indeed, a hypercalcemia in this condition. The present findings of an increased rate of phospholipid formation as measured by the uptake of P^{32} are consistent with the increased rate of bone production. Thus, in hypervitaminosis D, since the rate of matrix formation is greater than normal, it may be that the rate of calcification is inhibited by the presence of excess lipids and in particular phospholipids. It may be that calcification cannot occur until the phospholipids are removed. This could account for the decreased mineral content of the skeleton in hypervitaminosis D. It seems unlikely that any particular phospholipid is specifically involved since the present study showed that all phospholipids were increased proportionately.

Thompson and DeLuca(15) and Hoyosha (16) have shown that the vitamin D-deficient state leads to a diminished incorporation of P^{32} into the phospholipids of rat mitochondria from intestine and kidney but not from liver. The present work clearly indicates an increased incorporation of P^{32} into the phospholipids of bone in the presence of excess vitamin D. Thus, vitamin D status of the animal has significant effects on the phospholipid metabolism of tissues which are influenced by this vitamin, *i.e.*, kidney, intestine and skeleton.

Summary. The phospholipids of the diaphyseal and metaphyseal portion of the long bones of rats have been analyzed. Lecithin, phosphatidylethanolamine, sphingomyelin and lysolecithin constitute the major phospholipids with small amounts of cardiolipin and phosphatidic acid also being present. Hypervitaminosis D was found to cause a significant increase in all phospholipids without a selective action on any one component. The incorporation of P^{32} into the phospholipids was found to be increased indicating an increased synthesis rather than a decreased break-down. It is suggested that the accumulation of lipid material may be related to the failure of the osteoid in hypervitaminosis D to calcify properly.

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Nutritional Studies with the Guinea Pig. VIII. Thiamine. (32354)

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In a previous report from this laboratory, the effects of thiamine deficiency in the guinea pig were briefly described(1). The present report gives results of additional experiments designed to determine the approximate requirement of this animal for thiamine.

Methods. The methods described previously as to age, sex (male), feeding and care of the animals were followed(2). A purified diet, No. 13(2), containing 30% of vitamin-free casein was prepared weekly. Salt mixture A of Briggs *et al*(3) at a 6% level, was used in the first of the experiments and salt mixture N of Fox and Briggs(4) at a 6% level in a later study.

Results and discussion. A total of 143 guinea pigs, 3 to 5 days old, were used in tests with diets containing salt mixture A(3) with thiamine levels varying from zero to 16 mg/kg. With a few exceptions, from 3 to 5 tests were made for each level of intake. The absence of dietary thiamine resulted in impaired appetite, poor growth, weakness, tremors, lack of balance, retraction of the head, and eventual death as noted in the previous report(1). There was a high incidence of mortality in groups receiving 1, 2 or 3 mg thiamine per kg of diet, and some animals at the 6 mg level showed neurological symptoms. The response to higher levels of thiamine was erratic and it appeared that the requirement was inordinately high. It was suspected that destruction of the vitamin may have occurred during storage of the diets. To answer this question several diets were analyzed for thiamine by the thiochrome method on the day that they were prepared and again after 7 days storage at +5°C (Table I). It is evident that a marked destruction of the vitamin had occurred. It seemed probable that the salt mixture A, which contains 18% of K_2HPO_4, may have been responsible for the change. Waibel *et al* (5) have shown that K_2HPO_4 was primarily responsible for the loss of thiamine from purified diets.

TABLE I. Thiamine Content of Diets Prepared with Salt Mixtures A and N After Storage for 7 Days at + 5°C.*

Thiamine HCl added to diet (mg/kg)	Thiamine HCl found (mg/kg) Salts A	Salts N
1	.10	.95
2	.14	1.75
4	.24	3.33

* The manufacturer's (Nutritional Biochemicals Corp.) assay of the casein used in these studies was 0.14 μg thiamine·HCl/kg.

TABLE II. Thiamine Requirement with a 30% Casein Diet.

