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# THE HEMATOLOGIC RESPONSE IN DOGS TO THE ADMINISTRATION OF ANTI SPLEEN SERUM

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SINCE the early days of medicine, when each organ had its real or fancied attributes, the idea of restoring function to a deranged part by the administration of tissue from a normal organ has seemed attractive. However little the effort then availed, the principle has in recent years given organotherapy many important agents such as insulin and liver extract.

With the development of the science of immunology it seemed only natural that, instead of using organs *per se*, attempts be made to produce an antiserum to each. Certainly if such a preparation had any effect, either stimulant or depressant, there would be opened up a new field of almost unlimited possibilities.

The problem was defined by Metchnikoff,[34] who called such antisera "cytotoxic" in the belief that their effect on the cells of the body was a toxic one. He injected splenic tissue into animals in an attempt to make an antispleen serum, but soon abandoned this work, primarily because of the difficulties in determining what, if any, titer of antiserum he was working with. Funck,[19] Flexner,[18] and Bunting[10] used other tissues, such as lymph nodes and bone marrow, as antigens with indifferent results. More recently, in 1936, Chew, Stephens, and Lawrence[13] reported that an antileukocytic serum produced in some guinea pigs an almost complete reduction in the number of neutrophil leukocytes in the peripheral blood. Earlier work by Ledingham and Bedson,[24] Bedson,[1] Lindstrom,[26] Okita,[35] and Matsuno[29] had given essentially similar results. Although this effect was striking, a satisfactory technic for titrating the antisera was still not available.

The present study was stimulated by the recently reported Russian work in which a new technic for the titration of "cytotoxic" serum was used. Marchuk,[27, 28] Bogomolets and his colleagues have named this serum "anti-reticular cytotoxic serum." The claims made for its therapeutic application are many.[2–9, 11, 12, 14, 17, 20, 22, 23, 25, 30–33, 36, 37]. Although they are yet to be confirmed, and the mode of action elucidated, the standardization of a method of preparation of antisera and for their titration offers renewed hope that fundamental problems of hematology and allied fields can be attacked with this new tool.

## MATERIALS AND METHODS

### *I. Experimental Animals*

Dogs and rabbits were used for this study. The animals were kept in individual cages, the temperature and humidity in the animal quarters controlled at all times. When first obtained, all animals were kept in "isolation" quarters for a period of two weeks and observed for infection. Healthy animals were then moved to the

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This work has been supported by the University-Watts Hospital Research Fund.



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main quarters, placed on a standard diet, and again observed for several weeks. During this time those animals were chosen for the experiment which ate the diet well, maintained body weight, and exhibited good vitality. During the period of observation, these animals were free from any known infection, and remained so during the experiment.

*Dogs.*—Male hounds 1–2 years of age and weighing 5–10 Kg. were selected. The standard diet was Purina Dog Chow. Water was given ad lib.

TABLE 1. *The Effect of Dosage and Route of Administration of Antigen on Titer of Antiserum Obtained-Rabbit Anti-Dog-Spleen Serum*

| Rabbit | Weight (*Kg.*) | Antigen | Route | Dosage—Gm. of Tissue Equivalents | Titer of Antiserum Obtained 14 Days after Last Injection |
|---|---|---|---|---|---|
| 44-2 | 2.80 | Spleen | Intraperitoneal | Day 1—0.1<br>Day 6—0.2<br>Day 11—0.3<br>Day 16—0.4<br>Day 21—0.6 | 1:160 |
| 44-10 | 3.13 | Spleen | Intravenous | Day 1—0.01<br>Day 6—0.02<br>Day 11—0.04<br>Day 16—0.04<br>Day 21—0.08 | 1:640 |
| 44-11 | 3.24 | Spleen | Intravenous plus Intraperitoneal | Day 1—0.01 i.v. 0.12 i.p.<br>Day 6—0.02 i.v. 0.20 i.p.<br>Day 11—0.02 i.v. 0.30 i.p.<br>Day 16—0.02 i.v. 0.40 i.p.<br>Day 21—0.04 i.v. 0.50 i.p. | 1:320 |
| 44-12 | 3.36 | Spleen | Intravenous | Day 1—0.01<br>Day 6—0.02<br>Day 11—0.04<br>Day 16—0.04<br>Day 21—0.08 | 1:400 |

*Rabbits.*—White litter-mate chinchilla rabbits about 1 year of age and weighing 2–3 Kg. were used. They were fed Rockland Rabbit Diet with added vegetables.

