

A STUDY OF THE MECHANISM OF NUCLEINATE-INDUCED
LEUCOPENIC AND LEUCOCYTIC STATES, WITH
SPECIAL REFERENCE TO THE RELATIVE
RÔLES OF LIVER, SPLEEN, AND
BONE MARROW.

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PLATES 21 AND 22.

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The newer concept that there is normally a wide range of fluctuation in the total number of circulating red and white cells (1-5), reopens the whole question of the meaning of variations in blood counts. To many conditions, such as digestion and exercise, and to many chemical substances have been attributed specific changes in the blood count, which now are known to fall within the more recently established limits of the normal. Hence, in distinguishing between the delivery of new cells from marrow or lymph glands, and changes in distribution of cells already within the circulation, it becomes necessary to study all the factors that might be involved. Each particular stimulus under consideration must be known with reference to its particular point and mode of activity. As will be demonstrated in this paper the subject can only be analyzed in the living animal, for the position of cells in the blood vessels after death is no indication of their distribution during life. In the problem it is essential to study the influence of vasomotor reactions on the distribution of blood cells, to consider possible changes in blood volume, and especially to analyze the rôle of various organs on the peripheral concentration of the cells.

The observation by Sabin (6), in a survey of normal living blood cells, of certain "non-motile" polymorphonuclear neutrophils, and the

later determination (1), of their occurrence in showers in the peripheral blood, led to the hypothesis that the effective normal stimulus might be the liberated products from disintegrating cells. This could occur either directly or through the medium of the phagocytic group of cells reducing the debris. The correlation of the showers of "non-motile" cells in human pathological conditions with a subsequent increase of young motile neutrophils (7) further supported the concept. This seemed to point to a thorough restudy of the effect of nucleic acid and its derivatives on the leucopoietic system, inasmuch as these substances must be constantly produced physiologically in the body.

Nucleic acid was first isolated by Altmann (1877) from Miescher's nuclein. Miescher worked chiefly with pus, so readily obtained in those days of septic surgery, and since pus consisted mostly of nuclei, and his minimal residue was cytoplasmic, he reasoned that the material he obtained, which was high in phosphorous content, came from the nucleus, and hence the name "nuclein" (8).

Following the emphasis placed by Metchnikoff (9) on the "phagocytic power" of the polymorphonuclear leucocytes as the *modus operandi* for destroying bacteria, Ames and Huntley (10) were among the first to study nucleic acid experimentally from the standpoint of its apparent production of a leucocytosis. They concluded from the hypodermic injection of nuclein solution into dogs that there was an increase in the number of leucocytes in the central and peripheral circulation, and, further, since they found an increase of young cells, that the response was a true delivery of cells from the bone marrow. Milroy and Malcolm (11), using the sodium salts of yeast and thymus nucleic acid and their decomposition products, thymic acid, adenine, guanine, and cystosine, found after subcutaneous injections in rabbits and guinea pigs what they interpreted as a specific hyperleucocytosis, but the increases were from 5500 to 6800, or from 6000 to 7500, a range of variation hardly permissible of such interpretation today.

The first advocacy of nuclein therapy was on purely theoretical grounds, the claim being made, for instance by Vaughan, Novy, and McClintock (12), that by its use the germicidal power of the blood could be increased and hence the resistance heightened to diseases of microbic origin. However, the effectiveness of nucleic acid, in certain clinical conditions, through its apparent stimulation of an increase in the white count, has been periodically suggested by both physicians and surgeons (13-18), though the increases in white cells, which have been reported, are equivocal, and, for the most part, fall within the physiological range for the white count. Thus Habetin (19), in 1923, advocated the injection of 10 cc. of a 5 per cent solution of sodium nucleinate subcutaneously as a clinical test of bone marrow function, describing the reaction in healthy individuals as an increase of from 40 to 60 per cent of cells, whereas, as is now known, the normal range of

fluctuation is 100 per cent in 24 hours (1, 2). Larsell, Jones, Nokes, and Phillips (20, 21) recently reported an experimental and clinical study of the hemopoietic effects of intravenously injected nucleic acids. In rabbits they found hemopoiesis stimulated by the nucleic acid from the nuclei of bird's cells, and in selected human cases of anemia they describe a comparable but temporary hemopoietic stimulation. Robertson, Hicks, and Marston (22), on the other hand, found no leucocytosis, either relative or absolute, in human subjects on an otherwise purine-free diet, following the oral ingestion of nucleic acids of vegetable or animal origin.

Löwit (23) ascribed the "leucopenia" he found following injections of peptone, nucleins, and sodium urate to a leucolytic phenomenon in the inner organs; the leucocytosis which followed he thought a function of this lysis reflected in a direct response from the bone marrow. These leucopenic-leucocytic fluctuations of the white cells have been the subject of study and conjecture ever since, the mechanism having been in turn attributed to lysis (23), redistribution phenomena (24, 25, 26), positive and negative chemotaxis either within the circulation only (27) or between circulation and marrow.

That additional data relative to the fundamental mechanism of the changes ascribed to nucleinate might be available, the series of experimental observations here reported were begun in the fall of 1924.

Technical Detail.

Sodium nucleinate (Merck) from yeast nucleic acid was made up in fresh, glass-distilled water so that each cc. contained 100 mg. There was no essential difference in the action of two different lots from this source nor in a sample obtained from Dr. Henry Jackson, Jr. The biuret reaction for protein was negative when the test was applied to the sodium nucleinate of these experiments. The adenine and guanine nucleotides first used were prepared by the autoclave method of Jones (28) and made available also through the kindness of Dr. Jackson of the Thorndike Laboratory. Chemically pure, crystalline adenylic and guanylic acids were later supplied through the generosity of Dr. P. A. Levene of The Rockefeller Institute, and used with results entirely comparable in every respect with those obtained from the nucleotide products from the first source. The solutions before injection were brought to pH 7.8 by the addition of N/10 sodium hydroxide. A relatively large dosage was employed, in the majority of instances 1 gm. in 10 cc., in order that the effects, if any, might be decided enough for detection and analysis of the mechanism. Clinically there was considerable variation in the severity of the reaction in rabbits to the intravenous injections of 1 gm. of the sodium nucleinate, a more or less marked vasoconstriction occurring for from 15 minutes to $\frac{1}{2}$ hour, followed by vasodilatation and transitory diarrhea; the temperature registered at times 105°F. (normal for rabbits 102-104°). The injection of 2 gm. of sodium nucleinate intravenously in Cat 1, respiratory and myocardiographic tracings be-

ing recorded on the kymograph, showed a very transitory increase in the rate, but not in the amplitude of the respirations, and a moderate rise in the blood pressure maintained for a somewhat longer period. As the nucleotides are less soluble than the nucleinate in distilled water and tend to form a gel if the proper pH is not maintained, care is necessary in their preparation for intravenous use. However, given in the proper dilutions the guanine and adenine nucleotides did not produce the clinical symptoms of toxicity and fever noted with sodium nucleinate in certain animals.

All counts were made with Bureau of Standards equipment. The hemoglobins were read in a Duboscq colorimeter with the Newcomer standard. The hematocrit determinations were made with the Van Allen pipette. Refractometric readings were made for the serum protein estimations. For the Armeth differential counts cover-slip preparations were stained with Wright's blood stain and 100 cells counted from each slip and the average percentages taken. In some of the experiments the differential counts of the white cells were made with the supravital technic (6) using vital neutral red. The usual routine was employed in the taking of the various consecutive samples (7).

In the experiments in which comparable observations were made from ear vein, heart, and liver, the preparations were taken by three people simultaneously. Through the anterior mediastinum a needle was introduced into the right ventricle; at the same time a small triangle of liver tissue was removed through a small abdominal incision and fixed at once in Helly's fluid for histological study, the count being taken from the blood welling forth from the freshly cut surface. A warm sponge controlled the hemorrhage from the liver, and each succeeding sample was taken from a fresh area far enough removed (2 cm.) so that the former manipulation could not affect the local concentration of white cells. Similar consecutive samples of the spleen were taken for fixation and studied histologically in series, total counts from the splenic pulp being impossible for comparative studies. All such experiments were performed under general anesthesia with sodium-barbital, 0.4 gm. per kilo being injected intravenously prior to operation. Repeated control counts from the peripheral blood were taken before and after the sodium-barbital and during the operative procedures, in order to establish their influence on the cellular equilibrium of the individual animal before any special substances were introduced.

The rabbits undergoing splenectomy were operated upon with aseptic surgical technic, under ether anesthesia, and peripheral blood counts as well as other clinical observations were used as an index of their condition before special procedures were undertaken. In only one instance was appreciable leucocytosis noted following, and apparently related to, splenectomy.

Through the courtesy of Dr. Joseph T. Wearn, it was possible to carry out experiments with the oncometer in which changes in the volume of the spleen, during the reaction to sodium nucleinate, were recorded on the kymograph. Because of the small calibre of the vessels entering and leaving the spleen in the rabbit, it is difficult to secure excursions of systole and diastole, such, for example,

as are observed with the kidney; nevertheless, the pulsations of the arteries entering the spleen were plainly visible through the glass oncometer at all times, the return flow through the veins was unobstructed, a constant temperature was maintained, and the tracings recorded were in every particular identical with those reproduced by Roy (29), Schäfer and Moore (30), and others. Therefore, it is believed that these observations may be taken as at least tending to support the general conclusions with reference to the spleen, that have been reached by the other avenues of approach to the problem.

Forty-one rabbits were studied. Many of them were used for repeated experiments for comparison and contrast of the same and different substances in the same individuals, as may be seen from the charts. Six experiments were performed, in which a survey of peripheral blood, heart, and liver after sodium nucleinate was made every 15 minutes, repeated samples of liver and spleen being removed for histological study. Uncomplicated studies of the peripheral blood after sodium nucleinate were made in six rabbits, after guanine nucleotide in six rabbits, and after adenine nucleotide in three. Splenectomy was performed in seven rabbits; and tracings with the spleen in the oncometer were taken in ten animals to correlate with cellular data, from the peripheral blood, after sodium nucleinate. Correlation of repeated differential counts from peripheral blood and splenic puncture, taken before and after sodium nucleinate, was made in two animals (Table I).

The Bone Marrow during the Reaction to Sodium Nucleinate.

