Section of the splanchnics in the dog causes a decrease of approximately 50% in the blood glutathione, but no effect on the *experimental diabetes* following *pancreatectomy*. Acetone given by mouth in dosages of 6 cc. per kilo of body weight causes this glutathione-like substance to increase approximately 200% above normal and does not return to normal for approximately 3 days. Removal of the pancreas on the blood glutathione has been studied by Dott D. Ferrari.[2] Our results are at variance with his findings. Blood glutathione determinations before pancreatectomy in his dogs are not published.

## 7205 P

### Influence of Bleeding, Diet, Distemper, and Starvation on Serum Phosphatase Activity.

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In the course of a study of serum phosphatase activity in the rabbit, dog and rat, it was found that bleeding, diet, distemper, and starvation exert a marked influence upon the values obtained. Bodansky and Jaffe[1] have pointed out that a relationship exists between the type of diet, the nutritional state of the animal, and the plasma phosphatase activity. For convenience of presentation, data is given under the 4 respective headings. Serum phosphatase activity was determined according to the method of Bodansky.[2]

1. The influence of bleeding upon serum phosphatase activity was determined in adult rabbits. The serum obtained from 25 cc. of blood, drawn from the heart of rabbits, gave average values in a group of 14 animals for calcium of 13.96 mg./100 cc., for inorganic phosphorus 5.04 mg./100 cc., and for phosphatase activity 4.14 mg./100 cc. A second 25 cc. sample of blood drawn 8 to 15 days later showed the influence of the previous bleeding upon serum constituents of otherwise normal rabbits maintained on the same diet. The values for serum calcium averaged 14.13 mg./100 cc., for inorganic phosphorus 3.74 mg./100 cc., and for phosphatase

[2] Ferrari, Dott R., *Boll de Soc. Med.-Chir.*, Pavia, 1933, **47**, 305.

[1] Bodansky, A., and Jaffe, H. L., PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 199.

[2] Bodansky, A., PROC. SOC. EXP. BIOL. AND MED., 1930, **28**, 760.

activity 2.25 mg./100 cc. These data show that bleeding caused a marked decrease in serum phosphatase.

2. The influence of a high protein or carbohydrate diet on the serum phosphatase activity was studied. The diet of a number of dogs was alternated from bread and meal mash to one of beef hearts. The influence of these 2 diets on the serum phosphatase and inorganic phosphorus is shown in Table I.

TABLE I.

Influence of Diet on Serum Inorganic Phosphorus and Phosphatase.

| Dog No. | Inorganic Phosphorus mg. | Phosphatase Activity mg. | Days on Diet | Diet |
|---|---|---|---|---|
| 9 | 4.05 | 14.00 | 18 | bread and meal |
| | 4.92 | 3.86 | 13 | beef hearts |
| | 3.82 | 6.81 | 14 | bread and meal |
| | 3.18 | 12.51 | 13 | " " " |
| | 3.85 | 3.27 | 14 | beef hearts |
| | 4.76 | 11.22 | 15 | bread and meal |
| 10 | 7.30 | 15.25 | 15 | " " " |
| | 6.75 | 4.77 | 14 | beef hearts |
| | 6.75 | 20.62 | 14 | bread and meal |
| | 6.77 | 4.61 | 15 | beef hearts |
| | 5.12 | 6.94 | 14 | bread and meal |
| | 5.46 | 16.47 | 15 | " " " |

These results show that a high serum phosphatase activity accompanies feeding a bread and meal diet (high carbohydrate), while a beef heart (high protein) diet induces a low phosphatase activity. (The serum inorganic phosphorus sometimes varied directly and at other times inversely with the phosphatase activity.) Dogs when upon the lean meat diet invariably gained weight.

The influence of diet on the serum phosphatase of rats was also studied in an attempt to repeat the observations of Bodansky and Jaffe,[1] who found that rats maintained on a lean meat diet for 30 days showed a higher plasma phosphatase activity than those fed Sherman's Stock Diet the same length of time. Two groups of 8 rats each were placed on respective diets. Rats 1 to 8, inclusive, were placed on Sherman's Stock Diet No. 13, and rats 9 to 16, inclusive, were placed on the following diet:

2500 gm. ground beef hearts
25 gm. calcium carbonate
30 cc. cod liver oil
200 cc. tomato juice

All animals received as much food as they would eat. After 30 days on the 2 rations, the rats were killed, and the blood and serum

analyzed. On the serum, calcium, inorganic phosphorus, and phosphatase were estimated, while the hematocrit reading, organic and inorganic phosphorus values were determined on whole blood. The weight increase, as well as the analytical values, were practically identical for the 2 groups with the exception of the phosphatase activity, which was 46.40 for the group maintained on Sherman's Stock Diet as compared to 36.80 for those fed lean meat.

