

In the few instances (18) where the amount of serum available was sufficient, adequately controlled complement-fixation tests were performed by the method of Krah and Witebsky,¹⁰ using a lecithinized alcoholic extract of a mixture of 8 known virulent strains of *C. diphtheriae*. Five of these sera gave complete fixation. After absorption with known diphtheria organisms they showed no fixation, suggesting the presence of a specific antibody for *C. diphtheriae*.

Since it has been suggested that immunity to diphtheria is genetically associated with blood groups² we secured Schick data and blood groupings in 17 families (83 persons), but our evidence does not support the view of such a linkage.

Our findings indicate that resistance to diphtheria among Eskimos in these localities depends on the presence of antitoxin and suggest that carrier conditions, as well as latent specific infections, are the probable sources of this immunity.

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Nucleinate-Induced Extramedullary Myelopoiesis.

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Experimental studies¹ established the direct action of the nucleinate molecule and of the nucleotides comprising it on the myeloid centers in bone marrow. Peripheral leucocytoses sometimes reaching 100,000 per cu. mm. and persisting for 3 to 4 days followed large single intravenous dosages (1 gm.) of these materials in normal rabbits.

More recent studies² have shown that daily injections of 0.35 gm. each of the pentose nucleotides in rabbits for a brief period (5 to 7 days) will produce an hyperplasia of the myeloid tissues in marrow, as proved by serial biopsies, with a definite absolute increase in granulocytes in the general circulation. During the succeeding 7 weeks the peripheral count returned gradually to the preinjection level and the bone marrow resumed its normal myeloplasia.

¹⁰ Krah and Witebsky, *Z. f. Immunität.*, 1930, **66**, 59.

¹ Doan, C. A., Zervas, L. G., Warren, S., and Ames, O., *J. Exp. Med.*, 1928, **47**, 403.

² Doan, C. A., *J. Am. Med. Assn.*, 1932, in press.

The nucleinate molecule, thus apparently possessing the property of direct myelopoietic stimulation, has been utilized to determine the inherent capacity of normal rabbit's marrow to respond under circumstances of long continued maximal stimulation. It was desired to test especially the stability of the normal mechanism of maturation and delivery and to see if an uncontrolled marrow hyperplasia with a leukemic arrhythmia in the peripheral blood could be made to supplant the more orderly processes observed in the acute leucocytic responses.³ Or, it was thought that under such a continuous bombardment it might be possible to exhaust the ability of the marrow to respond with the gradual development of a relative hypoplasia or aplasia of the myeloid elements.

After observing 2 normal female rabbits (0-80 and 0-81) for one month, while adequate base line determinations of temperature, weight and blood cytology assured their suitability for this study, the daily intravenous injection of sodium nucleinate was started. The solutions were made up each day in normal saline prepared from fresh glass distilled water. Beginning with a dosage of 50 mg., maintained for one week, the amount was increased to 100 mg. Thereafter the daily dosage was increased 100 mg. each 7 days until 500 mg. was reached. After a 2 weeks' period at this level, the dose was raised by 100 mg. each week until the daily dose was 1 gm. Rabbit 0-81 received in all 56 gm. of sodium nucleinate over a period of 102 days and Rabbit 0-80 received 68 gm. over a period of 116 days before death ended the observations (May 1931). Both animals remained in apparent health throughout the experiment, gaining consistently in weight, in one instance from 1600 to 2300 gm. (0-80) and in the other from 1490 to 2030 gm. (0-81). The rectal temperatures at all times were within the limits of normal for the rabbit (100-102°). From a preliminary level of 3200 to 3900 neutrophilic granulocytes with a total count ranging between 8000 and 10,000 per cu. mm., the highest number of leucocytes recorded in Rabbit 0-80 was 58,300 with 49,500 neutrophils. The preliminary zonal range of neutrophils in Rabbit 0-81 was 960 to 2010 in a total leucocyte count of 6700 to 7400. The highest recorded count of white blood cells in this animal was 45,950 with 36,300 neutrophilic granulocytes. There was no eosinophilia or basophilia in either animal at any time and at no time were myelocytes found in the peripheral circulation. Nor was arrhythmia noted in the delivery of the cells to the blood stream. A more or less marked degree of neutrophilic leucocytosis was maintained through-

