ate oxidation above the amounts expected from the formulae. It should be stressed that standard conditions must be developed to ensure the same percentage oxidation each time.

Up to the present 35 proteins have been analyzed for leucine, isoleucine, and valine (cf. [4], Table II for some of the results).

11657

Rapid Absorption of Substances Injected into the Bone Marrow.

L. M. TOCANTINS

From the Division of Hematology, Jefferson Medical College and Hospital, Philadelphia.

Substances injected into the marrow cavity of the tibia of the rabbit and of the sternum of man appear to find their way immediately into the general circulation. That material so administered is taken up and utilized as rapidly as if it had been injected intravenously has been demonstrated in the following ways:

1. Blood replacement by intramedullary injection. A rabbit was bled of 20% of its calculated blood volume, by puncturing the heart and aspirating the blood slowly into a solution of sodium citrate. Twenty-four hours afterwards a needle with bevelled stylet was placed into the marrow cavity of the upper portion of the tibia, and blood, freshly removed from another animal, was introduced in an amount equivalent to that withdrawn the preceding day. The injection was allowed to proceed at the rate of about 5-7 cc per minute. Slight twitching of the leg after the injection started was the only disturbance observed in the animal. Seven animals were treated as described; 2 other animals were bled and allowed to recover spontaneously. Of the 7 treated animals 4 recovered their original (previous to the bleeding) erythrocyte and hemoglobin level within 24 hours after the intramedullary injection, 2 within 48 hours, and one died as a result of a hemopericardium. The last mentioned animal was the only fatality among all the animals that received various substances by the intramedullary route. None of the other animals showed any sign of distress during or after the experiments. In Fig. 1 is illustrated the response of the erythrocytes, hemoglobin and reticulocytes of a treated and an untreated animal.

2. Injections of glucose in experimental hypoglycemia. Four rabbits were rendered hypoglycemic by intravenous injections of a dose

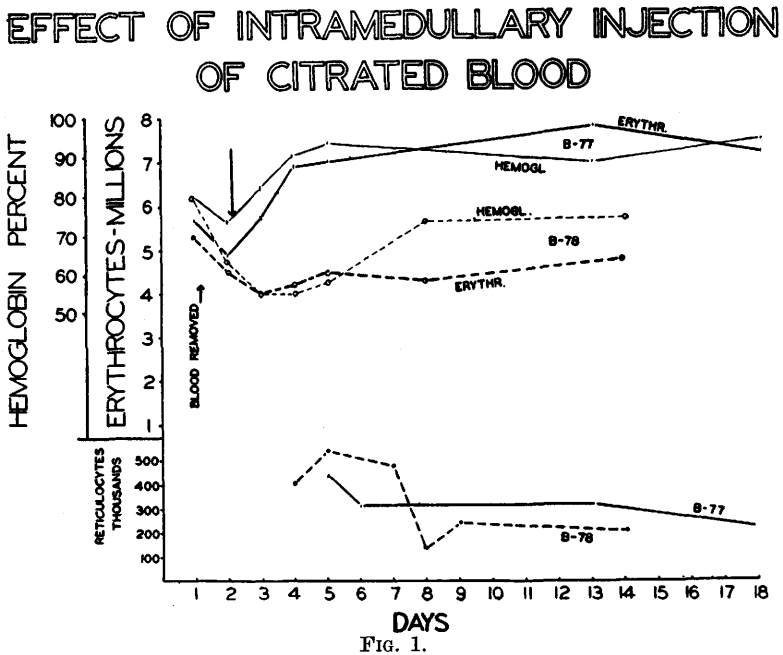

FIG. 1.

Blood was removed by heart puncture from both animals (short arrow). 24 hours later an amount of freshly collected blood equivalent to that removed was injected (long arrow) into the tibial marrow of Rabbit B-77.

of insulin equivalent to 12 units per kilogram of body weight. Immediately after the appearance of a convulsion (usually within 3 hours after the injection) a 25 or 30% solution of dextrose (2-3 g per kilo body weight) was injected into the marrow cavity of the upper portion of the tibia at the average rate of 8 cc per minute. The abdominal reflex which was always absent during the convulsive period returned immediately after the end of the injection and there were no further convulsions. All dextrose treated animals recovered from the hypoglycemic reaction. An additional animal to which the dextrose injection was not given died 37 minutes after the first convulsion.

