

ovariectomized female rat. It also effects a rapid hypertrophy of the adrenals and thyroid. These adrenal effects are lacking in the hypophysectomized rat but the uterine stimulation is unaffected either by hypophysectomy or adrenalectomy. The compound also pos-

sesses an acute toxicity which is characterized by marked narcosis leading to death. The maximum effective dose is approximately two-thirds of the lethal dose.

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Failure of Cockerel Comb and Testis Development on Sesame Meal and its Prevention by Vitamin B₁₂.^{*†} (18287)

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During the course of a nutrition experiment designed by one of us (A.R.P.), it was observed that when the ration contained 70% sesame meal there was marked failure of development of combs and testes. This condition was prevented by adding Vit. B₁₂ to the diet. The source of the Vit. B₁₂ was Merck APF Supplement No. 3.

Methods and materials. Single comb White Leghorn cockerels were fed a Vit. B₁₂ depletion diet based on soy bean (sodium proteinate) which contained 0.05% iodinated casein (Protamone). These birds were distributed evenly by weight into groups of 15 each, eliminating extremes in weight. The experimental rations consisting of sesame meal 70%, yellow corn meal 26%, dicalcium phosphate 2%, pulverized limestone 1%, salt mixture 0.5%, choline chloride 0.17%, Vit. A, D₃, E, and K in oil 0.25%, B-complex Vitamins (except B₁₂) in starch 0.10%, and sufficient iodized calcium carbonate to supply 1 mg iodine per lb were fed for 2 weeks. For purposes of comparison, a group of chicks depleted of Vit. B₁₂ was also fed a standard chick starter. Because the basal diet was deficient in lysine(1,2), certain groups were

fed additional lysine in the form of DL-lysine monohydrochloride monohydrate to provide 0.2% L-lysine. Since the requirement for Vit. B₁₂ on high protein diets is increased(3), sufficient APF supplement was added to provide for this deficiency (25 µg B₁₂ per lb of ration). This amount supplied approximately 10 times more Vit. B₁₂ than the standard chick starter mash used for comparison.

Results. Table I gives the gross effects of the various supplements at four weeks of age. During the experiment it was observed that the birds on the high sesame meal-Vit. B₁₂ deficient diet (Group 2) had underdeveloped, pale combs, whereas the combs of chicks receiving the APF supplement with or without lysine (Groups 3 and 5) showed normal red color and size comparable to those receiving the standard chick starter (Group 1).

Since comb growth and color are influenced by testicular function, the testes were examined to determine the state of their development. Testes produced on the basal diet were subnormal in weight, whereas the ones produced by an addition of both lysine and Vit. B₁₂ showed an increase in weight of 88% over those on the basal diet. The histological differences were even more remarkable. As expected, the testes from chicks on

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TABLE I. Effect on Comb, Testis, and Body Weight of Supplementing a Sesame Meal Diet with APF and with DL-Lysine Hydrochloride.

Group	Diet and supplement	Body wt 4 wk (g)	(Adjusted means)*		(Adjusted means)*	
			Comb wt, mg	% diff. from control	Testis wt, mg	% diff. from control
1	Chick starter control	311	1028.3	0	95.7	0
2	70% sesame meal basal	211	514.6	-50	65.1	-32
3	Basal + APF	245	1060.6	+ 3	109.2	+14
4	Basal + lysine	203	653.8	-36	72.4	-24
5	Basal + APF + lysine	213	828.7	-20	121.8	+27
Min. diff. required for significance						
at 5% level			312.7		21.9	
at 1% level			412.8		28.9	

* Means adjusted for body wt by covariance analysis.

the standard starter ration appeared normal (Fig. 1). In the testes produced on the sesame meal basal (Fig. 2) the seminiferous tubules were markedly decreased in number and size and they were separated by increased intertubular spaces. Leydig cells were fewer in number and many showed pyknotic nuclei. The condition resembled that of early hypophysectomy in the chick(4). Addition of Vit. B₁₂ resulted in normal testicular weight due to enlarged tubules with epithelium of greater height, normal intertubular spaces and apparently normal, functional Leydig cells (Fig. 3). This response suggested an increased action of pituitary gonadotrophin, particularly of the type that stimulates growth of Leydig cells.

