Comments and Communications

Starvation and Responsiveness of Some Laboratory Animals

BULLFROGS, in a recent shipment, were quite lethargic after storage and reacted only when they were strongly stimulated. It seemed possible that they were sluggish from starvation; therefore, they were injected with small amounts of a high concentration of glucose in Ringer's solution. The frogs staged a dramatic comeback. After a day, the screen lid of the tank in which they were kept had to be weighted down because of their vigorous hopping. It has been observed over many years that frogs are not readily induced to eat in the laboratory or even in outdoor tanks unless placed under conditions closely simulating nature. Prolonged starvation is therefore usually the result of storage. However, no tests were made to see whether the glycogen in the liver and muscle was depleted.

To get some idea of the degree of absorption of injected sugar, a series of graded injections was made and the urine was tested for overflow of glucose. Urine was accumulated in the bladder by superficially inserting a very fine insect pin on each side of the cloaca and tying the anal pore with thread. When a sample was desired the thread was released. After a number of trials, it was found that a 1.5-ml injection of 8% glucose into a 49-g grass-frog gave a positive test for reducing sugar in the urine in 1 hr. Tests for reducing sugar at various periods of time showed that about one-third of the sugar injected appeared in the urine within 7 hr, after which no more was voided. Undoubtedly, the amount of sugar absorbed depends upon the degree of starvation as well as upon the size of the animal. Presumably excess sugar is voided by the kidney, and therefore excessive amounts injected into frogs should not hurt them.

A somewhat similar observation was recently made with the sipunculid worm, *Phascolosoma agassizi*. Proboscis retractor muscles excised from freshly collected worms served as excellent though small experimental objects, but after a few days of starvation, contractions of such muscles were visible only under a magnifier and were insufficient to lift a blade of grass used as a lever for marking the kymograph. Muscles excised several hours after injection of glucose into the body cavity of the worm showed considerably more vigorous contractions. This was particularly true if the muscles were stored overnight in 0.1% glucose in sea water at 5° C. The results are reported because they may remind others of the possible depletion of sugar reserves in various animals stored under unnatural conditions while awaiting use in classroom experiments and the decreased responsiveness which may be reversed by supplying glucose.

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Radioactive Gold

IN the September 4, 1953, issue of SCIENCE, a brief communication appeared concerning "the danger of radioactive colloids" by Elemer R. Gabrieli of the Yale University School of Medicine. In this communication, reference is made to an article by Dr. Yuhl and myself (*Nucleonics*, **11**, 54 [1953]), which concerned the development of a new technique for recording the anatomic configuration of the liver by means of intravenously administered radioactive colloidal gold (Au^{198}). This was an experimental study carried out on animals. In this article reference was made to instrument developments in this laboratory in an effort to reduce the quantity of radioactive gold to a safe level for human application. This work has been completed and the method applied to the diagnosis of space-occupying lesions of the liver in humans.

It should be emphasized that the dosage of radiogold used in this procedure does not exceed 300 microcuries, which in an average patient delivers less than 15.2 equivalent roentgens to the liver. The total body radiation from this tracer dose of Au^{198} is approximately 0.32 equivalent roentgens. The application of this procedure has been limited to those patients with known primary malignant neoplasms elsewhere in the body who are suspected of harboring hepatic metastases from such primary lesions. There has been absolutely no evidence of radiation injury to the liver or hemopoietic tissue in these patients following the administration of a single tracer dose of 300 microcuries of radiogold.

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The Production of Heinz Bodies in Normal Human Erythrocytes by Metabisulfite

THE observation was previously made, and recently extended, that Heinz bodies formed in nonsickling erythrocytes of children and adults at pH 4.0 when exposed to a 2% aqueous solution of sodium bisulfite, prepared from reagent grade crystals or from 3 grain tablets of sodium metabisulfite.[1] The same results were obtained when 0.9% sodium chloride solution was used as the solvent (pH 3.6). These structures were not present when the wet mount was made, and appeared within the time limit (15 min) set for observing the sickling preparation. They were visible only at magnifications of 950–1000 ×, and were never seen within the sickled erythrocytes of patients with either sickle cell anemia or the sickle cell trait. No previous description of these changes could be found in the literature. The Heinz bodies went through the usual stages of formation at the periphery of the erythrocyte, coalescence, extrusion from the cell (with stalks),

[1] Supplied by Eli Lilly and Co.

and Brownian movement. This was followed by irregular linear distortion beginning at the central pallor and ending in incomplete red cell fragmentation.