### II. *Preparation and Titration of Antisera*

The method will be outlined in detail only for the preparation of antispleen serum as it is the same for heart, bone marrow, and other antigens.

Under ether anesthesia the dogs were bled out and spleens removed. The splenic capsule was trimmed away, 5.0 grams of spleen sliced thin with a razor and washed gently in five changes of physiological saline to remove as much blood as possible.

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The tissue was then thoroughly ground in a mortar with sterile, pure sea sand, resuspended in 25.0 cc. of physiological saline, and centrifuged for 4 minutes at 1000 rpm. The top fatty layer was pipetted off and discarded, and the supernate used as antigen. This material was used both for injecting rabbits and for the complement fixation test. Antigen so obtained was designated "undiluted antigen," and contained 0.2 gram of spleen/cc.

APPENDIX—*Titration of Antisera*

A. *Determination of Titer of Antigen*

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| Dilution of antigen | 1:1 | 1:2 | 1:4 | 1:8 | 1:16 | 1:32 | 1:64 | 1:128 |
| | 0.5 cc. | 0.5 cc. | 0.5 cc. | 0.5 cc. | 0.5 cc. | 0.5 cc. | 0.5 cc. | 0.5 cc. |
| Titered complement | 1.0 cc. | 1.0 cc. | 1.0 cc. | 1.0 cc. | 1.0 cc. | 1.0 cc. | 1.0 cc. | 1.0 cc. |
| Saline | 0.5 cc. | 0.5 cc. | 0.5 cc. | 0.5 cc. | 0.5 cc. | 0.5 cc. | 0.5 cc. | 0.5 cc. |
| 37° C. Water Bath—45 Minutes | | | | | | | | |
| Sensitized sheep cells | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| 37° C. Water Bath—30 Minutes | | | | | | | | |

Note 1. Antigen is prepared as for immunization.
Note 2. Complement previously titered to contain 2 full units in 1.0 cc.
Note 3. Sensitized sheep cells: 2% suspension with previously titered hemolysin.
Note 4. Titer of antigen to be used is the smallest quantity which gives complete fixation of complement.

B. *Determination of Titer of Antiserum*

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Antiserum | 1:20 | 1:50 | 1:100 | 1:200 | 1:300 | 1:400 | 1:500 | 1:600 | 1:700 | 1:800 | 1:900 | 1:1000 | 1:2000 | 1:3000 |
| | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| Titered antigen | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| Titered complement | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| 37° C. Water Bath—45 Minutes | | | | | | | | | | | | | | |
| Sensitized sheep cells 2% | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| 37° C. Water Bath—30 Minutes | | | | | | | | | | | | | | |

Note 1. Serum is inactivated at 56° C. for 30 minutes before the test is carried out.
Note 2. Titer of antiserum is highest dilution causing complete fixation of complement.
Note 3. Standard controls are set up.
Note 4. The hemolytic titer of the antiserum is also determined and sera with titers of 1:16 or more are not used.

Rabbits were injected by various routes, but the highest titers of antiserum were obtained when the antigen was given intravenously (table 1). Every fifth day the dose was increased (table 1), and each time fresh spleen was used in preparing the antigen. Fourteen days after the last injection 5.0 cc. of blood was obtained by cardiac puncture and the antispleen titer of the serum determined by a modification of Marchuk's method (Appendix).

Rabbits showing a high titer were bled out and the serum separated with careful consideration for a sterile technic. It was found that allowing the blood to clot and storing it in the refrigerator overnight gave the highest yield of serum. This

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was pipetted off and stored in sterile 1 cc. glass ampules at 4 degrees centigrade. Sterility of the serum was checked several times, and always before using.

### *III. Administration of Antiserum*

The serum was diluted 1:10 with sterile physiological saline 48 hours before administration and stored in the refrigerator while sterility was being checked by the inoculation of veal infusion broth and blood agar culture media. In this study the serum was given intravenously. The dosage was calculated on the basis of 0.1 cc. of 1:10 diluted serum/10 Kg. of body weight for the first dose, the second dose being three times the first.

