

PRELIMINARY REPORT ON PHOSPHATASE AND NUCLEOTIDE STUDIES IN THE PERIPHERAL BLOOD (NOV. 15 to 29th)

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Methods used:

A. Phosphatase stain - Blood smears were fixed in 95% ethyl alcohol, passed through ether-alcohol and coated with thin celloidin. The celloidin coat was hardened in 95% ethyl alcohol, and the smears passed into distilled water. They were then incubated for 1 1/2 hours at 37° C in the following substrate - Beta-glycerophosphate, calcium nitrate, and sodium barbital, buffered at approximately pH 9.3. After incubation, the smears were rinsed in dilute calcium nitrate solution and treated with ultra-violet ray in a 1% silver nitrate solution. The phosphatase appears as a golden brown precipitate where present. Some smears were counterstained with Delafield's Hematoxylin to give nuclear detail.

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B. Nucleotide stain - Blood smears were fixed in 100% methyl alcohol, stained for 3 minutes in Pyronine-methyl green stain, washed in distilled water, blotted, and mounted. The nucleotide stains a bright pink.

Blood studies:

1. ██████████ (Hospital) - studied every day, except Sundays, from November 15th to 29th. There appeared a positive phosphatase stain in certain of the polymorph leukocytes. These measured on the average of 15 mu in diameter. From November 15th to 20th the smears appeared constant. On November 21st there appeared to be a diminution in the number of polymorphonuclear cells showing a positive phosphatase stain. Many showing the stain had only a small area near the nuclei. Such cells measured on the average of 12 mu in diameter. This same picture occurred again on November 22nd. Smears for November 23rd, 24th, and 25th were characteristic of the first ones made. On November 27th and 28th there were negative results for phosphatase, and on November 29th there again appeared a few of the large sized polymorphonuclear cells with positive phosphatase.

The nucleotide stain for the same period appeared as follows:
 (Pink stain in cytoplasm rated as "0" for normal and "+" - up to 4 - for lack of normal staining.)

<u>Date</u>	<u>Polymorphs</u>	<u>Monocytes</u>	<u>Lymphocytes</u>
11/15	Clear cytoplasm	3 +	3 +
11/16	" "	3 +	3 +
11/17	" "	3 +	3 +
11/18	" "	2 +	2 +
11/20	" "	3 +	3 +
11/21	" "	3 +	3 +
11/22	" "	4 +	4 +
11/23	" "	3 +	3 + (Cytoplasm of lymphocytes scant.)

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 Name (ADD) - Organization

Date 1-4-85

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11/24	Clear cytoplasm	1 +	1 + (Scant cytoplasm to lymphs)
11/25	" "	3 +	3 +
11/27	" "	3 +	3 + (Many lymphs and monocytes shows 4 + cytoplasm)
11/28	" "	1 +	4 + (A few lymphs show a 3 + cytoplasm)

2. [redacted] (Hospital) - Phosphatase staining for November 15th and 16th were negative; on November 17th one polymorph was positive. November 19th was again negative. On November 20th and 21st, one polymorph showed a small amount of phosphatase stain near the nucleus. On November 22nd, five polymorphs were positive. The patient was reported deceased on November 23rd.

The nucleotide stain for the same period was

Date	Polymorphs	Monocytes	Lymphocytes
11/15	Clear cytoplasm	4 +	4 +
11/16	" "	4 +	4 + (A few large lymphocytes have 2 + cytoplasm)
11/17	" "	Part show 4 + " " 2 +	4 +
11/18	" "	2 +	Majority 4 + Some 2 +
11/20	" "	2 +	Majority 4 + 1 or 2 "0"
11/21	" "	4 +	4 +
11/22	" "	4 +	4 +

3. [redacted] (Hospital) - One set of blood smears was obtained on November 21st. The phosphatase stained a small area of the cytoplasm of two polymorphs on this smear, while the nucleotide method demonstrated a clear cytoplasm in the polymorphs, with 1 + stain in the monocyte cytoplasm and "0" in most lymphocytes. These latter had very little cytoplasm. There was many lymphocytes with a greater amount of cytoplasm which was 4 +.

