and 11 times as effective in the hypophysectomized. At this level, intraperitoneally administered PMS yielded ovaries of approximately the same weight in both groups. When the same total dose, namely 24 units, was distributed over a 4-day period, subcutaneous injection proved equally as effective as intraperitoneal in both normal and hypophysectomized rats. As a tentative explanation, it is suggested that the rate of absorption may be one of several factors involved in this mechanism.

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Effect of A.T.10 (Dihydrotachysterol) on Various Types of Experimental Rickets in Rats.

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A.T.10 (dihydrotachysterol) is, according to Holtz,[1] its discoverer, a reduction product of irradiated ergosterol. It has been claimed that it has a special capacity to raise the blood serum calcium concentration. Because of this remarkable property it has been used extensively in the treatment of parathyroid tetany. Albright, Bloomberg, Drake and Sulkowitch[2] have reviewed the literature and have studied the effects of this agent in comparison with vitamin D in cases of idiopathic hypoparathyroidism. They concluded that vitamin D has two fundamental actions: it increases the amount of calcium absorbed from the gastrointestinal tract and also the amount of phosphate excreted in the urine. A.T.10 acts similarly, but to a different degree; it has less effect on calcium absorption and greater effect on the excretion of phosphate in the urine.

A.T.10 has been reported not to cure experimental rickets in rats, except perhaps in toxic doses.[3] We have been unable to find a detailed account of Holtz's experiments on experimental rickets. We surmise that he used the classical high-calcium-low-phosphorus type of ricketogenic diets. If A.T.10 has the action described by Albright *et al.*, it is logical to postulate that it would be particularly unfavorable to the healing of rickets caused by low-phosphorus diets

1 Holtz, F., Gissel, H., and Rossmann, E., *Deutsche Z. f. Chir.*, 1934, **242**, 521.

2 Albright, F., Bloomberg, E., Drake, T., and Sulkowitch, H. W., *J. Clin. Invest.*, 1938, **17**, 317; *Ibid.*, 1939, **18**, 165.

3 Harnapp, G. O., *Monatschr. f. Kinderh.*, 1935, **63**, 262.

because it would increase the excretion of phosphate when phosphate must be conserved for deposition in bone. On the other hand, with a ricketogenic diet low in calcium and relatively high in phosphorus A.T.10 might be more effective because any increase in calcium absorption and any increase in phosphorus excretion would tend to alter conditions toward the normal. The present study concerns the problem of whether A.T.10 acts differently with various types of experimental rickets in rats, and not whether there is some slight antiricketic activity in the preparation, due either to a contaminant or to the A.T.10 itself.

There is no good way of standardizing dihydrotachysterol in rat units, as is possible for vitamin D. Holtz has used as a unit the "toxische grenz Dose" (the minimal toxic dose), which he defines as the amount which, given daily, will cause a 15% loss of weight in 16-18 g male mice in 10 days, or the death of 50% of the animals. It is calculated that this minimal toxic dose contains 25μg (0.025 mg) of dihydrotachysterol.

The material used in this experiment was supplied by the Department of Medical Research of the Winthrop Chemical Co. It contained 200 minimal toxic doses per cc. Before feeding to the animals it was diluted with cottonseed oil which had been tested and found to contain no appreciable amount of vitamin D. The concentration was adjusted so that the desired dose could be given by putting one drop of the material directly into the mouth of the rat.

The rats were bred in our laboratory. The breeding animals were fed Sherman Diet B (⅔ wheat and ⅓ dried milk plus 1.3% of NaCl) and the young were weaned at 21 days of age and were continued on this diet until 28 days of age. They were then divided into 10 groups of 4 rats each and fed one of the 3 following basal diets: a normal diet (Sherman Diet B); the high-calcium-low-phosphorus ricketogenic diet No. 2965 of Steenbock and Black (79% ground whole yellow corn, 20% gluten and 1% NaCl, plus 3% $CaCO_3$); or a low-calcium ricketogenic diet (the same as diet No. 2965 with $CaCO_3$ omitted).[4] The calcium contents of these diets were, by analysis,[4] respectively, 0.33, 1.16, 0.016%; the phosphorus contents, 0.50, 0.30, and 0.32%. The Sherman Diet B had a Ca/P ratio of 0.66/1. Although the phosphorus contents of the 2 ricketogenic diets were identical, it is proper to speak of the Steenbock diet as a high-calcium-low-phosphorus diet because the Ca/P ratio was 4.0/1, and of the other as a low-calcium-high-phosphorus diet because the Ca/P ratio was 0.05/1. Group 1 received the normal

[4] Shohl, A. T., and Wolbach, S. B., *J. Nutrition*, 1936, **11**, 275.

