monia. HCl exerted a destructive action on violacein in alcoholic solution. In an attempt to prepare an ash-free preparation, 172 mg. of the pigment was dissolved in 300 cc. of 95% ethyl alcohol and HCl gas was passed in until the violet color had changed to green. On addition of 500 cc. of water, a dark violet precipitate separated from a brown supernatant liquid. After filtration, washing, and drying, the weight of the precipitate was only 97 mg. indicating that over 40% had been destroyed by the HCl. Although the ash was reduced, the percent of nitrogen dropped to 3.43 indicating a partial decomposition of the molecule. By passing an excess of HCl into an alcoholic solution, the violet color was completely destroyed and could not be restored by neutralization.

The production of pyrrolic products by several different methods of reduction indicates that violacein contains one or more pyrrol rings in its molecule. The fact that the pyrrolic products exhibit little volatility with steam and are soluble in ethyl and petroleum ethers but insoluble in ethyl alcohol, suggests that the pyrrolic ring or rings may possess hydrocarbon side-chains. Probably violacein is chemically similar to prodigiosin, the red pigment of *Bacillus prodigiosus* which has been shown to be a tripyrrylmethane derivative.[10] The presence of iron is of possible significance in view of the supposed respiratory function of the pigment, and may indicate an analogy to hemoglobin, chlorophyll, and other metal-pyrrol compounds.

8726 C

Influence of Certain Liver Poisons on Action of Parathyroid Extract.

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In 1932 when practically nothing was known of the mechanism of the action of the parathyroid hormone in raising the blood calcium level, this investigation was undertaken to test the hypothesis that some other tissue, particularly the liver, might be involved as an intermediary in the action of the hormone. Since then Selye,[1]

[10] Wrede, F., and Rothhaas, A., *Z. physiol. Chem.*, 1933, **222**, 203.

[1] Selye, H., *Arch. Path.*, 1932, **14**, 60; *Endocrinology*, 1932, **16**, 547.

and Thompson and Pugsley[2] have proposed that the hormone acts directly on bone by stimulating an osteoclastic reaction. While there is much in favor of this view, the hypothesis of an intermediating action by some other tissue has by no means been completely eliminated.

Thus, so far, no one has been able to demonstrate a direct action of the parathyroid hormone on bone by *in vitro* experiments. Robison and Rosenheim,[3] for example, failed to find any effect by parathyroid extracts on the *in vitro* calcification of bone.

Furthermore, certain substances which produce liver damage, markedly reduce the rise in serum calcium normally produced by injections of parathyroid extract. In the early stages of this work[4] it was observed that acute intoxication with elementary phosphorus practically obliterated the serum calcium raising effect of the parathyroid hormone. This observation was also made independently and reported at about the same time by Nitzescu.[5]

The effects of the liver poisons carbon tetrachloride and hydrazine as well as of phosphorus have now been determined. The results obtained show that while phosphorus intoxication almost obliterates the increase in serum calcium normally produced by the injection of parathyroid extract, hydrazine intoxication causes a less marked although definite retardation and carbon tetrachloride causes no appreciable decrease.

It has been suggested by Thomson and Collip[6] that the action of phosphorus might well be the result of a toxic injury to the bone cells. The experiments with benzol were undertaken to subject this idea to an experimental test, since benzol is a well known bone marrow poison.