Following the intravenous administration of 1 gm. of sodium nucleinate into normal rabbits, there developed immediately in practically every instance a leucopenia which lasted for several hours (Charts 1 to 3 A). No leucolytic effect could be observed in *in vitro* tests made according to a method previously described (31) and the transitory vasomotor disturbances in the peripheral vessels passed off much sooner than the leucopenia. A leucocytosis, usually of considerable magnitude (50,000 to 100,000), but varying with the individual, succeeded this leucopenic period and in some instances lasted into the 2nd, 3rd, and 4th days (Chart 1). During the leucopenia the fall in total number of cells was primarily due to a decrease in the neutrophilic group (Chart 3), while the leucocytosis reflected greatly increased numbers of this cell type. The basophilic and eosinophilic leucocytes followed the fluctuations of the neutrophilic cells in miniature, disappearing entirely in the leucopenic phase and showing a slight increase in absolute number during the leucocytosis. The lymphocytes, in our experience, may or may not decrease in

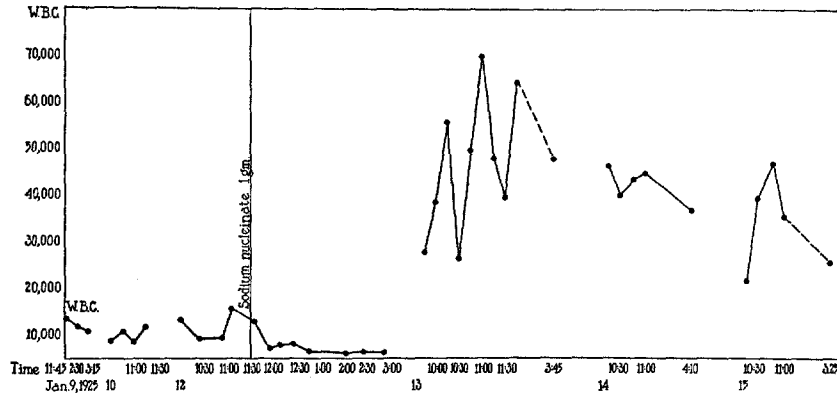


CHART 1. Rabbit 1, T 28. Leucopenia (minimum 2280) induced by 1 gm. of sodium nucleinate, and followed by a leucocytosis (maximum 69,700) on the 2nd, 3rd, and 4th days thereafter.

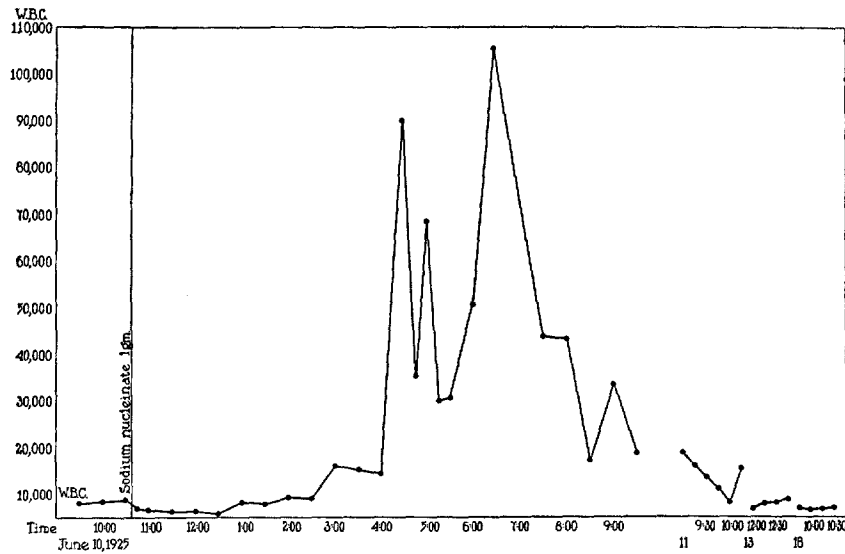


CHART 2. Rabbit 2, T 80. Leucopenia (minimum 1900) after sodium nucleinate which lasted for about 5 hours, with a leucocytosis reaching a maximum of 105,100 8 hours following the injection.

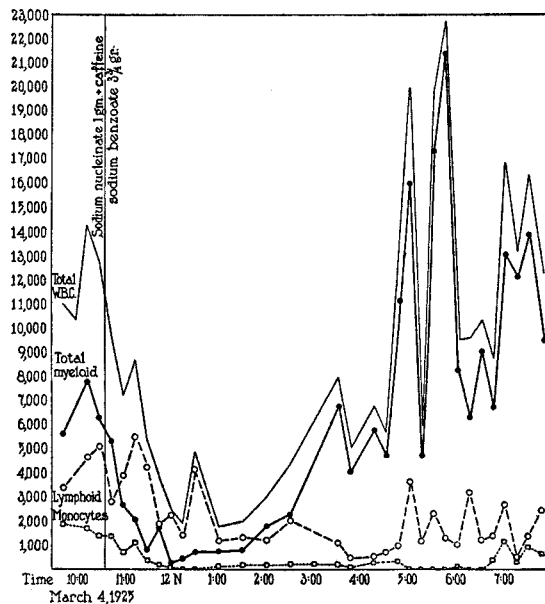


CHART 3A. Rabbit 3, T 35. There is here a depression of lymphocytes and monocytes following sodium nucleinate, but with only a return to normal thereafter, and some animals have not shown the depression in these groups. Both leucopenia and leucocytosis are primarily a reflection of variations in the myeloid group of cells originating in the bone marrow. Differential count of white cells made from cover slip films stained with Wright's blood stain.

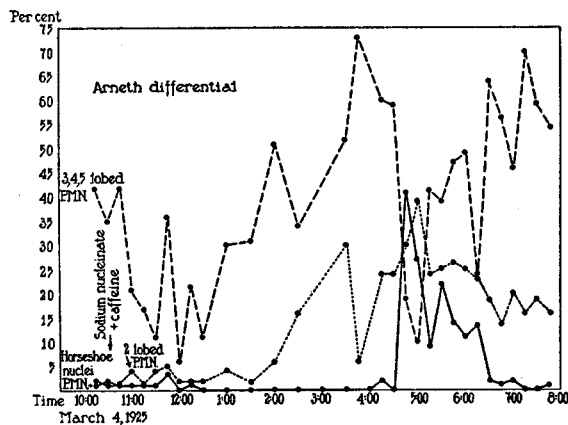


CHART 3B. Rabbit 3, T 35. There is a "shift to the left" in the Arneht pattern first noted 4 hours after sodium nucleinate and coincident with the onset of leucocytosis. This "shift" is indicative of the large part played by the bone marrow in the reaction.

absolute number during the leucopenia, but we have never observed a subsequent lymphocytosis of any moment in this series. The same may be said of the monocytes. A "shift to the left" in the Arneht pattern (Charts 3B, 10B) of the nuclei in the polymorphonuclear neutrophilic leucocytes indicated an increased delivery of cells from the bone marrow as at least one factor of importance in the phenomenon. Histological examination of the bone marrow during the hours just following the sodium nucleinate showed a markedly speeded up process of maturation of Myelocytes "C" (32, 33) into motile leucocytes; a mobilization of these cells about patent sinuses together with active diapedesis characterized the marrow in every instance.

Fig. 1 is a photomicrograph of the marrow from Rabbit 6 (Chart 6) 2 hours after the injection of 1 gm. of sodium nucleinate, while the period of leucopenia still existed in the general circulation, as indicated by counts from liver, right ventricle, and periphery. The sinus is surrounded by a continuous border of encroaching neutrophilic leucocytes in active diapedesis, the open spaces to right and left indicating the gradual depletion of the myeloid reserve of that focus. This appearance is identical with that observed after the injection of inactivated typhoid organisms (Figs. 22 to 24 in a former paper (34)). Fig. 2 shows a marrow after two injections of sodium nucleinate, the second having been given on the 4th day following the first, when the count had regained the normal level. The depletion of the myeloid elements with the beginning displacement of the fat cells preliminary to reparative metaplasia reveals a latent response on the part of the marrow, similar to that when a demand is made for increased cells from the late myelocyte level without the accompanying presence of a maturation factor (33, 35).

Analysis seemed to indicate that the response from the bone marrow to nucleinate preceded the subsidence of the leucopenia. To measure the response and to find if possible the location of the white cells during the leucopenic phase, surveys of certain of the abdominal viscera were undertaken.

The Liver and the Lungs during the Reaction to Sodium Nucleinate.

In none of the experiments was there observed a rise in the cells in the liver during a fall in the peripheral blood, while the circulation of the animal was maintained unimpaired.

Charts 4 to 6 give the concentration of white cells in ear vein, right heart, and liver with the animal under sodium-barbital anesthesia and the abdomen opened

under aseptic precautions. In each instance the leucopenia following sodium nucleinate was registered in the right ventricle and liver as well as in the peripheral blood, with no gross evidence of dilatation of the vessels of the splanchnic area. In both Rabbits 5 (Chart 5) and 6 (Chart 6) the counts in the peripheral blood

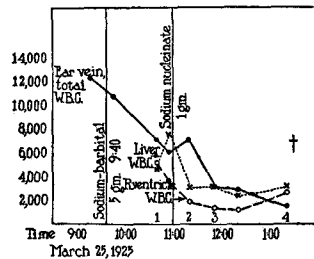


CHART 4.

CHART 4. Rabbit 4, T 49. The leucopenia following sodium nucleinate is the expression of a depression in the total white cells in the general circulation (counts from peripheral vein, right ventricle, and liver). The situation of the digits along the abscissa in Charts 4, 5, and 6 indicates the points at which liver and spleen tissue were removed for histological study.

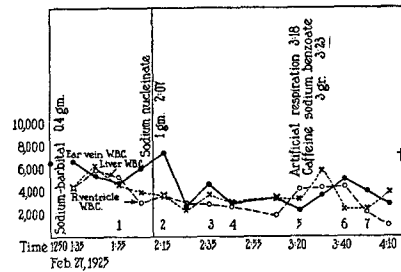


CHART 5.

CHART 5. Rabbit 5, T 37. The changes in total white cells following sodium nucleinate, the institution of artificial respiration, and the administration of caffeine are similar; specimens from periphery, right ventricle, and liver.

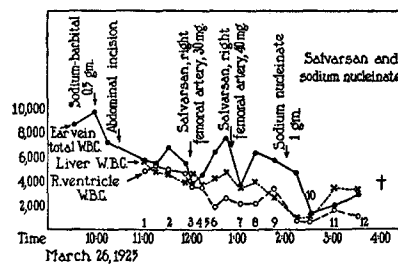


CHART 6. Rabbit 6, T 50. The leucopenia after salvarsan or sodium nucleinate occurs both in the central and peripheral blood; successive simultaneous counts from ear vein, right heart, and liver.

were higher, not lower than the other counts taken simultaneously, but the general trend of the total counts was in the same direction. Two injections of salvarsan in Rabbit 6 gave temporary depressions in the total cells without an increase in cells in the liver, though the transitory leucopenia following such injections has been attributed to this organ (36). The figures along the abscissa in each chart

indicate the points at which tissues from spleen and liver were removed for fixation. The microscopic study of sections of the liver from all the animals of the series showed that there had been no accumulation of leucocytes in the capillary bed of the organ during leucopenia.

Most of the estimations of the local concentration of cells, heretofore, have been made from histological sections of postmortem material or from counts from the liver after the circulation had ceased. In following successive counts from the liver both before and after death the explanation may be seen, perhaps, for the conception, generally current, of the large part played by the liver as a storehouse for the white cells in leucopenia. The profound disturbance in distribution of cells in the circulation at the moment of death makes it essential to take all preparations for total and differential counts of blood cells from organs while the animal is still alive and in good condition, that is to say, not in a state of shock.

In Chart 7 it will be seen that during the leucopenia the white cells in the liver remained low and only began to rise gradually coincident with the rise of cells in the general circulation. However, within 2 minutes of the death of the animal, the total count of white cells in the liver had risen from 11,000 to 37,000; the differential counts of the white cells, on the other hand, at 4:20 and at 4:24 were identical, a finding quite at variance with the increase as analyzed in the peripheral blood during the leucocytosis following sodium nucleinate (Chart 3). In this animal red cell counts were taken from periphery and liver at the same time that the white counts were being noted, in order that any changes in blood volume might be indirectly estimated. The remarkable constancy of the variations in the number of red cells in liver and periphery shows the specificity of the fluctuations in white blood cells.

