Dichromography—a Method for in vivo Quantitative Analysis of Certain Elements

Abstract. The method is based on absorption measurements of monochromatic x-rays. The radiation dose given to the patient is low, and thus the method is nondestructive. The physiological content of iodine in the human thyroid can be determined, as well as the distribution in the body of small amounts of roentgenological contrast substances.

A nondestructive method for the quantitative analysis of elements inside a living person would be of value for determining the function of organs in diagnostic and physiological studies. The use of monochromatic x-rays makes it possible to determine certain heavy elements as well as ensuring an optimum amount of contrast per dose given to the patient when producing radiographic images.

Any one element can be quantitatively determined according to the Beer law by measuring the attenuation of one monochromatic x-ray beam (*1*). Similarly, two elements can be analyzed with two monochromatic rays, provided that the ratios of the mass absorption coefficients of the elements are sufficiently different at the two wavelengths. To the first approximation the human body is composed of soft tissue, bone salt, and iodine (normally present in the thyroid or otherwise employed as a roentgenological contrast medium). If bone structures are avoided, two wavelengths are therefore sufficient for an in vivo analysis of the amount of iodine and soft tissue in a body section. Preferably the two wavelengths are chosen on each side of the absorption edge of iodine at 0.37 A (*2*).

The equipment comprises an x-ray tube for the production of monochromatic radiation through secondary emission, a system composed of two servo-controlled absorption wedges, a scintillator photomultiplier unit sensing the x-ray intensity, and an electronic feedback loop from the mutliplier to the two servomotors (*3*). One wedge is composed of material equivalent to soft tissue (for example, water); the other, of iodine. The two wedges are automatically kept in such a position that the intensities at the scintillator are constant. When the patient is placed in the beam, the two wedges are withdrawn a certain distance corresponding to the amount of soft tissue and iodine in the patient. Thus, the displacements of the two wedges are quantitative measures of the amounts of these substances. A scanning process is employed when producing images, showing the quantitative distribution.

At present the accuracy of the method is within ± 0.2 mg of iodine per square centimeter for static measurements and ± 0.5 mg/cm² when scanning is employed at a rate of 0.5 cm/sec and with a beam cross section of 0.25 cm². The accuracy is limited by statistical fluctuations of the number of quanta in the x-ray beam and by photomultiplier drift. Another error not included in the values given is caused by the presence of fat in the tissues. Fat has an absorption characteristic slightly different from that of the soft tissue wedge (water). Thus, 1 g of fat per square centimeter simulates the presence of 0.5 mg of iodine per square centimeter.

An additional advantage of the soft-tissue wedge is that the dose given to the patient is at a theoretical minimum. The beam is constantly attenuated so as to allow no more quanta to pass the patient than those necessary for the desired statistical accuracy. During a typical investigation of the thyroid, the dose becomes less than 1 mr.

The iodine distribution in a normal thyroid can be seen in Fig. 1. Each plotted curve represents the mean value of the ink recordings of two adjacent scanning lines. Deviations on the base level, representing the zero iodine values, are due to statistical fluctuations in the beam. It should be noted that no iodine, stable or radioactive, has been given to the patient before the investigation. The method is thus entirely different from that employed in radioiodine thyroid function tests.

The method has also been used for function tests of the liver, kidneys, and lungs, or of localized parts of these organs, in human beings and other animals, performed by studying the amount of iodine present in the organs after intravenous injections of contrast substances containing iodine (*4*). Circulation tests are also possible, as is the determination of the amount of blood in certain parts of the body.

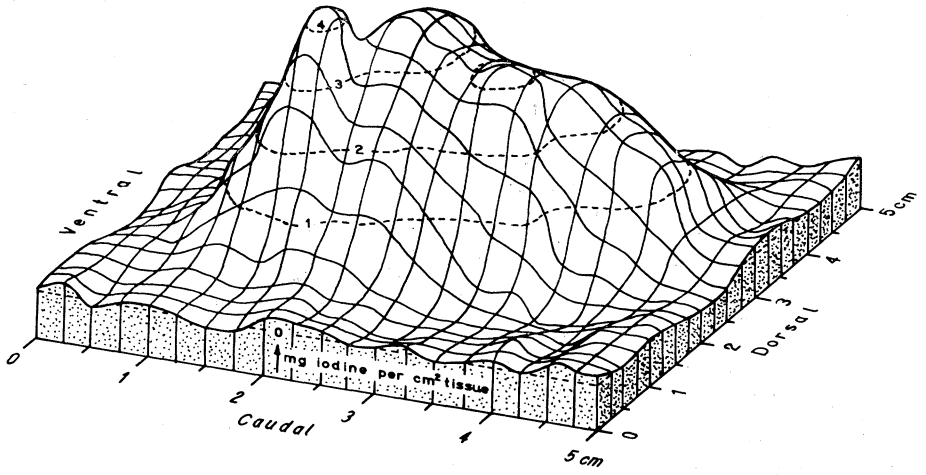

Fig. 1. Stereogram showing the distribution of iodine in a normal human thyroid. The peak concentration of 4 mg of I per square centimeter in the caudoventral part corresponds to the thyroid isthmus. The total amount of iodine in this thyroid was 23.5 mg. No iodine had been given to the patient prior to the determination.

