CELLULAR REACTIONS TO POLYSACCHARIDES FROM
TUBERCLE BACILLI AND FROM PNEUMOCOCCI

By F. R. Sabin, M.D., A. L. Joyner, M.D., and K. C. Smithburn, M.D.

(From the Laboratories of The Rockefeller Institute for Medical Research)

PLATE 24

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It has been reported previously that tuberculo-polysaccharides have some toxicity for tuberculous guinea pigs (1–3), but our present studies have convinced us that the polysaccharides by themselves are not toxic to normal animals and in comparison with tuberculo-protein are relatively innocuous to tuberculous animals. In regard to cellular reactions, they give rise to one constant phenomenon, namely, a draining of neutrophilic leucocytes from the bone marrow. In our early experiments all the tuberculous guinea pigs that died after having received the polysaccharide had extensive disease and had shown a profound fall in temperature, as in tuberculin shock, after receiving the polysaccharide. It was our opinion at the time that this fall in temperature might have been referable to the presence of traces of protein in the preparations of the polysaccharides since they were not free from nitrogen (3–5). Recent studies by Dr. A. Cournand of the Tuberculosis Service of Bellevue Hospital tend to confirm this view. In 1935 Cournand and Lester (6) reported from a study of clinical tests that tuberculo-polysaccharides induced mixed reactions, immediate and delayed. The polysaccharides used had been prepared by Drs. Heidelberger and Menzel and were not free from nitrogen (7). Drs. Heidelberger and Menzel have since submitted the polysaccharide to trypic digestion, which abolishes the reactivity of the protein in the precipitin test. With this material Dr. Cournand has found that the delayed reactions with the polysaccharide have been eliminated in large measure. This work will soon be published and we are permitted to cite it.

In the present studies we first submitted normal and tuberculous
guinea pigs to massive intracardiac and intraperitoneal injections of tuberculo-polysaccharide without any deaths that could be ascribed to the material, thus eliminating the possibility of a lethal effect. Second, we followed the immediate effect on the blood cells and the delayed effect, after 24 hours, on the cells of the peritoneal exudates in rabbits following intraperitoneal injections of small amounts of polysaccharides from tubercle bacilli and from pneumococci. Third, we have studied the effect on the bone marrow and on the cells of the blood in rabbits after intraperitoneal injections of small amounts of tuberculo-polysaccharide repeated daily over long periods of time.

Materials and Methods

We are indebted to Dr. R. J. Anderson of Yale University for the polysaccharides from tubercle bacilli. The use of all the materials from tubercle bacilli in these studies has been under plans for cooperative research of the Committee on Research of the National Tuberculosis Association, of which Dr. William Charles White is Chairman. Dr. Anderson gave us three different preparations of polysaccharide. For the first experiment the material used had been separated from the ether-alcohol extract of tubercle bacilli, human strain A-10. Two preparations, also from the human strain A-10, were used for the second experiment. The first was obtained by Dr. Anderson directly from defatted bacilli, that is, from bacilli which had been treated both with ether-alcohol and with chloroform. The second preparation was obtained from analysis of a so called "unfilterable lipid" which had been separated by treating the defatted bacilli with an acid. The chemical studies on these materials will be published by Dr. Anderson in the near future. For the third experiment we used a polysaccharide obtained by Drs. Anderson and Roberts from the ether-alcohol extract of bovine tubercle bacilli (8).

We are indebted to Dr. Michael Heidelberger of the Presbyterian Hospital, New York, for the polysaccharide from pneumococci, Type I, and for the non-specific, group polysaccharide designated C from pneumococci. The material from the Type I organisms was prepared without heating and was highly purified. It was received in a concentrated solution and was divided into two parts. One part was diluted with distilled water; the other with saline so that 5 cc. contained 10 mg. of the polysaccharide. To Dr. Walther Goebel of the Hospital of The Rockefeller Institute we are indebted for the preparation of the polysaccharide from pneumococci, Type III. It was also prepared without any heating and was highly purified. It was received in saline. The dextrose, trehalose, and the soluble starch were commercial preparations. The soluble starch was given in suspension in distilled water at room temperature. The distilled water used as a diluent came from a metal still and had never been in contact with rubber tubing. It was boiled and then cooled immediately before using.
The blood cells, the peritoneal exudates, and films of omentum were studied both by the supravital technique and after staining with Wright's methylene blue-eosin.

RESULTS

Experiment 1.—Tests for Toxicity of Tuberculo-Polysaccharides.

(a) In Normal Guinea Pigs.—The polysaccharide used in these experiments was separated from the ether-alcohol extract of human tubercle bacilli, strain A-10, by Dr. Anderson. The material was acid and for some of the injections was adjusted to pH 7 with NaOH. We did not detect differences in the symptoms according to whether the reaction was acid or neutral.

