

treatment, and that these differences would have been present regardless of the institution of treatment. Nevertheless, the cellular reaction, whether due to treatment or to the element of time, was identical with that occurring in rabbits which had developed an active immunity and had effectively suppressed the disease without treatment.

6123

Serum Phosphatase Changes in Calcium Deficiency and in Ammonium Chloride Osteoporosis.

AARON BODANSKY, HENRY L. JAFFE AND J. P. CHANDLER.

From the Laboratory Division, Hospital for Joint Diseases, New York City.

It was shown by Kay¹ that the plasma phosphatase increases in certain clinically observed cases of bone diseases. We have confirmed and extended these observations.² In an investigation of experimentally produced bone lesions we have reported the effects of chronic and acute hyperparathyroidism.³ In view of the many analogies observed in bone resorption, whether produced by parathormone or by other agents, it was desirable to extend our observations to the osteoporoses produced by low-calcium diets and by ammonium chloride administration to animals on low and high calcium diets.

Four litters of dogs were used—3, 6, 8, and 18 months old, respectively. The experiment was continued for about 11 weeks. The animals received a diet of fresh, lean horse meat supplemented with cod liver oil and tomato juice. This is a low-calcium diet. Some of the animals received a calcium supplement (2.5 gm. each of bone meal and calcium lactate per kilo of food) equivalent to between 0.5 and 2.5 gm. of calcium daily. On this diet the controls grew rapidly; the bones were normal upon autopsy. On the low-calcium diet, the growth was equally rapid; the bones, however, were thinned. Ammonium chloride was administered by stomach tube in a 1% solution. At the end of the experiment some dogs received daily as much as 1 gm. per kilo. In the youngest litter bone softening

¹ Kay, H. D., *J. Biol. Chem.*, 1930, **89**, 249.

² To be published.

³ Bodansky, A., and Jaffe, H. L., *Proc. Am. Soc. Biol. Chem.*, Montreal, Canada, April 8-11, *J. Biol. Chem.*, 1931, **92**, xvi.

and deformity resulted on a low-calcium diet; in all dogs administration of ammonium chloride generally caused loss of appetite.

The method for determination of phosphatase has been described.^{4, 5} Serum phosphatase rather than plasma phosphatase has been determined in this series of tests to avoid the inhibition of phosphatase activity by oxalate.⁵ Phosphatase values are influenced by diet, malnutrition and fasting.⁵ Depending on the duration of the fast, plasma phosphatase decreased in fasted dogs, rats and guinea pigs to between one-half and one-quarter of the values found in normal controls. These findings must be considered in the interpretation of results in long experiments in which satisfactory nutrition and growth cannot be maintained.

In the 3-month litter the average initial serum phosphatase was 11.0 units per 100 cc. On an adequate calcium diet final values (11 weeks later) in 2 controls were 7.6 and 6.8; on ammonium chloride treatment (1 puppy) the final value was 14.3. On a low-calcium diet plus ammonium chloride (1 puppy) the final phosphatase value was 14.8 units. Thus, in the 3-month litter, ammonium chloride, on an adequate as well as on calcium-poor diet, in spite of malnutrition, caused an upward trend of serum phosphatase instead of the downward trend observed in normal growing puppies.

In the 6-month litter the initial group average was 7.6 units per 100 cc. of serum (minimum 6.2, maximum 9.7). On the calcium-adequate diet 1 control showed a practically constant serum phosphatase (initial 7.2 units, final 7.8—the usual trend at this age is downward); 2 dogs receiving ammonium chloride showed a decreased serum phosphatase (initial 6.9 and 6.8, final 5.4 and 3.7 units). On the low-calcium diet, however, 1 control showed a rise from the initial 6.2 units to a final value of 9.0 units, and ammonium chloride administration (2 dogs) resulted in a rise to final values of 11.6 and 14.1 units (from initial values of 9.2 and 9.7 respectively). In this litter ammonium chloride did not reverse the usual downward trend of the serum phosphatase when the calcium intake was adequate. On the calcium-poor diet, however, the downward trend of the phosphatase was reversed in spite of some loss of appetite.

The 8-month litter (3 *bull terriers*) (initial phosphatase 3.8 units) showed no marked changes due to ammonium chloride either on the high or low calcium diet.

⁴ Bodansky, A., *Proc. Soc. Exp. Biol. and Med.*, 1931, **28**, 760.

⁵ Bodansky, A., and Jaffe, H. L., *Proc. Soc. Exp. Biol. and Med.*, 1931, **29**, 199.

In the 18-month litter the initial group average was 2.4 (minimum 2.3, maximum 2.7). On an adequate diet plus ammonium chloride (2 dogs) the final phosphatase values decreased to 1.5 and 1.8. On a low calcium diet, on the contrary, both with and without ammonium chloride treatment, the downward trend was not shown, the final phosphatase values remaining practically unchanged at 2.6 and 2.5, respectively.

The results of these experiments may be summarized as follows:

In growing animals the serum phosphatase normally decreases with age. On an experimental regime involving loss of appetite, further decrease of serum phosphatase would be expected. Therefore the serum phosphatase rise in the two younger litters is all the more convincing evidence of the greater susceptibility of young animals to the effects of experimental calcium deficiency and ammonium chloride acidosis. In older dogs, ammonium chloride is relatively ineffective in producing decalcification and serum phosphatase changes when an adequate calcium supplement is given.

6124

Pernicious Anemia: Method Whereby Therapeutic Efficacy of Liver and Liver Fractions May be Substantially Increased.

GEORGE B. WALDEN AND G. H. A. CLOWES.

From the Lilly Research Laboratories, Indianapolis.

Following the discovery of Minot and Murphy that raw liver is highly effective in the treatment of pernicious anemia, liver fractionation was undertaken by Cohn, Minot and their associates. The Cohn liver fraction G, in which approximately two-thirds to three-quarters of the potency of the original liver is recovered in 3 to 4% of its weight, has been generally employed in the oral treatment of pernicious anemia. Use of this preparation, involving the consumption of 10 to 14 gm. of material daily, entails some inconvenience to the patient. In attempting to isolate and identify the active principle Cohn, West and others have made very highly concentrated preparations but the procedures employed have not found practical application on account of their complexity and the losses of active principle entailed.

We here describe a method whereby the bulk of material administered orally to pernicious anemia cases to produce a given thera-