Dogs weighing between 12 and 15 kilos were used in the work. With each animal a control experiment was first performed by subcutaneously injecting 500 Hansen units of parathyroid extract, Lilly,* and then following the serum calcium curve for the next 24 hours. Acute intoxication by phosphorus, carbon tetrachloride and hydrazine was induced in the manner described below. Phosphorus intoxication was produced by injecting daily 5 to 10 mg. of

2 Thomson, D. L., and Pugsley, L. I., *Am. J. Physiol.*, 1932, **102**, 350.

3 Robison, R., and Rosenheim, A. H., *Biochem. J.*, 1934, **28**, 684.

4 Greenberg, D. M., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 721.

5 Nitzescu, I. I., *Compt. rend. soc. biol.*, 1932, **110**, 1141.

6 Thomson, D. L., and Collip, J. B., *Ann. Rev. Biochem.*, 1933, **2**, 231.

* The Parathyroid Extract used in these experiments was generously furnished by Eli Lilly and Co.

yellow phosphorus in oil (10 mg. P per ml. of olive oil) subcutaneously for a period of from 2 to 4 days prior to the injection of 500 Hansen units of Parathyroid Extract, Lilly, and then following the change in the serum calcium curve.

As recommended by Minot and Cutler,[7] the carbon tetrachloride was administered by stomach tube, daily doses of 40 ml. each being given for periods of 2 days. Hydrazine intoxication was produced by the subcutaneous daily injection of 10 ml. portions of 2% aqueous hydrazine sulfate for periods of 6 days prior to the administration of parathyroid extract. Each of the liver poisons was usually administered for several intoxication periods spaced about two weeks apart. In the course of these intoxications, the animals developed jaundice, bile appearing in the blood and urine. Autopsy revealed manifestations of liver damage in each instance.

Benzol intoxication was induced after the manner described by McGavack[8] by injecting 4 ml. of a mixture of equal parts of benzol and olive oil twice daily for 4 days prior to the administration of parathyroid extract. It was not feasible to repeat these experiments on the same animal as was done with the other toxic materials, because huge open sores developed on the skin at the sites of injection. The benzol intoxicated animals became dropsical, fluid being found at autopsy in the chest cavity. No significant drop in the red corpuscle count was observed during the duration of these experiments.

Representative experimental results obtained with each of the toxic substances subjected to test, are plotted in Fig. 1. In the block figures, the initial serum calcium level at the start of an experimental period is indicated by the white portion and the maximum serum calcium level produced following injection of parathyroid extract, by the cross hatched portion. The control periods are distinguished from the intoxication periods by the reversed direction of the cross hatching. The lettering along the abscissa refers to the designation assigned to the experimental dogs.

From Fig. 1 it is apparent that phosphorus profoundly checks the rise in serum calcium normally produced by the parathyroid hormone. With dog Bl, in the third intoxication period, injection of parathyroid extract actually lowered the serum calcium level. Hydrazine also exerts a considerable and consistent effect in lowering the magnitude of the increase in the serum calcium level. On

[7] Minot, A. S., and Cutler, J. T., *J. Clin. Invest.*, 1928, **6**, 369.

[8] McGavack, T. H., *J. Am. Institute of Homeopathy*, 1928, **21**, 461.

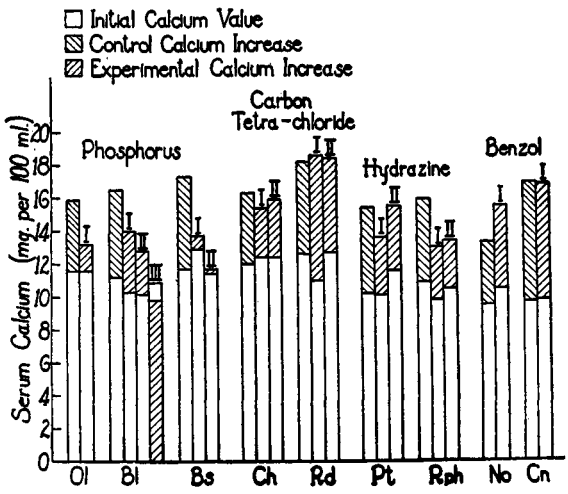

FIG. 1.

The Effect of Certain Liver Poisons on Parathyroid Action.

