50 of which are homozygous for a new mutation to short ear (*se*). Ten of these *sese* are 12/6 (the reduction in rib number being associated with *se* [*4*]), but otherwise the CBA mice are like the C3H sublines of Strong and Bittner.

No explanation of the observed difference is known. On the assumption that the difference is genetic, the following are possibilities. (1) The original C3H mice may have been heterozygous for one or more pairs of genes affecting skeletal type. By inbreeding, different genetic combinations may have become fixed in different sublines. The heterozygosity would have had to continue in the line at least until the Andervont subline was established in 1930. (2) One or more mutations of skeletal genes may have occurred following the separation of the Andervont subline, leading thereby to the establishment of different types in the existing sublines. (3) One of the sublines may have been genetically contaminated by an accidental and unrecorded mating outside the line. (4) Some other strain may erroneously have been labeled as C3H.

Pursuit of the origin of the difference appears fruitless. The important point is that, pending further information, at least two distinct types of C3H mice must be recognized. They may be designated as C3H/St or C3H/Bi for Strong's and Bittner's sublines and as C3H/He for the Andervont (Heston) subline.

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Manuscript received July 11, 1952.

Extraction of the Hyperglycemic Factor (HGF) of the Pancreas with Liquid Ammonia[1]

Piero P. Foă, Sheldon Berger, Leonida Santamaria,[2] Jay A. Smith, and Harriet R. Weinstein

Department of Physiology and Pharmacology, The Chicago Medical School[3]

The intravenous injection of most commercial insulin preparations is followed by a short period of hyperglycemia which reaches a maximum in 10–15 min, then gradually gives way to the typical insulin hypoglycemia. This phenomenon was known to early investigators who, working with pancreatic extracts but being mainly concerned with the purification of insulin, attributed it to an undesirable "contaminant," difficult to eliminate. Later investigators became interested in this "contaminant" per se, separated it from insulin, and called it "glucagon" or the "hyperglycemic-glycogenolytic factor (HGF)." Its properties have been extensively reviewed (*1–4*) and have led some investigators to suggest that HGF is a second pancreatic hormone, possibly secreted by the alpha cells of the islets of Langerhans (*5–8*). Other investigators (*9, 10*) have cautioned against the premature acceptance of this conclusion and have pointed out that, although HGF might actually be a hormone, direct proof of its secretion *in vivo* is not yet available. Indeed, the possibility exists that HGF might be a cleavage product of the insulin molecule, since most HGF preparations have been obtained by the rather drastic procedure of destroying insulin with alkali at 39° C (*11, 12*). In a few instances insulin has been inactivated by reducing its —S—S— linkages with cysteine, but the complete inactivation requires a ratio of cysteine to insulin of 40 : 1 by weight (*13*), and the physiological properties of such preparations are hard to evaluate in view of the hyperglycemic effect of cysteine itself (*14*). A method for the preparation of HGF that would avoid these difficulties would therefore be desirable.

Liquid ammonia dissolves many proteins, including insulin, with minimum denaturation and little or no loss of physiological activity (*13, 15, 16*). Furthermore, insulin dissolved in liquid ammonia is inactivated by a ratio of cysteine to insulin of only 1 : 1 by weight (*17*). Since insulin and HGF have very similar chemical properties, an attempt was made to extract them both with liquid ammonia. Insulin could then be inactivated by an amount of cysteine calculated on the basis of the estimated insulin content of the pancreas used.

Granulated lyophilized pork pancreas was ground in a Waring blendor; 200 g of the powder was placed in a transparent 1-liter Dewar flask graduated in 100-ml divisions and extracted with five successive 200-ml portions of liquid ammonia delivered directly from the original commercial cylinder. Each portion was left in contact with the pancreas for about 30 min; during this period the boiling of the ammonia kept the suspenison under continuous mild agitation. A second 1-liter Dewar flask, containing 50 mg of cysteine hydrochloride, was connected with the first one by means of a glass tube attached to a glass wool filter. At the end of each 30-min period, the clear pink solution was pumped from the first to the second flask by means of a rubber bulb. The cysteine hydrochloride was placed in the receiving flask before the extraction was started, to minimize handling and because the addition of the crystals to the liquid ammonia causes a violent boiling over of the solution. The cysteine readily dissolved in liquid ammonia and was found sufficient to inactivate completely the insulin extracted from the 200 g of pancreas powder. Care was taken to avoid leakage of water into the flasks; water, which constantly condensed and froze on the rubber and glass connections, would have caused the formation of ammonium hydroxide, and this might have dena-

[1] Supported by a grant from Eli Lilly & Company.

