tion of the gall bladder. In the experiments in which the duodenum is stimulated to secrete more cholecystokinin through the administration of hydrochloric acid, the concentration of the gall-bladder–contracting factor in the urine is higher. If the chief source of cholecystokinin is excluded by duodenectomy, the concentration of the gall-bladder-contracting factor in the urine is lower.

Greengard *et al.* (*6*) showed that the blood serum inactivates cholecystokinin. As more than 120 minutes are necessary for total inactivation, it is possible that cholecystokinin reaches the kidneys in an active form.

The term *urocholecystokinin* is suggested for the factor, present in urine, which causes contraction of the gall bladder without altering the blood pressure and duodenal motility.

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References

1. A. C. Ivy and E. Oldberg, *Am. J. Physiol.* 86, 599 (1928).
2. W. J. Snape, *Gastroenterology* 10, 496 (1948).
3. A. Svatos, *Ceskoslov. farmacie* 6, 39 (1957.)
4. P. A. Katzman and E. A. Doisy, *J. Biol. Chem.* 106, 125 (1934).
5. P. Sandblom, *Acta Radiol.* 14, 249 (1933).
6. H. Greengard *et al.*, *Am. J. Physiol.* 134, 733 (1941).

25 July 1958

Metabolic Dissociation of Short-Lived Barium-137m from Its Cesium-137 Parent

Abstract. At 4 to 7 days after Cs^{137} administration, Ba^{137m} was found to exceed Cs^{137}-Ba^{137m} equilibrium proportions in bone and plasma by factors of 3 and 14, respectively, in the living animal. This demonstrated the in vivo escape of Ba^{137m} from sites of Cs^{137} accumulation. Liver tissue was slightly deficient in Ba^{137m}, whereas tissues such as bone marrow and spleen showed little or no deviation from equilibrium.

Cesium-137 is one of the more important potential hazards of fallout. Although the metabolism of radiocesium in biological systems has been studied in appreciable detail (*1*), no consideration has been given to the consequences of the differential behavior of Ba^{137m}, the radioactive daughter of Cs^{137}. The decay scheme is as follows:

$$Cs^{137} \xrightarrow[T\frac{1}{2} = 33\ \text{yr}]{0.52\text{-Mev } \beta} Ba^{137m} \xrightarrow[T\frac{1}{2} = 2.6\ \text{min}]{0.66\text{-Mev } \gamma} Ba^{137}$$

Cesium-137 decays by beta emission with a 33-year half-life to Ba^{137m} which in turn decays by gamma emission with a half-life of 2.6 minutes to a stable state.

In the organism, Cs^{137} would be expected to have a metabolic behavior similar to that of potassium, as both are alkali metals; Ba^{137m}, however, would be expected to behave more like the other alkaline earths, calcium and strontium (*2*). The metabolic dissociation of Ba^{137m} from the parent Cs^{137} has probably been overlooked experimentally because tissues are usually not measured within minutes after removal from the organism; this delay permits the reestablishment of equilibrium conditions in the tissue samples (the rate of establishment of equilibrium is governed by the half-life of the daughter, 2.6 minutes).

In the work reported here (*3*), the observations were made within minutes after removal of tissues; this permitted an estimation of the differential behavior of parent and daughter and an evaluation of the concentrations of the isotopes as they exist in the living animal.

Twelve normal albino rats of body weight about 150 g were injected intraperitoneally with about 60 μc of Cs^{137}-Ba^{137m}. At 4 to 7 days, the rats were anesthetized with sodium pentobarbital, and samples of blood, bone, and liver were removed from the living animal for radioassay. For each sample, an accurate time record was kept, starting at the time of removal from the body. The whole blood and liver were placed directly in test tubes, and radioactivity measurements were started within 1 to 2 minutes after removal. The femur diaphysis was used for the bone sample and was cleaned of extraneous tissue and marrow as rapidly as possible; measurements were usually started about 2 minutes after removal. Three blood samples were drawn into hematocrit capillaries, centrifuged, and the plasma and red blood cells separated so that radioassay was started on each fraction about 4 minutes after removal of blood from the animal. The samples were all counted in the usual well-type scintillation detector. In order to follow the expected decay or growth, a count-rate meter was employed with a recorder to give continuous reading of the counting rate over the 10 to 20 minutes required for attainment of equilibrium. Tissue weights were obtained after completion of the radioactivity measurements. The values given for liver and blood represent an average of tissues from four animals and, for bone, an average of tissues from eight animals. The results are expressed as follows: A, observed counts per minute per milligram of tissue; A_{eq}, counts per minute per milligram of tissue at equilibrium.