TABLE 2.—*Data on a Dog (44-23) Receiving Antispleen Serum AS-10 Intravenously*

| Date | Time | Weight (Kg.) | HGB. (G. %) | RBC (millions)/ cu. mm. | WBC/cu. mm. | % Lymphocytes | % Monocytes | Lymphocytes/cu. mm. | Monocytes/cu. mm. | Total Mononu- clears/cu. mm. | Sedimentation Rate mm./hr. | Volume Packed RBC % | Reticulocyte % | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 11/29/44 | 2:00 P.M. | 6.45 | 18.0 | 8.5 | 10,500 | 22 | 3 | 2300 | 310 | 2610 | 1 mm. | 52 | 1% | (Platelet counts, blood chemistry values, etc., were normal and are not shown.) |
| 11/30/44 | 2:30 P.M. | 6.45 | 18.5 | 9.1 | 9,100 | 25 | 5 | 2280 | 455 | 2735 | 1 mm. | 55 | <1% | |
| 12/ 1/44 | 3:30 P.M. | 6.37 | 17.5 | 8.8 | 9,850 | 18 | 4 | 1775 | 395 | 2170 | — | 53 | — | |
| 12/ 2/44 | 1:45 P.M. | 6.54 | 18.0 | 8.8 | 11,100 | 22 | 6 | 2440 | 666 | 3106 | — | — | — | |
| 12/ 3/44 | 2:00 P.M. | 6.30 | 18.0 | 9.5 | 11,250 | 21 | 5 | 2360 | 565 | 2925 | 1 mm. | 51.5 | <1% | |
| 12/ 4/44 | 2:30 P.M. | 6.38 | 17.5 | 9.3 | 10,000 | 26 | 6 | 2600 | 600 | 3200 | — | 50 | 1% | 0.1 cc. 1:10 AS-10 i.v. at 5 P.M. |
| 12/ 5/44 | 2:30 P.M. | 6.46 | 17.4 | 8.46 | 12,900 | 35 | 3 | 4500 | 378 | 4878 | — | 50 | — | |
| 12/ 6/44 | 2:00 P.M. | — | — | — | 12,150 | 35 | 7 | 4250 | 850 | 5110 | 1 mm. | 51 | <1% | 0.3 cc. 1:10 AS-10 i.v. at 5 P.M. |
| 12/ 7/44 | 2:15 P.M. | 6.40 | 17.5 | 8.48 | 10,850 | 30 | 5 | 3260 | 545 | 3805 | — | 49 | — | |
| 12/ 8/44 | 2:15 P.M. | 6.35 | 15.2 | 7.54 | 10,750 | 37 | 4 | 3460 | 430 | 3890 | — | — | — | |
| 12/ 9-44 | 2:30 P.M. | 6.28 | 16.0 | 8.39 | 10,250 | 49 | 6 | 5025 | 615 | 5640 | 1 mm. | 48 | <1% | |
| 12/11/44 | 2:15 P.M. | 6.18 | — | — | 10,250 | 34 | 6 | 3480 | 615 | 4095 | 1 mm. | — | — | |
| 12/13/44 | 2:00 P.M. | 6.39 | 16.8 | 9.44 | 10,250 | 35 | 8 | 3600 | 820 | 4420 | — | 48 | <1% | |
| 12/15/44 | 2:30 P.M. | 6.38 | 17.8 | 8.81 | 10,150 | 28 | 7 | 2840 | 710 | 3550 | — | — | — | |
| 12/18/44 | 2:30 P.M. | 6.16 | 17.6 | 8.89 | 10,050 | 31 | 3 | 3120 | 300 | 3420 | — | 47 | 1% | |
| 1/ 3/45 | 3:00 P.M. | 6.39 | 17.5 | 8.52 | 8,250 | 24 | 4 | 1980 | 330 | 2310 | — | 47 | — | |
| 2/ 7/45 | 2:00 P.M. | 6.4 | 17.0 | 8.98 | 10,000 | 23 | 4 | 2300 | 400 | 2700 | 1 mm. | 47 | <1% | |

### *IV. Experimental Procedures*

All food was taken out of the dog cages in the evening and studies were carried out at about 2 o'clock the following afternoon before the dogs were fed again. Dogs were easily trained to lie quietly on the table. Blood samples were obtained by jugular puncture, care being taken to avoid venostasis. Smears on cover slips and supravital preparations were immediately made with fresh blood, and 10.0 cc. of blood was oxalated with dry potassium and ammonium oxalate as recommended by Heller and Paul.[21] The fixed blood smears were stained routinely with Wright's blood stain, and occasionally with special stains where indicated. The supravital technic employed combined Janus green and neutral red as the stain. Differential