4. [redacted] (Hospital) - One set of blood smears was obtained, November 24th - the phosphatase stain was negative. The nucleotide stain left the cytoplasm of the polymorphs unstained and the cytoplasm of both the monocytes and lymphocytes were 2 +.

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5. [REDACTED] (Hospital) - One set of blood smears was obtained. November 22nd - the phosphatase stain was negative. The nucleotide stain did not tint the cytoplasm of the polymorphs, while it gave a 1+ stain to the cytoplasm of the monocytes and a normal cytoplasmic stain to most of the lymphocytes. There were many lymphocytes, however, that had 4+ staining.

Each day blood smears were obtained from persons believed to be normal. These were made of personnel around the laboratory. Part of these "controls" were obtained from Dr. Bryan's office, and the rest were taken from members of the Pathology Department. A total of fifteen different people have been studied in this group. Of these, two were of questionable nature. One of them, [REDACTED] is reported to be suffering from "Infectious Mononucleosis". This did not give a positive phosphatase stain, but both the monocytes and lymphocytes gave a 2+ nucleotide stain. These lymphocytes are small with scant cytoplasm.

The other questionable person was [REDACTED] who had a positive phosphatase stain in the cytoplasm of certain of the polymorphs. At the time the smear was taken she felt ill and complained of a headache. The next day she was absent from work. The polymorphs staining with phosphatase averaged 15 μ in diameter, while the unstained ones averaged 8 μ in diameter. The nucleotide stain, however, was normal.

To date, thirty dogs have also been studied. One was a known leukemic (4 DA 4²) and gave consistently positive phosphatase stains and an abnormal nucleotide stain. The rest of the dogs were either normal or irradiated, without any evident leukemia. Those have all given negative phosphatase staining. The nucleotide slides of these animals have not been completely studied.

A series of ten rats was studied, but both normal and irradiated ones gave positive phosphatase stains in the cytoplasm of the polymorphs. Neither gave constant nucleotide results. It was decided to spend no further time studying rats in the present survey.

One control monkey done showed a negative phosphatase and a normal nucleotide stain.

Summary:

The phosphatase stain appears to be more constant in nature than the nucleotide stain; however, it does not look at present to be specific for leukemic blood.

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November 15th to 29th, 1944.

The constant staining by the phosphatase method in the [redacted] case and also that of leukemic dog (4 DA 4²) seemed to show promise. This, however, had some doubt cast upon it when it showed a positive reaction on the blood smear of [redacted] and was negative for [redacted] (diagnosed a leukemic).

One point of interest is the constant large size of the polymorphs which do show a positive phosphatase stain, in contrast to those of the same smear which are negative. This is an average of 5 mu difference in diameter as determined by an ocular micrometer. These observations suggested testing bone marrow smears by the phosphatase method, in order to determine what forming elements might stain positively. Three dogs have been studied to date and have shown positive phosphatase only in what appears to be the myelocytes. Juvenile cells and polymorphs, as well as the red cell series, are negative. A pencil of bone marrow from the femur of a normal dog is in the process of being embedded in celloidin in order that a more careful and detailed study of the phosphatase staining may be carried out.

The polymorphs of the blood smears which do show a positive phosphatase stain may be physiologically immature cells which are thrown into the circulating blood by bone marrow under certain definite periods of stress.

The Nucleotide stain appears to have no constant variation in staining properties for a given disorder. However, it does seem to indicate an abnormal physiology of the blood. Beyond this, at present, one cannot make assumptions.

It is contemplated to report next on the dog colony survey. This should be out in a week or so. After this we contemplate surveying a group of normal rats and then try to evaluate the nucleotide smears on the RM group.

We would like to continue with study of normal humans. We are desirous of obtaining smears from an acute leukemia and a chronic leukemia and studying them before and after treatment.

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