mal bone. The second precipitation with the molybdic acid reagent does not interfere with the accuracy of the method; the values for the ratio residual Ca/P so obtained are normal within the experimental error. When phosphorus values obtained from the first filtrate are employed, low ratios result.

In studying the method 11 specimens of normal calcification and 7 specimens of pathological calcification were analyzed. In all cases the value of the ratio was 1.96 within the experimental error; the theoretical value from $Ca_3(PO_4)_2$ is 1.94. One type of exception was noted. Three specimens of calcified fibroid of the uterus gave ratios of 2.23, 2.23, and 2.18 respectively.

The normal adult rats analyzed contained about 15% carbonate calcium, *i. e.*, carbonate Ca/total Ca × 100 was about 15. In the young rats analyzed the carbonate calcium was only from 8 to 10% of the total calcium. This indicates that the composition of bone in rats is not constant, but changes with age.

[1] Shear, M. J., and Kramer, B., *J. Biol. Chem.*, 1927, lxxiv, 9.

## 3740

## Immunization Against Pneumococcus by Feeding Desiccated or Milk Suspended Organisms.

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The author previously demonstrated that white rats can be protected against many lethal doses of pneumococci, intraperitoneally injected, if these animals are first fed either the tissues of animals killed by this germ,[1] living pneumococci,[2] or HCL killed germs.[3] It was next decided to determine whether the acid killed germ when dried and mixed with cracker meal and the acid killed germ when still moist and suspended in milk could also be used. The object was to simplify the method of administering the germs as far as possible, consistent with successful immunization. At the same time success or failure accompanying a given modification gradually helps toward an explanation of oral immunization with the pneumococcus.

The organisms were grown, killed and centrifuged as described elsewhere.[3] A thin layer of germs was spread on a watch crystal and warmed on a water bath, so that they were at no time heated above 37° C. Desiccation was complete in 15 minutes. For 20

days each rat was fed the dried germs, 50 cc. of culture per day, mixed with moistened cracker meal. For testing the resistance of control and treated animals, a 24 hour virulent culture was diluted serially and the desired amount injected intraperitoneally in a volume of 0.20 cc. Table I contains the data for this experiment, and gives the rat number, whether control or experimental, its weight, the dose injected and the result. These data show clearly that the rats which were fed the dried pneumococci are considerably more resistant to the virulent germ than the control animals. The experiment was repeated, using larger rats, which responded less regularly than rats of the weights shown in Table I, although to the same degree.

TABLE I.

*Effect of feeding desiccated, acid killed pneumococci (Type I) on the resistance of rats to intraperitoneal injection of the living germ.*

| Date 1926 | Rat | Wt. gm. | Dose cc. | Result | Date 1926 | Rat | Wt. gm. | Dose cc. | Result |
|---|---|---|---|---|---|---|---|---|---|
| Oct. 1 | C | 117 | $10^{-6}$ | D2 | Oct. 11 | C | 166 | $10^{-5}$ | D2 |
| | E | 120 | $10^{-6}$ | S | | E | 115 | $10^{-6}$ | D4* |
| Oct. 4 | C | 114 | $10^{-8}$ | S | | E | 147 | $10^{-5}$ | S |
| | C | 119 | $10^{-7}$ | S | | E | 143 | $10^{-5}$ | S |
| | C | 130 | $10^{-6}$ | D2 | | E | 143 | $10^{-5}$ | S |
| | C | 132 | $10^{-5}$ | D2 | | E | 163 | $10^{-4}$ | S |
| | E | 120 | $10^{-6}$ | S | | E | 150 | $10^{-4}$ | S |
| | E | 129 | $10^{-5}$ | S | Oct. 13 | C | 130 | $10^{-8}$ | D2 |
| | E | 124 | $10^{-5}$ | S | | C | 130 | $10^{-7}$ | D2 |
| | E | 132 | $10^{-4}$ | D3 | | C | 132 | $10^{-6}$ | D2 |
| Oct. 7 | C | 118 | $10^{-8}$ | S | | E | 143 | $10^{-5}$ | S |
| | C | 124 | $10^{-7}$ | D2 | | E | 130 | $10^{-5}$ | D2 |
| | C | 132 | $10^{-6}$ | D2 | | E | 149 | $10^{-4}$ | D2 |
| | C | 148 | $10^{-5}$ | D2 | | E | 147 | $10^{-4}$ | D2 |
| | E | 113 | $10^{-6}$ | S | Oct. 14 | C | 130 | $10^{-8}$ | D2 |
| | E | 135 | $10^{-5}$ | S | | C | 132 | $10^{-7}$ | D5 |
| | E | 139 | $10^{-5}$ | S | | C | 157 | $10^{-6}$ | D2 |
| | E | 137 | $10^{-5}$ | S | | C | 160 | $10^{-5}$ | D3 |
| | E | 141 | $10^{-4}$ | S | | E | 120 | $10^{-6}$ | D2 |
| | E | 146 | $10^{-4}$ | D4 | | E | 120 | $10^{-5}$ | S |
| Oct. 11 | C | 120 | $10^{-8}$ | S | | E | 137 | $10^{-5}$ | S |
| | C | 130 | $10^{-7}$ | D2 | | E | 148 | $10^{-4}$ | D1 |
| | C | 137 | $10^{-6}$ | D2 | | E | 155 | $10^{-4}$ | S |

