and his associates(14) and others, preparations of diphtheria toxin, by chemical and physico-chemical standards considered as pure, may display a considerable immunological inhomogeneity when analyzed in gel precipitation tests. This fact is probably partly explained by the sensitivity of the immunological method by which minute traces of impurities otherwise not demonstrable can be detected. This might not be the whole truth, however. According to the reports quoted above several major components could be distinguished in such presumably pure toxins. If so, the selectivity and resolving power of immunoprecipitation in gels must obviously considerably exceed that of the chemical methods. It is tempting, then, to apply the former method in attempts to further fractionate chemically purified, biologically active substances. Experiments along these lines are in progress.

Summary. Experiments on 3 immunological systems showed that antigens, when combined with antibodies in a gel retain their affinity for certain dyes. This affords the possibility of studying not only the chemical nature of certain antigens, but also their immunological behavior in complex mixtures.

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Leucocyte and Thymus Changes in the Pyridoxine-Deficient Young and Adult Male Rat.* (20910)

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The lymphopenic response is a well established index of the increase of 17-hydroxysteroids in the blood and of lymphoid tissue involution. During pyridoxine deficiency lymphopenia, usually accompanied by granulocytosis, has been seen in dogs(1,2), rats(2), monkeys(3,4), and mice(5). When pyridoxine was administered to pyridoxine-deficient animals, the lymphopenia and granulocytosis were reversed in some cases(2,5) but not in others(1,4,2). In adrenalectomized pyridoxine-deficient rodents, involution of the thymus(6) and lymphopenia(7) have been reported. These latter phenomena were presumably independent of the intermediation of the adrenal hormones and were attributed specifically to the lack of pyridoxine. Inanition could also induce thymic atrophy(8,9) and lymphopenia(10,11) in the intact animal, but these alterations did not occur during inanition in the absence of the adrenals(9,12). Some of the above findings were studied in this laboratory and have been confirmed.

Procedure. The total number of leucocytes in tail vein blood was counted from duplicate pipettes. One smear per animal was stained

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with Wright's stain and 200 cells were counted for the numbers of granulocytes and lymphocytes. Pair weighed as well as *ad libitum* controls were observed along with the deficient group in order to detect any changes attributable to inanition. Long-Evans strain rats were employed in all experiments. Young animals at 15 days of age as well as adults at 105 days of age were used. The diets and vitamin supplements were as follows: Diet 1. 36% casein, 4% hydrogenated vegetable oil, 4% salt mix(14), 54% sucrose, 1% B-vitamin mixture, and 1% oil soluble vitamin mixture. Each kg of diet supplied the following vitamins: thiamine hydrochloride, riboflavin, pyridoxine and folic acid, 2 mg each, calcium pantothenate and para-aminobenzoic acid, 10 mg each, niacin amide, 6.7 mg; inositol, 250 mg; biotin, 0.27 mg; choline, 500 mg; mixed tocopherols, 100 mg; vit. A, 10000 I.U., and vit. D, 1000 I.U. Diet 2. 50% casein, 5% hydrogenated vegetable oil, 4% salt mix(14), and 41% sucrose. Vitamin supplement: daily dose of thiamine hydrochloride, riboflavin, pyridoxine and folic acid, .02 mg each; calcium pantothenate and para-aminobenzoic acid, .10 mg each; niacin amide, 0.7 mg; inositol, 2.5 mg; biotin, .002 mg, and choline, 5.0 mg. Vit. A and D and mixed tocopherols were supplied separately in amounts of 1000 I.U., 100 I.U., and 3 mg/rat/week Diet 3. 24% casein, 10% hydrogenated vegetable oil, 4% salt mix(14), and 62% sucrose. Vitamins were fed separately in the same amounts given with Diet 2. On the 15th day of age litters were divided and two-thirds of the males were segregated with the mothers. Both received Diet 1. The remaining mothers and young were given the same ration without pyridoxine. On the 21st day of age the young rats were placed in individual metabolic cages and were fed Diet 2. The vitamin supplement was fed separately with pyridoxine omitted from the mixture given to the deficient group. Adult males were fed Diet 3. There were 3 groups, deficient, pair weighed controls and *ad libitum* controls. The deficient group received 30 mg of desoxypyridoxine per kg diet and pyridoxine was omitted from the vitamin mixture. In a second series of 2 groups of adult animals,