Rabbit B-85. Weight 3.3 Kg. Fasting 24 hours.

7-25-40 11:00 A. M.: Blood sugar (Benedict's method): 102 mg per 100 ml of blood.

11:02 A.M.: 22 units of crystalline zinc insulin intravenously.

12:33 P.M.: 18 units of zinc insulin intravenously.

12:42 P.M.: 2 convulsive seizures; abdominal and corneal reflexes absent. Blood sugar: 64 mg.

12:50-12:52 P.M.: 20 cc of 25% glucose injected into the marrow of the tibia. Abdominal and corneal reflexes present at the end of the injection. No further convulsions.

12:54 P.M.: Blood sugar: 540 mg.

1:03 P.M.: Blood sugar: 363 mg. Animal alert and active.
3:50 P.M.:Blood sugar: 78 mg. Animal fed and watered.
7-26-40 9:00 A.M. Animal well. Blood sugar (not fasting): 152 mg.

3. Rapidity of spread of dye injected in the tibial marrow. A marrow puncture needle was inserted into the upper portion of the tibia of a rabbit. A plain 20 gauge needle was introduced into the heart and 1.5 cc of blood aspirated into 0.5 cc of 1.3% sodium oxalate. The needle was left in the heart and meanwhile 1.5 cc of a 1% solution of Congo Red was injected through the tibial needle into the marrow cavity. At 10, 20 and 60 second periods following the end of the injection (duration of injection 7″) blood was removed from the heart through the indwelling needle. The specimens were then centrifuged and the color of the plasmas noted. The plasma was colored in the very first specimen, indicating that it took ten seconds or less for the material to reach the heart from the tibia. Similar results were obtained with a solution of fluorescein.

4. Injection of mercury into the marrow cavity of the tibia of the rabbit and of the sternum of man. Fig. 2 represents an X-ray photograph of a human sternum and its collateral rib fragments removed at autopsy. A needle had been inserted at point A and about 1 cc of

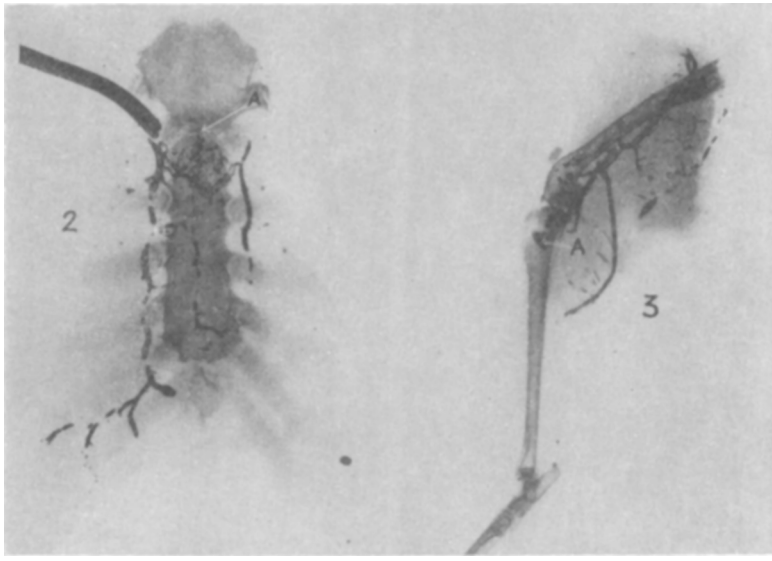

FIG. 2.
Sternum and collateral rib fragments of an adult negress. The thick line at the upper left corner is a hemostat clamping the cut end of the right internal mammary vein.

FIG. 3.
Lower extremity of a rabbit. Amputation was carried out before injection. Arrows indicate points of injections of the mercury.

mercury injected under slight pressure. Almost immediately after the beginning of the injection, mercury was running out of the severed ends of the internal mammary veins on the under surface of the sternum. The same technic was followed in an attempt to determine the path of outlet of mercury injected into the upper portion of the tibia of a rabbit (Fig. 3). Mercury so injected apparently finds its way through veins in the bone cortex into the deep femoral. Just as in the human sternum, the path of least resistance appears to be toward the venous system. In man the marrow tissue itself of the sternum, adjacent to the point of injection, was infiltrated to a limited extent, the mercury escaping chiefly into the outgoing venous channels; in the rabbit the rest of the tibia was entirely free of the injected material.

