ceeded to grow in a relatively normal manner. The reason for this preliminary inhibition with subsequent normal growth is not as yet clear to us. What seemed to be mutation forms similar to some described by Sweany[7] presented themselves in part of the cultures thus treated. That they were not contaminants seems to have been fully ruled out. These experiments are to be repeated shortly with the sodium salt of cholic acid added in varying percentages to glycerine agar.

In the guinea pigs, attempts to produce jaundice by tying off the common bile duct met with failure, since these animals invariably died of shock by the fifth day. This we found later had already been pointed out by Hewlett.[8] The injection of whole bile intravenously and intraperitoneally likewise proved futile. Rabbits, however, survived bile duct ligations nicely. Rabbits so jaundiced, and given intravenous injections of bovine tubercle bacilli, survived longer and at autopsy showed less in the way of lesions than the controls. Jaundiced rabbits inoculated with tubercle bacilli grown on varying percentages of cholesterol glycerine agar showed about as extensive lesions as the controls. Forty-five rabbits and 16 guinea pigs were used in these studies.

The studies are being continued, both with reference to the influence of jaundice on experimental *B. tuberculosis* infections and the influence of bile and bile derivatives on cultures in the test tube. A special point will be made to also study the influence of artificially induced hyper-cholesteria in rabbits on the course of experimental tubercle bacillus infections in these animals.

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Relation of Calcium and Phosphorus of Diet to Toxicity of Viosterol.*

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The discrepancies apparent in the literature as to the toxicity of viosterol may be attributed to (1) differences in the potency of the

[7] Sweany, H. C., *J. Am. Med. Assn.*, 1926, **87**, 1206.

[8] Hewlett, A. W., Pathological Physiology of Internal Diseases. D. Appleton and Company, 1923, 336 pp.

* The work reported in this and the succeeding papers was made possible by a grant from the Mead Johnson Company, Evansville, Indiana.

viosterol, (2) the character of the diet in regard to its content of calcium and phosphorus, (3) the age of the animals at the beginning of the experiment and (4) the duration of the experiments. Thus, many published results are based on the use of viosterol of unknown potency and varying methods of assay; and the diets employed range between an undescribed so-called "normal diet", through the Steenbock and McCollum rachitogenic diets, to diets consisting of "table-left-overs".

In the present study an attempt was made to ascertain the relation of calcium and phosphorus of the diet to the toxicity of viosterol.

The rats used were of our own breeding stock and were put on the experimental diets at the time of weaning. The viosterol was incorporated in the diet; the dosage was calculated in cod liver oil equivalents, *i. e.*, the amount of viosterol which produced a line test in 5 days, when added to the Steenbock rachitogenic diet, equaled ¼% of the diet in terms of cod liver oil potency.

(a) Steenbock-Bills stock diet. Ca = 0.515, P = 0.450 gm. %.

The toxicity, duration of life and well-being of animals on this diet are directly proportional to the dose of viosterol. 40,000 to 80,000 times overdosage is very rapidly fatal, sometimes with little evidence of metastatic calcifications, especially the 80,000 times overdosage. On 10,000 and 20,000 times overdosage animals do well at first, then they begin to decline in health and finally die, showing calcification of most organs, especially of the vascular system. Those on 2,000 and 4,000 times overdosage were killed at 10 months. They had reproduced many times and had shown no calcification.

(b) High calcium, low phosphorus diet. (Steenbock rachitogenic diet.) Ca = 1.240, P = 0.243 gm. %.

Since the usual length of life of an animal on such a rachitic diet is about 2 to 3 months, and since they do not grow on this diet, the deaths in this group cannot be entirely attributed to smaller doses of viosterol. On the contrary, some of the animals' lives were prolonged for long periods by adding viosterol to the diet (3 animals lived on the Steenbock diet plus 2000 times overdosage for 10 months without evidence of hypercalcification of organs). In high dosages, however, the addition of viosterol is quite fatal, but very few of the animals developed hypercalcification, although they had hypercalcemia.

(c) Optimal† phosphorus, low calcium diet. (Ca = 0.012, P = 0.475 gm. %.)