Thiamine HCl (mg /kg diet)	Total No. animals A salts	N salts	Avg wt (g) after 4 wk A-salts	N-salts
None	15	13	139 (2)*	139 (2)
1	15	13	170 ± 29† (13)	155 ± 26 (10)
1.5	—	5	—	204 ± 12 (5)
2	22	13	218 ± 31 (18)	257 ± 32 (11)
4	24	12	240 ± 24 (23)	246 ± 39 (12)
6	16	12	242 ± 22 (11)	259 ± 25 (12)
8	23	5	246 ± 37 (22)	258 ± 15 (5)
16	16	5	254 ± 42 (15)	269 ± 10 (5)

* Numbers in parentheses indicate No. of survivors.
† Standard deviation.

A considerably different response of guinea pigs to varying levels of thiamine was obtained when salt mixture N(4),* which contains no K_2HPO_4, was substituted for the A salts. With the N salts, in contrast to the A mixture, only a slight loss of thiamine occurred during one week of storage (Table I). The results of the feeding experiments using salts N (Table II) clearly indicated that 2 mg of thiamine/kg of diet are sufficient for growth, survival and prevention of deficiency symptoms. The actual requirement may be slightly less than 2 mg, but the 1 mg level was definitely inadequate with respect to both growth and survival. At the 1.5 mg level all of the 5 animals survived and growth was fairly good, but two of them were unsteady and showed lack of balance. The thiamine requirement of the guinea pig thus appears to be about the same as that of the rat and mouse(7).

The maximal weight achieved in 4 weeks (about 260 g) by the groups receiving adequate thiamine compares favorably with that previously reported for this complete diet, but is about 10% lower than maximal weight

* This salt mixture was devised after a thorough study(6) of the individual components of salt mixtures which cause oxidative rancidity in diets.

produced by a natural pelleted diet(2).

Conclusion. The thiamine requirement of the guinea pig was estimated with a purified diet prepared with two different salt mixtures. In the first experiment an apparently high requirement for thiamine was traceable to a rapid destruction of the vitamin with the diet stored at +5°C. With a different salt mixture, designed to minimize the storage changes, a requirement of 2 mg of thiamine/kg of diet was found.

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Effect of Thymectomy on Rous Virus Tumor Growth Induced in Chickens. (32355)

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Neonatal thymectomy has a pronounced inhibiting or stimulating effect on the tumor growth induced by various oncogenic viruses (2,3,6,7,9-15,21). Our objective was to investigate the role of the thymus on the induction and growth rate of Rous virus sarcoma in chickens.

Material and methods. Chickens were thymectomized during the first 72 hours of life. Thymectomy was done by the technique described by Peterson *et al*(15). As controls we used intact or sham-operated chickens of the same age as the thymectomized ones. The experimental and control birds were simultaneously inoculated with the Rous sarcome virus (strain Carr-Zilber) at the age of 10-12 days. As a source of Rous sarcoma virus we used a cell-free tumor extract, which was stored in the frozen state (at −70°C) for 6 months. The virus titer, which was estimated by means of virus injection into 1-day chicks, was 1×10^6 LD_{50}/ml. All experiments were made with the same pool of tumor extract. Each thymectomized and control chicken was inoculated with the virus in a dilution of 10^{-5} or 10^{-6} intracutaneously in both wings. All chickens were observed for 30 days after virus injection. During this period we examined the chickens for tumors 3 times each week and autopsied every dead chicken. All birds which remained alive to the end of the experiment were sacrificed and autopsied.

Results. The results of our experiments are summarized in Table I and Fig. 1 and 2.

The average time of tumor appearance in the thymectomized chickens was 10-12 days. In most control birds the tumors appeared 3-4 days later. The 10^{-5} dilution of virus caused tumors in each of the 42 thymectomized chicks (100%) and in 44 of the 52 controls (84.6%). The 10^{-6} dilution of virus caused tumors in 20 of 42 thymectomized chicks (47.6%) and in 13 of the 52 control birds (25.0%). Among the thymectomized chickens, in addition to the increased frequency of tumors, the tumors also grew more rapidly and killed sooner. By the end of the experiment there had been 36 deaths due to tumors among the 42 thymectomized chickens (85.7%) and in only 10 of 44 control chickens (22.8%). The probability value (P) by Chi^2 analysis was $P<0.001$.

The primary intracutaneous tumors in the