Two normal guinea pigs were given intracardiac injections of large amounts of the material adjusted to pH 7 and dissolved in 2 cc. saline. Guinea pig R 3668 received 170 mg. which was 50 mg. per 100 gm. body weight; and guinea pig R 3669 received 380 mg. which was 100 mg. per 100 gm. body weight. Neither animal showed marked symptoms. There was a fall in temperature during the day, about the same amount in the two animals, averaging 3.7°; the temperature had been taken hourly during 3 preceding days and varied about one degree. Four normal guinea pigs which had shown an average variation of 1.5° for the 3 preceding days were given the same amount of saline into the heart and showed an average fall in temperature of 1.5°. Guinea pig R 3668 was twice reinjected with the same polysaccharide but in acid form, 3½ months later. The first of these injections was of 10 mg., given intravenously, and the second of 200 mg., given intraperitoneally. These injections did not cause any symptoms; the following day the animal was sacrificed and showed that there had been a decrease in the number of neutrophilic leucocytes and of mature myelocytes in the bone marrow, and an increase in neutrophils and in macrophages containing neutrophils in the peritoneal exudate.

(b) In Tuberculous Guinea Pigs.—The tuberculous guinea pigs, thirty-one in all, which received the injections of the polysaccharide were divided into two groups according to the length of time which had elapsed after the inoculation. They had all been inoculated with 0.01 mg. of strain H-37 in the right groin.

We began the injections of polysaccharide in the first twenty animals 4 weeks after inoculation, when the tuberculin test made with 0.1 mg. of tuberculo-pro-

1 These are serial numbers covering the work of the laboratory for a term of years.
tein MA-100 had shown 2 and 3 plus reactions. The remaining eleven were not studied until they had been inoculated for 6 weeks. They had shown 3 and 4 plus tuberculin reactions and some of the group had already died.

Twelve of the tuberculous guinea pigs of the first group received 25 or 30 mg. of the polysaccharide in 2 cc. saline into the heart. Of these, six were given the sugar adjusted to pH 7 and six in the acid form. They all showed the same slight and transient symptoms,—some increase in respiratory rate, moderate chills, and a loss of muscle tone. The temperature fell immediately an average of 1.5° and then rose an average of 3.7°. The range of change in temperature was about the same in all the animals. Two of the animals were found dead the next morning with hemorrhage into the pleura due to a puncture of the tuberculous lung overlying the heart. Eight of the guinea pigs were reinjected into the heart after an interval varying from 2 to 3 weeks with no deaths, except that one of them succumbed immediately from heart block due to puncturing the His bundle.

The remaining eight of the first group received 30 mg. of the polysaccharide intraperitoneally. Six of them were reinjected 2 weeks later. The symptoms were the same as after the intracardiac injections except that there was no loss of muscle tone.

The eleven guinea pigs studied later in the course of their disease were injected intraperitoneally. Three of them received 20 mg. and the rest of them 50 mg. The symptoms were not more marked than in the other group. The temperature, instead of falling, as after the intracardiac injections, rose an average of 3.5°. One of this group died during the following night due to a rupture of the spleen from a needle puncture of the enormously enlarged organ.

In summary, thirty-one tuberculous guinea pigs received injections of from 20 to 50 mg. of the polysaccharide either into the heart or into the peritoneal cavity. Fourteen of the number were reinjected 2 or 3 weeks later with the same amounts. There was no increase in symptoms on reinjection, so there was no sign of any sensitization. After these forty-five injections there were only four deaths, all of them clearly accidental, two from puncture of the lung, one from puncture of the spleen, and one from heart block. These results indicate that the polysaccharides used cannot be considered as having lethal power for tuberculous guinea pigs.

**Experiment 2.**—**Peritoneal Exudates and Blood Cells of Normal Rabbits Receiving Bacterial Polysaccharides.**—It has long been known that a wide range of materials,—bacterial proteins, peptones, and various salts,—injected parenterally, call neutrophilic leucocytes from the vessels into the tissues and bring about characteristic changes in
the proportions of the white blood cells. Beard and Beard (9), who counted the blood cells as soon as 10 minutes after intraperitoneal injection of saline, showed that the fall in the number of circulating white blood cells begins at once and involves granulocytes, lymphocytes, and monocytes. This leucopenia is followed by a leucocytosis during the next few hours, which is due to a rise in neutrophilic leukocytes, since both monocytes and lymphocytes continue to fall. The lymphocytes are in the last group of the white blood cells to return to their original level (Sabin et al., 3).

We have made further studies of these phenomena, analyzing the qualitative changes in the neutrophilic leukocytes in the blood stream and following in the peritoneal exudates the reactions that are corre-

![Chart 1. Rabbit R 6043.](chart)

related with these changes. We have used five kinds of bacterial polysaccharides, three from pneumococci and two from tubercle bacilli, in comparison with two simple sugars, dextrose and trehalose, as well as soluble starch given in suspension, and certain diluents,—distilled water, saline, and Tyrode solution.