Rabbit 8 (T 67) was splenectomized. The counts in liver and periphery after sodium nucleinate were 5200 and 8800 respectively. During the experiment the animal died and 2 minutes after death the white cells in the liver were 23,700 per c.mm. In this instance also red cells from liver and ear vein were taken, as in Rabbit 7, and showed the same gradual fall in total numbers per cc. In Rabbit 15 (T 101), uninjected and normal, the total count from the liver just prior to death by air embolism was 6300, 1 minute after death it was 10,300, and 6 minutes after, 19,000. Rabbit 16 (T 91) showed 8000 cells in the liver just before death by air embolism, at the end of an experiment with the spleen in an oncometer during sodium nucleinate leucopenia and leucocytosis, while 2 minutes after death the total white cells in the same organ were 24,000 per c.mm.

Thus, whether under normal conditions, or in the splenectomized animal, whether during the height of the leucopenia or during the

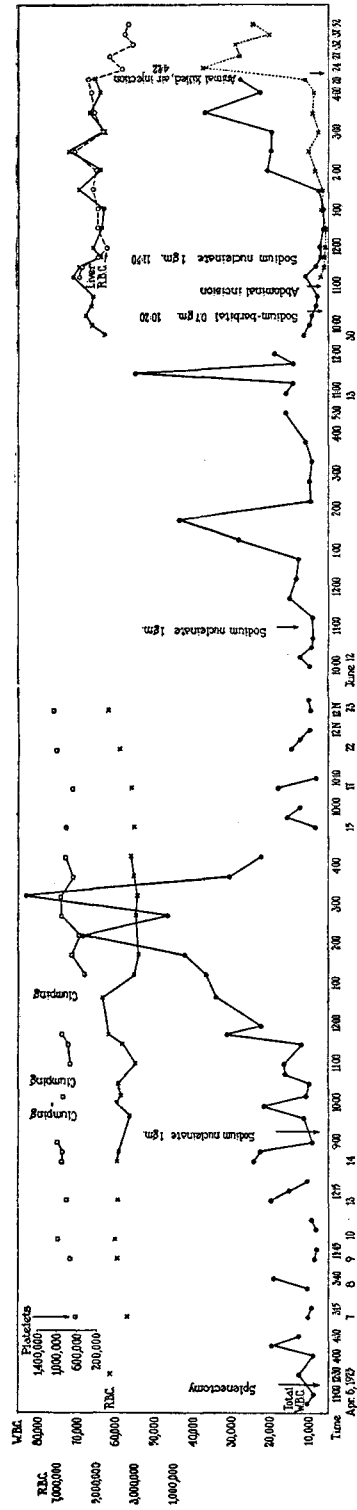


CHART 7. Rabbit 7, T 54. On three successive occasions over a period of 3 months leucocytosis followed within 2½ hours the injection of 1 gm. of sodium nucleinate into the splenectomized rabbit. Platelets and red cells are charted for comparison. On the last occasion red and white cells from liver and periphery were counted and the experiment terminated by air embolism. The immediate change in the concentration of the white cells in the liver after cessation of the circulation is graphically recorded.

leucocytosis after sodium nucleinate, consecutive counts taken from the liver before and after death show the same marked relative discrepancy in total numbers, though not in differential counts. In other words, the sudden increase in total white cells in the liver immediately after death is due to a redistribution of the white cells in the circulation at the moment of death, and not to a sudden influx of new cells from the bone marrow or spleen, as when a true increase in available cells of any type occurs. It would seem, therefore, that all estimates of redistribution phenomena must be made with the circulation unimpaired, and that, under the conditions of the experiments here cited, the changes within the liver blood do not initiate but reflect the leucocytosis following leucopenia after sodium nucleinate and salvarsan. There is within the organ no concentration of cells during the period of depression other than that found in the general circulation. All sections studied in this series showing increased numbers of granulocytes in the capillaries of the liver were taken at a time when there was a leucocytosis in the peripheral blood also. All tissue was taken from the anesthetized living animal and fixed immediately.

Since these observations were made, A. F. B. Shaw (2) and Webb (37) have reported similar studies on leucocytic distribution. Shaw found in normal rabbits and dogs under chloretone a uniform distribution of leucocytes at any given moment throughout the body, *viz.*, periphery, brain, liver, kidney, lung, mesenteric vein, and both sides of the heart. He emphasized the dangers in prolonged experiments of maldistribution of the leucocytes, induced, presumably, by circulatory disturbances following shock, and reported profound inequality in body distribution of leucocytes immediately after death. The problem of leucocytic distribution in the dog's body during horse serum anaphylaxis was studied by Webb, who found the transitory decrease in total cells from the capillary blood of liver, intestines, kidney, spleen, bone marrow, and from portal vein to parallel that of the periphery. This was controlled by the study of sections which revealed no aggregations of leucocytes within the capillaries of these organs. However, from the pulmonary vein during acute anaphylactic shock he obtained fewer leucocytes than from the pulmonary artery, and sections showed enormous numbers of leucocytes in the capillaries of the lungs. This confirms the earlier findings for the lung of Goldscheider and Jacob (24), Bruce (38), Andrewes (39), and others working with protein and bacterial antigens. However, Schilling (40), in studying Oeller's (41) tissues after anaphylaxis, analyzed this finding of specific accumulations of leucocytes in capillaries of the lung as secondary to the enormous local swelling of the endothelial cells. This would seem, therefore, to be a special

phenomenon associated with anaphylactoid sensitivity, the leucopenia being secondary to the mechanical factors involved in local swelling of the endothelium of the pulmonary capillaries. On the other hand, a study of the lungs during the leucopenic phase after sodium nucleinate, or as Wells (42) found after bacterial injections, showed no such accumulations of leucocytes to account for the peripheral leucopenia. Wells studied the leucopenia in rabbits following bacterial injections, and reported the finding of uniformly low counts from the blood of splenic and mesenteric arteries, splenic, portal, hepatic, and superior mesenteric veins, the parenchyma of the lung, both ventricles of the heart, and the marginal ear vein. In sharp contrast were the number of leucocytes obtained from bone marrow and liver, and especially from the blood of the parenchyma of the spleen.

TABLE I.
Relationship of Myeloid Cells in Peripheral Blood and Spleen Following Sodium Nucleinate.

Rabbit No.	Time	W.B.C. peripheral blood				Spleen puncture			
		Total	Myeloid	Lymphoid	Monocytes	Myeloid	Lymphoid	Monocytes	Clasmato-cytes
17 T98	Before Na nucleinate	7000	66	24	10	19	78	2	1
	1½ hrs. after Na nucleinate	6000	47	45	8	40	56	4	0
18 T99	Before Na nucleinate	18000	64	31	5	16	78	1.5	4.5
	1 hr. after Na nucleinate	8400	30	68	2	30	66	2	2
	2½ hrs. after Na nucleinate	35000	82	14	4	48	48	1	3

The Spleen during the Reaction to Sodium Nucleinate.

Having demonstrated that the leucopenia was not due to a stasis of leucocytes in the liver, lungs, or splanchnic area we turned more directly to the spleen as the organ of all the abdominal viscera most likely to be involved in the general phenomena in which we were interested, both from the nature of its anatomical structure and from what is known of its function. Because of the large number of cells in the spleen not in circulation at any given moment, total counts to compare with those from heart and liver were not attempted, but instead the differential count of cells, obtained by puncture with a fine capillary pipette, was made with the supravital technic (43), and compared with the findings in the peripheral blood. With this method

successive splenic punctures during the leucopenic-leucocytic periods following sodium nucleinate showed a gradual but definite increase in percentage of neutrophils in the splenic parenchyma.

It will be seen from Table I that the myeloid cells may be considered to make up from 15 to 20 per cent of the cells obtained under the conditions of splenic puncture. In Rabbit 17 (T 98), 1½ hours after sodium nucleinate, they had increased 21 per cent, or to 40 per cent of all the cells, while the myeloid cells in the peripheral blood had decreased from 66 to 47 per cent. In Rabbit 18 (T 99) the increase of myeloid cells in the spleen was from 16 to 30 per cent in 1 hour after sodium nucleinate, and to 48 per cent in 2½ hours, while the peripheral count fell from 18,000 to 8400 and myeloid cells from 64 to 30 per cent the 1st hour. The total number of lymphocytes in the peripheral blood throughout remained relatively constant though showing percentage fluctuations of 31 to 68 to 14 per cent, again emphasizing the specificity of the effect of the nucleinate on the neutrophilic group of white cells.

The histological evidence from the fixed sections of spleen, removed in series at the times when the specimens of the liver, previously mentioned, were taken, shows a gradual increase in neutrophilic leucocytes in the parenchyma of the spleen following sodium nucleinate.

In Charts 4 to 6 the points at which splenic tissue was removed for section are indicated by the digits along the abscissæ. Fig. 3 represents the normal spleen of Rabbit 4. Fig. 4 shows another portion of the same spleen 1 hour after sodium nucleinate (see Points 1 and 3 as marked on Chart 4). The beginning accumulation of small foci of granulocytes in Fig. 4, more particularly, at this early stage after the nucleinate, near the vessels, may be seen. The diffuse scattering of increased numbers of individual neutrophils throughout the parenchyma of the organ is not so apparent at this magnification. Fig. 5 is a photomicrograph of a section of the spleen of Rabbit 19 (T 31), 8 hours after sodium nucleinate during a leucocytosis reaching 60,000 to 70,000, which had started 3 hours before, after a leucopenic period of 5 hours.

This is representative of several experiments tending to show that the leucocytosis following the leucopenia is not simply the releasing of the cells stored up during the leucopenic period, in other words a temporary redistribution phenomenon, but the result instead of an increased activity of the hemopoietic centers, initiated early and maintained for considerable periods.

The series of oncometer experiments for the study of changes in volume of the spleen during the leucopenia-leucocytosis reaction to sodium nucleinate reveal a definite increase in the size of this organ.

The kymograph records, covering periods of hours, are far too voluminous to reproduce except in very schematic form, but in all particulars they resemble those illustrated in similar studies on the spleen (29, 30). The relative changes in splenic volume, as recorded and synchronized with the total white count from the peripheral blood, are represented diagrammatically in Chart 15. The control period gave the well recognized inherent contractions of the spleen during the base line observations 45 minutes before 1 gm. of sodium nucleinate was injected into the ear vein. Coincident with the onset of leucopenia, the splenic volume started to increase; when the count first began to rise (5.25 p.m.), there was a slight contraction of the spleen, but as the count reached 50,000, the volume again increased, reaching an equilibrium. At a moment when the total count had fallen again to about 10,000, 4 cc. of a 1:1000 solution of adrenalin was introduced into the vein; this was followed immediately by a strong contraction of the spleen and a coincident rise in the white count to 26,400; after the return to the former volume and white count, mechanical stimulation of the saphenous nerve gave a similar synchronous fall in spleen volume and rise in peripheral white count, differing only quantitatively from that previously recorded.