Each group of block figures represents the results of an experimental series on a dog. The designation of each dog is given by the lettering under the blocks along the abscissa. The initial serum calcium level at the start of each experiment is represented by the height of the white portion of the block; the maximum rise in serum calcium produced by the injection of 500 Hansen units of parathyroid extract by the cross hatched portion. The control and intoxication periods, as shown above, are cross hatched in reverse directions.

The dogs employed in these experiments weighed between 12 and 15 kilos. Intoxication with each of the substances employed was induced in the following manner:

Phosphorus: Dog Ol, Bl, and Bs were subcutaneously injected with 5 to 10 mg. of yellow phosphorus in oil for two to four-day periods.

Carbon tetrachloride: 40 ml. doses per day of carbon tetrachloride were administered by stomach tube to dogs Ch and Rd for two-day periods.

Hydrazine: 10 ml. portions of 2 per cent aqueous hydrazine sulfate were subcutaneously injected into dogs Pt and Rph daily for six-day periods.

Benzol: Dogs No and Cn received twice daily injections of 4 ml. portions of a mixture of equal parts of benzol and olive oil for 4 days.

the other hand, it appears from Fig. 1, that carbon tetrachloride exerts none or only a slight reducing effect on the calcium rise.

No definite explanation is available for either the inhibiting effect of the substances inducing liver damage on the parathyroid action, nor for the differentiation in the action of the 3 test substances. Although profoundly toxic effects were produced by each of the 3, the possibility exists that different degrees of liver damage were produced by phosphorus, hydrazine and carbon tetrachloride. Also possibly different liver cells of the liver are injured by the action of phosphorus than by carbon tetrachloride. This suggestion is supported by other experimenters.[9, 10] Haloff[9] states that chloroform

[9] Haloff, G. A., *Arch. Exp. Path. Pharm.*, 1928, **134**, 168.

[10] Marshall, E. K., and Rowntree, L. G., *J. Exp. Med.*, 1915, **22**, 333.

and carbon tetrachloride produce a fatty degeneration of the liver, while phosphorus attacks the Kupfer cells more.

The observation that benzol intoxication does not interfere with the rise in serum calcium induced by parathyroid extract, indicates that the mechanism of action of phosphorus and hydrazine is not through an injurious action on the bone cells.

Summary. 1. In acute intoxication experiments with the liver poisons, phosphorus, hydrazine and carbon tetrachloride it was found that phosphorus intoxication almost eliminates the serum calcium rise normally produced by injection of parathyroid extract, hydrazine causes a less marked rise and carbon tetrachloride has no appreciable retarding effect. 2. Since the bone-marrow poison, benzol, causes no alteration in the blood calcium curve following injection of parathyroid extract, the action of phosphorus and hydrazine appears not to be through an injurious action on the bone cells.

8727 C

Effect of Ultracentrifuging on Oxygen Consumption of the Eggs of *Ascaris suum*, Goeze.

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That the maintenance of structural integrity is of prime importance for the normal functioning of a large part of the oxidative processes of the cell has been demonstrated by a number of workers.[1-5] Bodine and Boell[6, 7] have shown that the normal activity of much of the so-called cyanide sensitive respiratory mechanism (indophenol oxidase system) is interfered with as the result of the structural disorganization of the cell. Recently Beams and King[8]

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1 Warburg, O., and Meyerhof, O., *Pflüg. Arch.*, 1912, **148**, 295.

2 Warburg, O., *Pflüg. Arch.*, 1914, **158**, 189.

3 Runnström, J., *Protoplasma*, 1930, **10**, 106.

4 Quastel, J. H., and Wheatley, A. H. M., *Bioch. J.*, 1932, **26**, 726.

5 Quastel, J. H., *Bioch. J.*, 1932, **26**, 1685.

6 Bodine, J. H., and Boell, E. J., *J. Cell. and Comp. Physiol.*, 1936, **7**, 455.

7 Bodine, J. H., and Boell, E. J., *J. Cell. and Comp. Physiol.*, 1936 (in press).

8 Beams, H. W., and King, R. L., 1936 (in press).