The amount of the sugars injected has been 10 mg. in every instance; the injections were all made intraperitoneally. In the earlier experiments we used 5 cc. of saline or distilled water as the diluent; later we found it better to use a small amount of distilled water, namely, 0.5 cc., since this amount gave the least reaction as a control. It would have been better had this diluent been used throughout the experiment, but it was not possible to repeat all of the injections and the results are still sufficient to show that the more complex bacterial polysaccharides, in comparison with saline and simple sugars, give rise to reactions that are greater in degree though the same in kind.
The effects of these injections were followed both in the peritoneum and in the bloodstream. The studies of the blood cells are illustrated in six graphs, two of which show the effect of the diluents—distilled water (0.5 cc.), Chart 1, and saline (5 cc.), Chart 2, while the effects of four different bacterial polysaccharides are illustrated as given in each of these diluents (Charts 3 to 6). The data used for these graphs were from the group recorded in Table I. The experimental
procedure was to obtain a base-line of the blood cells of the animal for a few days preceding the injections. These records are not included in the graphs since they were like the count made just before the injections. The counts of the total number of white blood cells per c.mm. were an average from two pipettes; the differential counts recorded in the graphs were all made from fixed films in order to determine the shift to the left (Arneth-Schilling) in the neutrophilic leukocytes.  

Our studies have shown that this shift to the left in the neutrophils of the blood stream is the most accurate measure of the amount of reaction to the peritoneal injections. We made a modified Schilling count, consistently dividing the neutrophils into two groups, those

\[ \text{Poly saccharide } ^{14} \text{C from pneumococcus} \]

\[ 10 \text{mg in } 0.5 \text{cc. distilled water} \]

\[ \text{Injection} \]

\[ \text{Total WBC} \]

\[ \text{Neutrophilic WBC} \]

\[ \text{Flameded WBC} \]

\[ \text{Non-flamed WBC} \]

\[ \text{Chart 5. Rabbit R 6340.} \]

\[ \text{Poly saccharide from unfilatereb lipid Tb A-10} \]

\[ 10 \text{mg in } 0.5 \text{cc. distilled water} \]

\[ \text{Injection} \]

\[ \text{Total WBC} \]

\[ \text{Neutrophilic WBC} \]

\[ \text{Flameded WBC} \]

\[ \text{Non-flamed WBC} \]

\[ \text{Chart 6. Rabbit R 6125.} \]

2 The term neutrophilic leucocyte is used for the pseudo-eosinophil or heterophil of rabbits' blood since this cell corresponds functionally to the neutrophil of human blood.
with nuclei which showed no filaments whatever, generally conceded to be young forms from the bone marrow, shown in a solid line on the graphs, and the more mature forms whose nuclei do show filaments, indicated by broken lines on the graphs. Differential counts of the

### TABLE I

*Fall in Lymphocytes in the Blood Stream during 10 to 12 Hours after Intraperitoneal Injections*

<table>
<thead>
<tr>
<th>Number of rabbits</th>
<th>Materials injected</th>
<th>Average number of lymphocytes per cc. before injections</th>
<th>Average number of lymphocytes per cc. after injections</th>
<th>Fall in lymphocytes per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>Distilled H₂O, 0.5 cc.</td>
<td>3327</td>
<td>1532</td>
<td>54</td>
</tr>
<tr>
<td>2</td>
<td>Saline, 5 cc.</td>
<td>2050</td>
<td>458</td>
<td>78</td>
</tr>
<tr>
<td>2</td>
<td>Dextrose in 5 cc. saline</td>
<td>2131</td>
<td>484</td>
<td>77</td>
</tr>
<tr>
<td>4</td>
<td>Tyrode</td>
<td>2584</td>
<td>1052</td>
<td>59</td>
</tr>
<tr>
<td>2</td>
<td>2–5 cc.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>2–0.5 cc.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Trehalose in 0.5 cc. distilled H₂O</td>
<td>3033</td>
<td>1361</td>
<td>55</td>
</tr>
<tr>
<td>2</td>
<td>Soluble starch in 0.5 cc. distilled H₂O</td>
<td>3979</td>
<td>1089</td>
<td>73</td>
</tr>
<tr>
<td>Average . . .</td>
<td></td>
<td>1846</td>
<td>605</td>
<td>67</td>
</tr>
<tr>
<td>2</td>
<td>Polysaccharide, pneumococcus I in 5 cc. saline</td>
<td>2622</td>
<td>1030</td>
<td>61</td>
</tr>
<tr>
<td>2</td>
<td>Polysaccharide, pneumococcus III in 5 cc. saline</td>
<td>5007</td>
<td>834</td>
<td>83</td>
</tr>
<tr>
<td>2</td>
<td>Polysaccharide, C pneumococci in 0.5 cc. distilled H₂O</td>
<td>3384</td>
<td>1274</td>
<td>62</td>
</tr>
<tr>
<td>2</td>
<td>Polysaccharide, unfilterable lipid tubercle bacilli in 0.5 cc. distilled H₂O</td>
<td>1118</td>
<td>324</td>
<td>71</td>
</tr>
<tr>
<td>Average . . .</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The cells were made just before the injections and then within 15 to 30 minutes afterward, followed by counts at hourly intervals throughout the day. In the case of the rabbits receiving distilled water, we counted the blood cells only in the afternoon (Chart 1). As the graphs
show, there was a slight immediate drop of the blood cells, followed
by a rise which was due to both forms of neutrophilic leucocytes. The
proportional rise of the immature to the mature forms was considered
as the index of the amount of the reaction. The immature forms, the
neutrophils whose nuclei showed no filaments, did not rise above the
mature leucocytes with either diluent, or with either of the simple
sugars, dextrose or trehalose. These immature forms rose above the
mature neutrophils in both of the animals which received the soluble
starch and after almost all of the injections with the bacterial poly-
saccharides. There were two exceptions, namely, one of the two
rabbits which received the C polysaccharide from pneumococci, as
shown in Chart 5, where the numbers became equal, and one of the
animals that received the polysaccharide from the unfilterable lipid
of tubercle bacilli. Thus, in general there was a greater reaction of
the neutrophils after the bacterial polysaccharides than after the
simple sugars, as will be evident also in the studies of the cells of the
peritoneal exudates.