Roy (29), who first adapted the plethysmograph to the investigation of changes in organ volume, described the rhythmic contractions of the normal spleen in dog, cat, and rabbit, and found that stimulation of the central end of a cut sensory nerve caused a rapid contraction of the spleen. Oliver and Schäfer (44) and Schäfer and Moore (30) confirmed this and described an enormous contraction of the spleen after the injection of extracts of the suprarenal gland.

Apparently in a state when the spleen is known to contain large numbers of myeloid cells, as illustrated in the case of Rabbit 19 (Fig. 5), any marked contraction of the organ might be expected to force out neutrophils, even as it does lymphocytes, under adrenalin stimulation in the normal (7). Barcroft's (45) conclusions with reference to the relation of spleen and the reserve of red cells would seem equally apropos for the white cells under the conditions just cited: "It (the spleen) has a function entirely in conformity with its muscular structure, being in fact a reservoir of corpuscles at once fitted by its reticulum to detain them, and by its musculature to expel them when required to do so." de Boer and Carroll (46) further deduced that the material expressed from the spleen was largely corpuscles (red cells) because the CO content of the blood in the splenic vein drawn during a contraction was intermediate between that of splenic artery and pulp.

A study of the platelets in the majority of the experiments here cited (Charts 7, 9, 14) revealed a clumping into large masses quite

impossible to separate for counting, during the period represented by the leucopenic phase. In the spleen an increase in the number of granulocytes phagocytized within the clasmatocytes was noted during their increasing infiltration of the parenchyma. These observations suggest that the nucleinate molecule in some way alters temporarily the surface of these cells, and of the platelets, sufficiently to permit of their adhesion and filtration in the parenchyma of the spleen, but not within the endothelial lined capillary beds of other organs. Those sufficiently injured are taken up by the phagocytic cells so abundant in the spleen normally, the remainder being returned to the circulation at a later time. That the majority of the white cells are not permanently injured or destroyed seems clear. Also the fact that lymphocytes and monocytes remain relatively undisturbed in many of the reactions militates against the concept of a purely mechanical factor in the leucopenia.

The Effect of Splenectomy on the Leucopenia Produced by Sodium Nucleinate.

The crucial test of the importance and extent of the rôle of the spleen in the leucopenia of sodium nucleinate origin is obviously to be found in observations after splenectomy. A series of splenectomies were accordingly performed before, during, and after sodium nucleinate administration.

No depression or shock, such as that described by Larsell, Nokes, and Phillips (20), was noted in the splenectomized rabbits after nucleic acid. Chart 8 shows the leucopenia following 1 gm. of sodium nucleinate with the removal of the spleen at the time which, in the majority of the animals, proved to be about the middle of the period of depression in the peripheral count. A leucocytosis reaching 52,000 was immediately precipitated. It will be seen from Charts 7, 9, and 10 A that aseptic splenectomy does not cause such a rise in the white count *per se*.

Partial splenectomy, as performed in Rabbit 9 (Chart 9), did not prevent the usual leucopenia, but after complete removal of the remaining portion of the organ at a later operation there was not the characteristic leucopenia with sodium nucleinate in the original dosage, and the leucocytosis followed more speedily, the highest count reaching 110,000. In this instance the lowered platelet count was due to the agglutination of the platelets and not to an actual decrease in their numbers, and the red counts indicate indirectly the relative constancy of the blood volume. Rabbit 7 (Chart 7) showed the onset of leucocytosis after splenectomy

within 2½ hours of the sodium nucleinate injection on three different occasions over a period of 3 months. On all three occasions the tendency of the white count to maintain its normal rhythmic equilibrium undisturbed until the real increase was apparent from the bone marrow was clear from the preliminary counts, which may be taken as an indication that the latent period of response for the hemopoietic organs under this stimulus in this individual was between 2 and 3 hours. Here again the clumping of the platelets was found but without an accompanying leucopenia as in the normal rabbits, and the close conformity of red cells from marginal ear vein and liver indirectly controls the estimation of blood volume and stasis factors.

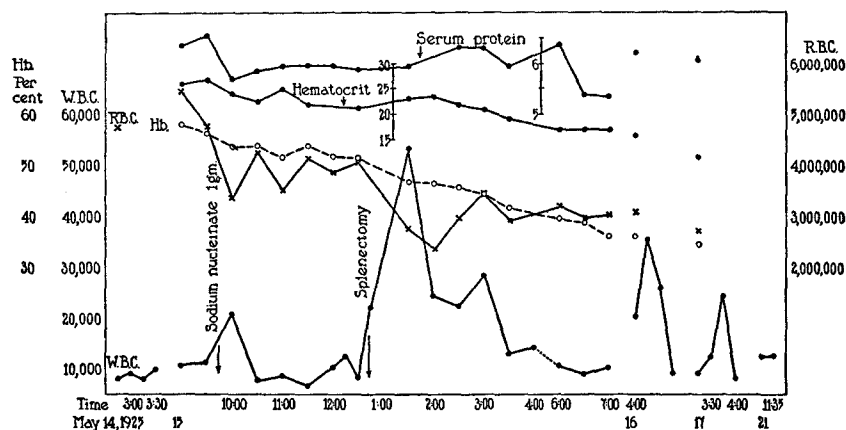


CHART 8. Rabbit 8, T 67. Splenectomy in the midst of the leucopenic period after sodium nucleinate was followed immediately by a leucocytosis reaching 55,000, a high zonal range for the white cells being maintained on the succeeding 2 days. Such a leucocytosis does not necessarily follow splenectomy, as indicated on Charts 7 and 9. Serum protein, plasma-cell ratio, hemoglobin, and total red cells are recorded as an indirect control of changes in the blood volume.

In Rabbit 10 (Chart 10A) the latent hemopoietic response to sodium nucleinate 3 days after splenectomy occurred in 1 hour and 40 minutes, and again, 18 days after, in 2½ hours, with no tendency to leucopenia comparable with that observed under identical conditions in the normal animal. Chart 10B shows the Arnetz differential count of the neutrophilic leucocytes during the first experiment with the nucleinate, the "shift to the left" being apparent within an hour and marked at 2 hours. Thus the response of the bone marrow to a chemotactic stimulus, to be discussed later, was here apparent in the total count within 2 hours and qualitatively within an even shorter period. On Chart 10A in the second experiment (May 5) are recorded the complete series of observations including serum protein, cell-plasma ratio, red cells, and hemoglobin taken coincidentally with the white cells

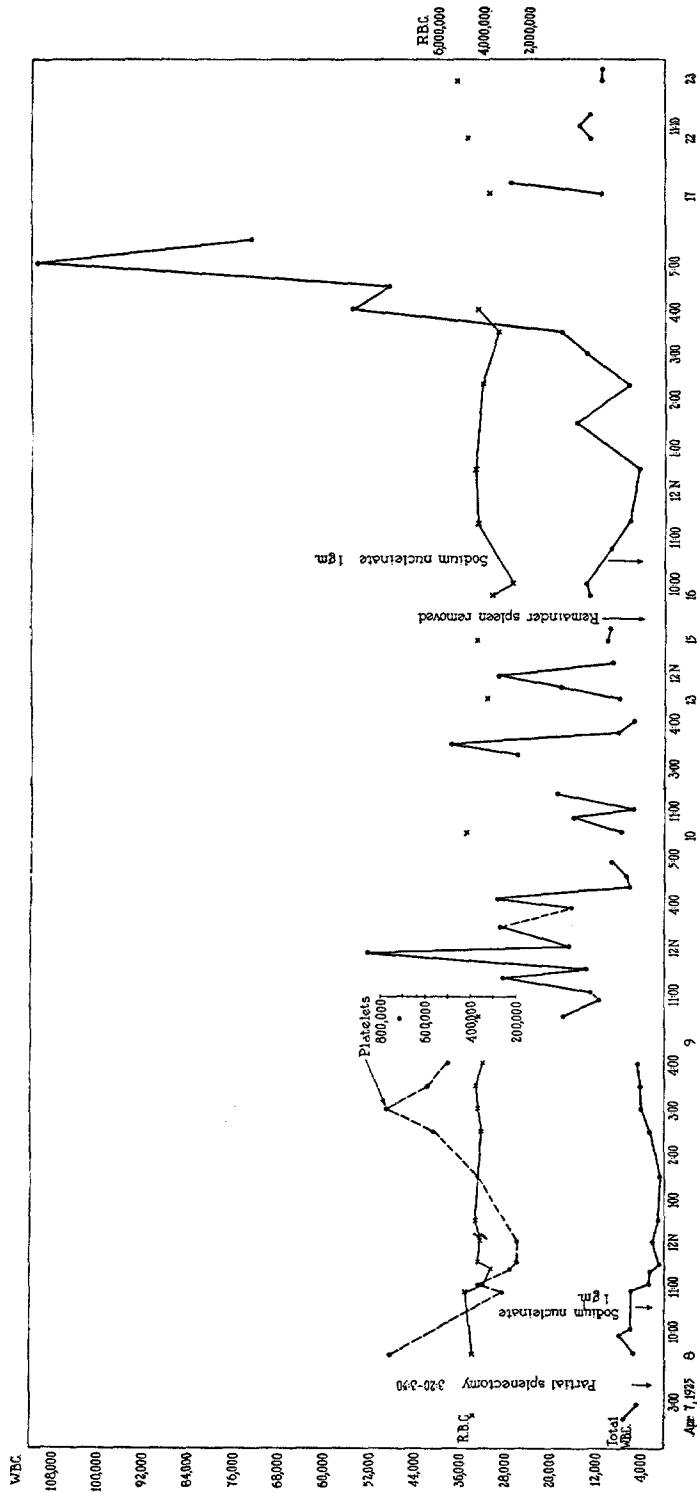


CHART 9. Rabbit 9, T 57. Partial splenectomy did not eliminate the leucopenic period of 6 hours after sodium nucleinate (minimum 1550), but removal of the remainder of the spleen at a second operation did. A total count of 111,000 was reached 6 hours after the same dosage of sodium nucleinate without the development of a comparable severe leucopenia preceding. The red cells, and the fall in platelets due to clumping, are recorded.

and indicating the specificity of the leucocytic response. In order that the condition existing during the leucocytosis so frequently observed on the day following the sodium nucleinate injections might be observed, an abdominal incision was made under sodium-barbital anesthesia, and total red and white counts taken simultaneously from liver, right ventricle, and ear vein. It will be seen that the red cells from the three sources showed comparable numbers and fluctuations, but that the white cells in the periphery showed fluctuations entirely independent of the liver, even in the absence of the spleen.

With the spleen eliminated from the rabbit sodium nucleinate, under the conditions outlined, repeatedly produced a leucocytosis, without preceding leucopenia, within a period of time one-half to one-sixth of that required in animals in which the spleen was intact, the latter invariably showing a profound leucopenia immediately following the nucleinate injection.

