mechanism that resists invasion by foreign cells, which appears to be absent at birth and for a short time thereafter, but which then develops rapidly. The possibility of such a mechanism appears in the studies of Duran-Reynals(3) with birds, those of Gross with mice and in our own with rats.

Summary. Lymphatic leukemia induced in Wistar rats by the gastric instillation of methylcholanthrene, was transferred to 40-day-old animals of our random bred Wistar colony with only indifferent success. When transfers were made to animals not older than 7 days, these were successful in 100% of the animals. Transfer of the leukemia was also made uniformly to equally young animals of the Sherman and Long-Evans strains. The intraperitoneal injection of leukemic spleen cells was used in these experiments.

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Effect of Crystalline Vitamin B_{12} on Experimental Anemia in Mice.* (18332)

P. K. VIJAYARAGHAVAN† AND MAX S. DUNN.

From the Chemical Laboratory, University of California, Los Angeles.

Crystalline vitamin B_{12} has been shown to produce favorable hematological response and general clinical improvement in cases of Addisonian pernicious anemia. It has been reported(1) that vit. B_{12}, when effective, is active in smaller doses than folic acid or thymine but the interrelations between these antianemic substances have not been clearly established. The potency of vit. B_{12} preparations has been determined on the basis of the clinical response of pernicious anemia patients(2,3) and assays have been made based on the growth of microorganisms (*Lactobacillus leichmannii* and *Lactobacillus lactis* Dorner)(4-8).

The experiments reported here were performed to determine the effect of vit. B_{12} on the regeneration of red blood cells and hemoglobin in mice made anemic by the intraperitoneal injection of phenylhydrazine. Phenylhydrazine has been employed as a mild hemolytic agent in *Polycythemia vera*(9). According to Long(10) the mature circulating red cells of laboratory animals and humans are hemolyzed but the erythroblastic elements of the bone marrow are not injured by this drug. In other studies on the hemopoietic activity of proteins and amino acids phenylhydrazine was used to induce severe hemolytic anemia in rats and rabbits(11,12). Damodaran and Vijayaraghavan(13) observed that liver extract (Cohn's fraction G) caused a

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† Permanent address: Assistant Research Officer, Nutrition Research Laboratories, Coonoor, India.

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marked increase in erythrocytes, but not hemoglobin, of rats rendered severely anemic with phenylhydrazine. More recently Nichol *et al.*(14) reported that neither liver extract nor vit. B_{12} stimulated hemoglobin regeneration in chicks which were depleted of folic acid and made anemic with phenylhydrazine. A stimulatory effect was noted when folic acid was administered.

Experimental. The basal diet was of the following composition: Vitamin test casein (General Biochemicals' product) 15, sucrose 71, lard (Swiftning) 8, and salt mixture 6 (15). The mg of vitamin supplements added per 100 g of diet were thiamine .5, riboflavin 1, pyridoxine .5, niacin 10, *p*-aminobenzoic acid 20, inositol 100, folic acid .02, biotin .006, ascorbic acid 40, calcium pantothenate 2, choline chloride 50, vit. K (Menadione) .1, alpha tocopherol 5, vit. A 2000 (U.S.P. units) and vit. D 200 (U.S.P. units). It is probable that vit. B_{12} was present, but at a relatively low level, in this diet.

Adult mice from A strain parents were conditioned by maintaining them on the basal diet for 3 days prior to the injection of phenylhydrazine hydrochloride. On the following (fourth) day the tail was clipped and blood was collected discarding the first 1 or 2 drops. Red blood cell counts were made on blood samples diluted 1:200 using Hayem's fluid and Spencer's bright line counting chamber. Hemoglobin was determined by the alkaline oxyhemoglobin method(16) using a photoelectric colorimeter (Lumetron, Model 400-A).

Anemia was produced by a single intraperitoneal injection of .5% aqueous solution of phenylhydrazine hydrochloride in the dosage (6 to 8 mg per 100 g of body weight) required to reduce the red blood cell count and the hemoglobin concentration to 50 and 60%, respectively, of their normal values.‡ The comparable dosage for rats is 2 to 3 mg. In the first experiment on red blood cell and hemoglobin regeneration 3 μg of vit. B_{12} (Squibb's crystalline Rubramin) were injected (intraperitoneally) daily for 10 days into each anemic mouse (6 control and 7 experimental). In the second experiment 1 to 4 μg of vit. B_{12} (Merck's crystalline product) were injected (intraperitoneally) daily for 4 days into each anemic mouse (5 control and, at each level of vit. B_{12}, 6 experimental).

Discussion. Food consumption averaged 3 to 5 g daily per mouse during the first 4 days following the injection of phenylhydrazine hydrochloride and 4 to 6 g thereafter. There was no significant difference in body weight or food consumption between the control and the test animals during the experimental period.