Preliminary experiments indicate the possibility of measuring the amount of bone salt simultaneously with determination of the iodine and soft-tissue values by means of three selected wavelengths and three wedges (*5*).

BERTIL JACOBSON
Karolinska Institutet, Stockholm, Sweden

References and Notes

1. For a review of such methods, see A. Engström, in *Physical Techniques in Biological Research*, G. Oster and A. W. Pollister, Eds. (Academic Press, New York, 1956), vol. 3, p. 489.
2. B. Jacobson, *Acta Radiol.* 39, 437 (1953).
3. ——— and R. S. Mackay, in *Advances in Biological and Medical Physics*, J. H. Lawrence and C. A. Tobias, Eds. (Academic Press, New York, in press), vol. 6.
4. A report on these tests, written in cooperation with P. Edholm, is in preparation.
5. The method described has been developed during the last three years by means of financial support from Knut och Alice Wallenbergs Stiftelse and from Harald och Greta Jeanssons Stiftelse.

16 June 1958

Effect of Growth Hormone on Lipid Aldehydes

Abstract. Female rats at the body weight plateau which responded to 13 daily injections of 0.4 mg of growth hormone by greater weight gain per gram of food had a decreased concentration of lipid aldehydes in blood plasma, an increased concentration in liver, and no change in intestine, compared with control rats given isotonic saline.

The administration of growth hormone (GH) decreases the rate of turnover of plasma phospholipids in man (*1*) and increases the content and the rate of turnover of the phospholipids of the liver

in the rat (2). The response of individual phospholipids to the hormone has not been demonstrated. The data presented here (3) indicate that the administration of growth hormone to the rat changed the lipid aldehyde concentration (plasmalogen) of the liver and blood plasma.

Twenty-six female Sprague-Dawley rats that had reached a body weight plateau were divided into two equal groups, and the food consumption of each rat was measured. One group received 13 daily intraperitoneal injections of 0.2 ml of a solution containing 2 mg/ml of Somar (Armour somatotropin) (4); the other received daily injections of 0.2 ml of sterile isotonic saline. By the 14th day, when the experiment was terminated, the rats that had been injected with growth hormone had gained an average of 115 mg body weight per gram of food, compared with 41 mg per gram for the controls ($p < .001$) (5).

The rats were killed by decapitation, and the blood was collected in citrate. The liver was removed, weighed, frozen, lyophile-dried, and ground. The whole intestine was removed, washed out, frozen and lyophile-dried. After centrifugation, a 2 ml portion of the blood plasma was introduced drop by drop into a solution of 95-percent ethyl alcohol–diethyl ether (3:1); the solution was allowed to stand at room temperature overnight, then raised to boiling, cooled, and made to volume; and the protein precipitate removed. An aliquot portion of the lipid extract was evaporated to dryness; the residue was treated with acetic acid and mercuric chloride followed by Schiff's reagent (6). The color was extracted with chloroform and read against a standard prepared in the same way or from a calibration curve, to obtain milligrams of stearaldehyde.

One gram of dry liver (or the whole dry intestine) was treated overnight with the alcohol-ether solution; the mixture was then heated to boiling, and the insoluble residue was centrifuged off and reextracted with the hot solvent. The combined extracts were evaporated to dryness, the lipid was extracted from the residue with petroleum ether–chloroform (5:1), and the extract was washed with an equal volume of 50-percent ethyl alcohol. After removal of the solvent the total lipid was dried, weighed, and redissolved in chloroform.

In order to estimate the amount of fat in the liver, values for phospholipid and cholesterol were determined in this total lipid extract. Phospholipid was precipitated by acetone and weighted; cholesterol was determined by the Liebermann-Burchard reaction. The difference between the sum of the values for the separately determined lipids and the total lipid fraction was designated as "fat."

Table 1. Lipid aldehyde concentration of blood plasma, liver, and intestine of normal rats and of rats injected with growth hormone (GH). The number of determinations is given in parentheses.

