oping muscle obtained from rat embryos of 14 days, fetuses of 19 days, rats of 1-2 days and 9-10 days of age. The activity was calculated in micrograms of phosphorus liberated in 15 min. at 37.5° per mg N.

The activity was determined for each of 4 fractions and the sum of these, the activity of the whole homogenate, was in close agreement with previous determinations of the whole homogenates.

All 4 fractions increase in activity during development; the M_1 fraction, containing the purest preparation of the myosin proteins develops in activity 6 times faster than the other fractions.

While the apyrase activity was formerly thought to be limited to the myosin protein fraction this contributes but a minor part of the total activity.

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Role of Pyridoxine in the Production of Leucocytes in Normal and Leukemic Mice.* (17467)

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Morgan, Groody, and Axelrod[1] showed that pyridoxine-deficient dogs developed a leucocytosis with an absolute increase in the numbers of circulating neutrophiles and an absolute decrease in the numbers of lymphocytes. These abnormalities could be corrected by giving adequate doses of pyridoxine. In the same year McCall, Waisman, Elvehjem, and Jones[2] described similar leucocyte changes in pyridoxine-deficient and in pantothenic acid-deficient monkeys. They stated that the reversal of the polymorphonuclear to lymphocyte ratio also occurred in riboflavin deficiency. In the pyridoxine-deficient animals the reversal could not be corrected completely by pyridoxine but could be corrected by whole liver. Wintrobe and his associates[3] observed a tendency of the granulocytes to increase in pyridoxine-deficient swine, but there was no reversal of the ratio of polymorphonuclears to lymphocytes. Stoerk, Eisen, and John[4] described thymus atrophy and a reduction in the number of fixed and circulating lymphocytes, and Stoerk[5] described lymphoid atrophy and regression of rat lymphosarcoma in pyridoxine-deficient rats. In order to facilitate the production of pyridoxine deficiency, Ott[6] investigated the antipyridoxine activity of desoxypyridoxine hydrochloride (2,4-dimethyl-3-hydroxy-5-hydroxymethylpyridine). He found that two molecules of the analogue were sufficient to offset the vitamin activity of one molecule of pyridoxine, as measured by chick growth. Under other conditions[7] much larger quantities of the analogue are necessary. These observations prompted us to investigate the effect of both pyridoxine deficiency and pyridoxine excess on the circulating leucocytes and hematopoietic organs of normal mice and of mice having various abnormalities of the leucocyte pattern.

Pyridoxine Deficiency in Normal Mice.

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1 Morgan, A. F., Groody, M., and Axelrod, H. E., *Am. J. Physiol.*, 1946, **146**, 723.

2 McCall, K. B., Waisman, H. A., Elvehjem, C. A., and Jones, E. S., *J. Nutrition*, 1946, **31**, 685.

3 Wintrobe, M. W., Follis, R. H., Miller, M. H., Stein, H. J., Alcayaga, R., Humphreys, S., Suksta, A., and Cartwright, G. E., *Johns Hopkins Hosp. Bull.*, 1943, **72**, 1.

