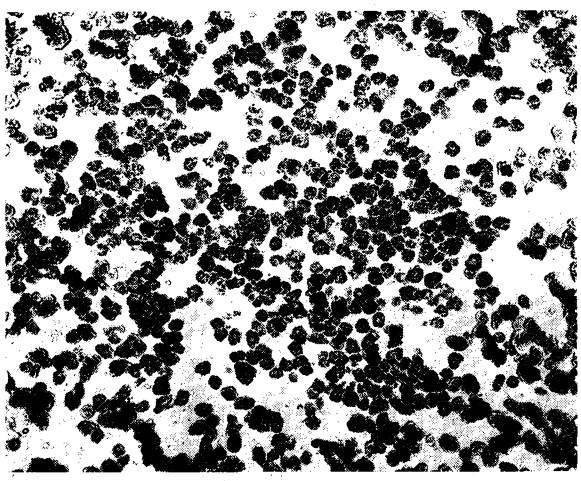

FIG. 1. Whole liver cells prepared from liver perfused with 0.027 *M* sodium citrate in calcium-free Locke's solution. Homogenization and purification carried out in calcium-free Locke's solution.

sum of the whole cells and the calculated broken cells.

The results obtained with preparations made from 25 rats are shown in Table 1. In the first two experiments shown (citrate), omission of back perfusion via the hepatic vein probably accounts for the low yield. Back perfusion with a pressure of 120 cm of water distends the liver considerably and appears to help separate the liver cells. However, similar back perfusion with complete Locke's solution did not increase the whole-cell yield. Although no extended experiments using rats of different ages have been made, it appears that older rats (350 g) give lower yields of whole cells.

Citrate, pyrophosphate, Versene, and ATP were all almost equally effective; glycerophosphate was less efficient in preventing cell breakage. Considerable variation in whole-cell yield in different preparations made with the same perfusion medium were noted.

Purified suspensions were prepared by sedimenting the whole cells by centrifuging 4 min at $110 \times g$ at 0° C, resuspending in calcium-free Locke's solution and resedimenting twice, using the same centrifugal field. A preparation prepared by perfusion with 0.027 *M* citrate is shown in Fig. 1.

The practical advantage of using suspensions of whole cells in place of slices are many and have been previously discussed by Elliott and Libet (*14*). It should be emphasized, however, that numerous alterations may well have occurred during the procedures described here, and the synthetic potentialities of such free cells may well differ considerably from those in the intact organ. It should be noted that Versene has recently been found to prevent the decline in oxidative phosphorylation otherwise seen in isolated heart mitochondria (*15*) and may have a similar effect here.

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Purification and Crystallization of Hyperglycemic Glycogenolytic Factor (HGF)

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Murlin *et al.* in 1923 (*1*) first noted a hyperglycemic response obtainable with certain pancreatic extracts and suggested the name glucagon for the causative agent. Interest in material causing hyperglycemic action was increased by the finding that insulin preparations exhibited a hyperglycemic response which appeared as an initial and transient hyperglycemia following intravenous administration of the insulin preparation (*2, 3*).

Several workers (*2, 4*) have attempted unsuccessfully to isolate a highly purified material responsible for hyperglycemic action either from pancreas or from commercial amorphous insulin. In this communication we report the preparation of a novel crystalline compound (HGF) having a high degree of hyperglycemic activity.

Precautions were exercised in the isolation procedure to avoid steps involving denaturation or inactivation of contaminating proteins, principally insulin. In this way it was possible to use insulin assays to follow the removal of this particular contamination in various preparations made in the course of the work. The utilization of mild procedures also prevented decomposition of the hyperglycemic material.

The hyperglycemic activity was determined by noting the increase in blood sugar after intravenous injection of the test material into anesthetized cats. Blood sugar was determined on blood samples withdrawn at 5-min intervals during the first 25 min after the injection.

An amorphous fraction obtained during the com-

[1] We are pleased to acknowledge the assistance of H. J. Wick in providing the determinations of biological activities, and of H. A. Rose in photographing and characterizing the crystals.