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TABLE 3.—*Summary of Results*

| Dog | Experiment | Serum and Titer | Dosage (cc. undiluted serum) | Lymphocytes/cu. mm. | | | | | Monocytes/cu. mm. | | | | | Total Mononuclears/cu. mm. | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Average (control period) | Average first 6 days | % Change Average | Peak Level | % Change Peak | Average (control period) | Average first 6 days | % Change Average | Peak Level | % Change Peak | Average (control period) | Average first 6 days | % Change Average | Peak Level | % Change Peak |
| 44-1 | Antispleen serum i.v. | AS-10 1:500 | 0.015—Day 6<br>0.045—Day 8<br>0.135—Day 11<br>0.405—Day 13<br>1.21—Day 15 | 1590 | 3291 | +107 | 4780 | +200 | 411 | 414 | 0 | 1020 | +148 | 2000 | 3673 | +84 | 4694 | +134 |
| 44-23 | Antispleen serum i.v. | AS-10 1:500 | 0.01—Day 6<br>0.03—Day 8 | 2290 | 4100 | +79 | 5025 | +119 | 500 | 565 | +11 | 850 | +70 | 2790 | 4664 | +67 | 5640 | +98 |
| 44-26 | Antispleen serum i.v. | AS-10 1:500 | 0.012—Day 6<br>0.036—Day 8 | 2113 | 3389 | +61 | 3825 | +72 | 915 | 2046 | +123 | 2360 | +147 | 3028 | 5435 | +80 | 6210 | +105 |
| 44-18 | Antispleen serum i.v. | AS-10 1:500 | 0.012—Day 6<br>0.036—Day 8 | 2965 | 5115 | +71 | 6000 | +102 | 540 | 1231 | +127 | 1680 | +211 | 3500 | 6346 | +82 | 7660 | +119 |
| 44-31 | Antispleen serum i.v. | AS-10 1:320 | 0.015—Day 6<br>0.045—Day 8 | 2023 | 2368 | +17 | 2620 | +30 | 543 | 1064 | +96 | 1311 | +141 | 2656 | 3432 | +29 | 4045 | +58 |
| 45-2 | Antispleen serum i.v. | AS-12 1:400 | 0.012—Day 6<br>0.036—Day 8 | 2940 | 3235 | +10 | 3920 | +33 | 277 | 708 | +155 | 1660 | +500 | 3217 | 3943 | +23 | 4920 | +53 |
| 45-4 | Antispleen serum i.v. | AS-12 1:400 | 0.01—Day 6<br>0.03—Day 8 | 3263 | 2880 | −13 | 3380 | +4 | 266 | 1096 | +310 | 1435 | +440 | 3529 | 3976 | +13 | 4590 | +30 |
| 44-29 | Control normal rabbit serum i.v. | R-15 0 | 0.0135—Day 6<br>0.0405—Day 8 | 3874 | 3626 | −7 | 4370 | +13 | 915 | 930 | +2 | 1090 | +2 | 4789 | 4556 | −5 | 4920 | +3 |
| 44-35 | Control normal rabbit serum i.v. | R-17 0 | 0.015—Day 6<br>0.045—Day 8 | 3372 | 3022 | −11 | 3780 | +12 | 621 | 625 | 0 | 830 | +34 | 3993 | 3646 | −9 | 4340 | +9 |
| 44-30 | Control normal rabbit serum i.v. | R-21 0 | 0.016—Day 6<br>0.048—Day 8 | 2876 | 2900 | +1 | 3285 | +14 | 428 | 400 | −7 | 825 | +93 | 3304 | 3300 | 0 | 3655 | +11 |
| 44-36 | Control antiheart serum i.v. | AH-1 1:500 | 0.01—Day 6<br>0.03—Day 8 | 4575 | 4370 | −5 | 4990 | +7 | 464 | 545 | +17 | 910 | +96 | 5039 | 4515 | −12 | 5534 | +10 |
| 44-37 | Control antiheart serum i.v. | AH-1 1:500 | 0.01—Day 6<br>0.03—Day 8 | 3722 | 3444 | −8 | 3950 | +4 | 744 | 761 | +2 | 980 | +32 | 4466 | 4205 | −6 | 5020 | +15 |
| 45-18 | Antimarrow serum i.v. | AM-1 1:400 | 0.006—Day 6<br>0.018—Day 8 | 2780 | 2540 | −9 | 3000 | +8 | 612 | 1131 | +85 | 1760 | +187 | 3392 | 3671 | +8 | 4180 | +23 |
| 44-22 | Large dose antispleen serum i.v. | AS-10 1:500 | 2.25—Day 6 | 4780 | 3400 | −41 | 1900 | −153 | 625 | 490 | −28 | 136 | −360 | 5490 | 3890 | −42 | 2036 | −123 |

counts on 200 leukocytes were done on each preparation. Blood cell counts were carried out in duplicate and sometimes triplicate, and for each dog the same pipets

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and chambers were used at all times. The type of data obtained for each dog is shown in table 2.