Scheunert and Krzywanek (47), following the work of Barcroft and his associates, have shown that for 1 year after splenectomy there is no change in erythrocyte volume after exercise, and they conclude that the spleen is the only organ which regulates the number of circulating erythrocytes. With reference to the white cells, Viale (48) has recently reported experiments with direct electric stimulation of the spleen of the dog exposed at laparotomy under chloral narcosis. The occurrence, immediately following faradic stimulation, of a leucopenia in the blood of the splenic vein with the differential count unchanged, and without changes comparable in direction or degree in the red cells, led him to ascribe the phenomenon to a contraction of the spleen with adherence of white cells to the vessel walls. Direct mechanical massage of the spleen gave no changes in red or white cells. He describes the spleen as an organ of mechanical regulation of the number of both red and white cells. Soler (49) found, on the basis of postsplenectomy leucocytosis, a capacity inherent in the spleen for selective regulation of the circulatory corpuscular elements. He proposes the term "sissoressi" from the Greek meaning "I accumulate." However, no critical analysis was made of the mechanism or cause of the leucocytosis he observed, and in our own experience aseptic splenectomy is not followed necessarily by a leucocytosis.

That the spleen may, under certain conditions, act as a temporary reservoir for myeloid white cells and thus exert something of a regulatory function, more or less beneficial, over their availability to the general circulation and tissues seems clear. As Krumbhaar (50) has pointed out in reviewing the anatomy of the spleen, "the shunts through the pulp offer splendid opportunities for storage and cellular metabolic mechanisms and in fact clinical evidence indicates that at times

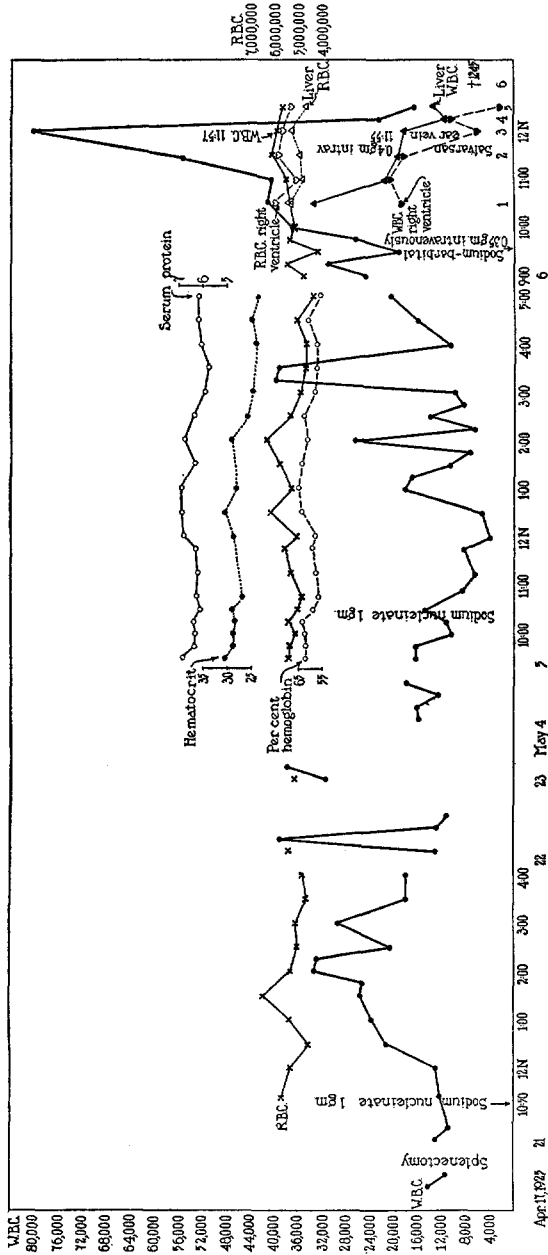


CHART 10A. Rabbit 10, T 62. Within 2 hours sodium nucleinate had given rise to a leucocytosis without preceding leucopenia in this splenectomized rabbit. Fourteen days later a similar leucocytic response was elicited within 2½ hours, the maximum counts, however, not being reached until the 2nd day. At this time successive total red and white counts were taken simultaneously from periphery, right ventricle, and liver. The counts on the red cells from the three sources run closely parallel, but those of the white cells from the peripheral vein show much greater fluctuations than those from ventricle and liver.

the backwaters thus afforded may become a positive disadvantage." The leucopenia of splenic origin after sodium nucleinate represents such a "positive disadvantage." On the other hand, it is just as clear that the spleen is not the sole regulatory mechanism for white cells in the body and that its function in this direction is accessory rather than primary. In the case of the leucopenia following injections of sodium nucleinate in the normal rabbit, the spleen seems to be the only organ involved, but that this may not be true under different conditions,

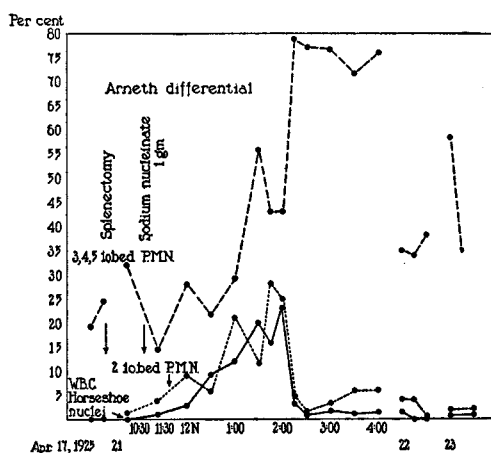


CHART 10B. Rabbit 10, T 62. In this instance the "shift to the left" in the Arneth pattern for the neutrophilic leucocytes is evident sooner than it was in the normal rabbit (see Chart 3B), and coincides with the earlier appearance of leucocytosis in the splenectomized animal. The speed of the response from the bone marrow may be deduced from the "shift" in the Arneth curve, in animals in which the spleen does not act as a reservoir for the white cells.

has been shown in the work already cited on the mechanism of leucopenia in anaphylaxis.

The Effect of Adenine and Guanine Nucleotides.

With the thought that the factor responsible for this stimulus to the neutrophilic leucocytes and apparently acting directly on the hemopoietic tissues, might reside in one of the nucleotides of which the more complex nucleinate molecule is composed, and that the cause, mechanical or chemical, of the leucopenia might be eliminated, experiments

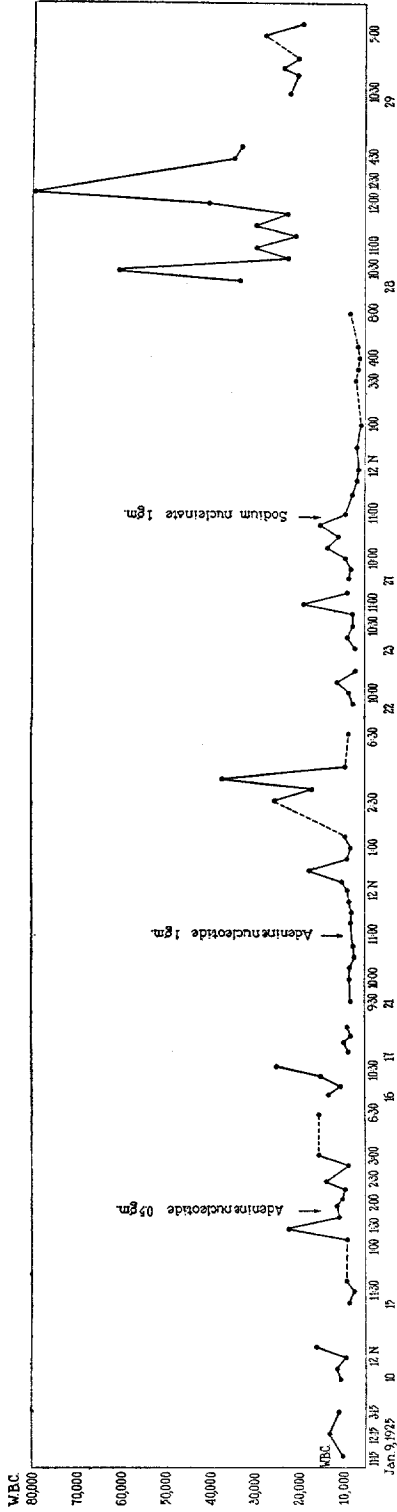


CHART 11. Rabbit 11, T 27. Adenine nucleotide (Dr. Henry Jackson, Jr.) in a dosage of 0.5 gm. was ineffective. One gm. in the same animal 6 days later produced an appreciable rise over the preliminary level within 1½ hours, and after 4 hours a count of 37,100. After another 6 days 1 gm. of sodium nucleinate induced a typical leucopenia (minimum 2900) lasting for more than 9 hours, a leucocytosis (maximum 79,500) following on the succeeding 2 days.

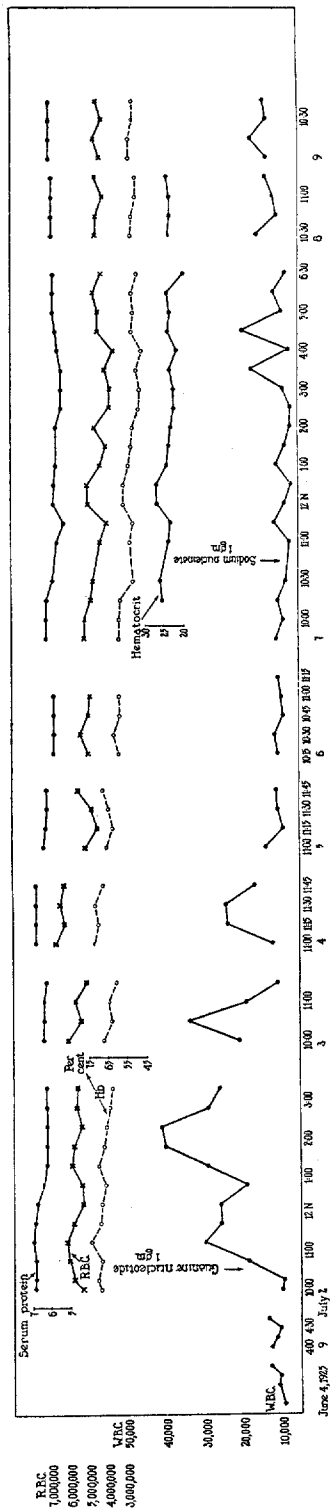


CHART 13. Rabbit 12, T 77. One gm. of guanine nucleotide produced an immediate rise in the total white count, a maximum of 41,000 being reached in 3¼ hours, an increase maintained for the 2 days following. One gm. of sodium nucleinate 5 days later gave the usual leucopenia (minimum 4700), but the leucocytosis following was only moderate in degree because of slow replacement of Myelocytes C after the preceding depletion. Both the magnitude of the initial response and the rapidity of replacement of myeloid cells show variations with individual animals. Observations on the total red cells, hemoglobin, cell-plasma ratio, and serum protein show indirectly that the blood volume did not change.

were undertaken with adenine and guanine nucleotides, the only immediate split products of nucleic acid available at the moment.