The observed radioactivities (A) in the various tissue samples as a function of time after removal from the body are presented as a semilog plot in Fig. 1. It is immediately apparent that the gamma activity in bone and blood decreased markedly with time to an equilibrium value, whereas that in liver increased slightly. Graphic analysis of these curves indicated that they represented two exponential rate processes: there was a short-lived component and a component of long enough half-life that decay could be neglected over this time scale. The short half-life was calculated in the usual way by plotting A–A_{eq} (irrespective of sign) versus time on a semilog scale (Fig. 1). As expected, a half-life of about 2.5 minutes for blood, bone, and liver was obtained; this is in agreement with that reported for Ba^{137m}.

The observations on plasma and erythrocytes are presented in Fig. 2. For convenience, the values are expressed in terms of A/A_{eq}. It is obvious that there was no appreciable change in the radioactivity of the red blood cells during the 20-minute counting period; the possible slight decline at the early time periods may have been caused by trapped plasma. The radioactivity in the plasma, however, decreased significantly with time, with a half-life of about 2.7 minutes.

There is little question that the Ba^{137m}

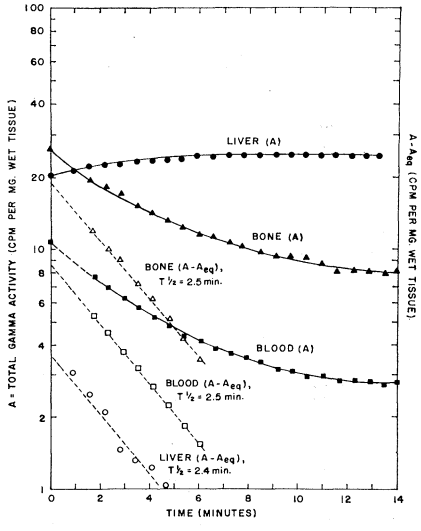

Fig. 1. Change in the total gamma activity (A) in liver, bone, and blood after removal from rats previously injected with Cs^{137}-Ba^{137m}, and the plot of their corresponding short-lived component ($A - A_{eq}$) with calculated halftimes ($T\frac{1}{2}$) given.

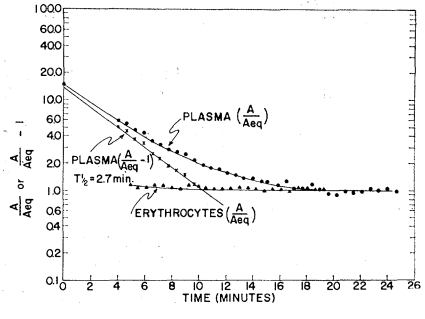

Fig. 2. Partition of excess Ba^{137m} between plasma and erythrocytes in the rat (see text for definition of terms).

daughter produced within the body from Cs^{137} is differentially metabolized so that, in the living animal, various tissues may have a deficiency or excess of Ba^{137m} as compared with the equilibrium mixture. It can be calculated from the data presented thus far that the gamma levels in the liver, bone, blood, and plasma of living animals in these experiments were 0.8, 3.3, 3.9 and 14 times the values as determined by usual tissue analysis.

In another study, other tissues were examined by the same procedure to see whether there was an excess or deficiency of Ba^{137m}. It was observed that the gamma activity in blood and bone decreased with time as noted before. No deviations from the equilibrium proportions were seen for bone marrow, testes, and brain; there was a suggestion of an excess Ba^{137m} in spleen and a deficiency of Ba^{137m} in kidney. The significance of these results is obscured because of the difficulty of detecting slight deviations from equilibrium against the high levels of Cs^{137}-Ba^{137m} in these tissues.

The source of excess Ba^{137m} in the plasma is most likely the soft tissues. Although the red blood cells probably contribute to the plasma excess, they can only be a minor source since the blood as a whole shows a considerable excess of the Ba^{137m}. The excess of Ba^{137m} in bone is probably the result of two mechanisms: a reflection of the increased plasma levels and a selective accumulation of barium by the bone. Evidently any depletion of the plasma barium by selective accumulation in bone is far outweighed by the entry of barium into the plasma from the tissues.

The fact that there is an excess or deficiency of Ba^{137m} in the living animal and that it can be measured raises some interesting considerations: (i) Since the gamma activities in tissues of the living animal are different from those expected from usual radioassay procedures, the radiation dosage may be different from that calculated from available Cs^{137} levels. (ii) Barium-137m is produced within cells and the opportunity is presented for studying the mechanism by means of which this element is extruded from the cell. (iii) Steady-state levels of Ba^{137m} in clinical cases after Cs^{137} administration may indicate both the state of the circulatory system and tissue metabolism; these factors, among others, should influence the level of excess Ba^{137m} in the blood. (iv) The excess Ba^{137m} in the skeleton offers the possibility of studying the processes of rapid incorporation of alkaline earths into bone independent of the slower process of growth. For example, from a comparison of the excess Ba^{137m} to calcium ratios in the plasma and bone, it was calculated that the exchangeable calcium in the femur diaphyses was 4 to 5 percent; this is in general agreement with other values in the literature as determined by other methods (*4*).