[2] Fulbright Fellow. Present address: Istituto di patologia generale, Università di Perugia, Italy.

[3] 710 S. Wolcott Ave., Chicago 12, Ill.

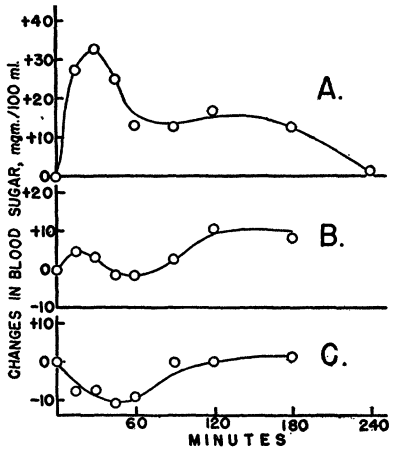

FIG. 1. Effect on the blood sugar of the rabbit of intravenous injection of liquid ammonia extracts of pancreas (*A*), of cysteine hydrochloride (*B*), and of saline (*C*).

tured the HGF. When the five 200-ml fractions had been collected in the second flask, they were allowed to evaporate through a mercury seal. Complete evaporation required about 36 hr. When all the liquid ammonia had evaporated, the receiving flask was opened, the residual gas was blown off with air, and about 20 g of a rose-colored paste was collected. This paste was extracted with saline (10 ml/g paste), the pH adjusted to 7.4, and the suspension centrifuged.

About half the solids dissolved into the saline, and this was our "crude preparation." This was divided into five equal portions: solution A was used as prepared, solution B was acidified to pH 4.5, solution C was treated with sodium chloride to make a 25% solution, solution D was treated with ammonium sulfate to make a saturated solution, and solution E was treated with ethyl alcohol to make an 80% solution (*11, 12, 18, 19*). In all cases a yellowish precipitate was obtained, collected in a tared centrifuge tube, and dried. All materials were dissolved in 10 ml of saline at pH 7.4. Three kilogram rabbits were used as test animals. After collecting a control blood sample from the marginal vein of the ear, the material to be tested was injected intravenously, and additional venous blood samples were taken at intervals after the injection. Blood glucose was determined in duplicate following the method of Folin and Malmrose (*20*). Three rabbits received 10 ml of solution A, the "crude" preparation containing the solids extracted from 10 g of the original pancreas powder; 3 rabbits received the material obtained from 10 ml of solution E; 2 rabbits each received the material obtained from 10 ml of solutions B, C, and D, respectively. All solutions produced a hyperglycemia of comparable intensity and duration; the results, therefore, were averaged. Fig. 1 *A* shows that the rabbits' blood sugar started to rise within 15 min, reached a maximum in about 30 min, and returned to normal 3–4 hr after the injection.

To rule out the possibility that this hyperglycemia may have been due to the added cysteine, cysteine hydrochloride in doses of 2 mg/kg in 10 ml of saline was injected into 2 rabbits. No significant changes in blood sugar were obtained (Fig. 1 *B*). This dose of cysteine is about 2.5 times greater than the maximum total amount of 2.5 mg which 10 ml of "crude" extract would have contained had all the cysteine hydrochloride originally added remained unmodified. This is unlikely, for most of it is probably oxidized as insulin is reduced. The possibility that the hyperglycemia might have been due to a nonspecific response of the animals to manipulation was ruled out not only by the above experiments with cysteine but also by two experiments in which the rabbits received 10 ml of saline under identical conditions (Fig. 1 *C*).

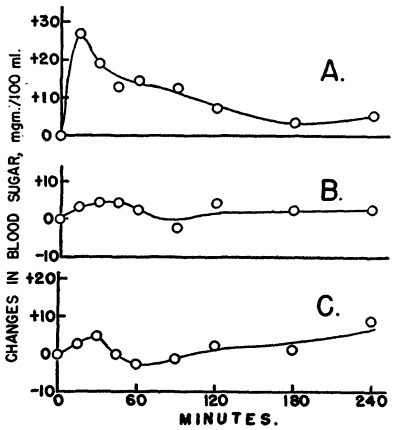

FIG. 2. Effect on the blood sugar of the rabbit of intravenous injection of liquid ammonia extracts of stomach (*A*), kidney (*B*), and liver (*C*).