4 Stoerk, H. C., Eisen, H. N., and John, H. M., *J. Exp. Med.*, 1947, **85**, 365.

5 Stoerk, H. C., *Fed. Proc.*, 1948, **7**, 281.

6 Ott, W. H., PROC. SOC. EXP. BIOL. AND MED., 1946, **61**, 125.

7 Umbreit, W. W., and Waddell, J. G., PROC. SOC. EXP. BIOL. AND MED., 1949, **70**, 293.

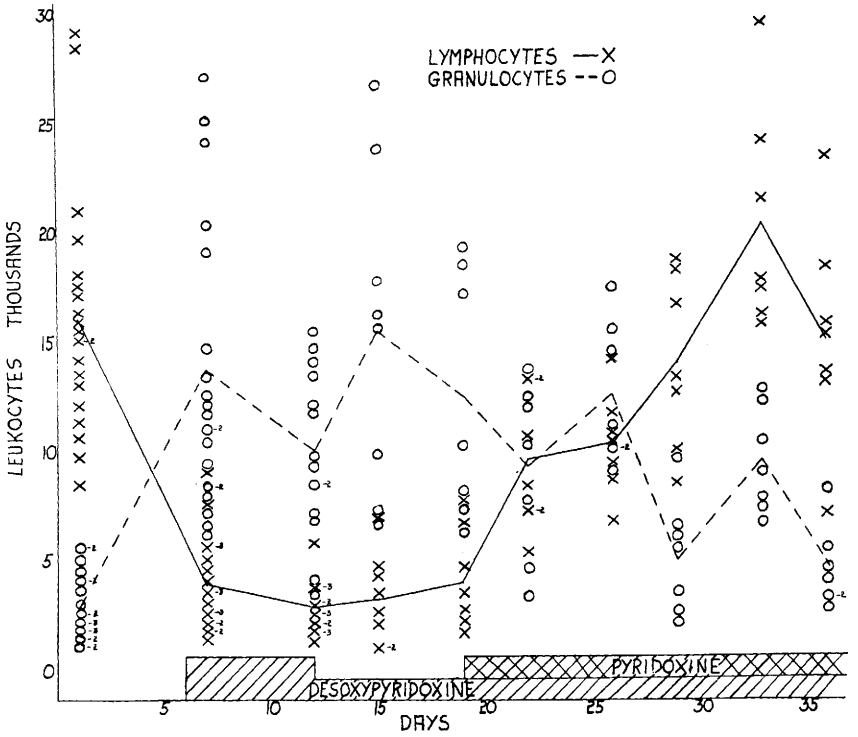

FIG. 1.
Leucocyte changes in pyridoxine-deficient normal mice.

Weanling mice of a Swiss strain were placed in cages with wire-mesh bottoms. They were fed *ad libitum* a modification of a pteroylglutamic acid-deficient diet which has been described previously;[8] pyridoxine and succinylsulfathiazole were not included. The animals were given pteroylglutamic acid intraperitoneally in daily doses of 20 μg. In addition desoxypyridoxine† was given subcutaneously as a 2% solution of the hydrochloride. In a preliminary experiment, daily doses in excess of 1 mg were fatal within ten days. On a 1 mg dose the mean survival time was 20 days and weight loss was marked. Many of the animals developed the expected granulocytosis and lymphopenia but several did not. In a second experiment the animals were given 2 mg daily for a week and then 1 mg daily. Again the leucocyte response was variable and weight loss occurred rapidly. Also the leucocyte changes could not be corrected completely by the administration of pyridoxine. These unsatisfactory results using a purified diet suggested a trial of a crude diet (Purina dog chow) supplemented by larger amounts of desoxypyridoxine given parenterally, as a possible means of producing the desired alteration in the leucocyte pattern. This approach proved successful. Twenty Swiss mice were fed Purina dog chow *ad libitum*. On the 6th day subcutaneous injections of desoxypyridoxine HCl in a 2% solution were started. From the 6th to the 12th day 8 mg were given daily in 2 doses of 4 mg each at an interval of 8 hours. Because of a high mortality in the animals the doses were then halved to supply 4 mg daily, and this was continued to the end of the experiment; during this period only one mouse died. Supplementary pyridoxine HCl (0.2%) was added to the ground diet on the 19th day. Since the mice ate from 3 to 6 g of dog chow daily, they were supplied with 6 to 12 mg of supplementary vitamin daily. Smaller amounts used in preliminary experiments had failed to restore the

[8] Weir, D. R., Heinle, R. W., and Welch, A. D., PROC. SOC. EXP. BIOL. AND MED., 1948, **69**, 211.

† Kindly furnished by Merck & Co., Inc.

leucocyte pattern to normal. Total and differential leucocyte counts were done twice weekly. At autopsy, sections of liver, spleen, marrow, thymus, lymph nodes, and both adrenals were taken for histologic study.

As shown in Fig. 1, a complete reversal of the ratio of granulocytes to leucocytes occurred very promptly after desoxypyridoxine was started, with development of absolute granulocytosis and lymphopenia. Within 3 days after the pyridoxine supplement was started restoration of the normal leucocyte pattern had begun, and was complete 14 days later (36th day). After the reduction of the dose of desoxypyridoxine during the initial period, only one animal died and the others remained in apparent good health. There was no significant weight loss, and they did not develop ruffled fur, convulsions, or obvious neurologic disturbances. Red blood cell counts were not done in this experiment, but, in a preliminary experiment which was conducted under identical conditions except for a lower dose of supplementary pyridoxine, the mean erythrocyte count did not change significantly.

At autopsy on the 36th day no gross or microscopic abnormality could be detected in the tissues. In order to obtain material for histologic study at a time when the reversal of the granulocyte to lymphocyte ratio was established, 20 mice were maintained on the regimen described above, but were not given the supplementary pyridoxine. Fifteen of the 20 survived to be killed on the 37th day, at which time granulocytosis and lymphopenia, with a complete reversal of the ratio, had been present for 32 days. None of these animals showed any evidence of lymphoid atrophy. The thymus glands and lymph nodes were normal in appearance, and the splenic follicles were, if anything, slightly hyperactive. There was no increase in the size of the adrenal glands or demonstrable change in their cytological structure. The marrows showed possible increase in cellularity due to increased numbers of granulocytes without increase in immature cells, although this change was not marked. The liver of one animal that died early showed extensive hemorrhagic necrosis. The liver of another that survived to be killed showed a few small foci of necrosis. There is reason to believe that a generalized tissue deficiency of pyridoxine had not been established. Growth was not impaired, convulsions did not occur, there was very slight anemia, and lymphoid regression or atrophy was not present at autopsy. It is likely that a partial deficiency is sufficient to produce the leucocyte shift without producing other demonstrable changes.