TABLE I.

Group	Basal diet	Daily supplement	Avg gain in body wt, 21 days, g	Ash of extracted femur, %	Blood serum		Evidence of rickets*	
					Ca, mg/100 cc	Phosphate, mg of P/100 cc	X-ray	Histology
1	Normal	—	55	56.3	11.4	8.9	—	—
2	High-Ca-low-P	—	17	35.7	10.3	2.9	++++	++++
3	" "	Cod liver oil†	14	45.7	10.6	6.5	—	—
4	" "	A.T.10, 1/8‡	18	41.0	11.3	4.4	++	++++
5	Low-Ca-high-P	—	5	38.9	6.4	8.3	—	+++
6	" "	Cod liver oil†	20	42.3	8.5	8.1	++	±
7	" "	A.T.10, 1/800‡	6	39.4	7.3	7.9	—	+++
8	" "	A.T. 10, 1/80‡	5	37.6	7.0	9.2	—	±
9	" "	A.T.10, 1/8‡	12	41.9	5.9	10.8	—	—
10§	" "	A.T.10, 1/8‡	3	39.9	5.1	8.5	—	+

* — = no rickets; ± = borderline rickets; + = rickets. The degree of rickets is indicated by the number of plus signs, from one, which is mild, to 4, which is most severe rickets.

† One drop of cod liver oil, equivalent to 9 units of vitamin D.

‡ The fractions are in terms of the "minimal toxic dose."

§ The supplement was added to the basal diet of this group at the end of 21 days, and the experiment continued for 7 days longer.

diet and served as a normal control. Groups 2, 3 and 4 were given the high-calcium-low-phosphorus ricketogenic diet; group 3 received a supplement of cod liver oil and group 4 of A.T.10. Groups 5, 6, 7, 8 and 9 were fed the low-calcium-high-phosphorus ricketogenic diet; group 6 received a supplement of cod liver oil equivalent to 9 units of vitamin D; and groups 7, 8 and 9, three different amounts of A.T.10 respectively. All supplements were given daily. The duration of this experiment was 21 days. This procedure constitutes a test of protection against development of rickets. The rats of group 10 were fed the low-calcium-high-phosphorus rickets-producing diet for 21 days and then given a supplement of A.T.10 for 7 days. The purpose of this experiment was to test the curative effect of A.T.10.

At the end of the experiments the animals were X-rayed, weighed and bled from the femoral artery under ether anesthesia. The blood serum was analyzed for calcium and inorganic phosphate. The bones of one leg were preserved in 10% formalin for histologic examination; those of the other were used for determinations of the percentage ash of the dried bones after extraction with alcohol and ether. The methods of analysis used have been previously described.[4] The relevant data are presented in Table I.

Results. The criteria for interpretation of both roentgenograms and microscopic anatomy have been described in detail in a previous study.[4] The rats fed the high-calcium-low-phosphorus diet alone (group 2) showed the usual classical findings of rickets both by X-ray and by histologic examination of decalcified sections of the bones. The rats fed the same diet plus cod liver oil (group 3) showed complete protection by both methods of examination. The rats fed the same diet plus A.T.10 (group 4) were not protected by even the largest dosage used in this study.

The X-ray pictures of the bones of the low-calcium-high-phosphorus groups are very difficult if not impossible to interpret, because this type of diet results in bones with a very narrow zone of cartilage. The diagnosis of rickets depends upon interpretation of the histology of bone. When so examined, the bones of rats which were fed low-calcium-high-phosphorus diets in group 5 (without supplement) showed marked rickets; those in group 6 (with cod liver oil) showed little or no rickets; and those in groups 7, 8 and 9 showed progressively less rickets as the dose of A.T.10 was increased, and absence of rickets when ⅛ minimal toxic dose was given.