Lysine alone did not substantially change the weight of the testes. The histological appearance, however, indicates that supplementation with lysine actually had a detrimental effect as illustrated by the tubules filled with debris and the marked lymphocytic infiltration in intertubular spaces (Fig. 4). The Leydig cells were compressed in narrow intertubular spaces and many displayed the pyknotic nuclei previously mentioned as characteristic of hypophysectomy.

Supplementation with lysine and Vit. B₁₂ produced slightly greater testicular growth and differentiation of the seminiferous epithelium (Fig. 5 and 6) than seen in the normal controls (Fig. 1). These birds showed testes with larger tubules, increased height of ger-

mal epithelium and scattered mitotic figures. The intertubular spaces were enlarged and filled with an increased number of Leydig cells that appeared to be functional.

Discussion. The data obtained in this study suggest that Vit. B₁₂ plays a role in the development and function of the reproductive organs. This view is supported by the work of several investigators. Hartman, Dryden and Cary(3) demonstrated a stimulatory effect of Vit. B₁₂ on the reproductive organs of the female rat deficient in that vitamin. An analogous relationship was shown to exist by Hertz and Tullner(5) with respect to the need for pteroylglutamic acid to maintain sensitivity of the chick oviduct to estrogen, apparently by stimulating protein synthesis. Similarly, Vit. B₁₂ seems to accelerate the utilization of amino acids for protein synthesis (6). The view that chicks deficient in Vit. B₁₂ are unable to synthesize the necessary protein for gonadal response to gonadotrophin is further supported by the work of Chow(7) who demonstrated a lack of response to this hormone by Vit. B₁₂-deficient rats of both sexes.

Summary. Feeding cockerels depleted of

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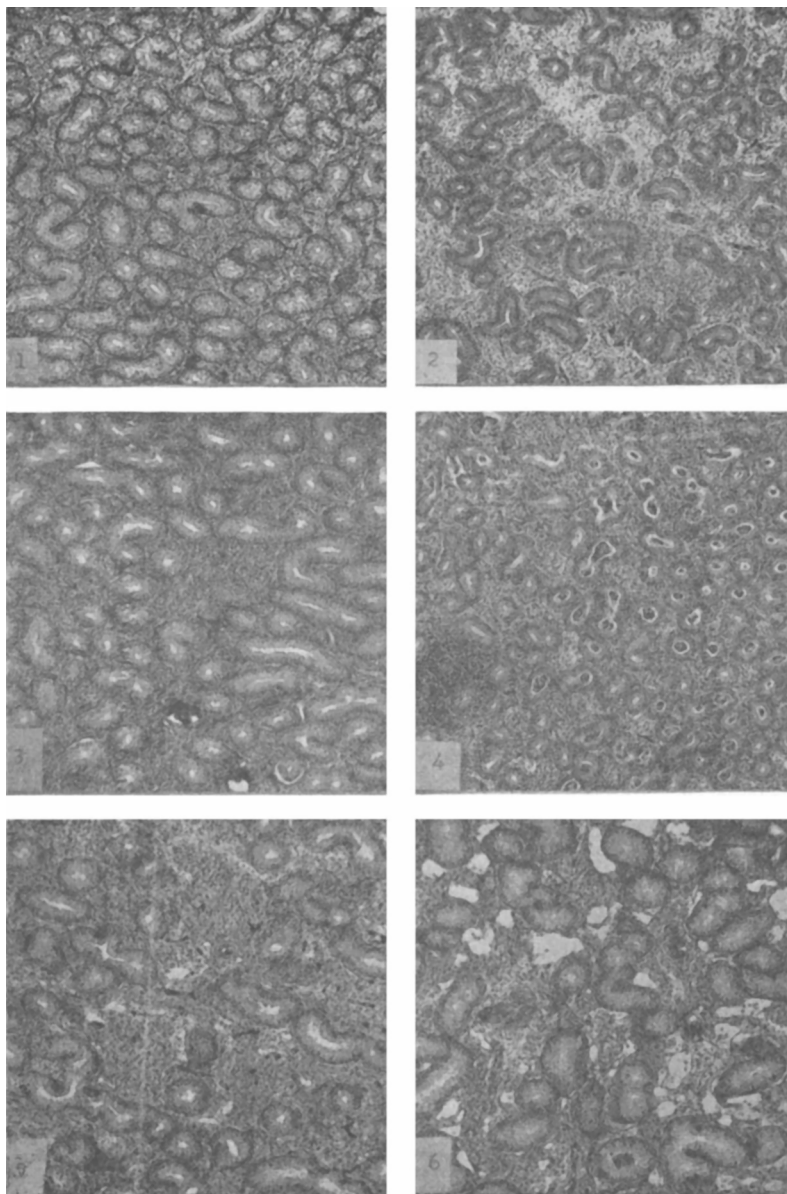


