THE EFFECT OF INJECTED LEUCOCYTES UPON THE DEVELOPMENT OF A TUBERCULOUS LESION.*

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The purpose of the experiments which will be described has been to determine the effect of injected leucocytes upon the development of a tuberculous lesion. For the experiments the dog, which is somewhat insusceptible to tuberculosis has been selected for two reasons. Preceding studies have demonstrated methods by which it is possible to obtain sterile leucocytes in great quantity almost wholly free from the inflammatory irritant which has been used to cause their accumulation. Of equal importance for the purpose of the experiments is the fact that the development of the tuberculous lesions produced by injection of tubercle bacilli into the pleural cavity of the dog can be followed with considerable accuracy by percussion of the animal's chest.

The insusceptibility of the dog to tuberculosis has been exaggerated and its apparent immunity to the disease is doubtless dependent in part upon the fact that its habits do not expose it to infection. Freedom from spontaneous infection is not an accurate index of susceptibility, for the guinea-pig, in which tuberculosis develops with great readiness, is rarely subjected to spontaneous infection. Spontaneous tuberculosis in dogs has been studied especially by Jensen (twenty-eight cases), Cadiot (forty cases) and Eber1 (eleven cases). The lungs are the primary seat of infection in a large proportion of the cases. The pleura and mediastinal lymphatic glands are implicated. Jensen has described the sarcoma-like appearance of tuberculous tissue in the dog and thinks that tuberculosis of various organs in this animal closely resembles the same lesion in cattle.

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1Eber, Lubarsch and Ostertags Ergebnisse der allg. Path., 1897, iv, 859.
Effect of Injected Leucocytes upon a Tuberculous Lesion.

Injection of a suspension of tubercle bacilli into the pleural cavity of dogs causes tuberculosis which is almost constantly fatal. Fluid accumulates in the cavity; flat nodules of grayish white tuberculosis tissue are formed upon the pleura of the chest wall and diaphragm and occasionally upon the surface of the lungs. The lesion is bilateral and fluid accumulates in both the right and left pleural cavities, but the disease usually progresses more rapidly in the cavity which has received the injection. Rounded masses of firm gray-white tissue appear in the mediastinum (Fig. 1) and when the lesion is advanced may grow together, forming a continuous mass extending the whole length of the sternum. The tissue is at first succulent.
grayish white and homogeneous, having an appearance suggesting sarcoma, but later becomes opaque and yellow where caseation occurs. Similar masses of tuberculosis tissue (Fig. 2), often continuous with those in the mediastinum, occupy the membranes which in the dog extend from each side of the pericardium to the diaphragm and enclose a cavity; into this cavity fits a lobe of the right lung. The mediastinal lymphatic glands (Figs. 1 and 2), situated behind the upper end of the sternum, quickly enlarge, become hard and undergo caseation.

Dissemination of tubercle bacilli occurs at an early period of the disease and ten days after inoculation tubercles may be found in the liver. After many weeks tubercles may be found in the lungs, spleen and kidneys. Extension by way of the lymphatic system occurs rapidly and evidence of tuberculosis appears successively in the retropleural glands just above the diaphragm, in glands near the duodenal part of the pancreas and in the retroperitoneal glands which are near the adrenals.

Death frequently occurs as the result of broncho-pneumonia due to secondary infection. After several weeks the animal may cough and there is abundant purulent discharge from the nose. Associated with this condition conjunctivitis and ulceration of the cornea may occur.

The experiments which have been described have been possible only because the course of the disease can be followed during life by percussion of the chest. In the normal animal standing in its usual position there is relative dullness two or three centimeters to the right of the median line caused by projection of the heart to the right. The median line of the animal is marked and the upper limits of relative and absolute dullness accurately measured immediately behind the fore-leg; figures thus obtained are accurate within less than half a centimeter and afford the only available means of measuring during life changes in the fluid and solid contents of the chest.