	Normal		GH		p values
	Av.	S.D.*	Av.	S.D.*	
Blood plasma (mg %)	3.10(11)	1.01	1.35(12)	0.52	< 0.001
Liver (dry tissue, %)	0.33(13)	0.06	0.43(12)	0.11	< 0.02; > 0.01
Intestine (dry tissue, %)	0.21(10)		0.23(12)		

* Standard deviation of the arithmetic mean (Av).

The acetone-insoluble and the acetone-soluble fractions were treated like the blood extract for development of color with the Schiff's reagent (6). The sum of the aldehyde of each fraction gave the total aldehyde of the dry tissue.

As is shown in Table 1, the administration of growth hormone decreased the lipid aldehyde concentration of the blood plasma, from a normal value of 3.10 to 1.35 mg percent aldehyde ($p < 0.001$). This decrease is more marked than that for the plasma phospholipid phosphorus of normal patients given growth hormone (1).

In the liver, growth hormone caused a significant increase in total aldehyde, not only in concentration but in the amount per total liver, since the liver-to-body-weight ratio was the same in the treated and untreated groups. The increase in total aldehyde of the tissue following administration of growth hormone may indicate a change in molecular type of the individual phospholipids from those containing both fatty acids in esterified linkage to those containing only one esterified acid plus a potential fatty acid aldehyde in ether linkage (7). Such a change may have resulted from a diminished supply of fatty acids. The decreased ability of the livers of GH-treated rats to synthesize fatty acids from either pyruvate or acetate has been shown (8). Our finding of only 1.1 percent "fat" in the livers of the GH-treated rats compared with 2.4 percent for the normal ($p < 0.05$; > 0.02), in the absence of any change in amounts of phospholipid or cholesterol, supports this explanation for the increased aldehyde content. A similar inverse relationship between total lipid (mainly fat) and plasmalogens has been found in the adipose tissue of the young rat (9).

In the intestines, unlike the liver, the lipid aldehyde concentration of the tissue did not change following treatment with growth hormone.

According to the recently proposed structure for plasmalogens (7), these contain an α,β-unsaturated ether linkage, which is capable of taking part in a wide variety of chemical reactions. The decrease in lipid aldehyde concentration of the blood following administration of growth hormone and the increase in the liver shown in this experiment seem to link the plasmalogens as aldehyde precursors with the action of this hormone.

FRANCES L. HAVEN, W. R. BLOOR, ALICE A. GREENE,* WILLIAM D. MAYER†
Biochemistry Department, University of Rochester School of Medicine and Dentistry, Rochester, New York

References and Notes

1. E. A. McCulloch, A. Britton, C. J. Bardawill, K. J. R. Wightman, *Science* 123, 1084 (1956).
2. A. L. Greenbaum and P. McLean, *Biochem. J.* 54, 407 (1953); A. L. Greenbaum, C. N. Graymore, T. F. Slater, *J. Endocrinol.* 15, 235 (1957).
3. This investigation was supported by research grant C669(C7) and fellowship CF-5936 from the National Cancer Institute, National Institutes of Health, U.S. Public Health Service; and by an American Cancer Society grant (Instr. 57E).
4. Countesy of Dr. Sanford L. Steelman, Armour Laboratories, Chicago, Ill.
5. C. H. Goulden, *Methods of Statistical Analysis* (Wiley, New York, 1939), p. 40; R. A. Fisher and F. Yates, *Statistical Tables for Biological, Agricultural and Medical Research* (Oliver and Boyd, London, 1938), p. 26.
6. W. F. Neuman, Ph.D. thesis, University of Rochester.
7. G. V. Marinetti, J. Erbland, E. Stotz, *J. Am. Chem. Soc.* 80, 1624 (1958); M. M. Rapport and R. E. Franzl, *J. Neurochem.* 1, 303 (1957).
8. A. L. Greenbaum and R. F. Glascock, *Biochem. J.* 67, 360 (1957).
9. C. L. Yarbro and C. E. Anderson, *Proc. Soc. Exptl. Biol. Med.* 91, 408 (1956).

* Present address: Bureau of Biological Research, Rutgers University, New Brunswick, N.J.
† Summer research fellow, American Cancer Society, 1956.

18 June 1958

Effect of Oxidation and Reduction upon the Biological Activity of Parathyroid Hormone

Abstract. Parathyroid hormone loses biological activity upon oxidation with hydrogen peroxide. Part or all of this lost activity can be regained by subsequent reduction with cysteine. The extent and reversibility of this oxidation is dependent upon *p*H.

During work upon the isolation of the calcium-mobilizing principle from bovine parathyroid glands, a frequent decrease or disappearance of hormonal activity has been observed. This has been particularly true when material that has been subjected to countercurrent distribution was assayed. Because many of the