FIG. 1. Normal testes from chicks fed the control chick starter mash. 200X.

FIG. 2. Testes from chicks on deficient sesame meal basal diet. These show a decrease in diameter of tubules and an increase in the intertubular space, but with fewer Leydig cells in the intertubular spaces. 200X.

FIG. 3. Testis from a chick fed sesame meal basal diet and APSF supplement. The number of tubules and the tubular diameter resembles strongly that seen in the control. The interstitial spaces are not excessive and have a normal number of Leydig cells. 200X.

FIG. 4. Testis from a chick fed sesame meal basal with lysine supplementation. This testis reflects histologically the failure of lysine to restore normal morphology of the testes. The tubules are of small diameter with low epithelium and have, in addition, intratubular debris. Also, the interstitial tissue, which has few Leydig cells, shows infiltration with lymphocytes. 200X.

FIG. 5 and 6. Two different areas of a testis from a chick fed the sesame meal basal diet supplemented with both lysine and APSF. The striking increase in weight of the experimental testes over that of the control testes is borne out by the appearance of this gonad. The tubules are of greater diameter and show an increased epithelial thickness. There is an increase in the intertubular spaces which are filled with well-formed Leydig cells. 200X.

Vit. B₁₂ from the second to fourth weeks of age on a 70% sesame meal diet deficient in Vit. B₁₂ and lysine resulted in a failure of morphological differentiation of testicular tissue accompanied by a decrease in comb growth. Supplementation with Vit. B₁₂ and lysine produced markedly greater testicular growth and differentiation over that in the normal controls.

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Presence of Myelokentric and Lymphokentric Acid in Sera of Patients with Lymphomatoid Diseases. (18288)

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The urine of patients with leukemia and other lymphomatoid diseases has been shown to contain substances(1) that we have named myelokentric and lymphokentric acid(2). These substances seem to play a part in the proliferation and possibly the maturation of myeloid and lymphoid cells. These substances have been found also in the organs(3) of patients with these diseases and in normal beef liver(4).

Heinle and his co-workers(5) have shown that relatively small amounts of material extracted by methods other than ours from similar urinary sources showed considerable potency for proliferation of myeloid cells. From this work we have considered the possibility that small amounts* of plasma or serum from patients with these diseases might contain sufficient conjugated material to produce reactions in the organs of animals. There-

fore, the sera from three types of leukemia, Hodgkin's disease and normal individuals have been given to guinea pigs, and at various intervals after injection the animals were sacrificed and the organs studied.

Material and methods. Under sterile precautions 20 to 40 ml of blood was removed from each subject, placed in a sterile tube, allowed to clot, and later centrifuged for ½ hour at 3000 r.p.m. For injection, 5 ml of the serum was drawn off and introduced into the peritoneal cavity of a guinea pig. Usually two animals were used, one of them being sacrificed after 24 hours, the other after 48 hours; in 2 experiments the interval was 8 hours. Sections were made of liver, spleen, lymph nodes, adrenal, kidney and bone marrow.

Many of the sera from normal individuals were collected at the time of donations of blood for transfusions. To obviate any discrepancy due to the alarm mechanism which might occur incidental to the removal of blood, samples in 3 instances were taken at the start of the donation as well as at the end. Samples of serum were obtained from 9 patients with chronic myeloid leukemia, 6 patients with chronic lymphoid leukemia, 6 patients with monocytic leukemia, 4 patients with Hodgkin's disease and 12 normal individuals. Table I shows the distribution of

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* Urine dose = 500 ml to 7.1. Versus serum dose = 5 ml.