5. Gravity infusion of physiological salt solution into the sternum of man.* The sternal bodies of 3 adult men were punctured with a specially built gauge 15 needle, holding within it a gauge 18 needle with a bevelled stylet. Marrow was aspirated through the smaller gauge needle which was then removed. The lumen of the larger needle was then filled with physiological salt solution and connected with a salt solution infusion apparatus. In one man, complaining of bone pains, it was not possible to infuse any solution by this method; in the other 2, salt solution ran in by gravity readily, without discomfort, the infusion being maintained at rates varying between 5-10 cc per minute, for periods as long as 30 minutes when the experiment was discontinued.

Josefson[1] injected as much as 5 cc of liver extract into the sternal marrow cavity of patients with pernicious anemia with the object of stimulating the bone marrow directly. A transient headache, sometimes severe, accompanied by vomiting, often followed these injections. These symptoms were probably caused by the massive rapid absorption of the drug, for they may be observed also after intravenous injections of liver extract. Radio opaque substances have been injected into the sternum of living patients with the object of studying structural changes in the marrow; the method succeeded only partially, because the injected material escaped into the general circulation before adequate photographs could be obtained.[2] The medulla of bones may, therefore, offer one more route for parenteral therapy when quick absorption is desired and prevailing circumstances (poor or obliterated veins, extensive burns or mutilations) make it difficult or impossible to use the common paths.

* From the wards of Medical Service B, Pennsylvania Hospital.

1 Josefson, A., *Acta Medica Scand.*, 1934, **81**, 550.

2 Berthet, G., Benda, R., Orinstein, E., and Depitre, *Sang.*, 1940, **14**, 172.

Summary. Substances injected into the marrow cavity of the tibia of the rabbit and of the sternum of man are almost immediately absorbed into the general circulation. Blood and glucose solutions respectively, by intramedullary injection, corrected rapidly experimental anemia and hypoglycemia induced in rabbits.

11658

Formation of the Anti Egg-White-Injury Factor (Biotin) in the Rumen of the Cow.

L. W. McELROY AND T. H. JUKES

From the Divisions of Animal Husbandry and Poultry Husbandry, University of California, Davis.

When raw egg white is added to a nutritionally adequate diet, characteristic skin symptoms are produced in various species. The symptoms may be prevented or cured by supplementation with a vitamin-like factor.[1] Evidence has recently been presented[2] that this "anti egg-white-injury" factor is identical with biotin.

It has recently been shown that thiamin, nicotinic acid, riboflavin, pantothenic acid, pyridoxine and vitamin K may be formed in the rumen of the cow.[3] The present experiments indicate that the anti egg-white-injury factor (biotin) is also formed under the same conditions.

In the present experiments, the characteristic symptoms of egg-white-injury were produced in chicks by feeding the following basal diet (Diet ED): Yellow cornmeal, 55 g; wheat middlings, 20; dried skim milk, 10; commercial casein, 10; ground limestone, 2; steamed bonemeal, 2; alfalfa meal, 1; NaCl, 0.5; $MnSO_4$, 0.05; fresh raw egg white, 30 cc. The wet mixture was spread in thin layers and allowed to dry at room temperature, after which 0.3 g of fish oil blend (3000-A, 400-D) was added. Chicks were placed on this diet at hatching. They developed the characteristic symptoms of the syndrome in 3 to 4 weeks. The dermatitis appeared simultaneously at

[1] Boas, M. A., *Biochem. J.*, 1927, **21**, 712; György, P., *J. Biol. Chem.*, 1939, **131**, 733.

[2] György, P., Melville, D. B., Burk, D., and du Vigneaud, V., *Science*, 1940, **91**, 243; du Vigneaud, V., Melville, D. B., György, P., and Rose, C. S., *Science*, 1940, **92**, 62.

[3] McElroy, L. W., and Goss, H., *J. Biol. Chem.*, 1940, **133**, lxv; *J. Nutrition*, in press.