When A.T.10 was administered after low-calcium-high-phosphorus rickets had developed (group 10), the healing was far advanced after 7 days' administration. Thus the evidence for protec-

tion and cure both show the effectiveness of A.T.10 as an antiricketic agent in this type of rickets.

The animals fed the low-calcium-high-phosphorus diets, except those receiving cod liver oil, seemed more lethargic than normal animals or even than those which were given the high-calcium-low-phosphorus ricketogenic diets.

The average gains in weight of rats fed the ricketogenic diets, especially the low-calcium-high-phosphorus diet, were far less than those of the normal controls. Neither cod liver oil nor A.T.10 effected increases with the high-calcium-low-phosphorus ricketogenic diet. Both caused gains in weight with the low-calcium-high-phosphorus diet; the gains with A.T.10 were greatest when the amount given afforded complete protection against rickets.

In the dosages used the effects of A.T.10 on the concentrations of calcium and inorganic phosphate in the blood serum were not striking. Whatever may be its action in raising serum calcium after removal of the parathyroid glands, A.T.10 did not, under the given conditions, cause an increase of calcium concentration in the blood serum. It caused a slight increase in the serum phosphate in high-calcium-low-phosphorus rickets. The usual values for calcium and phosphate were found in the controls.

When protection against the development of rickets with A.T.10 was accomplished, it occurred without significant increase in either the absolute amount or percentage of ash, water, or organic matter in the femurs. This is in contrast to the effect of vitamin D on high-calcium-low-phosphorus rickets in which deposition of minerals is marked. When cure was initiated with A.T.10 a large increase in the amount of fat occurred (not shown in table).

The data show that in the dosage used the effects of A.T.10 can be interpreted only by a study of the bone histology; the roentgenograms, blood serum and bone ash show but slight changes from the normal. The histologic studies are, however, conclusive. They demonstrate that the action of A.T.10 can be sharply differentiated from that of cod liver oil. The latter is effective in the prevention and cure of rickets with both types of diets, but an amount of A.T.10 which is without demonstrable effect in prevention of rickets in rats on the usual high-calcium-low-phosphorus diet, protects them from developing rickets when they are fed a low-calcium-high-phosphorus diet. Even if the slight increase in serum phosphate and modification of rickets as shown by X-ray are attributed to an antiricketic action of A.T.10 upon rats fed the high-calcium-low-phosphorus type of diet, this effect is much less marked than upon those fed the low-

calcium-high-phosphorus diet. Therefore, even if the material used is contaminated with vitamin D, or in itself has antiricketic properties when used in massive doses, the difference in its action upon the two types of rickets is manifest. Although the exact composition of the preparation is not known, it is obvious that it does not contain a large amount of vitamin D, for it did not cure rickets in rats resulting from high-calcium-low-phosphorus intake, even with the largest doses used. Furthermore, the cure of the rickets in rats resulting from low-calcium-high-phosphorus diets was not effected by the toxic factor, for the blood serum calcium was not raised nor was pathological calcification produced in the soft tissues. It is reasonable to conclude, as do Albright *et al.*, that A.T.10 and vitamin D affect calcium and phosphorus metabolism to a different degree, and that A.T.10 is more effective in preventing rickets induced by the low-calcium-high-phosphorus diet because it facilitates the elimination of phosphate in the urine and hence renders this type of diet less ricketogenic.

Summary. The effect of A.T.10 on prevention of rickets in rats differs according to the ricketogenic diet fed. Rickets caused by high-calcium-low-phosphorus diets was not prevented by a non-toxic amount of A.T.10 which protected rats fed low-calcium-high-phosphorus diets.

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Resistance of Young Dogs to Acute Arrest of the Cephalic Circulation.*†

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The observation has been made by several investigators that the young animal is much less susceptible to asphyxia than the adult.[1, 2]

* Aided by a grant from the Committee on Scientific Research of the American Medical Association.

† Assistance in the preparation of these materials was furnished by the personnel of Works Progress Administration, Official Project No. 665-71-3-69, subproject No. 309.

1 Reiss, M., *Z. f. d. ges. exp. Med.*, 1931, **79**, 345.

2 Avery, R. C., and Johlin, J. M., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 1184.