In Table I are shown the changes in the lymphocytes following
these injections. These records are also taken from the differential
counts made from fixed films. After every injection, whether of
diluent or sugars, there was a fall of the lymphocytes in the blood
stream. The prolonged fall of the lymphocytes and their slow recovery
are also illustrated on the graphs, except that the line was not included
on Chart 3 where the other lines were so close together. In Table I
are presented the total number of lymphocytes per c. mm. before
and the lowest number of lymphocytes after the injections. As the
graphs show, the lowest number of lymphocytes was reached 4 to 6
hours after the injections. The amount of the fall of lymphocytes in
the blood shown was practically the same for each animal and there-
fore was the same for the two groups, those that received only the
diluents and simple sugars (fall of 66 per cent) as contrasted with those
that received the bacterial polysaccharides (fall of 69 per cent).

In Table II is shown a study of the cells of the peritoneal exudates
arranged approximately in accordance with the percentage of free
neutrophils. It will be noted that in the data from the effects of the
polysaccharide from the unfilterable lipid, the cells from two animals
(R 6125 and R 6126) are recorded separately. This was because
### Table II

**Cells of Peritoneal Exudates**

<table>
<thead>
<tr>
<th>Rabbit No.</th>
<th>Materials Injected</th>
<th>Amounts and Diluents</th>
<th>Total Number of Cells per c.mm.</th>
<th>PMN</th>
<th>Lymphocytes</th>
<th>Monocytes</th>
<th>Amount of Monocytes with Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>R 6285</td>
<td>None</td>
<td>—</td>
<td>1700</td>
<td>0</td>
<td>5</td>
<td>92</td>
<td>—</td>
</tr>
<tr>
<td>R 6286</td>
<td>Distilled water</td>
<td>0.5 cc.</td>
<td>5100</td>
<td>14</td>
<td>12</td>
<td>61</td>
<td>12 Slight</td>
</tr>
<tr>
<td>R 6038</td>
<td>Tyrode</td>
<td>2-0.5 cc.</td>
<td>5200</td>
<td>17</td>
<td>5</td>
<td>57</td>
<td>21 **</td>
</tr>
<tr>
<td>R 6039</td>
<td>Tyrode</td>
<td>2-5.0 cc.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R 6040</td>
<td>Trehalose</td>
<td>10 mg., 2-in 0.5 cc.</td>
<td>4916</td>
<td>32</td>
<td>1</td>
<td>57</td>
<td>9 **</td>
</tr>
<tr>
<td>R 6041</td>
<td>Trehalose</td>
<td>1-in 5 cc. saline</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R 6042</td>
<td>Polysaccharide</td>
<td>5.0 cc.</td>
<td>6925</td>
<td>34</td>
<td>3</td>
<td>42</td>
<td>21 Moderate</td>
</tr>
<tr>
<td>R 6043</td>
<td>Soluble starch</td>
<td>10 mg. in 0.5 cc.</td>
<td>4850+ clots</td>
<td>42</td>
<td>4</td>
<td>36</td>
<td>18 Marked</td>
</tr>
<tr>
<td>R 6113</td>
<td>Dextrose</td>
<td>10 mg. in 5 cc.</td>
<td>6800</td>
<td>56</td>
<td>1</td>
<td>31</td>
<td>11 **</td>
</tr>
<tr>
<td>R 6120</td>
<td>Polysaccharide</td>
<td>10 mg., 1-in 5 cc.</td>
<td>7712</td>
<td>50</td>
<td>2</td>
<td>36</td>
<td>11 Moderate</td>
</tr>
</tbody>
</table>