Effect of Pyridoxine Deficiency on the Leukemia of Ak Mice. In mice of the Ak strain the intravenous injection of cells from diseased older animals produces leukemia in 100% of younger animals.[9] In the animals now being bred in this laboratory this is believed to be a mixed type of leukemia, in which both adult granulocytes and adult lymphocytes participate, with the appearance of small numbers of immature blastic cells. About 10 to 15 days after transplanting, the total leucocyte count begins to rise. It remains elevated for 10 to 20 days and then falls toward normal levels shortly before the animal dies. Death usually occurs 25 to 35 days after the transplant. Differential counts show that the initial rise is due to an increase of granulocytes. The ratio of these cells to lymphocytes may be as great as 4 to 1. As the leukemia progresses the lymphocytes increase and, coincidentally with this rise, the granulocytes begin to fall and reach normal levels when the lymphocytes are still elevated. At death any elevation of counts above normal is due to increased numbers of lymphocytes. The immature blastic cells appear in small numbers at the height of the leucocytosis and persist until death. The effect of pyridoxine deficiency on this leucocyte pattern was investigated. Fourteen weanling Ak mice were placed on a diet of Purina dog chow. On the first day all animals were given intravenous injections of a suspension of cells from lymph node and spleen of a diseased older animal. On the 8th day a group of 9 was started on desoxypyridoxine hydrochloride. Each was given 1.5 mg subcutaneously twice daily. Total and differential leucocyte counts were done at the end of the first week and twice weekly

[9] Weir, D. R., and Heinle, R. W., PROC. SOC. EXP. BIOL. AND MED., 1947, **66**, 268.

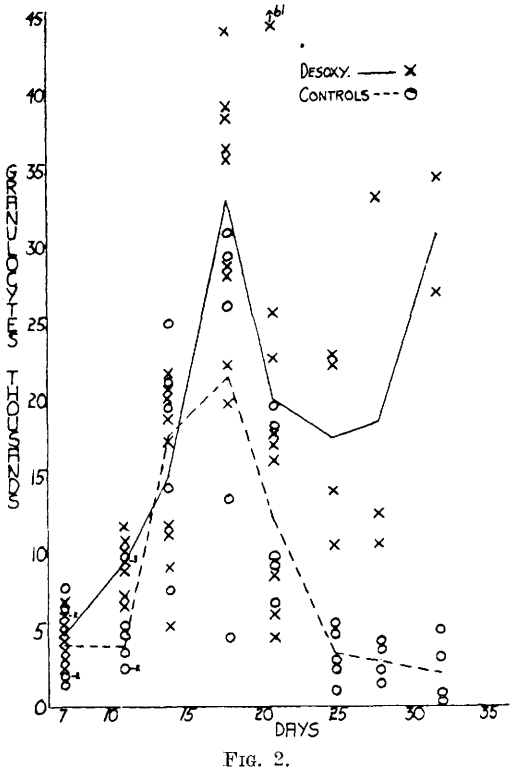

FIG. 2.
The effect of pyridoxine deficiency on the granulocytosis of Ak mouse leukemia.

thereafter. The mean survival time of the animals receiving desoxypyridoxine was 24.6 days as compared to 31.8 days for the controls. This decrease in survival time is statistically significant. The value for P is less than 0.02.[10] The leucocyte patterns are shown in Fig. 2. In the early stages of the leukemia the granulocyte counts of the experimental animals were generally higher than those of the controls. On the 25th day and thereafter all the granulocyte counts of the experimental animals were 2 to 6 times as great as the highest count shown by the controls during the same period. The lymphocyte counts of the experimental animals were generally lower than those of the controls but not significantly so. Histologic study of sections of liver, spleen, bone marrow and lymph nodes taken at autopsy failed to reveal any difference between the experimental and control groups.

[10] Fischer, R. A., Statistical Methods for Research Workers, Oliver and Boyd, Edinburgh, 1934.