After a preliminary period of six days for base line determinations, 7 dogs received antispleen serum, 3 dogs received normal rabbit serum having no antispleen titer, and 2 dogs received antiheart serum. One dog received antimarrow serum, and 1 dog received a single large dose of antispleen serum (table 3, col. 2).

## RESULTS

The results are summarized in table 3.

The only marked effect noted was a relative and absolute increase in the mononuclear cells of the peripheral blood. A marked lymphocytosis was seen, and also an increase in monocytes. From fixed smears it was sometimes impossible to classify some of the mononuclear cells as either lymphocytes or monocytes, and parallel supravital studies showed the cells in question to have the large mitochondria of lymphocytes and the neutral red rosette typical of monocytes. For the purpose of analyzing the results, therefore, these cells were considered together as "mononuclear cells."

As will be seen from table 3, the 4 dogs receiving rabbit anti-dog-spleen serum AS-10 with an antispleen titer of 1:500 showed increases in absolute numbers of circulating mononuclears of 134 per cent, 98 per cent, 105 per cent, and 119 per cent. Dog 44-31 received the same serum which, after it had been stored at 4° C. for 5 months, showed diminution of titer to 1:320. The mononuclear response in this case was only +58 per cent. Dogs 45-2 and 45-4 received a second lot of antispleen serum, AS-12, made in the same way and having a titer of 1:400. These showed increases of 53 per cent and 30 per cent.

The control animals which received normal rabbit serum having no antispleen titer, and those receiving an antiheart serum (AH-1) having an antiheart titer of 1:500 and an antispleen titer of 0, showed no corresponding mononuclear response. The maximum peak reached was 15 per cent above the base line.

The rise in circulating mononuclears in very prompt, occurring within 24–48 hours of the first dose of serum, and under the conditions of the experiment there is a return to the base line figure in about 20 days. The major portion of the rise is due to cells which are typically lymphocytic, and it was noted that there were relatively more of the large variety during the height of the response. Later an increase in typical monocytes was observed, while the period in between was characterized by cells smaller than monocytes and having the ambiguous features already noted. In 1 dog (44-1) these cells of doubtful classification reached at one time a level of 12 per cent. An inconstant feature was the occasional appearance during the height of the mononuclear response of large blast cells which lacked sufficient criteria for classification.

There were no other significant changes. The hematocrit values remained constant or became slightly lower, and there was never any evidence of hemoconcentration. Occasionally moderate decreases in the red blood cell count were seen, accompanied by lower hemoglobin values, but the icterus index did not increase

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and there was no change in the reticulocyte count. In some dogs the titer of complement in the blood was followed through the period of mononuclear response without any increase being noted. In 2 dogs daily determinations of the heterophil antibody titer failed to show any rise. Blood chemistry values (NPN, albumin, globulin, fibrinogen) remained constant. Sedimentation rates were normal in all experimental dogs and did not change during the experiments. The white blood count showed occasional slight rises during the mononuclear response, while with equal frequency it became somewhat lower. There was no significant increase in blood platelets, although 1 dog in the antispleen group showed at one time a platelet count of 4.5 millions.

The effect of a large dose of antispleen serum was studied in dog 44-22. This dog was given 22.5 cc. of 1:10 diluted serum, equivalent to 5 cc./10 Kg. This dose was somewhat toxic, as evidenced by vomiting produced two hours after it was given intravenously. The dog refused to eat his diet that evening, but seemed to be well the next morning. In this case the lymphocyte percentage in the peripheral blood dropped from a base line average of 27 per cent to 7 per cent two days later, and at no time showed the rise in mononuclears seen in the dogs receiving small doses. Calculated on the basis of absolute numbers of mononuclear cells, the change in this case was −123 per cent. At the same time a marked eosinophilia was noted.

## DISCUSSION

The future significance of these findings is necessarily in the realm of speculation. Two principles, however, the specificity of antisera and the opposite effects of small and large doses, are supported by the data obtained.