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References and Notes

1. S. L. Hood and C. L. Comar, *Univ. Tenn.-AEC Agr. Research Program* ORO-91 (1953); S. L. Hood and C. L. Comar, *Arch. Biochem. Biophys.* 45, 423 (1953).
2. G. C. H. Bauer, A. Carlsson, B. Lindquist, *Biochem. J.* 63, 535 (1956).
3. This investigation was supported by the Armed Forces Special Weapons Project through contract DA-49-007-MD-897 administered by the Surgeon General, Department of the Army. The technical assistance and aid of Dr. D. N. Tapper, Lorna Shaw, and L. A. Cape are gratefully acknowledged.
4. W. F. Neuman and M. W. Neuman, *The Chemical Dynamics of Bone Mineral* (Univ. of Chicago Press, Chicago, Ill., 1958).

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Effects of Thyroxin on Amino Acid Incorporation into Protein

Abstract. The effect of thyroxin on the in vitro incorporation of DL-leucine-1-C^{14} into the protein of rat liver homogenates has been investigated. Both thyroxin pretreatment in vivo and thyroxin in vitro at a concentration of $1 \times 10^{-5}M$ were found to increase the rate of amino acid incorporation. The increased activity following the thyroxin pretreatment in vivo was found to be localized in the mitochondrial fraction. It is suggested that the acceleration of metabolic rate characteristic of thyroxin action may be secondary to the stimulation of energy-requiring reactions such as protein synthesis.

Recent concepts of the mechanism of action of thyroxin have emphasized its uncoupling effect on oxidative phosphorylation (*1, 2*). This effect, however, is observed only with relatively high concentrations of thyroxin, occurs equally well with both the D- and L- forms (*1*), and, except for changes in oxidative metabolism, explains few of the physiological effects of the thyroid hormone. Many clinical features of thyroid disease suggest a major, if not primary, role of the thyroid hormone in protein metabolism. In immature animals it is involved in growth; in adults it causes pronounced changes in nitrogen metabolism. Furthermore, in the adult brain and testis—organs in which the quantities of protein and lipid turned over per unit time are apparently negligible compared with turnover of carbohydrate, as evidenced by a respiratory quotient of approximately 1 (*3*)—the characteristic acceleration of metabolic rate that is observed in almost all other tissues is absent in hyperthyroidism (*4*).

Previous observations (*5*) have indicated that thyroxin pretreatment in vivo stimulates amino acid uptake into the protein of rat liver slices. To investigate further the apparent relationship between thyroid function and protein synthesis, studies were undertaken to determine the effects of in vivo and in vitro thyroxin administration on the in vitro incorporation of DL-leucine-1-C^{14} into the proteins of rat liver homogenates. Livers from 90- to 150-g fasting, male Sprague-Dawley rats were homogenized by means of glass homogenizers in 5 ml of $0.25M$ sucrose solution per gram of tissue. Homogenization was performed at 0° to 2°C, and tissue fractions were maintained at that temperature through all subsequent operations until final incubation. Intact cells, nuclei, and cell debris were removed by centrifugation at 700*g* for 10 minutes. The supernatant fluid was spun at 54,000*g* for 60 minutes in a Spinco model L ultracentrifuge. The

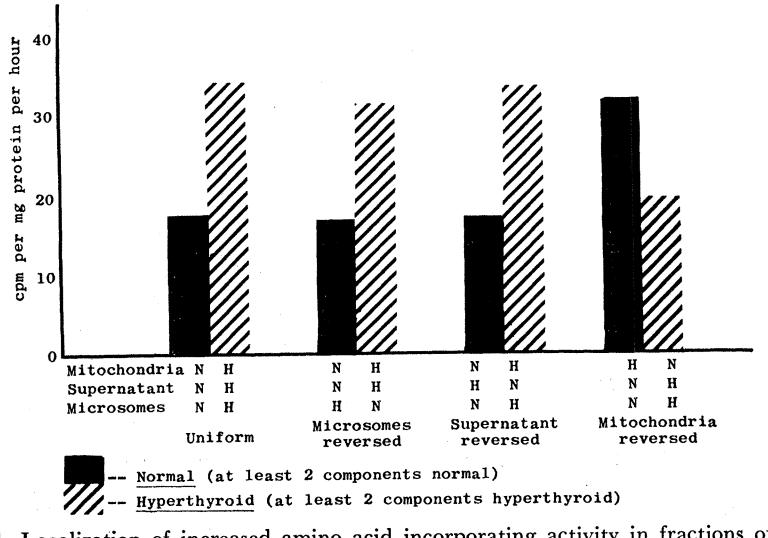

Fig. 1. Localization of increased amino acid incorporating activity in fractions of liver homogenates from rats pretreated with thyroxin. Results are representative of seven such experiments.