are fundamental to the leukemic process from those associated with a more uncontrolled malignancy.

Summary. Ribosomes were prepared from normal human granulocytes, acute and chronic leukemias and rat liver. These preparations were analyzed for polyribosome (PRS) profile by means of sucrose density gradient centrifugation. None of the leukemic cell preparations had as high a proportion of PRS as did rat liver. Normal granulocytes and chronic granulocytic leukemic (CGL) cells had a greater fraction of PRS than did acute granulocytic (AGL) or chronic lymphocytic leukemic (CLL) cells. Most of the AGL and CLL particles were ribosomal subunits, monomers, and dimers. With 30′ of uridine-^{3}H labeling of all types of leukemic cells, the highest specific activity was in the subunits and PRS regions with low specific activity in the monomer-dimer regions. The data suggests that the monomers and dimers are not intermediates for the formation of PRS.

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Vitamin D Deficiency in Adult Quail and Chickens and Effects of Estrogen And Testosterone Treatments.*† (31943)

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The effects of vitamin D deficiency on various aspects of the reproductive processes in mature hens have been described by Turk and McGinnis(1,2). These workers, using Single Comb White Leghorn hens, observed that vitamin D deficient hens remained in good physical condition, even though they laid very few eggs. The limited number of eggs laid by these hens had thinner shells and were smaller in size. Taylor and Hertelendy (3) suggested that vit. D is required for estrogen to increase plasma calcium, phospholipid and blood total lipids in mature roosters.

In a continuation of studies on vitamin D

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deficient hens using commercial White Leghorn pullets bred for high egg production, typical symptoms of osteomalacia were observed when 20-week-old pullets were fed a vitamin D deficient diet for 4 months. High mortality also occurred. In a separate study with mature Japanese quail, high mortality occurred in vitamin D deficient females, while a very low mortality occurred in deficient males. Turk(4) observed that vitamin D deficient roosters had larger testes than vitamin D supplemented roosters, but based on artificial insemination and natural mating studies, there was no difference in reproductive efficiency accompanying this enlargement. Whether greater testosterone production in the vitamin D deficient male bird is a factor which reduces mortality has not been established. Experiment 1 was conducted to study whether high mortality in the female quail was related to changes in calcium retention. Experiment 2 was conducted to determine whether injections of testosterone, estradiol, or a combination of both would modify the development of vitamin D deficiency signs in adult chicken hens.

Materials and methods. Experiment 1. Forty-eight male and 48 female quail were fed the following basal diet from time of hatching to 7 weeks of age, when the females began to lay: ground barley, 30.0%; ground milo, 30.0%; soybean meal, 30.0%; limestone, 5.0%; dicalcium phosphate, 3.0%; iodized salt, 0.5%; trace mineral mixture, 0.1%; vitamin mixture, 1.5%. The trace mineral mixture provided the following per kg diet: manganese, 100 mg; iron, 100 mg; calcium, 100 mg; copper, 10 mg; zinc, 100 mg; iodine, 3.3 mg; and cobalt, 1 mg. The vitamin mixture provided the following per kg diet: vitamin A, 6,000 IU; riboflavin, 4 mg; calcium pantothenate, 12 mg; vitamin B_{12} (1 mg/g dissolved in approximately 10 ml of distilled water), 0.002 mg; niacin, 29 mg; choline, 650 mg; vitamin K (Klotogen F), 0.9 mg; and vitamin D_3, 1,200 ICU.

At this time the quail were divided by sex into 4 groups, 2 of which were fed the basal diet deficient in vitamin D and the others the same diet supplemented with 1,200 ICU vitamin D/kg diet. Females fed the deficient diet to 4 months of age ceased laying eggs, whereas the vitamin D supplemented females were still laying eggs at the rate of 90%. At this time, 24 quail of the 4 groups were divided again into 3 pens (8 birds per pen) and continued on the same diets containing 0.3% chromic oxide for 3 weeks to study calcium retention. Fecal collections were made from a collecting pan beneath the metabolism cage in which the quail were housed. Feed and feces were analyzed for dry matter and chromic oxide by the methods of Anderson and Hill(5), and calcium was determined by the AOAC method(6). After this phase of the study was completed, the birds were returned to the experimental diets for a year to observe mortality and egg production.