After injection of tubercle bacilli increase of relative dullness over the ventral part of the right chest is evident within one or two days and this impaired dullness increases continuously. Usually within a week or ten days absolute dullness makes its appearance.
Changes in the extent of dullness on percussion are referable in part to accumulation of fluid, in part to solid tuberculosis masses in the mediastinum and adjacent membranes. Observations made by puncture of the animal's chest and by autopsy indicate that absolute dullness is caused by the presence of fluid. Not infrequently in inoculated animals absolute dullness disappears although increased relative dullness persists and the disease continues with undiminished severity. In such instances disappearance of absolute dullness is doubtless due to absorption of pleural effusion.

The following experiments illustrate the effect on dogs of intrapleural injection of the strain of tubercle bacillus employed in the greater number of the experiments which will be described. The organism had the characters of the human type of tubercle bacillus and was of only moderate virulence, killing guinea-pigs by intraperitoneal injection after from three to four weeks; it did not kill rabbits after injection into the peritoneal cavity, but caused their death six or seven weeks after intrapleural inoculation.

**EXPERIMENT 1.**—Dog, wt. 4,150 grm. Into the right pleural cavity was injected 0.5 c.c. of a suspension of B. tuberculosis. Relative and absolute dullness over the right side of the chest gradually increased so that at the end of about a month relative dullness measured 8.5 cm. and absolute dullness 6.6 cm. Below the skin at the point of injection a nodular mass fixed to the chest wall made its appearance two weeks after inoculation, increased in size, and opened spontaneously; it remained as an open wound until death which occurred with increasing emaciation at the end of 50 days after inoculation.

**Autopsy.**—Each pleural cavity contains about 150 c.c. of almost clear yellow fluid which on standing forms a transparent coagulum. Upon both visceral and parietal pleurae are flat, yellowish-white nodules. Masses of hard, partly caseous tuberculous tissue occur in the mediastinum and in the membranes extending from pericardium to diaphragm. The substernal lymphatic glands are greatly enlarged and caseous. The liver contains an immense number of miliary tubercles which occupy about one half the area of the section prepared for microscopic examination. The spleen and kidney contain tubercles in small number.

**EXPERIMENT 2.**—Dog, wt. 4,850 grm. Into the right pleural cavity was injected 1 c.c. of a suspension of B. tuberculosis. Dullness over the ventral part of the thorax on the right side increased so that at the end of two weeks the upper level of relative dullness was 7.6 cm. from the mid line; of absolute dullness, 3 cm. During this time the body weight had been maintained. The animal became thin and death occurred at the end of 20 days after the inoculation.

**Autopsy.**—The right pleural cavity contains 75 c.c. of almost clear yellow fluid; the left cavity contains 80 c.c. Upon the surface of both lungs are flat gray-white projections usually less than 1 mm. across. The mediastinum which is thickened contains large masses of newly formed hard grayish white tissue;
a similar mass is in contact with the diaphragm. The lymphatic glands below
the cephalic end of the sternum are greatly enlarged and caseous. The liver
contains an immense number of tubercles which occupy at least a third of the
section for microscopic examination. The spleen contains tubercles; none are
found in the kidneys.

Inoculation with 0.5 c.c. of a suspension of tubercle bacilli caused
death in fifty days whereas twice this amount of the same suspen-

The leucocytes used for injection have been obtained from dogs
by repeated injection of turpentine into the pleural cavity. One or
two cubic centimeters of turpentine have been injected into the
right pleural cavity; when after three days the resulting inflamma-
tory exudate has reached a maximum a second similar injection
is made. Fluid continues to accumulate and may be serous, sero-

Leucocytes obtained one or two days either after injection of tur-
pentine or after aspiration are separated by centrifugalization from
the serum of the exudate and twice washed by centrifugalization
with normal salt solution. The leucocytes after removal of the
overlying salt solution readily pass through the coarse needle of a
syringe. The quantities injected represent volumes of leucocytes
packed together by centrifugalization.

A coarse needle with blunt beveled point and with an opening at
the side a short distance from the end has proved convenient for
intrapleural injection. The skin is punctured with a sharp instru-
ment and the needle of the syringe is inserted obliquely in such posi-
tion that the beveled surface of the end is parallel with the chest wall.