Liquid ammonia extracts of whole stomach powder were found to contain a hyperglycemic factor (Fig. 2 *A*), confirming the observations of others who found HGF in extracts of gastric mucosa (*11*). Kidney and liver extracts,[4] on the other hand, were inactive (Fig. 2 *B* and *C*). Since all extracts were prepared in the same manner from equal weights of starting material, contained the same amount of cysteine, and were used under identical conditions, these negative results further indicate that the hyperglycemia obtained with pancreatic and gastric extracts is due to HGF and not to cysteine or to a nonspecific response.

Experiments designed to purify the HGF further and assay its potency are in progress.

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Manuscript received July 10, 1952.

Aragonite Rafts in Carlsbad Caverns, New Mexico

Donald M. Black[1]
U. S. National Park Service, Grand Canyon, Arizona

The Left Hand Tunnel of Carlsbad Caverns consists of a maze of rooms and corridors that have developed in limestone along two series of vertical joints intersecting at nearly right angles. Water has dissolved away the walls to form deep crevices, in many of which basins have been formed between adjacent deposits of flowstone (*1*). It was in one such basin that rafts of aragonite were first noticed (Fig. 1). The surface of

FIG. 1. Positive water basin in the Left Hand Tunnel (about 4 ft deep). *A*, floating key-shaped mineral raft; *B*, water level; *C*, alcove with piece of mineral sheet attached at water level; *D*, gasoline lantern; *E*, lantern reflections. Note small floating fragments between large raft and *E*.

one raft was more than a square foot in area; small ones several inches square were removed from the water without breaking. When edges of the rafts were submerged, they sank; and in view of aragonite's specific gravity, the floating phenomenon must be attributed to surface tension.

Small fragments of mineral films attached to the shore line indicated that the rafts were first formed across small alcoves, and were later broken into fragments composed of small crystals the long axes of which parallel the surface of the water. Richard T. Moore (*2*) reports:

> This material [the rafts] is aragonite; the orthorhombic calcium carbonate. This identification is based upon the optical data obtained on the sample. . . .
> All crystals: Prism
> Brachypinacoid (side)
> Some crystals: Very steep dipyramid
> Some crystals: Medium flat brachydome. . . .

Evaporation from the surface of an undisturbed pool of water causes the mineral matter in solution to concentrate in the surface tension film. When the downward dispersion of the mineral is not fast enough to lower the concentration, the solution becomes saturated. Should the air pressure or temperature decrease, supersaturation initiates mineral deposition on the walls and calcareous projections (i.e., carbonate crystals). The distribution of solution, combined with surface tension, facilitates the development of thin crystal films on the water surface. These sheets, or films, have very little strength. With any appreciable drop in water level the weight of the minerals pulls the films free of their shoreline contacts, and fragments remain on the surface as rafts. Continued crystal growth ultimately causes the rafts to sink of their own weight.

It is not unlikely that some mineral sheets form within a few hours or a few days. Their brittleness and their attachment to the shore line could not withstand more than a very small drop in water level. With an average mineral content of 400 ppm (higher than average for most pools in the caverns), 6250 cu in. of water would have to evaporate to deposit 2.5 cu in. of minerals. This would be sufficient to form a mineral film .000496 in. thick over a pool having 35 sq ft of surface area (about equal to the pool observed). If all these minerals were collected to form a raft 1 ft square, its average thickness would be about .017 in. Those in Carlsbad averaged .06; this would indicate the surface level would have to drop nearly 4.4 in. to form this volume of minerals, or that additional minerals were brought into the pool by seep water.

Wm. J. Foster (*3*), of the National Speleological Society, reports that he has observed floating mineral films in the following caves: Nestles Quarry Cave near Martinsburg, W. Va.; Melrose Caverns near Harrisonburg, Va.; Onondaga Caverns near Sullivan, Mo.; Marvel Cave near Reeds Spring, Mo. The mineral films he observed in the first three caves were all in "rimstone" pools; in the last-mentioned, the deposit was along the edges of one of the rather extensive lakes that occur in the lower level.

George W. Moore (*4*) and others (*5*) have noted "calcite crystals" and a "thin film of crystalline calcite . . . so thin that the surface tension supports

[1] The writer wishes to express his gratitude to the superintendent, guides, and naturalists of Carlsbad Caverns for conducting field trips and assisting in the collection of data; to Wm. J. Foster for criticism and for the data on caves in which he has seen floating sheets of minerals; and to the various members of the National Park Service who have given helpful criticism in preparation of this article.