Experiment 2. A total of 294 White Leghorn pullets, 20 weeks of age, were randomly distributed into individual laying cages. Half of them were fed a vitamin D deficient diet and the others were fed the same diet supplemented with 1,200 ICU vitamin D_3/kg. Composition of the diet (Ration A) and housing conditions were similar to those described by Turk and McGinnis(1). After the experimental diets were fed for 10 months, approximately 57% mortality had occurred in the vitamin deficient hens, while only 14% mortality had occurred in the vitamin supplemented hens. At this time 30 hens from the vitamin deficient groups and 30 hens from the vitamin supplemented treatment were randomly selected and divided into 5 treatments, 6 hens per treatment, as follows:

Treatment 1 ..... Control (injection of 1 ml of oil-carrier)

Treatment 2 ..... A capsule of 5,000 ICU D_3/hen

Treatment 3 ..... 1 mg testosterone propionate in oil/hen/day

Treatment 4 ..... 1 mg estradiol cypionate in oil/hen/day

Treatment 5 ..... 1 mg testosterone plus 1 mg estradiol in oil/hen/day

These treatments were continued for 5 weeks, at which time the hens were sacrificed and the tibia, sternum, and femur were removed for determinations of bone ash and bone density by X-ray. At the same time, about 10 ml of blood were taken from each bird for deter-

TABLE I. Influence of Vitamin D_3 on Egg Production, Calcium Retention, and Mortality in Mature Male and Female Japanese Quail.

Added vitamin D	Sex	% Egg production	% Calcium retention*	% Mortality†
None	♂	—	— 1.5 ± .3	16
	♀	0	— 4.5 ± .4	90
1200 ICU D_3/kg	♂	—	— 7.1 ± .5	17
	♀	90‡	+38.7 ± .7	30

* Value represents mean ± standard error of mean. % calcium retention does not take into account the calcium deposited in the eggs.
† Total mortality to one year of age.
‡ Average egg production for 3 weeks.

mination of plasma calcium. Oviducts were removed and their weights recorded immediately. Bone ash was determined by the standard AOAC(6) method and plasma calcium was determined by the procedure of Pappenhagen and Jackson(7).

Results. Experiment 1. Vitamin D deficient male and female quail, as well as vitamin D supplemented males, were all in slight negative calcium balance (Table I). However, a high mortality was observed only in the vitamin D deficient female quail. It was noted that some of the vitamin D deficient female quail were laying a few eggs without shells or membranes at the start of the calcium retention study.

Experiment 2. Influences of vitamin D, testosterone, and estradiol on egg production, body weight, plasma calcium, and oviduct weight are shown in Table II. The most striking response was to the injection of testosterone in vitamin D deficient hens. The injection alleviated the debility caused by the vitamin D deficiency; that is, they regained strength within a week, as evidenced by their ability to stand, and gained an average of 268 g in body weight in 5 weeks, while the vitamin D deficient controls continuel to lose weight. The injection of testosterone did not influence egg production in the vitamin D deficient hens, but completely inhibited egg production in vitamin D supplemented hens within 3 days and caused the hens to begin molting in 10 days. Injection of estradiol into vitamin D deficient and supplemented hens did not influence egg production or apparent physical condition of the hens. The same effect of estradiol on plasma calcium of vitamin D deficient hens has been observed repeatedly in this laboratory. Synergistic effects of testosterone and estradiol were shown in both groups based on the significantly reduced oviduct size and plasma calcium. The combined injection into the vitamin D deficient hens appeared to improve the physical condition somewhat, but not as much as the in-

TABLE II. Influences of Vitamin D_3, Testosterone, and Estradiol on Egg Production, Body Weight Gain, Plasma Calcium and Oviduct Weight in Laying Hens.