What is the mode of action of "cytotoxic" serum? That such small doses should have any action whatever seems remarkable, and yet many substances necessary for cellular integrity and function are measurable as traces rather than in grams. The vitamins, for instance, acting as activators, catalysts, or even precursors, exert effects out of proportion to the extremely small amounts in which they are active. This is even more striking in the case of the "trace" elements and the hormones. Considerable evidence has accumulated to show that large amounts are unnecessary, and may even be toxic.

The first conclusion to be drawn from the data presented, supported by previous reports on antisera of other types, is that, if the serum is active at all, the action is fairly specific. Only one cell type, or one group of related cells, is affected, and the results depend on the antigen used. Thus Chew, Stephens, and Lawrence[13] made an antileukocytic serum by injecting cells from experimentally produced peritoneal exudates into rabbits. This antigen consisted of 75–90 per cent neutrophils, 1–4 per cent lymphocytes, the remainder being large mononuclear cells. Proportionately large doses of this serum (the antileukocytic titer in vitro was not determined) when given intravenously to guinea pigs produced in a few hours an almost complete disappearance of neutrophils from the peripheral blood, the other cells being relatively unaffected. Yamamoto[39] using serum from a goose immunized against rabbit neutrophils also found the effect to be entirely on the neutrophils. Experi-

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ence with antiplatelet serum by many observers has shown that it is specific for platelets. In the present study it is notable that the lymphocytes, monocytes, and mononuclear cells were affected, corresponding to the main cellular population of the spleen. Serum made against heart muscle had, by contrast, no effect on these cells.

The second conclusion is that the dosage of antiserum given is of special importance, and that small doses have a different effect from large doses. When rabbit anti-dog-spleen serum is given in very small doses (total of 0.04 cc./10 Kg.) the mononuclear cells in the peripheral blood show increases of 100 per cent or more. If a large dose is given (5 cc./10 Kg.) a reduction of 123 per cent results. It should be emphasized that in the previously quoted work on antileukocytic serum,[13] relatively large doses of serum were used to produce depression of the neutrophils (1.3–2.3 cc./Kg.). The work of Bogomolets is based on the assumption that small doses are "stimulating" while large doses are "blocking" in effect. The difference in response in dogs to the administration of small and large quantities of antispleen serum is partial confirmation of this.

If this assumption is correct, it should be possible to either stimulate or depress a cellular system at will by administering small or large doses, respectively, of a specific antiserum. The therapeutic applications would be many. For example, an increase in mononuclear cells in the peripheral blood is often a good prognostic sign in acute infectious diseases. The same is generally true in chronic infections. In tuberculosis, for example, not only is the lymphocyte/monocyte ratio important, but also the polynuclear/mononuclear ratio. Ratios and indices, such as those of Sabin and Medlar, are attempts to express this numerically. The large mononuclear cells of the blood and fixed tissues are responsible for the formation of the inner cellular portion of the tubercle, the small lymphocytes mark its outer border, while resolution or fibrosis is probably a function of the same mononuclear cells. Therefore, any agent which stimulates this group of cells to greater activity might be an adjuvant to general or specific therapy.

In the field of acute infections, any agent which stimulates antibody production in the infected host is beneficial. Interesting in this connection are the recent reports by Dougherty, White, and Chase,[15] Ehrich,[16] and White[38] indicating that antibodies are probably made or stored in the lymphocytes. Again it might be hoped that stimulation of these cells would result in increased antibody formation.

Many other possibilities suggest themselves. The lack of specificity of morphological methods has, it is well known, led to considerable confusion in the interpretation of cellular origins, relations, and reactions. After many years of careful study by outstanding investigators, the "reticulo-endothelial system," for example, is still the subject of spirited controversy. The same may be said of the various theories on the origins of blood and tissue cells. It is hoped that specific stimulation or inhibition by means of antisera may be the biological "tool" which is needed to clarify these fields.

In making this preliminary investigation of antispleen serum, many questions have been raised. It is to be hoped that further work will provide the answers.

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SUMMARY

1. Intravenous injection into dogs of rabbit anti-dog-spleen serum in doses of 0.04 cc./10 Kg. results in a significant increase in the mononuclear cells of the peripheral blood.

2. The rise in circulating mononuclears occurs promptly, with 24–48 hours, and is sustained, generally for 20 days.

3. A large dose of the same serum, 5.4 cc./10 Kg., exerted an opposite effect, producing a significant decrease in circulating mononuclears.

4. The possible significance of these findings is discussed.

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