*Weak at the beginning. C = Control. E = Experimental. D = died-days. Last day of feeding germs, October 1. S = Survived.

In connection with this study of the effect of different methods of oral administration of germs, an experiment was done in which the freshly prepared, acid killed, centrifuged pneumococci were suspended in milk and given by medicine dropper directly into the mouths of the rats. For 19 days each animal received the bacteria from 50 cc. culture per day. Table II gives figures for this test. The results were excellent. The treated animals tolerated 1,000 to 10,000 fatal doses.

TABLE II.

*Effect of feeding moist acid killed pneumococci (Type I) suspended in milk on the resistance of rats to intraperitoneal injection of the living germ.*

| Date, 1926 | Rat | Wt. gm. | Dose cc. | Result |
|---|---|---|---|---|
| Nov. 12 | E | 128 | $10^{-5}$ | S |
| | E | 107 | $10^{-6}$ | S |
| | C | 167 | $10^{-5}$ | D2 |
| | C | 147 | $10^{-6}$ | D4 |
| | C | 125 | $10^{-7}$ | D2 |
| | C | 117 | $10^{-8}$ | D2 |
| Nov. 13 | E | 136 | $10^{-4}$ | S |
| | E | 107 | $10^{-5}$ | S |
| | E | 113 | $10^{-5}$ | S |
| | E | 104 | $10^{-6}$ | S |
| | C | 130 | $10^{-6}$ | D2 |
| | C | 127 | $10^{-7}$ | S |
| | C | 121 | $10^{-8}$ | S |
| Nov. 16 | E | 138 | $10^{-4}$ | S |
| | E | 151 | $10^{-4}$ | S |
| | E | 133 | $10^{-5}$ | S |
| | E | 136 | $10^{-5}$ | S |
| | C | 158 | $10^{-5}$ | D2 |
| | C | 144 | $10^{-6}$ | D2 |
| | C | 143 | $10^{-7}$ | D2 |
| | C | 142 | $10^{-8}$ | D2 |

C = Control. E = Experimental. D = Died-days. Last day of feeding germs, November 12. S = Survived.

As a further extension of these modified methods of the oral administration of the pneumococci, we shall study the effect of (1) dissolving the organisms in bile, feeding this mixture either in liquid form or after drying the whole, (2) mechanically disrupting the organisms both moist and dry, and, (3) enzymically digesting the bacteria. Up to the present, the use of moist, acid killed pneumococci, freshly prepared and mixed with cracker meal or suspended in milk, appears to be the most desirable of the modifications employed.

[1] Ross, Victor, *J. Immunol.*, 1926, xii, 219-229.

[2] Ross, Victor, *J. Immunol.*, 1926, xii, 237-249.

[3] Ross, Victor, *J. Lab. and Clin. Med.*, 1927, xii, 566-572.