more rapid change occurs on irradiation of adsorbed material. The configurations *trans–cis–trans–trans* and *trans–cis–cis–trans* are suggested for the new isomers mentioned. As two minor isomers also appear, five out of the ten possible steric forms of diphenyloctatetraene have been observed. It is shown that the formation of substantial quantities of certain isomers is prevented by spatial conflicts.

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Does the Parathyroid Hormone Influence Phosphatase Activity?

BY THOMAS R. WOOD AND WILLIAM F. ROSS

It is well established that serum phosphatase is markedly increased in rickets, hyperparathyroidism and other diseases characterized by lesions in the bones.[1,2,3] It has even been suggested that the action of phosphatases on organic phosphate compounds in bone "is the factor that controls the direction and intensity of calcification in bone."[3] This concept is supported by the increased serum phosphatase activity after parathyroid hormone injection,[4] and by similar changes in bone phosphatase of rats following injection of the hormone.[5,6]

The question arises, as to whether there is an *in vitro* influence of the parathyroid hormone on the activity of phosphatase. Such an effect, if reasonably pronounced, would afford a simple, economical, and rapid assay method for the hormone, which is sorely needed at the present time. Heymann[7] reported that glycerophosphatase and hexosediphosphatase of bone are inhibited by parathyroid extract, but Bakwin and Bodansky,[8] drew the opposite conclusion, that rat and cattle bone phosphatases are not affected. Both of the above studies involved the use of very small amounts of impure parathyroid preparations.

The effect of a very active parathyroid extract[9] on the hydrolysis of glycerophosphate by a kidney phosphatase preparation has therefore been investigated. The hormone concentrate had a nitrogen potency of 300 units per mg. of nitrogen, thus being three times as active as any preparation hitherto reported,[10] and many times more active than those used in the phosphatase studies referred to above.[7,8] At the same time experiments were carried out in which two other proteins, thrice-crystallized egg albumin and crystalline, carbohydrate-free horse serum albumin[11] were substituted for the hormone. Other conditions such as *p*H, temperature, and magnesium ion concentration were identical in all experiments.

The data obtained from these studies give the curves of Fig. 1. Under our conditions each of the three proteins has an activating effect upon phosphatase, and the parathyroid hormone is not unlike the other two substances in any respect. Characteristic of the curves is the tendency to approach a constant maximum, which is not followed by a subsequent decline in activity.

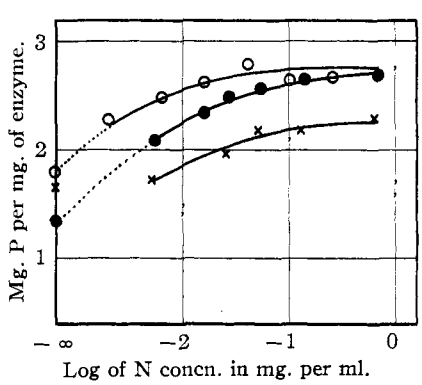

Fig. 1.—The *in vitro* effect of parathyroid hormone (O), horse serum albumin (●), and egg albumin (X) on phosphate liberation by kidney phosphatase.

This behavior of phosphatase recalls its activation by other nitrogen containing compounds, such as ammonia, amino acids and veronal. The activity in the presence of these substances, however, passes through a maximum and then decreases.

(1) Roe and Whitman, *Am. J. Clin. Path.*, **8**, 233 (1939).
(2) Bodansky and Jaffe, *Arch. Internal Med.*, **54**, 88 (1934).
(3) Peters, Robbins and Lavietes, *Ann. Rev. Biochem.*, **5**, 295 (1936).
(4) Page and Reside, *Biochem. Z.*, **226**, 273 (1930).
(5) Williams and Watson, *Endocrinology*, **29**, 250 (1941).
(6) Page, *Biochem. Z.*, **223**, 222 (1930).
(7) Heymann, *ibid.*, **227**, 1 (1930).
(8) Bakwin and Bodansky, *Proc. Soc. Exptl. Biol. Med.*, **31**, 64 (1933).
(9) Ross and Wood, *J. Biol. Chem.*, **146**, 49 (1942).
(10) Collip and Clark, *ibid.*, **66**, 133 (1925).
(11) McMeekin, THIS JOURNAL, **61**, 2884 (1939).