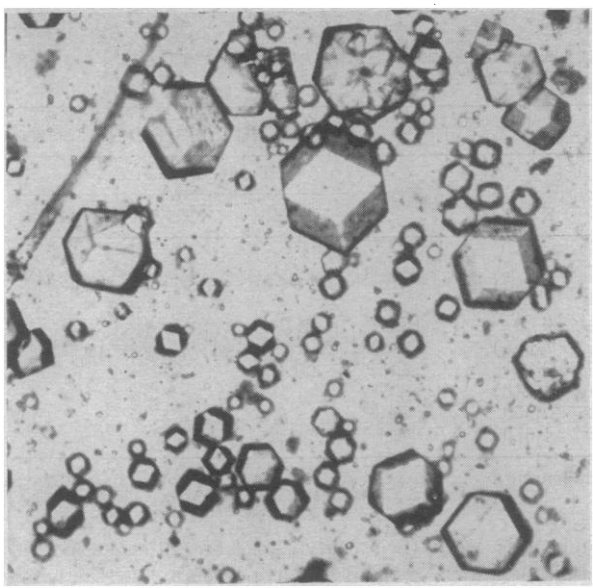

FIG. 1. Crystalline HGF.

mercial purification of insulin served as starting material. An initial precipitation at pH 6.7 yielded a material of considerable hyperglycemic activity and relatively free of insulin. Additional purification was achieved by collecting the fraction that separated between 50 and 76% acetone. Successive fractional precipitations at controlled pH in acetate and phosphate solutions resulted in a highly purified preparation that was relatively soluble in water but largely insoluble in presence of electrolytes. Administration of 0.15 μg/kg body weight of this material into cats gave a 30 mg% increase in blood sugar. A solubility curve indicated that the preparation was about 70% pure. Carboxyl-terminal amino acid analyses performed on the purified sample according to the method of Akabori (*5*) yielded essentially a single amino acid.

The highly purified preparation was dissolved in a buffer solution at alkaline pH. The precipitate obtained after centrifugation at 64,000 g was removed, and the supernatant solution was allowed to stand in the refrigerator overnight. Figure 1 shows a photomicrograph of crystals formed under these conditions.

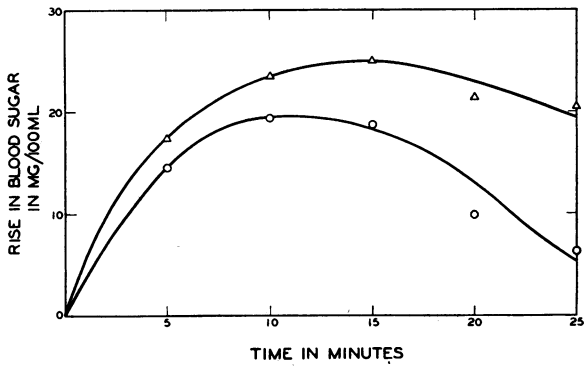

FIG. 2. Effect of HGF on blood sugar: triangles, average response of 6 cats after intravenous injection of 0.1 μg of HGF/kg body weight; circles, average response of rabbits after intravenous injection of 1.5 μg of HGF/kg body weight.

The crystalline material is relatively insoluble in cold water and gives positive biuret, Folin-Ciocalteu (phenol reagent), and Sakaguchi tests. The ultraviolet absorption curve shows a maximum at 278 mμ and a minimum at 250 mμ. The crystals belong to the isometric system and appear as rhombic dodecahedra. They contain only traces of zinc. Biological activity of the crystalline material was determined in both cats and rabbits. Results of these tests are presented graphically in Fig. 2.

Chemical and physical chemical properties, amino acid composition and analyses, and other characteristics are currently being determined with the crystalline material. This work along with detailed procedures for isolation will be published elsewhere.

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Failure of Cyanide to Inhibit β-Amylase

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According to Roy and Underkofler (*1*), treatment of malt extracts with 0.1% sodium cyanide at 30° for 1 hr had no effect on the α-amylase activities but lowered the saccharogenic activities of these extracts; NaCN completely inhibited the β-amylase activities of extracts from wheat, barley, and soybean, and of solutions of commercial β-amylase. The apparent survival of β-amylase activities in the malt extracts, according to them, was due to the limited dextrinase activities which resisted the cyanide treatment. In their experiments no attention was paid to the pH of the cyanide-treated enzyme solutions. Sodium cyanide is a strong base, and it can easily be supposed that the pH values of the NaCN-treated enzyme solutions would shift far to the alkaline side of the reaction. Furthermore, it is well known that in nonspecific inhibition the degree of inhibition is dependent on the relative concentrations of inhibitor and active centers in the enzyme molecule. From the foregoing considerations, it seemed of interest to reëxamine the effect of cyanide on the α- and β-amylase activities of various origins.

Potassium cyanide, in place of sodium cyanide, was used in this experiment. The control test was run simultaneously without added inhibitor, and tests were made with 0.1% HCN (neutralized to pH 5.0) and with 0.1% potassium carbonate, respectively. Potas-