Preliminary Studies of the Effects of Pyridoxine in Excess. Groups of normal mice were given parenteral pyridoxine HCl in divided daily doses of 8 and 16 mg for a period of 48 days. None of the animals showed any significant change in the leucocyte pattern. Three groups of 5 Ak mice were injected intravenously with a leukemic transplant. All were maintained on dog chow diet. One group received 2 mg of parenteral pyridoxine HCl in a single daily dose; another group received 4 mg; and the third group served as controls. The pyridoxine supplement failed to influence the course of the leukemia or to alter the histologic findings at autopsy. During the study of a myeloid metaplasia factor from human urine Heinle, Hirschmann, and Wearn[11] observed that the injection of certain extracts of urine from patients with chronic myeloid leukemia, produced a moderate to marked granulocytosis in mice. The effects of excess amounts of pyridoxine on this granulocytosis was studied. Ten adult Swiss mice were given for 10 days daily injections of a urine extract known to be potent. Five of these also were given pyridoxine hydrochloride parenterally in doses of 8 mg twice daily. The mean polymorphonuclear count of the 10 animals at the beginning of the experiment was 3370 per cu mm. At the end of the 10 day injection period the mean for the group receiving pyridoxine was 32,615; for those not receiving pyridoxine it was 43,544. The difference is not statistically significant.

Discussion. The results indicate that a deficiency of pyridoxine causes an increase in granulocyte production in normal mice and in mice dying from transplanted leukemia. It is known that vitamins of the B_6 group take part in transamination and decarboxylation,[12-14] and that the active compound is a phosphorylated form of pyridoxal acting as co-

[11] Heinle, R. W., Hirschmann, H., and Wearn, J. T., Approaches to Tumor Chemotherapy, 1947, 77.

[12] Schlenk, F., and Snell, E. E., *J. Biol. Chem.*, 1945, **157**, 425.

[13] Bellamy, W. D., and Gunsalus, I. C., *J. Bact.*, 1943, **46**, 573.

[14] Gunsalus, I. C., and Bellamy, W. D., *J. Bact.*, 1944, **47**, 413.

transaminase and co-decarboxylase.[15-18] In the intact normal animal the various compounds of the group presumably are freely interconvertible.[19,20] From these considerations, the hypothesis is suggested that the granulocytosis in pyridoxine-deficient mice is due to the failure on the part of the animals to transaminate, decarboxylate, or otherwise modify unneeded supplies of granulocyte-building substances, and that the animals thereupon utilize these unneeded supplies in producing excess numbers of granulocytes. It is not necessary to assume that the reverse would be true. A further hypothesis may be made that the granulocytosis of a disease state such as human myeloid leukemia may be due to a defect in the absorption or utilization of vitamins of the B_6 group. Although freely interconvertible in the intact animal, this may not be true of them in the diseased animal. Further investigations in mice and in leukemic human beings are in progress to test the validity of these hypotheses. It has been demonstrated that adrenocorticotropic and adrenal cortical hormones will cause granulocytosis and lymphopenia in animals.[21,22] In our histologic material we could not demonstrate evidence of adrenal cortical hyperplasia.

Summary. 1) Pyridoxine deficiency in mice produces granulocytosis and lymphopenia. 2). The leukemia of the Ak mice now being bred in this laboratory is participated in by both granulocytes and lymphocytes. In pyridoxine deficiency the leukemic granulocytosis is more severe and the survival time of the animals is decreased. 3) The hypothesis is advanced that the granulocytosis in pyridoxine-deficient mice is due to the failure on the part of the animals to perform metabolic functions in which pyridoxine derivatives play a catalytic role; thus, supplies of compounds used in the formation of granulocytes accumulate, and, as a result, the animals utilize this plethora in producing excess numbers of granulocytes. The implications of this hypothesis are discussed.

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16 Lichstein, H. C., Gunsalus, I. C., and Umbreit, W. W., *J. Biol. Chem.*, 1945, **161**, 311.

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21 Valentine, W. N., Craddock, C. G., Jr., and Lawrence, J. S., *Blood*, 1948, **3**, 729.

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Device for Measuring Blood Pressure in the Unanesthetized Rat. (17468)

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The simple technic for measuring blood pressure in the rat described here consists of placing a cuff permanently around the abdominal arorta and vena cava just above their bifurcations and measuring the blood pressure by means of an oscillometer and mercury manometer.

Description of the Apparatus and Its Use. The aortic cuff is shown in Fig. 1. It consists of a latex bulb with a short stem which is fitted on to a piece of plastic tubing, and a plastic shell into which the bulb is inserted.

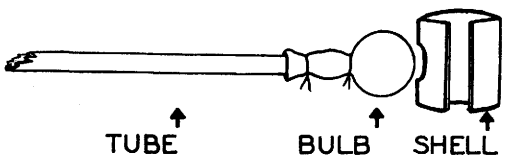

FIG. 1.
The aortic cuff unassembled.