As shown in Fig. 1 the normal red blood cell count of the control animals was regained on the eighth day and of the vit. B_{12}-treated group on the fourth day after the peak of

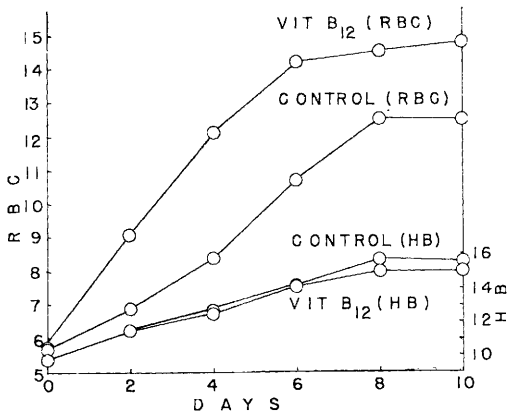

FIG. 1.

Rate of regeneration of red blood cells and hemoglobin in mice with and without vit. B_{12}. R.B.C.—Red blood cells expressed as millions per cu. mm. H.B.—Hemoglobin expressed as g per 100 ml. The values plotted are from the 4th day after injection of phenylhydrazine hydrochloride.

‡ The normal red blood cell count of the mouse was found to average 12.4 (12.0-12.7) millions per cu mm compared to 7.5-8.0 for the rat. The hemoglobin concentration (14.5-15.5 g per 100 ml) is the same for both animals.

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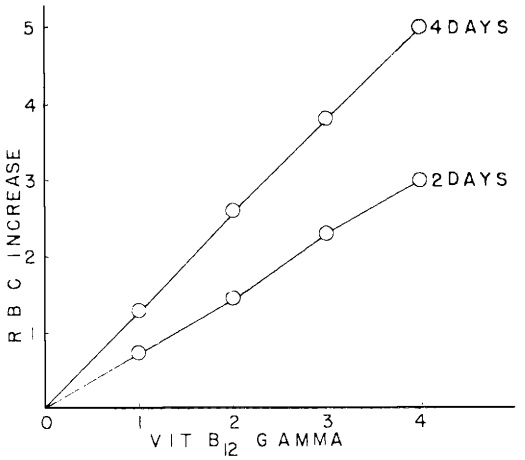

FIG. 2.

Increase in R.B.C. (red blood cells) over control at different levels of vit. B_{12}. R.B.C. count expressed as millions per cu. mm. Each value represents the average from a group of 6 animals.

the anemia. It is of interest that on the sixth day the red blood cell count of the test animals increased to values higher than normal. As indicated the concentration of hemoglobin was not influenced by the administration of vit. B_{12}. Presumably the size of the erythrocytes newly formed under the influence of vitamin B_{12}, the hemoglobin content of the cells, the total blood volume and possibly other factors account for these findings which are in striking contrast to the effects of vit. B_{12} noted in pernicious anemia as well as other types of megaloblastic anemias.

The increases in numbers of red blood cells observed in the test animals at each of the 4 levels of vit. B_{12} for periods of 2 and 4 days are shown in Fig. 2. At 4 days particularly it may be noted that the increase in red cell counts was directly proportional to the concentration of vit. B_{12} administered. These results suggest that an assay procedure for the determination of vit. B_{12} could be developed in terms of the increase in red blood cell counts at different dosages of vit. B_{12}. Experiments toward this end are in progress.

Summary. The number of red blood cells per unit volume, but not hemoglobin concentration, was increased by crystalline vit. B_{12} injected intraperitoneally into mice rendered anemic by the administration of phenylhydrazine hydrochloride. The mice were maintained prior to and during the experiment on a chemically-defined diet probably containing a relatively small amount of vit. B_{12} but adequate quantities of other hematopoietic substances. The increase in red blood cells, particularly during the first 4 days of severe anemia, was found to be directly proportional to the amount of vit. B_{12} administered. These results suggested the possible development of an assay procedure for the determination of vit. B_{12}.

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Some Spectrophotometric Observations on Invertebrate Nerves and their Extracts.* (18333)

A. M. SHANES,† AND T. J. DEKORNFELD.

From the School of Medicine, Georgetown University, Washington, D. C. and the Marine Biological Laboratory, Woods Hole, Mass.

Recent experimental results have led to the suggestion that metabolic reactions may be intimately associated with the subliminal and threshold response of nerve. Optical methods, such as employed by Keilin(1), Urban and Peugnet(2) and Arvanitaki and Chalazonitis(3), appear to offer the means of fol-

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† Present address: Experimental Biology and Medicine Institute, National Institutes of Health, Bethesda, Md.

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