Chart 11 records graphically the reactions to different dosages of adenine nucleotide and to sodium nucleinate in Rabbit 11. One-half gm. of adenine gave no detectable increase of cells on the 1st day and only a slightly suggestive response on the 2nd day. One gm. of the same substance 6 days later showed an appreciable rise above the control counts within 1½ hours of the injection, and a leucocytosis reaching 40,000 between 2 and 4 hours after. The same animal, when

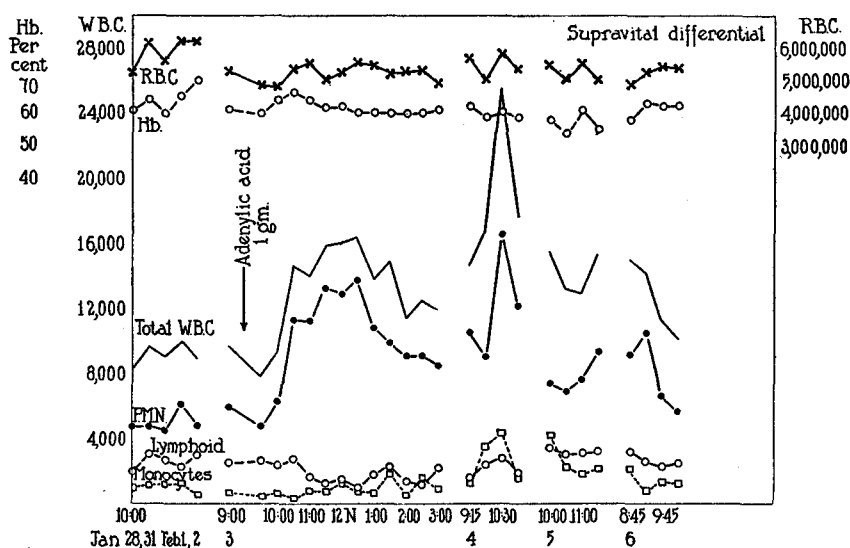


CHART 12. Rabbit 23, R 409. One gm. of adenylic acid (Dr. P. A. Levene) initiated a leucocytosis of neutrophilic origin within 1½ hours, the curves being identical in type with those produced by the guanine nucleotide in Charts 13 and 14. Differential counts of the white cells made with the supravital technic; slight lymphopenia, no lymphocytosis; monocytic rise an individual rather than a specific response probably; red cells and hemoglobin within normal limits.

given 1 gm. of sodium nucleinate 6 days after the second dose of adenine, showed a typical leucopenia lasting for more than 9 hours with a leucocytosis the 2nd day reaching 80,000.

One gm. of adenylic acid in 15 cc. of distilled water, with just enough $N/10$ NaOH to put it into solution, produced a slight increase in the neutrophilic leucocytes within 45 minutes that was well marked in 1½ hours (Rabbit 23, R 409, Chart 12). The leucocytosis was maintained during the succeeding 3 days, the highest count being recorded on the 2nd day. The differential count of the white cells made

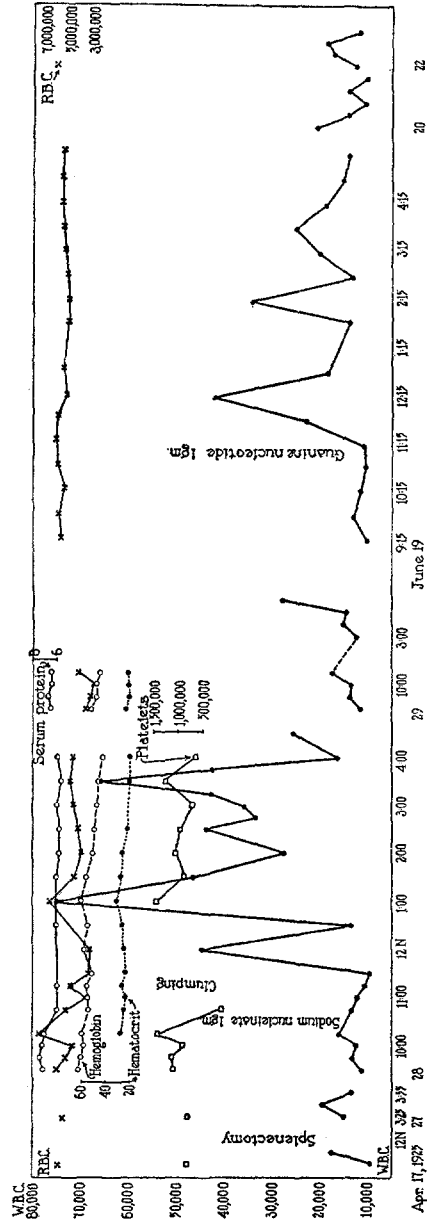


CHART 14. Rabbit 13, T 61. After splenectomy the response to sodium nucleinate and guanine nucleotide gave counts of 45,300 and 42,600 respectively within 1 1/2 hours. The nucleinate gave the highest counts (maximum 75,200). Note the clumping of the platelets after sodium nucleinate during the time when leucopenia is usual in non-splenectomized animals.

with the supravital technic showed the specificity of the effect on the myeloid cells with the maximum a threefold increase in polymorphonuclear neutrophils. The neutrophilic leucocytes showed unusually active motility, there was a definite increase in the percentage of younger forms, and Myelocytes C and metamyelocytes were recorded in four of the counts on the day of the injection and in one count on the 2nd day. The lymphocytes showed a slight depression on the 1st day, but no lymphocytosis followed. The monocytes in this instance were increased in total number, particularly on the 2nd and 3rd days, but this would appear to be an individual idiosyncrasy of the animal instead of a part of the usual response. Red cells and hemoglobin remained within the normal limits of variation. There was no temperature reaction.

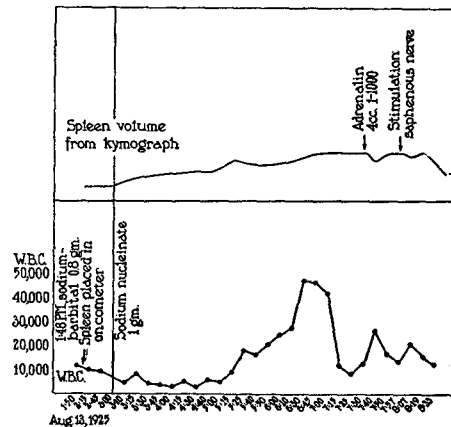


CHART 15. Rabbit 14, T 108. During the leucopenia following sodium nucleinate the spleen increases in volume. This increase is maintained through the initial period of leucocytosis, at least, and correlates with the numerical and histological evidence of greatly increased numbers of neutrophilic leucocytes in the splenic pulp. Coincident with the splenic contraction after adrenalin and after mechanical stimulation of a sensory nerve there was, in each case, a transitory increase in the white cells.

On Chart 13 may be followed the contrast in the effects of guanine nucleotide and sodium nucleinate in the same individual. The response after the administration of 1 gm. of guanine nucleotide was immediate, as evidenced by the control counts preceding. The leucocytosis reached 40,000 within 3 hours and a high count was maintained for the succeeding 2 days, the normal range being reached only on the 4th day. Five days after the guanine 1 gm. of sodium nucleinate was given, which resulted in a leucopenia lasting for more than 4 hours, the leucocytosis following being relatively low. This illustrates a latent period in the replacement of Myelocytes C after their depletion in response to the first chemotactic

substance, during which period a second chemotactic stimulus is relatively ineffective. Serum protein, red cells, hemoglobin, and cell-plasma ratio were followed in this animal, as in others, and showed no appreciable difference with the two substances. Guanylic acid in a dosage of 450 mg. (Rabbit 24, R 410) produced a proportionate immediate increase in the neutrophilic leucocytes identical with the curves shown on Charts 13 and 14 where guanine nucleotide was used. No temperature or constitutional reaction was noted.

Chart 14 shows similarity of the response to sodium nucleinate and guanine nucleotide in a splenectomized rabbit. Within $1\frac{1}{2}$ hours after sodium nucleinate the count was 45,000, while within 45 minutes after guanine nucleotide the count was 23,000, and at $1\frac{1}{4}$ hours 42,000. The highest point reached under the sodium nucleinate was 75,000, $2\frac{1}{2}$ hours after the injection, the highest point in this animal under guanine was 42,000, reached after $1\frac{1}{2}$ hours.

Both adenine and guanine nucleotides from two sources, in a dosage comparable to that used with sodium nucleinate, gave leucocytosis without preceding leucopenia, the effectiveness of the response being noted within the usual period of leucopenia after nucleinate. The leucopenia, described earlier, is, therefore, associated only with the more complex molecule, while the specificity of the stimulus to the neutrophilic leucocytes is retained by the less complex split products. In brief, the response of the splenectomized rabbit to sodium nucleinate is the same as that of the normal rabbit to the nucleotides.

The Effect of Sulfur and Peptone.

Since flowers of sulfur in olive oil given subcutaneously and peptone (Witte) given intravenously, have been used clinically to induce a leucocytosis, they were administered under controlled conditions to compare with the response to sodium nucleinate.

Fourteen mg. of flowers of sulfur in 2 cc. of olive oil were injected intramuscularly into Rabbit 20 (T 36). From a preliminary base line of 5500 to 7600, the counts rose to 10,000 and 11,000 1 hour and $1\frac{1}{4}$ hours, respectively, after the injection. These two counts were the only ones throughout the day going above 8700 and none were lower than 5500. This is well within the range of normal fluctuation for white cells in a rabbit in which counts are made every quarter hour during the day.

The injection of 1 gm. of Witte's peptone intravenously in Rabbit 21 (T 38) was followed by a fall in the white count from a preliminary zonal level of 9400-10,900 to 5200 in 7 minutes, coincident with a vasoconstriction; 15 minutes later, however, coincident with vasodilatation, the count was 10,400, and the count for the remainder of the day fluctuated between 6000 and 12,400.

The degree and the nature of the effects of nucleinate and nucleotides seem to be quite distinct from those observed in these last instances.

DISCUSSION.

The foregoing experiments reveal a prompt unequivocal response of the bone marrow, in the delivery of new mature neutrophilic leucocytes to the circulation, after the introduction intravenously of large doses of sodium nucleinate. However, there is a latent period in the replacement of the cells at the level of the Myelocyte C, in bone marrow, as shown through the inability to reproduce a comparable leucocytosis after too frequently repeated injections. Examination of the bone marrow confirms the rapid depletion of the myeloid foci followed by only a gradual replacement. Thus sodium nucleinate and adenine and guanine nucleotides contain stimuli capable of calling forth new neutrophilic cells so long as there is an adequate reserve of myeloid cells at the level of Myelocyte C, but they lack any factors specifically active for the maintenance of this level.

Ehrlich (51) defined chemotaxis as the attractive force, from microorganisms or toxins, for bringing cells from the large reserve always found in marrow. This term Cohnheim had acquired from the botanist Pfeiffer and carried over to the reaction involving the cells in inflammation (40). The first suggestion of a physiological factor with such potentialities was made by Horbaczewski (52) who correlated supposed digestion leucocytosis with an increase in the uric acid of the blood. However, Ewald (53) found uric acid relatively inactive in dogs in contrast to thymus and yeast nucleic acids and xanthine and guanine, the latter all inducing appreciable leucocytosis. Muir (54) assumed from his study of the marrow either a hyperplasia of myelocytes secondary to the exodus of leucocytes into the blood on the principle of overregeneration after repeated loss, as suggested by Weigert, or that the chemotactic substances directly stimulate cellular proliferation.