Washed leucocytes obtained by the method which has been de-
scribed cause a readily recognizable reaction when introduced into
the right pleural cavity of a normal dog. Ten cubic centimeters of
these cells cause an accumulation of fluid which is indicated by a
broad area of relative and usually of absolute dullness over the
dependent part of the cavity. This increased dullness reaches a
maximum on the day following injection and rapidly subsides, dis-
appearing after three or four days. The quantity of fluid which
accumulates (indicated by the amount of dullness) and the dura-
Effect of Injected Leucocytes upon a Tuberculous Lesion.

The pleural cavity is not permanently altered by the reaction which occurs. In an animal which had received four injections of leucocytes (10 to 25 c.c.) at intervals of about one week the pleural cavities were found to be normal and the mediastinum and adjacent membranes delicate.

Series B.—The effect of injections of leucocytes upon thoracic dullness increased by inoculation with Bacillus tuberculosis.

One half cubic centimeter of a suspension of Bacillus tuberculosis was injected into the right pleural cavity of six dogs. Dullness on percussion over the right side of the thorax underwent an increase, exhibiting in different animals considerable variation in rapidity. Two animals (weighing respectively 5,050 and 7,250 grm.) in which the disease was allowed to pursue its course served as control.

At the end of seven or eight days, when relative dullness was much increased and absolute dullness had made its appearance in all of the inoculated animals, leucocytes in quantities from twelve to twenty-five cubic centimeters were injected into the right pleural cavities of the remaining four dogs; the injections were repeated at intervals of about one week.

For extraneous reasons it was found necessary to discontinue injection of leucocytes at the end of one month after inoculation; at this time the area of dullness had in the four injected animals diminished considerably and was not much greater than that present before the onset of tuberculosis. It was thought possible that recovery might follow but examination of the chest on the fortieth day of the disease showed that in two animals there was increase of relative and reappearance of absolute dullness.

Experiment 3.—Dog, wt. 5,450 grm. The animal received three intrapleural injections of leucocytes; changes in thoracic dullness are indicated below. At the end of a week a small nodule was found below the skin at the point at which the inoculating needle had been inserted; ten days later leucocytes were injected into the nodule. It became smaller but finally broke upon the surface and remained as a discharging ulcer until death. Death occurred 57 days after inoculation.

<table>
<thead>
<tr>
<th>Day of Disease</th>
<th>Absolute Dullness</th>
<th>Relative Dullness</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>—</td>
<td>2.7</td>
</tr>
<tr>
<td>9</td>
<td>3.0</td>
<td>3.9</td>
</tr>
<tr>
<td>10</td>
<td>4.7</td>
<td>6.6</td>
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</tbody>
</table>

Inoculated with tubercle bacilli.

14 c.c. leucocytes injected.
Eugene L. Opie. 425

12 — 4.3
19 — 3.4  12 c.c. leucocytes injected.
20 — 3.4
27 — 5.3  10 c.c. leucocytes injected.
28 — 4.5
29 — 3.8
40 4.6  8.1
58

 Died.

Autopsy.—The pleural cavities are each distended with several hundred cubic centimeters of turbid fluid which compresses the lungs. The mediastinum and subpericardial membranes which are thickened and opaque contain large confluent gray white masses with a maximum thickness of 0.5 cm. The mediastinum is pouched to the right and much crinkled. Parietal and pulmonary pleurae are thickened and opaque. The subternal lymphatic glands are moderately enlarged and partially caseous. The liver is large and contains an immense number of tubercles. An occasional tubercle is found in the lungs.

Experiment 4.—Dog, wt. 6,850 grm. The animal was inoculated into the right pleural cavity with 0.5 c.c. of a suspension of B. tuberculosis. A nodule formed in the skin at the point of injection. The progress of the disease during which leucocytes were injected three times is shown by the following table. Death occurred after 68 days.