Treatment		% Egg production*	Body wt gain, g/hen	Plasma calcium, mg/100 ml	Oviduct wt, g
Pre-experimental	Experimental				
No D_3	None	4	—258	16.4 abc	35.7 ± 4.1†abc ‡
	D_3§	54	— 38	18.7 abcd	42.8 ± 7.0 bcd
	Testosterone	1	+268	17.4 abc	26.2 ± 6.4 a e
	Estradiol	4	+ 52	21.4 de	27.1 ± 6.6 ab e
	Test. + estradiol	0	+237	14.2 c	17.1 ± 3.2 e
1200 ICU D_3/kg diet	1200 ICU D_3/kg diet	61	+ 62	25.9 e	51.4 ± 4.9 cd
	D_3§	69	— 21	24.1 de	49.3 ± 2.9 cd
	Testosterone	2	— 48	17.3 abc	41.1 ± 4.1 abcd
	Estradiol	76	— 60	20.5 a de	55.5 ± 8.9 d
	Test. + estradiol	0	+ 92	15.1 bc	18.4 ± 3.5 e

* Egg production during 5-week period (6 hens/treatment).
† Values mean ± standard error of mean of 5 oviducts/treatment.
‡ Values followed by the same letters were not significantly different at the 5% level.
§ 5000 ICU D_3/hen was given once in a capsule and the hens were kept on the same diets.

TABLE III. Influences of Vitamin D_3, Testosterone, and Estradiol on Bone Ash and Its Density in Laying Hens.

Treatment		% Bone ash (dry, fat-free)*				Bone
Pre-experimental	Experimental	Tibia	Femur	Sternum	Average of three bones	density† (X-ray)
No D_3	None	47.3 ± .6a	39.8 ± 2.2a	37.3 ± 1.8a	41.5	‡1.3
	D_3	52.4 ± 2.5 b	50.2 ± 3.3 bc	41.5 ± 3.1ab	48.1	2.4
	Testosterone	52.2 ± .9 b	44.5 ± 1.6 b	42.2 ± 1.6 b	46.3	2.0
	Estradiol	54.0 ± 1.1 b	47.5 ± 1.4 bc	41.1 ± 2.6ab	47.6	2.1
	Test. + estradiol	52.4 ± .6 b	46.3 ± .9 b	45.8 ± 1.8 b	48.2	2.4
1200 ICU D_3/kg diet	1200 ICU D_3/kg	60.0 ± 1.8 c	57.0 ± 1.4 d	55.0 ± .9 c	57.7	3.4
	D_3	57.9 ± 1.6 c	53.8 ± 1.5 cd	45.4 ± 1.8 b	52.4	2.9
	Testosterone	61.3 ± 1.3 c	61.1 ± 1.3 d	52.6 ± 1.9 c	58.3	4.0
	Estradiol	60.0 ± 1.1 c	56.8 ± .8 d	52.4 ± 1.6 c	55.7	3.6
	Test. + estradiol	59.8 ± .3 c	57.4 ± .8 d	53.1 ± .9 c	56.8	3.7

* Mean ± standard error of mean of 5 bones/treatment.

† Bone density was determined by X-ray photo. We are indebted to Dr. J. E. Alexander at Radiology Department of Veterinary School who took the photos and scored the density as follows:

5 marked sclerosis	2 decrease in density
4 denser than normal	1 decrease in density and thin cortices
3 normal	0 most severely affected

‡ Average density score of 5 femurs and 5 sternums.

jection of testosterone alone. The combined injection into the vitamin D supplemented hens also inhibited egg production and caused them to molt in 2 weeks. The increases in bone ash of the deficient hens by testosterone and estradiol injection were statistically significant (Table III). Bone ash values agreed very closely with the results of bone-density determined by X-ray.