Sabin and Doan (33) have recently presented the reactions of bone marrow in terms of two factors, first a chemotactic factor (C) which acts directly on the myelocytes of Type C with an immediate response to a demand for increased leucocytes, such as that exemplified in the response to sodium nucleinate or guanine within 45 minutes to $2\frac{1}{2}$ hours as here described; second, a maturation factor (M), not necessarily in association with the chemotactic factor, but which is

necessary for a maintained leucocytosis, through an active regeneration of myelocytes in the marrow without the latent period involved in Weigert's principle. Sabin (35), in a current review on bone marrow, discusses these factors in detail and presents a new basis upon which to approach the whole problem of the chemical control of the maturation and delivery of red and white cells from the hemopoietic centers. On such a basis as she formulates, it may be that nucleic acid and its immediate derivatives will be found to be important chemotactic factors used physiologically by the body in the maintenance of the normal equilibrium between supply and demand and even in pathological states. That a so called maturation factor is lacking in this group of substances would be the only conclusion justified from the data thus far accumulated. The myelocytic replacement here seems dependent only upon the principle of "overregeneration after repeated loss." Bacon, Novy, and Eppler (55) ascribe the leucocytic response in infection to non-specific stimuli arising from a quantitative alteration in the body metabolism rather than directly from infecting organisms, in other words, a response to the same stimuli which on a smaller scale produce the variations of normal values in the absence of infection. Under such a concept the importance of nucleic acid and of all the possible physiological stimuli is obvious.

SUMMARY.

The leucopenia induced by sodium nucleinate has been followed by repeated counts made simultaneously from the blood of a peripheral vein and from the internal organs, combined with a study of histological sections of the same organs taken with the counts. Measurements with the oncometer of changes in volume of the spleen have been correlated with the leucopenia and the leucocytosis following sodium nucleinate. It has thus been determined that the leucopenia is not the result of a vasomotor phenomenon, or of a change in blood volume, nor is it secondary to a retention of the white cells in the capillaries of lung or liver; it is due to the accumulation of neutrophilic leucocytes in the parenchyma of the spleen. That the spleen is solely responsible for the temporary depression of white cells in the general circulation under these conditions has been shown by splenectomy.

In splenectomized rabbits no leucopenia developed, but instead a leucocytosis due to a direct action on the bone marrow.

A profound change occurs in the distribution of the cells in the circulation at the moment of death. The liver, within a minute of the cessation of the circulation, shows a three- to fourfold increase in the number of white cells per c.mm., the differential count remaining unchanged. Thus estimations of the physiological distribution and redistribution of cells in the living state may be made only with the circulation unimpaired.

The injection into normal rabbits of adenine and guanine nucleotides, split products of nucleic acid, gave immediate leucocytosis of bone marrow origin similar to that observed with the more complex molecule when the spleen was eliminated.

The response of the bone marrow to chemotactic stimuli, such as those here used, may be reflected in the general circulation, through an absolute increase of young neutrophilic leucocytes, within a period of less than 1 hour. Within this brief period there takes place maturation from Myelocyte C and metamyelocyte into the early motile leucocyte, and the delivery of these just matured cells into the circulation. The response to one injection of nucleinate or nucleotide may persist into the 3rd and 4th days with a gradual depletion of the normal reserve of Myelocytes C in the bone marrow.

BIBLIOGRAPHY.

1. Sabin, F. R., Cunningham, R. S., Doan, C. A., and Kindwall, J. A., The normal rhythm of the white blood cells, *Bull. Johns Hopkins Hosp.*, 1925, xxxvii, 14.
- 2, a. Shaw, A. F. B., The diurnal tides of the leucocytes of man, *J. Path. and Bact.*, 1927, xxx, 1.
- 2, b. Fletcher, E. G., and Mitchell, A. G., Physiologic variations in the leukocytes in infants and children, *Am. J. Dis. Child.*, 1927, xxxiv, 807.
3. Stetson, R. P., Normal variations in white blood cells under conditions of minimal metabolism, *Arch. Int. Med.*, 1927, xl, 488.
4. Isaacs, R., and Danielian, A. C., Maintenance of leucocyte level and changes during irradiation, *Am. J. Med. Sc.*, 1927, clxxiv, 70.
5. Bushnell, L. D., and Bangs, E. F., A study of the variation in number of blood cells of normal rabbits, *J. Infect. Dis.*, 1926, xxxix, 291.
6. Sabin, F. R., Studies of living human blood cells, *Bull. Johns Hopkins Hosp.*, 1923, xxxiv, 277.

7. Doan, C. A., and Zerfas, L. G., The rhythmic range of the white blood cells in human pathological leucopenic and leucocytic states, with a study of thirty-two human bone marrows, *J. Exp. Med.*, 1927, xlvi, 511.
8. Mathews, A. P., *Physiological chemistry*, 2nd edition, New York, 1919, 161.
9. Metchnikoff, *Pathologie comparée de l'inflammation*, Paris, 1892.
10. Ames, D., and Huntley, A., The nature of the leucocytosis produced by nucleic acid; a preliminary experimental study, *J. Am. Med. Assn.*, 1897, xxix, 472.
11. Milroy, T. H., and Malcolm, J., The metabolism of the nucleins, *J. Physiol.*, 1899, xxv, 105.
12. Vaughan, V. C., Novy, F. G., and McClintock, C. T., The germicidal properties of nucleins, *Med. News*, New York, 1893, lxii, 536.
13. de Paoli, E., and Calisti, A., Beobachtungen und experimentelle Untersuchungen über den Wert der Injektion des nukleinsauren Natriums bei der Prophylaxe der operativen Infektion des Peritoneums, *Mitt. Grenzgeb. Med. u. Chir.*, 1912, xxiv, 275.
14. Jacobi, W., Ueber intravenöse Injektionen hoher Dosen von Natrium nucleinicum beim Menschen, *Z. exp. Path. u. Therap.*, 1921, xxii, 266.
15. Medwin-Gardner, F. M., The precipitation of the crisis in the treatment of pneumonia, *Brit. Med. J.*, 1924, ccxlix, 748.
16. Daubenton, F., Severe case of lobar pneumonia successfully treated with sodium nucleinate, *South African Med. Rec.*, 1925, xxiii, 463.
17. Gotmakher-Redelman, A. M., New sign of Banti's disease, *Klinicheskaya Meditsma*, 1926, iv, 236 (abstract in *J. Am. Med. Assn.*, 1926, lxxxvii, 1428).
18. Antoncich, R., Bone marrow function test, *Crón. med.*, 1926, xliii, 3 and 65 (abstract in *J. Am. Med. Assn.*, 1926, lxxxvii, 1690).
19. Habetin, P., Über Funktionsprüfung des Knochenmarkes, *Wien. Arch. inn. Med.*, 1923-24, vii, 329.
20. Larsell, O., Nokes, H. T., and Phillips, B. I., Hemopoietic effect in rabbits of intravenous injection of cells, nuclei, and nucleic acids from blood of fowls, *Arch. Path. and Clin. Med.*, 1926, ii, 698.
21. Larsell, O., Jones, N. W., Nokes, H. T., and Phillips, B. I., The hemopoietic effects of intravenously injected nucleic acids, *J. Am. Med. Assn.*, 1927, lxxxix, 682.
22. Robertson, T. B., Hicks, C. S., and Marston, H. R., A comparison of the utilization of nucleic acids of animal and vegetable origin, *Wistar Inst. Bibl. Service Advance Abstr. Sheets*, 1927, No. 85, Nov. 14 (to appear in the *Australian J. Exp. Biol. and Med. Sc.*).
23. Löwit, M., *Studien zur Physiologie und Pathologie des Blutes und der Lymphe*, Jena, 1892.
24. Goldscheider, A., and Jacob, P., Ueber die Variationen der Leukocytose, *Z. klin. Med.*, 1894, xxv, 373.

25. Schultz, Experimentelle Untersuchungen ueber das Vorkommen und die diagnostische Bedeutung der Leukocytose, *Deutsch. Arch. klin. Med.*, 1892, li, 234.
26. Gräff, S., Leukozytenbewegung im Blute. Verschiebungsleukozytose—myelogene Leukozytose, *Berl. klin. Woch.*, 1921, i, 84.
27. Wells, W. A., Leucocytosis and immunity, with a critical analysis of the theory of nuclein-therapy, *Med. News*, New York, 1896, lxi, 425.
28. Jones, W., Nucleic acids, London and New York, 2nd edition, 1920.
29. Roy, C. S., Physiology and pathology of the spleen, *J. Physiol.*, 1881, iii, 203.
30. Schäfer, E. A., and Moore, B., On the contractility and innervation of the spleen, *J. Physiol.*, 1896, xx, 1.
31. Doan, C. A., The recognition of a biological differentiation in the white blood cells, *J. Am. Med. Assn.*, 1926, lxxxvi, 1593.
32. Sabin, F. R., Austrian, C. R., Cunningham, R. S., and Doan, C. A., Studies on the maturation of myeloblasts into myelocytes and on amitotic cell division in the peripheral blood in subacute myeloblastic leucemia, *J. Exp. Med.*, 1924, xl, 845.
33. Sabin, F. R., and Doan, C. A., Bone marrow as an organ, *Proc. Soc. Exp. Biol. and Med.*, 1927, xxv, 121.
34. Doan, C. A., Cunningham, R. S., and Sabin, F. R., Experimental studies on the origin and maturation of avian and mammalian red blood cells, *Carnegie Institution of Washington, Pub. No. 361, Contributions to Embryology*, 1925, xvi, 163.
35. Sabin, F. R., The bone marrow, *Physiol. Rev.*, 1928, viii, (April).
36. Mueller, E. F., and Myers, C. N., Biochemical studies on the behavior of the leucocytes after intravenous administration of alkalinized salvarsan, *Proc. Soc. Exp. Biol. and Med.*, 1924-25, xxii, 95.
37. Webb, R. A., The mechanism of anaphylactic leucopenia in dogs, *J. Path. and Bact.*, 1924, xxvii, 79.
38. Bruce, D., On the disappearance of the leucocytes from the blood after injection of peptone, *Proc. Roy. Soc. London*, 1894, lv, 295.
39. Andrewes, F. W., The behavior of the leucocytes in infection and immunity, *Lancet*, 1910, ii, 8.
40. Schilling, V., Das Knochenmark als Organ. III. Funktion des Markenparenchyms und Leukozytose, *Deutsch. med. Woch.*, 1925, li, 516.
41. Oeller, *Münch. med. Woch.*, 1922, ii, 1774; 1924, i, 218.
42. Wells, C. W., Leucopenia and leucocytosis in rabbits, *J. Infect. Dis.*, 1917, xx, 219.
43. Cunningham, R. S., Sabin, F. R., and Doan, C. A., The differentiation of two distinct types of phagocytic cells in the spleen of the rabbit, *Proc. Soc. Exp. Biol. and Med.*, 1924, xxi, 326.
44. Oliver, G., and Schäfer, E. A., Physiological effects of extracts of the suprarenal capsule, *J. Physiol.*, 1895, xviii, 230.