<table>
<thead>
<tr>
<th>Day of Disease.</th>
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<th>Description</th>
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<td>4.0</td>
<td>Inoculated with B. tuberculosis.</td>
</tr>
<tr>
<td>9</td>
<td>4.0</td>
<td>5.6</td>
<td>25 c.c. leucocytes injected.</td>
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<td>3.7</td>
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<tr>
<td>19</td>
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<td>3.7</td>
<td>11 c.c. leucocytes injected.</td>
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<td>20</td>
<td>3.3</td>
<td>7.0</td>
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<td>22</td>
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<td>4.8</td>
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<tr>
<td>27</td>
<td>—</td>
<td>4.8</td>
<td>10 c.c. leucocytes injected.</td>
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<td>28</td>
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<tr>
<td>40</td>
<td>—</td>
<td>4.6</td>
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</tr>
<tr>
<td>69</td>
<td></td>
<td></td>
<td>Died.</td>
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</table>

Autopsy.—The pleural cavities contain a large quantity of fluid which compresses the lungs. The mediastinum contains masses of newly formed tissue which is dense and fibrous and contains caseous patches. Masses of similar tissue occur in the membranes which extend from pericardium to diaphragm. The lungs are atelectatic and contain upon their surfaces and in their substance numerous tubercles. About one third of a section of liver consists of tuberculous tissue.

Experiment 5.—Dog, wt. 6,750 grm. The animal received into the right pleural cavity 0.5 c.c. of a suspension of tubercle bacilli. A nodule appeared at the point of inoculation about a week later. Leucocytes were injected into the nodule which subsequently diminished much in size. The animal received four injections of leucocytes into the pleural cavity, it became emaciated and died at the end of 89 days.
Effect of Injected Leucocytes upon a Tuberculous Lesion.

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<td>4.6</td>
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<td>4.9</td>
</tr>
<tr>
<td>33</td>
<td>—</td>
<td>4.0</td>
</tr>
<tr>
<td>40</td>
<td>2.8</td>
<td>6.8</td>
</tr>
<tr>
<td>90</td>
<td>—</td>
<td>Died</td>
</tr>
</tbody>
</table>

Inoculated with *B. tuberculosis.* 12 c.c. leucocytes injected.

22 c.c. leucocytes injected.

10 c.c. leucocytes injected.

10 c.c. leucocytes injected.

Autopsy.—Pleural cavities contain a large amount of fluid. The pleura is thickened and opaque and on its surface in places is a thin layer of fibrin. The

![Chart I](chart.png)

Chart I.—The progress of the disease in Experiment 6 and in subsequent experiments has been depicted by a chart which indicates the amount of absolute and relative thoracic dullness measured to the right of the mid line. Injection of leucocytes is indicated by heavy black perpendicular lines whose length (one square equals 5 c.c.) represents the quantity of cells injected. Relative dullness is represented by the lightly shaded zone; absolute dullness by the heavily shaded areas. In the normal animal there is no absolute dullness over the thorax to the right of the mid line but since projection of the heart to the right causes relative dullness two or three centimeters beyond the mid line a normal base line (dotted horizontal line) of relative dullness has been drawn for each animal. Weight is indicated by a dotted line at the upper part of each chart.
mediastinum is free from tuberculous masses save above the diaphragm where there is a mass of fibrous and caseous tissue; in the membranes which extend from pericardium to diaphragm are similar masses, that on the right being the larger. The mediastinal lymphatic glands are moderately enlarged and consist of caseous material surrounded by a fibrous capsule. The lungs and liver contain tubercles in immense number. In the liver they are surrounded by a thin fibrous capsule.

Experiment 6.—Dog, wt. 4,750 grm. Seven days after inoculation with B. tuberculosi as a time when relative dullness over the right chest had increased and absolute dullness had made its appearance leucocytes were injected. Three similar injections were subsequently given and at the end of 27 days dullness over the right chest was only slightly greater than that before inoculation; the changes are shown by Chart 1. During this period the body weight diminished slightly. The animal at the end of eight months is very active and apparently well, its weight being 2,050 grm. more than the weight at the time of inoculation. Percussion shows only a normal relative dullness over the right thorax.

Control Experiments.