† The minimal and optimal amounts for growth and maintenance.

Animals on this diet do not grow well, but seem to survive longer than animals getting a very high calcium and the same amount of phosphorus and viosterol in the diet. With very large doses of viosterol another factor is brought into play—that of removing calcium from the bones and shifting to the blood. These animals develop emaciation, extreme osteoporosis‡ and high serum calcium concentrations. Animals on intermediate doses of viosterol show slight calcic changes in aorta and kidneys if left on the diet for a long time. Animals on this diet plus 400 times overdosage were killed at 340 days and showed very slight changes in aorta while the kidneys were normal.

(d) High calcium, optimal phosphorus. Ca = 1.212, P = 0.475 gm. %. (The amount of phosphorus is twice as great as in the Steenbock rachitogenic diet.)

Animals on this diet receiving viosterol die much sooner than on the low calcium diets. Those on very high viosterol die too soon to show calcification of organs, but those on the smaller doses which survive longer show marked calcic changes in 150 days.

(e) High phosphorus, low calcium diet. Ca = 0.012, P = 1.780 gm. %.

Animals on this diet do not grow well. On the larger doses of viosterol they do not survive very long and show moderate or mild calcification. Rats on 20, 400, and 4,000 overdosage were killed at 220 days and showed only a few specks of calcification in the cortico-medually zones of the kidneys. These renal lesions were also seen in those animals receiving no viosterol, and are apparently due to the constant excretion of phosphate by way of the urine.

(f) High phosphorus, minimal† calcium diet. Ca = 0.412, P = 1.780 mg. %.

This group of animals was very susceptible to viosterol. They died very quickly and showed most profound calcification. The kidneys were literally made up of calcium. Even 400 times overdosage was quite fatal. With the exception of 0 and 20 times overdosage, all the animals of this series died or had to be killed because of extreme illness at 3 months or sooner. Those on 0 or 20 times were killed at 216 days and showed the kidney lesions as in the previous group.

These experiments indicate the following facts: The composition of the diets with respect to their calcium and phosphorus content is

‡ Some of the bones in this group show extreme porosity of the upper part of the tibia just below the cartilaginous zone and appear as a pseudo-rachitic metaphysis roentgenologically.

a determining factor in the toxicity and hypercalcifying property of viosterol. In the presence of a calcifying agent, increasing the amount of phosphorus in the diet renders the organism more susceptible to hypercalcification. With large doses of viosterol animals on certain diets may die without showing any signs of hypercalcification, although hypercalcemia may be present; while on other diets they may show hypercalcification without hypercalcemia.

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Effect of Calcium and Phosphorus of Diet on Tetany and Serum Calcium of Parathyroidectomized Rats.

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In the study of experimental hypoparathyroidism 2 factors frequently cause contradiction and confusion: (1) the anatomical relation of the parathyroid in the animals employed; and (2) the composition of the diet, especially with respect to its calcium and phosphorus content. In experiments with the dog or the monkey the extirpation of the parathyroids without destruction of thyroid tissue is frequently impossible and the complete operation of thyroparathyroidectomy must be used. That the thyroid alone has a profound influence on calcium and phosphorus metabolism has long been appreciated and recently Aub[1] and his associates have demonstrated this fact by metabolism experiments in human beings. Hence the removal of the thyroid along with the parathyroids may cause an alteration in calcium and phosphorus metabolism different from that observed when either of them alone is removed. The rat, on the other hand, has but 2 encapsulated and easily removable parathyroids. Erdheim[2] and his followers made use of this anatomical fact and produced tetany in rats by cauterizing the parathyroids, and causing little or no destruction of the adjacent thyroid tissue. Erdheim showed that parathyroidectomy in the rat produces faulty dentition, delays calcification of a callus and impoverishes the body of lime salts.

The experiments of Erdheim were conducted prior to our more

[1] Aub, J. C., Bauer, W., Heath, C., and Ropes, M., *J. Clin. Invest.*, 1929, **7**, 97.
[2] Erdheim, J., *Frankfurt f. Path.*, 1911, **7**, 175.