*In these exudates there was an average of 0.2 per cent PME and 0.5 per cent serosal cells which have been omitted from the table.*
<table>
<thead>
<tr>
<th>Rabbit No.</th>
<th>Materials injected</th>
<th>Amounts and diluents</th>
<th>Total number of cells per c.mm.</th>
<th>PMN per cent</th>
<th>Lymphocytes per cent</th>
<th>Monocytes per cent</th>
<th>Amount with PMN</th>
<th>Amount of cellular reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>R 6119</td>
<td>Polysaccharide from pneumococcus Type III</td>
<td>10 mg. in 5 cc. saline</td>
<td>7225+ clots</td>
<td>51</td>
<td>21</td>
<td>17</td>
<td>11</td>
<td>Marked</td>
</tr>
<tr>
<td>R 6264</td>
<td>Polysaccharide C pneumococcus</td>
<td>10 mg. in 0.5 cc. dist. H₂O</td>
<td>Clotted</td>
<td>61</td>
<td>1</td>
<td>16</td>
<td>22</td>
<td>&quot;</td>
</tr>
<tr>
<td>R 6340</td>
<td>Polysaccharide from defatted tubercle bacilli, human strain A-10</td>
<td>10 mg., 2-in 1 cc. saline 3-in 5 cc. saline</td>
<td>5550+ clots</td>
<td>58</td>
<td>3</td>
<td>29</td>
<td>10</td>
<td>&quot;</td>
</tr>
<tr>
<td>R 6341</td>
<td>Polysaccharide from unfilterable lipid</td>
<td>10 mg., 4-in 5 cc. saline 2-in 0.5 cc. dist. H₂O</td>
<td>7725</td>
<td>81</td>
<td>1</td>
<td>12</td>
<td>6</td>
<td>&quot;</td>
</tr>
<tr>
<td>R 6029</td>
<td>Polysaccharide from pneumococcus Type III</td>
<td>Clotted</td>
<td>24</td>
<td>6</td>
<td>38</td>
<td>30</td>
<td>&quot;</td>
<td></td>
</tr>
</tbody>
</table>

† This was the only instance in which there were marked differences in the differentials with different diluents and so they are recorded separately.

These two differential counts, which were alike, varied from the others in the group. The difference is, however, not so extreme if it be noted that the monocytes containing neutrophils were very high in these two animals. The total number of cells in the peritoneal fluid cannot be obtained accurately after the injections of the sugars because the fluid clots so quickly. The procedure has been to have the pipettes rinsed with a solution of heparin and then to fill them as soon as possible on opening the peritoneal cavity. If only small clots formed, the counts were made to obtain some approximation of the number of cells present. The constant reaction has been an increase in neu-
trophils in the exudate and the phagocytosis of them by monocytes. The phagocytic mononuclear cells (monocytes, clasmocytes, or macrophages) have been notably in the milk spots of the omentum, to some extent under the serosal lining of the peritoneum, in the sinuses of the retrosternal lymph nodes, and, to a marked degree, in the spleen. A few of the cells with phagocytized neutrophils wandered into the peritoneal exudate. In the last column in Table II is an estimate of the amount of these two reactions, the exudation of neutrophils and their phagocytosis in the omentum, as seen in films and in sections, and in the lymph nodes and spleen. As will be seen in Table II, the least reaction was obtained from distilled water, Tyrode solution, and the simple sugar, trehalose. On the other hand, soluble starch in suspension, dextrose, and all the bacterial polysaccharides except one induced marked reactions.

Two rabbits, R 6342 and R 6343, were studied 1 and 6 hours after the intraperitoneal injection. These animals received 10 mg. of tuberculo-polysaccharide from the unfilterable lipid in 0.5 cc. distilled water. Thus the experiment was like that recorded in Chart 6, in which it will be noted that the greatest fall in neutrophils was at the end of 1 hour and the greatest rise in them at the end of 6 hours after the injection. At the end of 1 hour (R 6343) there was a slight increase in the amount of fluid in the peritoneal cavity; the total number of cells per c.mm. of the exudate was 7450 and the fluid did not clot until it had stood for some time. The differential count showed 98 per cent active motile neutrophils, 1 per cent lymphocytes, and 0.5 per cent monocytes and 0.5 per cent serosal cells. At the end of 6 hours (R 6342) the fluid was scanty and clotted so quickly that the count of 8000 cells must be considered as too low; the differential count showed 94 per cent active motile neutrophils; 1.5 per cent rounded neutrophils, 2 per cent eosinophils, 1 per cent lymphocytes, and 1.5 per cent monocytes. There were no phagocytic cells containing neutrophils in the exudate and none were found in the omentum, in the retrosternal lymph nodes, and none in the spleen.