45. Barcroft, J., Recent advances in the knowledge of the spleen, *Lancet*, 1925, i, 319.
46. de Boer, S., and Carroll, D. C., The mechanism of the splenic reaction to general CO poisoning, *J. Physiol.*, 1924, lix, 312.
47. Scheunert, A., and Krzywanek, F. W., Weiteres über die Milz als Blutkörperchen-Reservoir, *Arch. ges. Physiol.*, 1927, ccxvii, 261.
48. Viale, G., La milza organo regolatore del numero dei corpuscoli rossi e bianchi circolanti, *Arch. fisiol.*, 1927, xxv, 380.
49. Soler, F., Contribucion al conocimiento de la funciones del bazo. Sissoreis esplanica, *Rev. med. latino-am.*, 1926, ii, 126.
50. Krumbhaar, E. B., Functions of the spleen, *Physiol. Rev.*, 1926, vi, 160.
51. Ehrlich, P., Die Anaemie, in *Nothnagel, H., Spec. Path. u. Therap.*, 1898, viii, 75.
52. Horbaczewski, J., Die Ausscheidung der Harnsäure und die Zahl der Leucocyten im menschlichen Blute nach Aufnahme von Chinin, Atropin, Pilocarpin, Antipyrin und Antifebrin, *Sitzungsber. k. Akad. Wissensch., Math.-naturw. Cl.*, 1891, vol. C, pt. 3, 101, Ref. B, abstracted in *Deutsch. med. Woch.*, 1892, lxxx, 772.
53. Ewald, G., Ueber intravenöse Verabreichung von Nucleinsäure und ihren Abbauprodukten beim Hund, *Z. exp. Path. u. Therap.*, 1913, xii, 348.
54. Muir, R., On the relations of the bone marrow to leucocyte production and leucocytosis, *J. Path. and Bact.*, 1901, vii, 161.
55. Bacon, D. K., Novy, F. O., and Eppler, H. H., Factors in leucocytosis, *Arch. Int. Med.*, 1922, xxx, 229.

EXPLANATION OF PLATES.

PLATE 21.

FIG. 1. Femoral bone marrow from Rabbit 6 (T 50) 1½ hours after 1 gm. of sodium nucleinate intravenously. The sinus is surrounded with neutrophilic leucocytes in active diapedesis and to right and left of the vessel may be seen cleared areas from which the myeloid elements have been withdrawn. See Chart 6. Section 10μ. Methylene blue-eosin. × about 750.

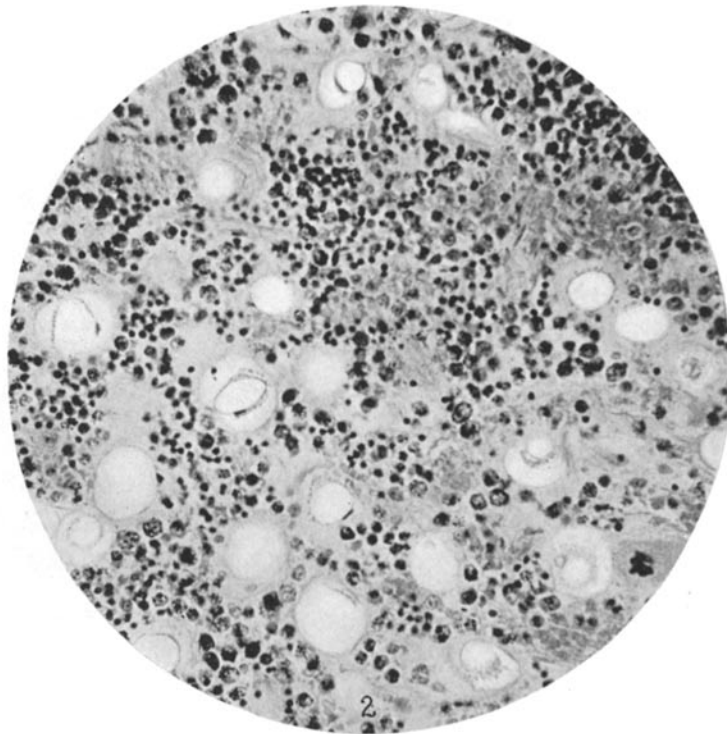
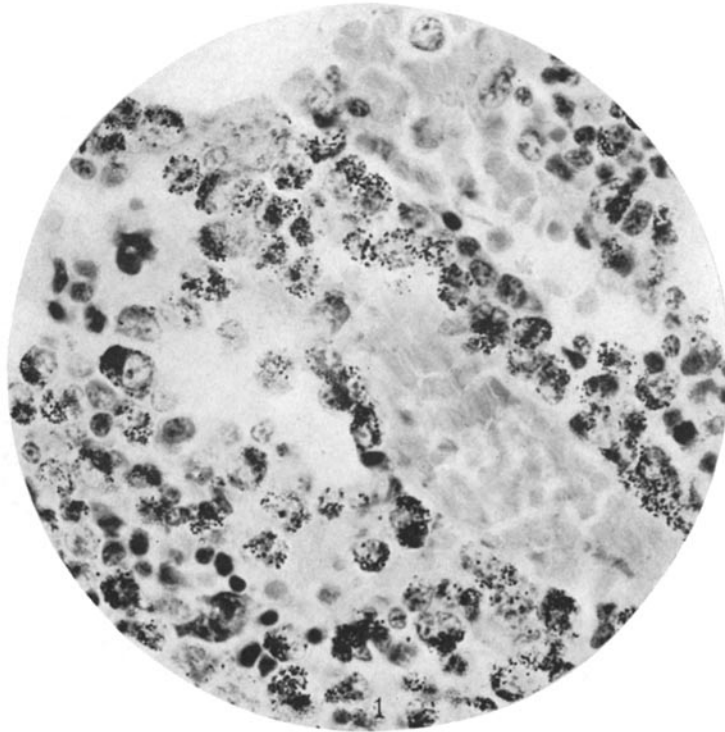
FIG. 2. Femoral bone marrow from Rabbit 22 (T 33) 18 hours after a second dose of 1 gm. of sodium nucleinate, the first having been given 4 days before. There is a decrease in size and number of fat cells in inverse proportion to the changes in the myeloid foci. It will be noted that the cells have not yet occupied all of the space made available for their development by the regression of the fat. Section 10μ. Methylene blue-eosin. × about 270.

PLATE 22.

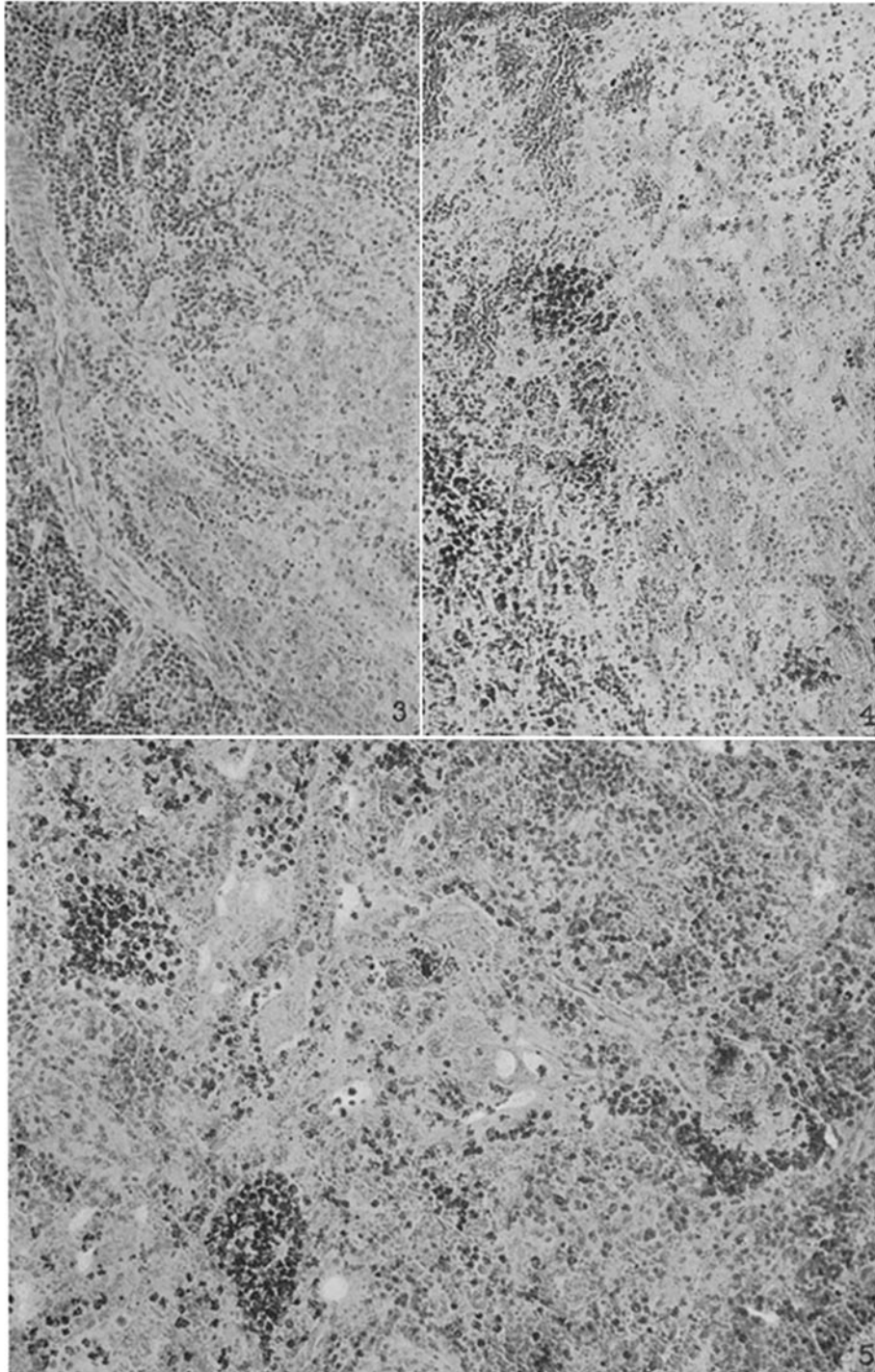
FIG. 3. Normal spleen from Rabbit 4 (T 49) 10 minutes before 1 gm. of sodium nucleinate was given intravenously. See Chart 4, Point 1. Section 10μ. Methylene blue-eosin. × 190.

FIG. 4. Sample of spleen from same experiment as Fig. 3, 1 hour after 1 gm. of sodium nucleinate intravenously. Note the beginning appearance of foci of neutrophilic leucocytes in the parenchyma. The darker cells are the neutrophilic leucocytes. The diffuse scattering of individual neutrophils throughout the pulp is not so easily recognized at this magnification. See Chart 4, Point 3. Section 10μ . Methylene blue-eosin. \times about 190.

FIG. 5. Sample of spleen from Rabbit 19 (T 31) 8 hours after 1 gm. of sodium nucleinate. Leucopenia had lasted for 5 hours, followed by 3 hours of leucocytosis. The maximum accumulation of neutrophilic leucocytes in the splenic parenchyma is still maintained. The section shows definite foci of leucocytes as well as numerous neutrophils scattered throughout the pulp. The darker cells are the neutrophilic leucocytes. 10μ . Methylene blue-eosin. \times about 190.



(Doan, Zerfas, Warren, and Ames: Leucopenic and leucocytic states.)



(Doan, Zerfas, Warren, and Ames: Leucopenic and leucocytic states.)