³ Doan, C. A., and Zervas, L. G., *J. Exp. Med.*, 1927, **46**, 511.

out the experiment without the development of a tolerance and subsequent lack of response to the later injections of the nucleinate. There was a relative and absolute lymphopenia of moderate grade with an occasional shower of monocytes. The erythrocyte and hemoglobin levels fell gradually in both rabbits from between 5,000,000 and 7,000,000 with 10 to 11.8 gm. of hemoglobin to between 3,000,000 and 4,000,000 and 6.6 to 7 gm. of hemoglobin. The color index at the beginning was 0.60 and at the end 0.66 in Rabbit 0-80 and 0.50 and 0.57 in Rabbit 0-81. That is, the anemia was due to a proportionate fall in hemoglobin and erythrocytes, indicative of mechanical limitation of erythropoietic foci in the bone marrow, such as that found in acute experimental tuberculosis.⁴ Microscopic study of the marrow revealed an extensive myeloid hyperplasia probably sufficient to account for the red cell phenomenon.

At postmortem no gross pathologic changes were noted in any organs or tissues except spleen and kidneys. The spleen in each animal weighed 8.5 gm. but the capsules were not tense and on cut surface there was not the parenchymal bulging with profuse hemorrhage characteristic of an acute splenic tumor. The usual scattered follicles were observed but were widely separated by solid, grayish-pink, cellular tissue.

The left kidney in Rabbit 0-80 weighed 17.5 gm. and the right 18.5 gm., as compared with 18 gm. and 19 gm., respectively, for Rabbit 0-81. The capsule stripped readily and there were no hemorrhages. The usual striations of the cortex were partially obliterated.

The bone marrow of the long bones was pale grayish-pink, solid, not fatty, easily removed in intact pencils for microscopic study. No lymphoid hyperplasia was apparent either in the regional lymph nodes or in the Peyer's patches and diffuse lymphoid tissue of the gastro-intestinal tract.

The bone marrow on microscopic examination showed myeloid hyperplasia with marked diminution of fat. The most striking finding in the spleen was the presence of multiple small foci of myeloblasts and early myelocytes scattered throughout the parenchyma. Sections from the kidneys, more especially those from Rabbit 0-80, likewise showed multiple foci of myelocytes large and small, perivascular and inter-tubular, associated with glomerulo-tubular nephritis. Fig. 1 shows one of the larger periarterial foci in the kidney, Fig. 2 being a higher power of the central portion of the focus

⁴ Doan, C. A., and Sabin, F. R., *J. Exp. Med.*, 1927, **46**, 315.



FIG. 1.

Extra-medullary focus of myelocytes in kidney parenchyma. Rabbit 0-80. $\times 150$.

showing agranular myeloblasts and early myelocytes with varying numbers of specific granules. Neither basophilic nor eosinophilic myelocytes or erythroblasts were observed in any of these ectopic myelopoietic foci. In attributing these findings to the nucleinate molecule it may be remarked that this phenomenon has not been encountered previously either as a spontaneous or induced occurrence in an experience covering 13 years of intensive experimentation upon the cytological reactions of the rabbit.

A careful microscopic survey of multiple sections from the blocks of tissue representing the mesenteric, right and left inguinal and axillary, submaxillary and popliteal lymph nodes, liver, lungs, ovaries, heart muscle, adrenal and thyroid glands, failed to reveal myelopoietic activity or cellular infiltrations. The evidence of definite lymphoid hypoplasia is being investigated further.

Our experiments would seem to suggest 3 tentative conclusions:

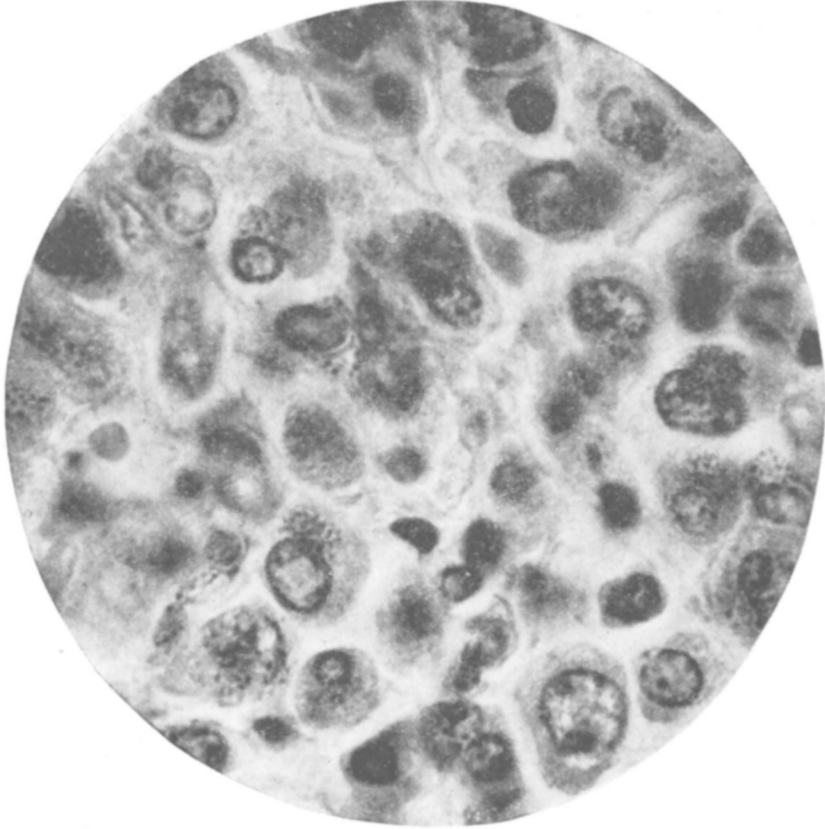


FIG. 2.
Individual myeloblasts and early myelocytes "A", "B", and "C" from the focus in Fig. 1. $\times 900$.

(1) nucleic acid and its degradation products exert an immediate chemotactic effect on normal myeloid foci with a prompt, effective increase in the delivery of mature neutrophils to the circulation under a controlled rhythmic mechanism; (2) a short course of injections of the pentose nucleotides has resulted in the multiplication and maturation of myelocytes in normal marrow, without injury to the integrity of this or other organs of the body, and this reaction has been responsible for the neutrophilic leucocytosis observed in the blood stream; (3) repeated large intravenous injections of the nucleinate molecule have neither tended to exhaust the myelopoietic potentialities of marrow nor to cause a malignant hyperplasia of these elements in normal rabbits. Under the conditions of these experiments, a sufficiently specific and intensive stimulus to myeloid proliferation was present to occasion not only an extensive degree

of marrow hyperplasia but also to actually initiate autochthonous extramedullary myelopoiesis in kidneys and spleen.

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Effect of Light on Rats Receiving a Complete Diet.*

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Conditions in our colony have been standardized as far as practicable. The diet is of the greatest simplicity and constancy.¹ The humidity is not controlled, but is recorded. The temperature is now maintained throughout the year at $26^{\circ} \pm 1^{\circ}$. The light is as uniform as natural light can be, coming through a window across the entire north wall of the colony. No direct sun enters the room.

Even with this uniformity of light from the north sky, it was thought that seasonal changes and location of cages might materially affect our experimental animals. At no time is there any ultra-violet light of antirachitic value in the room since the daylight is filtered through 2 thicknesses of window glass.

It is well known that light 2900-3100 A° in wave length has an effect on the metabolism of animals and is of very great importance if the animal is receiving a poor Ca:P ratio and lacks vitamin D in the diet. The recent work of Clausen² emphasizes the importance of a daily exposure of rats to infra-red radiation if the rats are rachitic. As little as 10 minutes a day of this light from a carbon arc with an energy value of only 0.122 cal/min/cm² was sufficient to markedly affect the rats.

The object of this experiment was to find out whether light materially affects the growth, food and water consumption, ovulation, lactation or bone analysis of rats maintained on our very simple but complete diets.

Albino rats (from our stock colony) were put in the experimental cages at weaning time (21 days after birth). They were kept in individual cages previously described.¹ The maintenance diet consists of pure casein 12.0%, sucrose 84.1%, salt mixture 3.9%,

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¹ Burr, G. O., and Burr, M. M., *J. Biol. Chem.*, 1929, **82**, 345.

² Clausen, E. M. L., *J. Nutr.*, 1929, **2**, 125.