In contrast to these peritoneal exudates of active neutrophils during the first 6 hours after intraperitoneal injection of a tuberculo-polysaccharide are the late reactions 24 hours after the injections. At this time the exudates are mixed and consist of both neutrophils and monocytes which have phagocytized them. This is true both after the simple materials and after the bacterial polysaccharides. These later reactions are shown in Figs. 1 to 4.
The exudates studied 24 hours after injection showed considerable evidence of damage, or at least of aging of the neutrophils. In the supravital films many of the neutrophils were round and showed neither streaming of granules nor movement of the cells; in fixed films it could be seen that many of them had fragmented and pyknotic nuclei. These points are clear in Fig. 1, which is from a photograph of a fixed film of a peritoneal exudate from a rabbit 24 hours after it had received 10 mg. of a tuberculo-polysaccharide from defatted tubercle bacilli. This photograph also shows monocytes both with and without phagocytized neutrophils, as well as many free neutrophils. Most of the actively phagocytic cells were found not free in the exudates but rather in the milk spots of the omentum, in the sinuses of the retrosternal lymph nodes, and in the spleen. Every animal showed a considerable reaction of the phagocytosis of neutrophils in the spleen. In Fig. 2 is shown a milk spot of the omentum of a rabbit (R 6040) 24 hours after an injection of 0.5 cc. of distilled water. The majority of the cells are monocytes and near the lower margin is a group of monocytes, one with two nuclei and one with two phagocytized neutrophils. In contrast to the small amount of reaction, both of free and phagocytized neutrophils in this photograph, is the reaction shown in Fig. 3, 24 hours after injection of 10 mg. of the C polysaccharide from pneumococci. This is also a milk spot and shows many free and phagocytized neutrophils. The neutrophils which had been phagocytized were not only those with pyknotic nuclei but also leucocytes which, though they were within monocytes, still looked normal. These phagocytized neutrophils were found in various stages of disintegration; in some of the monocytes there were only a few fragments of what seemed to be nuclear debris; in other phagocytic cells, especially after both forms of type-specific polysaccharides from pneumococci, were clumps of debris which we interpret as cytoplasmic. Such a cell is shown in the center of Fig. 4 from a film of omentum of a rabbit (R 6264) which had received 10 mg. of the polysaccharide from Type III pneumococci. This debris is in small clumps, vaguely suggesting platelets; it stained purple in Wright’s eosin-methylene blue after fixation with dioxane and methyl alcohol. After this fixative the metachromatic basophilic granules are well preserved and are easily discriminated from this material. Every transition between the cytoplasm of the neutrophils and this granular material can be made out in these omental films. These reactions indicate that the phagocytic mononuclear cells are able to disintegrate neutrophils quickly. Previous observations have shown that the reaction is practically complete in 48 hours. The two upper cells in Fig. 4 are serosal cells, one of which shows clear vacuoles. These correspond to refractile droplets of lipid, as seen in the living cell. They are a constant reaction of irritation in serosal cells.

**Experiment 3.—Effect of Daily Injections of Tuberculo-Polysaccharide on the Cells of the Blood and Bone Marrow.**—In the third experiment two normal rabbits received daily (except Sundays) intraperitoneal
injections of 10 mg. of tuberculo-polysaccharide obtained from bovine tubercle bacilli (8) in 1 cc. distilled water. Rabbit R 2178 received these injections for a period of 6 weeks; rabbit R 2179 for 6 months. This experiment was planned to ascertain the effect on the bone marrow of repeated injections of this material.

In these animals the blood cells were counted frequently and no effort was made to follow the reactions on the blood cells during the first 12 hours after the injections as in the preceding experiment. The differential counts were made with the supravital technique. During the period of the injections there were no important changes in the total number of white blood cells and in the total number of the neutrophils and lymphocytes per c.mm., as shown in Table III. When the animals were sacrificed 24 hours after the last injection, rabbit R 2178 showed some thickening of the omentum and a considerable number of neutrophils in the peritoneal exudate and in the omentum, both free and within monocytes or macrophages. There had also been active phagocytosis of the neutrophils in the spleen and the bone marrow was moderately hyperplastic. Rabbit R 2179, which had received the injections for a longer period, showed so much thickening of the omentum that a film could not be made for supravital study. Both the omentum and the peritoneal lining of the body wall showed layers of large monocytes, some of them containing nuclear debris, probably from phagocytized neu-