The published data for glycine[12,13] illustrate this quite satisfactorily, and we have obtained similar data for ammonia and veronal. On the basis of these results the nature of the activation by these simple organic compounds appears to differ from that caused by proteins, but the parathyroid hormone, even in the high concentrations employed (as much as 75 units per ml. of digest solution) has no individual and characteristic effect of its own.

Experimental

A concentrate of "alkaline" phosphatase was prepared from beef kidney cortex by the method of Albers and Albers.[14] The resulting solution was evaporated to dryness in the frozen state; such preparations are stable for months at 5°.

The digest solutions were 0.025 *M* in borate buffer of *p*H 9.75, 0.0005 *M* in magnesium chloride, and 0.015 *M* in sodium glycerophosphate (52% β); the enzyme was present in a concentration of 0.004%. The thoroughly dialyzed protein solutions were adjusted to *p*H 9.75 immediately before incorporation in the above mixture. The total volume was 10 ml.; hydrolysis was allowed to proceed at 30° for sixty minutes. The final values of *p*H were 9.55 ± 0.05 unit. Inorganic phosphate liberated during the digestion was estimated by the method of Fiske and Subbarow.[15] Control experiments in the presence of each of the three proteins demonstrated that no inorganic phosphate appeared if enzyme or substrate was omitted.

Summary

The parathyroid hormone, like two other typical proteins, egg and serum albumins, accelerates the liberation of phosphate by kidney phosphatase. It has no peculiar effect which can be attributed to its function as a hormone.

(12) Bodansky, *J. Biol. Chem.*, **115**, 101 (1936).
(13) Williams and Watson, *ibid.*, **135**, 337 (1940).
(14) Albers and Albers, *Z. physiol. Chem.*, **232**, 189 (1935).
(15) Fiske and Subbarow, *J. Biol. Chem.*, **66**, 375 (1925).

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The Structure of Riddelliine, the Alkaloid in *Senecio Riddellii*. I

BY ROGER ADAMS, K. E. HAMLIN, JR., C. F. JELINEK AND R. F. PHILLIPS

The similarity of the alkaloids occurring in the plant genera *Senecio*, *Trichodesma*, *Heliotropium*, and *Crotalaria* has previously been discussed.[1] As was pointed out, these alkaloids are esters which on alkaline hydrolysis yield an acid and an alkanolamine, retronecine or some other closely related bicyclic base.

As a result of investigations on the alkaloid Riddelliine, obtained by ethanolic extraction of *Senecio Riddellii*, it can be stated that this compound possesses the properties characteristic of the group. Riddelliine was first isolated from the plant by Manske,[2] who reported a molecular formula of $C_{18}H_{23}O_6N$. His directions for extraction and isolation were followed and as a result of analytical data on the pure riddelliine, its hydrochloride and methiodide, Manske's formula was confirmed. Hydrolysis of riddelliine indicated that this alkaloid was typical of the *Senecio* group. Alkaline cleavage yielded a basic product, which proved to be retronecine, and a crystalline acid, $C_{10}H_{14}O_6$, designated as riddellic acid.

$$C_{18}H_{23}O_6N + 2H_2O \xrightarrow{Ba(OH)_2} C_8H_{13}O_2N + C_{10}H_{14}O_6$$

(1) Adams and Rogers, THIS JOURNAL, **61**, 2815 (1939).
(2) Manske, *Can. J. Res.*, **B17**, 1 (1939).

From the above equation it is to be noted that in common with most of the other *Senecio* alkaloids, water enters into the reaction with no loss of carbon dioxide and that the acid product contains ten carbon atoms. The retronecine, which was isolated in a nearly quantitative yield, was identified by comparison with an authentic sample.

Riddellic acid was obtained in both an anhydrous and hydrated form. It is optically active and is shown by direct titration to be dibasic. However, by addition of excess alkali and back titration, evidence for a third carboxyl was found which indicates the probability of the presence of a lactone linkage. The acid formed a dimethyl ester on treatment with diazomethane. Molecular weight determinations in dioxane and benzene indicate that dimethyl riddellate is a monomer, although in the latter solvent apparently one molecule of benzene associates with a molecule of solute. Preliminary hydrogenation experiments show that with a platinum oxide catalyst, two moles of hydrogen are absorbed to give a product which as yet has not been obtained in a pure state. Significantly, when the dimethyl ester of riddellic acid was hydrogenated with platinum oxide, only