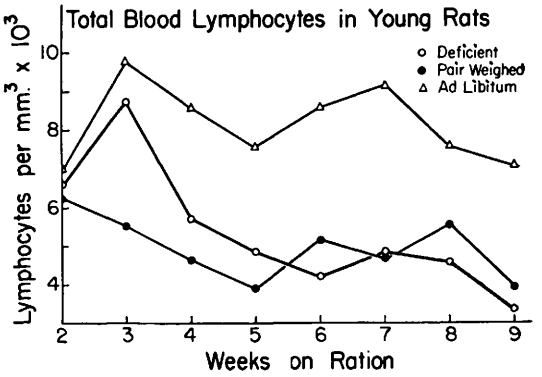

FIG. 1. Each point represents the mean value of 8 animals.

pair weighed and deficient, which had been on Diet 3 for 8 weeks, the group which had previously served as pair weighed controls, were permitted to eat *ad libitum* and at the same time, desoxypyridoxine was omitted from the diet of the deficient animals. The latter were given 5 times the control group level of pyridoxine in their vitamin mixture for a period of 2 weeks and the pyridoxine level thereafter reduced to the amount received by their controls. The 2 groups in the second series were thus restored to full feeding and vitamin adequacy.

Results. An absolute lymphopenia ($p = .01$) of equal magnitude in young pyridoxine-deficient rats and pair weighed controls occurred from the 4th and 3rd weeks respectively through the 9th week (Fig. 1). A less marked but comparable lymphopenia ($p = .03$ to $.05$) was also seen in similar groups of adult animals during the 7th and 8th weeks on the depletion regimen (Table I). The absolute numbers of lymphocytes in the young and adult *ad libitum* groups remained constant throughout the experimental period (Fig. 1, Table I).

An absolute increase of the number of granulocytes ($p = .01$) appeared in the young (Fig. 2) and adult (Table I) pyridoxine-deficient rats as compared with the respective pair weighed controls only at the termination of the experimental periods. In the adult (Table I) and young (Fig. 2) *ad libitum* groups the circulating levels of these cells did not fluctuate significantly at any time.

Thymus weights (Table II) were much less

TABLE I. Leucocyte Counts of Adult Pyridoxine-Deficient, Pair Weighed and Normal Control* Rats during Depletion and after Replacement of the Vitamin and Full Feeding.

Treatment	No. of rats	Weeks on diet	Lymphocytes/mm³ × 10³ Deficient	Lymphocytes/mm³ × 10³ Pair weighed	Granulocytes/mm³ × 10³ Deficient	Granulocytes/mm³ × 10³ Pair weighed
Depletion	8	7	6.8 ± .9	6.5 ± .6	5.9 ± .8	2.8 ± .3
	7	8	6.4 ± 1.2	6.9 ± 1.5	6.0 ± 1.7	2.5 ± .5
Replacement	7	1	8.1 ± 1.0	9.3 ± 1.3	4.5 ± .5	3.2 ± .5
	7	2	7.8 ± .8	8.4 ± .9	2.5 ± .3	3.2 ± .2
	7	3	8.4 ± .9	9.5 ± .2	2.4 ± .4	2.1 ± .4
	7	4	7.9 ± 1.0	8.7 ± .5	2.4 ± .4	2.1 ± .4
	7	7	8.8 ± 1.3	7.2 ± .4	3.0 ± .4	2.9 ± .4

* Values for normal *ad libitum* controls in 7th week were: lymphocytes, 9.0 ± .9 × 10³, granulocytes, 2.7 ± .2 × 10³.

in young and adult pyridoxine-deficient rats than in their respective pair weighed controls ($p = .01$), but thymus weights of both these groups were much less ($p = .01$) than were those of their *ad libitum* controls. Minimum thymus weights occurred at 5 weeks in the young deficient and pair weighed animals after which there was no further decrease.

When pyridoxine was administered to the adult deficient rats and the pair weighed animals were allowed free access to food, the total numbers of lymphocytes and granulocytes (Table I) were restored to normal values within 2 weeks and the thymus weights (Table II) by the 7th week.

Discussion. Dougherty and White(8,9) concluded that the granulocytes were not controlled by the adrenal or adrenocorticotrophic hormones. The present results suggest that pyridoxine may be one of the factors regulating the rate of production, delivery or removal of the granulocytes.