### TABLE III
**Means of Blood Cells before and during Period of Injection**

<table>
<thead>
<tr>
<th>Rabbit No.</th>
<th>White blood cells</th>
<th>PMN</th>
<th>Lymphocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before injection</td>
<td>During period of injection</td>
<td>Before injection</td>
</tr>
<tr>
<td>R 2178</td>
<td>10,275</td>
<td>12,864</td>
<td>4815</td>
</tr>
<tr>
<td>R 2179</td>
<td>11,300</td>
<td>10,189</td>
<td>7101</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Rabbit R 2178</th>
<th>Rabbit R 2179</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myeloid cells</td>
<td>71.2</td>
</tr>
<tr>
<td>Erythroid cells</td>
<td>17.9</td>
</tr>
<tr>
<td>Primitive cells</td>
<td>10.9</td>
</tr>
</tbody>
</table>

### TABLE IV
**Proportion of Myeloid to Erythroid Cells in the Bone Marrow after Repeated Injections of Tuberculo-Polysaccharide**
trophils. There were also a few giant cells. There were fewer neutrophils either free or phagocytized than in rabbit R 2178. The bone marrow was hyperplastic throughout, with, however, some fat cells remaining; there was well marked extramedullary formation of neutrophilic myelocytes in the spleen. The counts of the cells of the marrow of these animals showed an increased proportion of myeloid to erythroid cells in each instance, as shown in Table IV.

Discussion

The fact that such large amounts of tuberculo-polysaccharides as have been given not only to normal but to tuberculous guinea pigs did not elicit marked symptoms indicates that the harmful effects of these sugars are slight. Perhaps they cannot be considered as entirely non-toxic or innocuous since, as Courmand and Lester (6) have shown, a characteristic skin reaction can be elicited with certain polysaccharide fractions in tuberculous patients.

These studies have shown that polysaccharides from tubercle bacilli and from pneumococci introduced into the peritoneal cavity of rabbits call neutrophils from the circulation in larger numbers than the same amount of normal saline or simple sugars. This phenomenon in general is well known and has been submitted to extensive study both with bacterial products and with many different kinds of salts (3, 9, 10–16). As has been shown, there is an immediate fall in the white blood cells, including neutrophils, monocytes, and lymphocytes (9). The leucopenia becomes maximum in 1 hour, when the neutrophils begin to rise, while monocytes and lymphocytes continue to fall. The lymphocyte is the last of the three cells to rise and does not reach its original level for 24 hours (Sabin et al., 3).

In the present studies it has become clear that the lymphocytes react entirely differently from the neutrophilic leucocytes. There is a prolonged and steady fall in lymphocytes, lasting from 6 to 8 hours; during this period, as well as for 24 hours later, there is no exudation of lymphocytes into the peritoneal cavity. This is clear in the cells of the exudates of the rabbits studied on the day of the injections, where only 1 per cent of lymphocytes were found, as well as in the low percentages of lymphocytes in the exudates 24 hours after injection, as shown in Table II. Also careful studies of the condition of the lymphocytes in the films of blood cells have failed to reveal any signs of degeneration of lymphocytes. From these observations it seems
likely that even the slight irritation of the peritoneum brought about by these injections has retarded the delivery of lymphocytes into the circulation. This hypothesis might be tested by finding the rate of delivery of lymphocytes through the thoracic duct after such intraperitoneal injections.

The study of the reactions of the neutrophilic leucocytes, on the other hand, indicates that two entirely different mechanisms of the bone marrow are brought into play. There is, first, a mechanism for the quick delivery of neutrophils into the blood stream and, second, a building up of the bone marrow after depletion. The first mechanism comes into play about an hour after the injection and acts for from 6 to 10 hours. This is illustrated in the graphs, where the rise in neutrophils has usually lasted for 6 hours but in one instance for 10 hours (Chart 3). During this period no damage to the extravasated neutrophils was detected in peritoneal exudates and no phagocytosis nor digestion of them was found either in the omentum or in the spleen. Only one observation gives any suggestion of a possible chemical stimulus from the cells available during this period. By the 6th hour there is a marked and rapid clotting of the peritoneal exudate. From many observations on these peritoneal exudates we have found that such rapid clotting is correlated with the appearance in the exudates of fragments of the surface films of cells, usually of monocytes. Such cellular debris is shown in the lower edge of Fig. 1. Fragmentation of bits of the cytoplasm from the neutrophils is frequently seen in supravital films of blood cells and may have taken place in the peritoneal exudates in from 6 to 10 hours concomitantly with the increase in clotting time of the fluid. However, it seems to us that the speed with which the delivery of young neutrophils from the marrow to the blood stream takes place suggests rather that the materials injected, salts or sugars, which have attracted the neutrophils from the peripheral vessels into the tissues, when they arrive in the sinuses of the bone marrow, attract the young neutrophils from the marrow into the sinuses. It is well known that in the peripheral vessels the neutrophils move along the inner lining of the endothelium, while in the marrow the young neutrophils lie along the outer surface of the endothelium of the sinuses. Thus the conditions are the same, except that in the periphery the stimulus is
outside the vessels and by chemotaxis draws the cells into the tissues, while in the marrow the stimulus may be within the vessels. The marrow is the only place where there is a supply of neutrophils outside the vessels, so it is in this organ that the new neutrophils are drawn into the vessels. This phenomenon takes place before the disintegration of the extravasated neutrophils can be detected. Generally speaking, it is the reaction during the first 12 hours after the injection of foreign materials into the tissues. It is, of course, true that there may be some peripheral redistribution of cells in the blood vessels, as, for example, from the spleen during these early hours after intraperitoneal injections, but it seems to us that the most likely explanation of the phenomenon is that some of the materials injected reach the marrow before they can be eliminated by the kidneys and serve as the agent for the depletion of the marrow of young leucocytes.