Experiment 7.—Dog, wt. 5,050 grm. Control. The weight of the animal fell quickly after inoculation. Relative dullness over the right pleural cavity gradually increased and absolute dullness was present at the end of a week. Absolute dullness disappeared although relative dullness persisted until a short time before death. Death occurred after onset of cough and purulent nasal discharge at the end of 34 days.

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<td>25</td>
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<tr>
<td>28</td>
<td>—</td>
<td>6.1</td>
</tr>
<tr>
<td>35</td>
<td>—</td>
<td>Died.</td>
</tr>
</tbody>
</table>

Inoculated with B. tuberculo.

Autopsy.—The animal is emaciated. In the right chest wall at the site of inoculation is a mass of partially caseous tuberculous tissue which projects upon the parietal pleura. Each pleural cavity contains only about 25 c.c. of fluid. The thickened and injected mediastinum and adjacent membranes contain tuberculous masses. The substernal, retropleural and retroperitoneal lymphatic glands are much enlarged and tuberculous. There is bronchitis and patches of broncho-pneumonia. The liver is enlarged, and exhibits fatty degeneration; small caseous tubercles are numerous.

Experiment 8.—Dog, wt. 7,250 grm. Control. After inoculation the body weight rapidly fell; relative dullness over the right pleural cavity increased slightly and at the end of a week absolute dullness had made its appearance but subsequently disappeared. Death occurred at the end of 35 days.
Effect of Injected Leucocytes upon a Tuberculous Lesion.

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<tr>
<td>29</td>
<td>—</td>
<td>6.1</td>
</tr>
<tr>
<td>36</td>
<td>Died</td>
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</table>

Inoculated with B. tuberculosis.

Autopsy.—The animal is emaciated. Each pleural cavity contains about 150 c.c. of turbid yellowish fluid. The mediastinum and subpericardial membranes which are intensely injected contain caseous masses. The substernal lymphatic glands and lymphatic glands near the duodenum are enlarged and caseous. The lungs contain deep red patches of broncho-pneumonia and there is bronchitis. The liver contains numerous small caseous tubercles.

The first injection of leucocytes into a pleural cavity containing effusion, indicated by absolute and increased relative dullness, has in two instances (Experiments 5 and 6) been followed within twenty-four hours by diminution of dullness, doubtless the result of absorption of fluid. In one instance (Experiment 4) fall of the level of dullness was delayed at least twenty-four hours whereas in one instance (Experiment 3) a well-marked increase of dullness preceded the disappearance of absolute dullness, relative dullness, probably due to the presence of tuberculous masses, persisting. Injections of leucocytes were repeated at intervals of a week or ten days, dullness not infrequently increasing between injections and falling after them. These changes are illustrated by Chart I, in which relative and absolute thoracic dullness are charted.

In this series of experiments, which were begun eight months ago, two control animals died at the end of five weeks; two animals receiving three injections of leucocytes lived about two months; a third injected animal, receiving four injections, lived three months, and a fourth animal receiving the same number of injections is living and well.

It is noteworthy that injection of a large quantity of leucocytes (20 c.c.) has been usually followed by marked increase of dullness with subsequent fall. The following experiments show the effect of leucocytic injections repeated more frequently and in larger quantity than those previously employed.
Series B.—The effect of leucocytes injected into the pleural cavity at short intervals and in large quantity during the course of tuberculous pleurisy.

Two animals were inoculated intrapleurally with 0.5 cubic centimeter of a suspension of Bacillus tuberculosis. During the first ten days changes of thoracic dullness pursued an approximately parallel course in the two animals, relative dullness gradually increasing and absolute dullness appearing. The injected animal died at the end of fifty-six days and the control animal was immediately killed for comparison.