The leucocyte and thymus changes which occurred in the deficient adult animals did not result from a moribund condition or permanent damage to lymphopoiesis. Clearly, pyridoxine deficiency had a specific effect upon involution of the thymus in addition to any effect resulting from inanition.

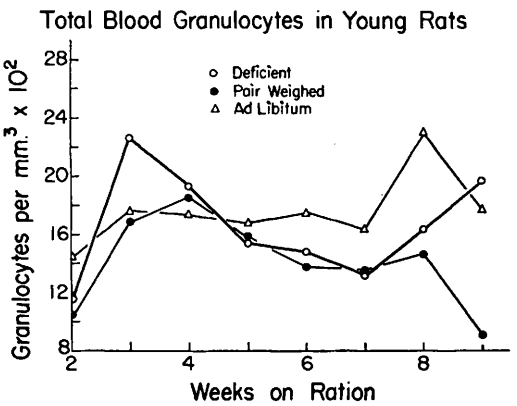

FIG. 2. Each point represents the mean value of 8 animals.

While there was a significant difference between thymus weights in the deficient and pair weighed groups, lymphopenia of the same degree occurred in both groups. There is no evidence, therefore, that pyridoxine deficiency was responsible for the lymphopenia in any of the animals. The lymphopenia in the emaciated pyridoxine-deficient animals was no greater than that caused by inanition alone. The lymphopenia in the deficient animals appears to have been initiated exclusively by inanition, possibly by augmented secretion of 17-hydroxyketosteroid hormones by the adrenal in a stress situation.

Supporting evidence for an increased output of adrenal hormones in similar groups of animals has been reported by us(13); an equally depressed circulating eosinophil level was found in young and adult deficient rats and their respective pair weighed controls. Furthermore, an unimpaired adrenal function in the pyridoxine-deficient and pair weighed adult animals as measured by eosinopenia was demonstrated(15). The possibility, however, that the lack of pyridoxine may have an additional, direct role in the production of lymphopenia is not excluded by the present evidence.

Summary. Specific involution of the thymus resulted from pyridoxine deficiency in rats and could be distinguished from the atrophy resulting from inanition alone. In-

TABLE II. Thymus Weights of Young and Adult Pyridoxine-Deficient Pair Weighed and Normal Rats.

Series	Treatment	Weeks on diet	*Ad libitum* normal		Pair weighed normal		Deficient	
			No. of rats	Wt of thymus, mg	No. of rats	Wt of thymus, mg	No. of rats	Wt of thymus, mg
Young	Depletion	5	11	323 ± 26	12	125 ± 10	9	31 ± 3
Adults I	"	9	5	309 ± 22	5	104 ± 12	5	30 ± 3
	"	8	10	190 ± 17	10	95 ± 9	10	38 ± 4
Adults II	Replacement	7*			7	205 ± 13	7	218 ± 12

* 7 weeks of replacement following 8 weeks of depletion.

anition induced lymphopenia of the same degree in young and adult pyridoxine-deficient animals and their respective pair weighed controls. Granulocytosis occurred only in the pyridoxine-deficient young and adult animals. All blood and thymus changes were rapidly restored to normal following realimentation of the deficient and pair weighed adult animals.

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Effect of Streptococcal Hyaluronic Acid (SHA) in Mice, Rats, Rabbits and Dogs. (20911)

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There are no published data on the toxicological effects of hyaluronic acid prepared from pathogenic bacteria, although pathological changes in tissues of the host have been attributed to the hyaluronic acid produced by invading organisms(1,4).

Materials and methods. The sodium salt of SHA was prepared by Dr. George H. Warren from a virulent strain of *Streptococcus pyogenes*, Group A Type 9 (T9/63/3) obtained from the Hospital of the Rockefeller Institute. It contained 3.67% N, and was free from S, P, and halogen. All solutions of SHA for injection were prepared with physiological salt solution. Autopsies were performed on all the animals listed in Table I, and on a similar number of controls. Sections of heart, lung, liver, kidney, spleen, thyroid, adrenal, pituitary, thymus, bone marrow, pancreas, salivary gland and lymph node from all animals were prepared for microscopic study. In addition, brain and aorta from