Entirely different from these reactions of the first 12 hours after intraperitoneal injections, are the phenomena to be made out during the next 12 hours. Between 12 and 24 hours there have been not only an aging of the extravasated neutrophils but a marked phagocytosis of them by monocytes. It is our opinion that the neutrophils which have emigrated from the vessels remain in the tissues where, if in not too overwhelming numbers, they are phagocytized and disintegrated within a short time. It was such observations that initiated the study of the action of nucleotides (Doan, Zerfas, Warren, and Ames, 17; Doan, 18) on the bone marrow. It seems likely to us that all of the material of the neutrophils, cytoplasmic as well as nuclear, is broken down by the phagocytic mononuclears and returned to the blood stream. The experiments involving injections of the polysaccharides over weeks and months suggest that this disintegration of extravasated neutrophils is correlated with the building up of the marrow after depletion, that is, with the cell division and the maturation of melocytes. The experiments with repeated injections of small amounts of polysaccharide are an example of a nice adjustment of the marrow by which the disintegration products of the leucocytes withdrawn from the circulation make the marrow just hyperplastic enough to replace them. The result was a normal blood count and a hyperplastic marrow.

Our observations indicate that there are two phases of the reactions
of the bone marrow to intraperitoneal injections of materials that are sufficiently innocuous to reveal a physiological mechanism. There is first a withdrawal of leucocytes from the peripheral vessels and a compensating draining of new neutrophils into the sinuses of the bone marrow. The stimulus for the first and probably for both of them is the material injected. In the periphery the stimulus is outside the vessels; if our theory is correct, in the marrow it is within the lumen of the sinuses. There is second a building up of the marrow through the products of disintegration of the extravasated neutrophils brought about by the phagocytic mononuclear cells. These two phenomena are separated in time. In both instances the forces involved were nicely balanced under the conditions of our experiments.

SUMMARY

1. Purified tuberculo-polysaccharides are relatively innocuous both to normal and to tuberculous guinea pigs.
2. Both tuberculo-polysaccharides and polysaccharides from pneumococci call larger numbers of leucocytes from the blood vessels than do saline and dextrose and trehalose.
3. The mechanisms controlling the delivery of lymphocytes and neutrophils into the blood stream are different.
4. Slight irritation of the peritoneal lining slows the delivery of lymphocytes to the blood stream.
5. There are two phases in the reaction of the bone marrow to intraperitoneal injections. Correlated with the draining of neutrophils from vessels to tissues, owing to the presence of foreign materials in the latter, there is a draining of young neutrophils from the marrow into the sinuses of the marrow as these same materials reach the sinuses. The subsequent disintegration of the neutrophils extravasated into the tissues is correlated with increased myeloid activity in the marrow.

BIBLIOGRAPHY

EXPLANATION OF PLATE 24

Fig. 1. Peritoneal exudate from rabbit R 5665, 24 hours after an intraperitoneal injection of 10 mg. of polysaccharide from defatted tubercle bacilli. Fixed for 2 minutes in dioxane, 30 parts, and methyl alcohol, 70 parts, and stained with Wright-Giemsa. × 1200.

Fig. 2. Film of omentum of rabbit R 6040, 24 hours after an intraperitoneal injection of 0.5 cc. distilled water. Fixed in dioxane, 30 parts, and methyl alcohol, 70 parts, for 3 minutes and then stained with Wright's methylene blue-eosin. × 550.

Fig. 3. Film of omentum of rabbit R 6340, 24 hours after an intraperitoneal injection of 10 mg. of C polysaccharide from pneumococci. Fixation and stain as in Fig. 2. × 1000.

Fig. 4. Film of omentum of rabbit R 6264, 24 hours after an intraperitoneal injection of 10 mg. of a polysaccharide from pneumococci Type III. Fixation and stain as in Fig. 2. × 1000.