These observations may be significant in further demonstrating the differences between the globin fractions of normal and sickle cell hemoglobins. It is now believed that Heinz bodies represent particles of denatured globin ("globan") (*1*). If so, their formation under the above conditions indicates a denaturing effect by the bisulfite on the intraerythrocytic globin. It has been assumed that the principal difference between normal and sickle cell globins lies in the folding or coiling of the polypeptide chains of the globin molecules (*2*). If this is so, it is conceivable that certain reactive groups (possibly —SH) in the normal globin molecule are available for combination with bisulfite (and subsequent denaturation of the globin), whereas the different molecular arrangement of the sickle cell globin does not lend itself to this reaction. Studies are now in progress to observe the effect of other reducing agents on this phenomenon and to determine whether quantitative differences are present in the reaction of erythrocytes from patients with sickle cell anemia as compared to those with the sickle cell trait.

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Who Discovered Vitamins?

EVERY writer who has given an account of the earliest conception that essential nutrients for mammals, other than the "albuminous, saccharine, and oleaginous principles" comprehended in 1827 by Prout, states that N. Lunn (*1*) in 1880, while working in the laboratory of G. v. Bunge, at Dorpat, was the first to provide proof that unidentified dietary essentials must exist in milk. The basis for Lunin's statement was his observation that mice could remain in good health during at least 60 days when restricted to milk as their sole food, whereas, when given a mixture of casein, lactose, milk fat, and milk ash, in the same proportions as in milk, they speedily declined and died. He wrote: "Mice can live well under these conditions when receiving suitable food (e.g., milk), but as the experiments show that they cannot subsist on proteins, fats and carbohydrates, salts and water, it follows that other substances indispensable for nutrition must be present in milk besides casein, lactose, fat and salts."

The present writer believes that the first person actually to express this belief was the distinguished French chemist, J. A. B. Dumas. Ten years before Lunin wrote the statement quoted above, Dumas published a paper on "The Constitution of Blood and Milk," which was published in English translation in 1871 (*2*). In this paper he described the effects of substitute foods on the infants of Paris during the Siege. Here, for the first time in history, a distinguished scientist interpreted his observations on the experiences of human subjects, restricted through the pressure arising from the siege, to a diet as simplified, in chemical terms, as any rigidly controlled animal experiment. Dumas told of the extremity of the people in Paris when they ran out of "comestibles and combustibles." He said ". . . to the scarcity of milk and eggs, the certain cause of the premature decease of a great number of young children . . . and finally, to the exhaustion of the supplies of corn, flour and meat, which, rendering the capitulation of Paris inevitable, marked the precise day for it. . . . Scientific men were asked urgently to find ways for obtaining heat without combustibles; to reconstruct food with mineral materials, without the cooperation of life . . . to reproduce, at least the essential food of man with nonalimentary materials."

"Was it possible," he continued, "to come to the assistance of new-born children by replacing milk, which could no longer be got, by some saccharine emulsion? In this case there was no question of creative chemistry, but only of culinary chemistry. Recipes were not wanting, all reproducing an albuminous liquid with sugar and an emulsion of a fatty body. As a provisional succedanum this artificial milk deserved to be welcomed. But sometimes there was such a conviction in the authors of these preparations that one was forced to dread for the future of the effects of their faith. This was of a nature to make many proselytes, to the great injury of the children at nurse. . . . How could the latter [the milk dealers] have the least scruple when they were taught to manufacture an emulsion which they saw recommended to consumers, as the real equivalent of milk?"

The disastrous effects of feeding infants and young children on such emulsions led Dumas to say: "For these reasons, and many more, for no conscientious chemist can assert that the analysis of milk has made known the products necessary to the life which that aliment contains, we must renounce for the present, the pretensions to make milk, and especially to abstain from identifying any emulsion with this product."

So far as I am aware, the observations of Dumas appear never to have been mentioned by investigators of nutrition. They deserve recognition as the earliest conclusive demonstration that unrecognized nutrients exist, which before had found no place in the philosophy of physiologists and chemists, and were not to be again suspected of existence until, a decade later, Lunin recorded his eventful conclusions.

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