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.

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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

Table 1. Biological activity of parathormone-B after oxidation and reduction.

Preparation	Initial potency* (U.S.P. units/mg)	*p*H† of oxidation	Potency after oxidation (% of initial)	Potency after reduction (% of initial)
PTH-B	150–200	3.8	40–65	110–180
PTH-B	160–200	4.8	35–60	110–140
PTH-B	160–200	7.0	5–20	40–70
Reagent control	0	3.8	0	0–10

* Determined on control solution (see text).
† The buffers employed were: 0.01*M* glycine acetate, *p*H 3.8; 0.06*M* sodium acetate – 0.01*M* glycine, *p*H 4.8; and 0.01*M* potassium phosphate, *p*H 7.0.

findings were closely analogous to those noted during the isolation of adrenocorticotropin (ACTH) (*1*), it seemed possible that parathyroid hormone might undergo oxidation and reduction, with accompanying loss and regain of biological activity, in a manner similar to that reported by Dedman *et al.* for ACTH. It has been known for some time (*2*) that parathyroid hormone activity is destroyed by oxidizing agents but not by reducing agents. There has never been any evidence reported, however, to indicate that the loss of activity brought about by oxidation could be regained subsequently by reduction.

Experiments have been undertaken, therefore, to see whether the effects of oxidation upon the biological activity of parathormone can be reversed by reduction. For these experiments, parathormone-B (PTH-B), prepared as previously described (*3*), was dissolved (1 mg/ml) in buffer solutions at *p*H 3.8, 4.8, or 7.0, respectively. As a control, an aliquot of the solution was removed for assay. A sufficient amount of 30-percent hydrogen peroxide was added to the remainder to make the solution 0.1*M*. The oxidation was allowed to proceed for 30 minutes at from 25° to 28°C, after which it was stopped by the addition of from 0.5 to 1.0 mg of catalase (*4*). The solutions were then brought to *p*H 3.8 by the addition of 0.1*M* acetic acid, and an aliquot was taken for biological assay. Cysteine hydrochloride (20 mg/ml) was added to the remaining solution, which was heated to 80°C in an oven and kept at this temperature for 6 hours. The solution stood for another 12 hours at 25°C before being assayed. A reagent control solution, containing no hormone, was carried through the same procedure and assayed at the same time. The control, the reagent control, peroxidized, and peroxidized-reduced preparations were then assayed for their calcium-mobilizing ability in parathyroidectomized rats by a modification of the method described by Munson (*5*). Young male rats weighing from 110 to 120 g were kept on a low calcium diet for 4 days before destruction of their parathyroids was carried out by electric cauterization. Immediately after operation they received an aliquot of the test solution, and 6 hours later a blood sample was obtained by cardiac puncture. The cardiac puncture, anesthesia, and calcium determinations were carried out as previously described (*6*). The results have been recorded in Table 1 as percentages of original potency, 100 percent being the response obtained with the control solution in each assay.

As can be seen in Table 1, parathormone-B loses biological activity upon oxidation and regains activity upon subsequent reduction. The loss of activity appears to be greater, and in part irreversible, when oxidation is carried out at the higher *p*H values. Sometimes the potency of the material treated with cysteine hydrochloride was significantly greater than that of the control. Also, it has been possible to reactivate by reduction a number of parathormone-B preparations which had spontaneously lost part (as high as from 25 to 70 percent) of their biological activity.

Since it seemed possible, but unlikely, that the large excess of cysteine employed caused reactivation by means other than reduction, attempts were made to produce a similar effect by adding equimolar amounts of NaCl (solution brought to *p*H 2.0 with HCl) or of glycine hydrochloride (*p*H 2.0) to the oxidized hormone. In neither instance was there recovery of the lost activity.

These results are quite similar to those obtained by Dedman *et al.* in their studies with ACTH (*1*). At present it is believed that ACTH contains no cystine or free sulfhydryl groups. The chemical groups involved in this unusual phenomenon remain to be elucidated. Similar properties have also been described for melanocyte-stimulating hormone (*7*), which also is thought to lack sulfhydryl groups and cystine. Amino acid analysis of a hydrochloric acid hydrolysate of a highly purified preparation of parathormone-B showed no significant amount of cystine (*8*). In addition, amperometric titration of parathormone-B in 8*M* urea, by the method of Benesch, Lardy, and Benesch (*9*), did not reveal any free sulfhydryl groups. Thus, the similarity between the oxidation-reduction properties of parathormone-B and those of the two pituitary hormones is quite striking.

The dependence of the biological activity of parathyroid hormone upon the state of oxidation or reduction has obvious practical implications for those engaged in work upon the isolation of parathyroid hormone. Whether it has any biological significance remains to be determined. However, it is interesting to note that a suggested mechanism for the action of both ACTH and parathormone involves a triphosphopyridine-nucleotide-linked oxidation-reduction system (*10*). It seems possible, therefore, that the unusual oxidation-reduction properties of these hormones may be of importance in their biological activity (*11*).

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