Discussion. The results of the quail study (Table I) demonstrate clearly that mature males did not need supplementary vitamin D to maintain normal physical condition and calcium equilibrium. These observations, coupled with the results of Turk(4) on vitamin D deficient male chickens, suggest that the requirement of adult male birds for vitamin D is either very low or non-existent. High mortality in vitamin D deficient female quail may be related to some function of vitamin D other than calcium metabolism because calcium retention was only slightly lowered. Whether the failure of vitamin D deficient birds to respond to estrogen is related to the high mortality in females as suggested by Taylor and Hertelendy(3), or whether an imbalance in hormones explains the difference between sexes is not known.

No explanations can be offered for the difference in responses of plasma calcium and oviduct weight to estradiol injection (Table II) in the deficient and supplemented hens. It may be speculated that the level of circulating estrogen migh be lower in the vitamin D deficient hens than in vitamin supplemented hens which could bring about an elevation of plasma calcium. The presence of vitamin D would not appear to be necessary for the action of estrogen in this respect. However, the injection of estradiol to vitamin D deficient hens did not increase oviduct size, but instead, caused a slight decrease in size. Whether it could be a nonspecific resistance to stimulation due to the concomitant inanition, or to a need of vitamin D for the action of estrogen on the oviduct, is not known. Whether vitamin D is required for the action of certain hormones during the reproductive processes or whether it is required for synthesis of yolk material in the liver of laying hens, as suggested by Hertelendy and Taylor(8) is not clear. Although the improved bone ash values of vitamin D deficient hens by hormone treatment was never equivalent to that of the hens fed vitamin D throughout the experiment (Table III), it was of great interest that the improvement was observed in all 3 bones in the absence of vitamin D. At present no satisfactory explanation can be offered for the increase of bone ash and bone density in the absence of vitamin D. These results emphasize the need for more work on the interrelation-

ship between hormones and vitamin D in adult animals.

Summary. Mature male quail remained in a good physical condition even though no vitamin D was given for a year. In contrast, high mortality occurred in the vitamin D deficient females, even though calcium balance was not different from that observed in the male quail. It was suggested that the requirement of adult male birds for vitamin D is either very low or non-existent. Injections of testosterone and estradiol into vitamin D deficient hens did not influence egg production, but significantly improved bone ash of tibia, sternum, and femur. Alleviation of debility caused by the vitamin D deficiency was observed in laying hens when testosterone was injected.

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Effect of Maternal Nicotine Intake on Fetal Weight and Length in Rats.* (31944)

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We have reported that addition of nicotine to the drinking water of pregnant rats resulted in lighter weight offspring and greater variability in liver lipid content than in control rats given distilled water(1). It was noted that the water intake was lower in the case of the pregnant rats drinking nicotine solution. Food intake was not measured, but the weight gain during the pregnancy was comparable to that of controls. To determine whether the fetal changes resulted from nicotine itself or decreased food intake, a different experimental design was sought in order to give nicotine in multiple small doses throughout the pregnancy with minimal disturbance or trauma to the gravid rat and, at the same time, to maintain a normal food intake.

After testing several types of feeding and drinking patterns, it was found that nicotine-diet mixtures were acceptable to the rats at concentrations that permit comparison with the results of our previously reported experiments. In this paper we present the results of experiments in which pregnant rats were maintained on normal food and water intake and yet took in nicotine in amounts comparable to the earlier study. Fetal weight and length were determined on the 20th day of gestation.

Materials and methods. Sprague-Dawley derived rats were used throughout. Virgin females were mated and time of conception was marked from the appearance of the vaginal plug. Distilled water was given *ad lib* and daily intake was recorded. The food was finely ground Purina Mouse Breeder Chow given *ad lib* in non-spillable food cups. Food intake was measured at 2-3-day intervals. Nicotine was added to food by injecting a solution containing 25 mg/ml (through a 25 gauge needle) into the powdered food while mixing. The mixture was then stirred mechanically for 2 hours. Diets were freshly prepared weekly and stored at 3-4°C.

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