3. The influence of distemper on the serum phosphatase activity was observed in 3 dogs upon which normal values had been obtained previously. Table II shows the increase in phosphatase activity which accompanied distemper in these animals.

TABLE II.

| Dog No. | Phosphatase Values | |
|---|---|---|
| | Normal mg./100 cc. | Distemper mg./100 cc. |
| 1 | 5.78 | 17.60 |
| 2 | 3.51 | 7.15 |
| 3 | 5.08 | 11.39 |

4. The influence of starvation on the serum phosphatase activity was observed in 3 dogs that had previously been on a diet of bread and meal mash. The serum phosphatase values prior to, and after, 5 days' starvation are given in Table III.

TABLE III.

| Dog No. | Phosphatase Values | |
|---|---|---|
| | Before Starvation mg./100 cc. | After 5 Days' Starvation mg./100 cc. |
| 6 | 7.04 | 3.95 |
| 10 | 16.47 | 12.71 |
| 9 | 11.22 | 6.30 |

Bodansky and Jaffe[1] reported a decrease in the plasma phosphatase activity of guinea pigs starved for 3 and 6 days, as well as of rats starved for 96 hours.

The results that we have obtained as to the influence of a lean meat diet on the serum phosphatase activity differ from those reported by Bodansky and Jaffe.[1] When dogs or rats are maintained on a high carbohydrate diet, the serum phosphatase activity is higher than when these animals are fed a diet rich in meat. The explanation of this fact probably exists in the metabolism of these 2 types of food. The decreased phosphatase activity observed in starvation may be due to an increase in endogenous protein metabolism. The serum phosphatase decrease which results from bleeding

may have a similar explanation, while the increase observed in distemper may be due to the liberation of this enzyme from disintegrating leucocytes, since Roche[4] has shown these cells to contain this enzyme.

## 7206 C

### Methemoglobin and Methylene Blue as Cyanide Antagonists.

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The action of methylene blue in restoring the respiration of cyanide inhibited tissues (Gerard[1]) has led to the clinical and apparently successful use of the dye as an antidote for cyanide poisoning (Brooks[2]). It has been urged that methylene blue acts in the intact organism not directly by substituting in the cells for the inactivated respiratory enzymes, but indirectly by removing the cyanide and releasing the enzyme. Cyanide would thus be removed by the formation of the stable cyanmethemoglobin, from it and methemoglobin; formed in turn from the blood pigment by methylene blue (Wendel[3]). An antagonism of methemoglobin and cyanide has been demonstrated on rat tissues (Rosenthal and Voegtlin[4]).

It seemed of interest, in this connection, to determine the action of methemoglobin and methylene blue, with cyanide, on isolated invertebrate tissues. Ciliated gill tissue of the quahog *(Venus mercenaria)* was isolated in sea water and its oxygen consumption followed at 22°C. in Warburg manometers. Alkali in the inset, by absorbing $CO_2$ and HCN, led to a rise in pH, but always less than 0.5.

The normal $Qo_2$ (cmm. $O_2$ per hour per gm. fresh weight) averaged 375 (26 experiments) during the first hour in sea water, about 10% less for the second. Cyanide led to typical inhibition, though rather high concentration was needed. As per cent of the normal, average (4 series of experiments) values in cyanide were: M/11,000,

[3] Sherman, H. C., and Munsell, H. E., *J. Am. Chem. Soc.*, 1925, **47**, 1639.

[4] Roche, J., *Bull. soc. chim. biol.*, 1931, **13**, 841.

[1] Gerard, R. W., *Physiol. Rev.*, 1932, **12**, 504.

[2] Brooks, M. M., *Am. J. Physiol.*, 1932, **102**, 145.

[3] Wendel, W. B., *J. Biol. Chem.*, 1933, **100**, Proceedings C.

[4] Rosenthal, S. M., and Voegtlin, C., *Public Health Reports*, 1931, **46**, 521.