Experiment 9.—Dog, wt. 5,800 grm. At the end of a week a nodular thickening had developed in the chest wall at the point of inoculation; leucocytes were injected into the nodule which after several weeks diminished in size. Leucocytes were injected into the pleural cavity ten days after inoculation and the animal received four injections within fifteen days. Mange made its appearance and was widely distributed upon the skin. The animal became thin and death occurred at the end of 56 days.
Antopsy.—The body is thin but not emaciated. The right pleural cavity is
distended with several hundred cubic centimeters of almost opaque whitish
fluid which compresses the lung. The parietal and pulmonary pleura is everywhere grayish white and thickened often to 1 mm. The left pleural cavity is
also distended and the pleura is grayish white but less thickened than on the right
side. The distribution of the lesions which are present is represented by Fig.
3; compare with Fig. 2 showing the lesions in the control. Upon the surface
of the mediastinum which is thickened and leathery are several flat yellowish white elevations. At the junction of the subpericardial membranes and diaphragm on each side are small scar-like masses which on section are composed of grayish white tissue.
The substernal lymphatic glands are moderately enlarged, measuring 1.4 cm. in long
diameter. The lungs, which are much compressed, contain numerous tubercles. The liver
contains a great number of large tubercles; tuberculous tissue occupies at least a third
of a section for microscopic examination.

Control Experiment.

Experiment 10.—Dog, wt. 5,650 grm. Control. Ten days after inoculation a nodule made its appearance immediately below the skin at the site of injection; it gradually increased in size and broke two weeks later. There was cough beginning about ten days after inoculation, Weight diminished gradually. The animal was killed at the end of 56 days, for comparison with that of the preceding experiment.

Autopsy.—The right pleural cavity contains about 100 c.c. of turbid fluid;
the left cavity contains about the same amount of fluid. The parietal and pulmonary pleura are not thickened. Situated in the mediastinum above the
diaphragm and extending into the subpericardial membrane is a very large mass of grayish white succulent, in places, caseous tissue (see Figs. 1 and 2); a similar mass which is smaller occupies the subpericardial membrane on the right side. Figs. 1 and 2 show the situation of these tuberculous masses. A large mass of tuberculous tissue is situated in the posterior mediastinum above the diaphragm. The substernal lymphatic glands are greatly enlarged, hard and caseous, measuring 1.6 cm. in long diameter. The lungs contain no tubercles. The liver contains small scattered tubercles.

Whereas increase of thoracic dullness proceeded uninterruptedly
in the control, the first intrapleural injection of leucocytes in the

treated animal was followed by a fall of relative and disappearance
of absolute dullness. A second injection three days after the first
was followed by an accumulation of fluid which showed no tendency to subside until a third injection was given. The fourth injection (30 c.c.) was given with the hope of influencing favorably by a large quantity of leucocytes what appeared from the extent of thoracic dullness to be a very severe infection. Fluid showed little indication of decrease and death resulted about one month later. The generalized chronic pleurisy with effusion, which doubtless caused or hastened death, has not been observed in any of the untreated tuberculous animals and is probably referable to the injections of leucocytes which were repeated at unusually short intervals and in unusual amount; for repeated observations have demonstrated that the intensity of the inflammatory reaction which follows injection of leucocytes bears a relation to the quantity injected.

Although the injection of leucocytes did not prolong the life of the animal nor exert a favorable influence upon the course of the disease, comparison of the lesion with that of a control animal inoculated with the same suspension and killed after the same interval has shown that the local tuberculous process has been markedly retarded. In the control animal (Fig. 1) the mediastinum and subpericardial membranes are occupied by enormous succulent partially caseous masses and the lymphatic glands adjacent to the pleural cavities are greatly enlarged and caseous. In the treated animal (Fig. 3) there are small scar-like masses almost wholly composed of fibrous tissue in the same situations and the adjacent lymphatic glands are moderately enlarged and show no caseation. Microscopic examination of the thickened pleura shows that it is composed of fibrous tissue with none of the characters of tuberculous new growth. Tuberculosis in the neighborhood of the injected pleural cavity had in large part disappeared.

Series C.—The effect of long-continued injections of leucocytes upon the course of experimental tuberculous pleurisy.

With the information derived from the foregoing experiments, an attempt was made to treat with leucocytic injections animals with experimental tuberculous pleurisy. Seven dogs received half a cubic centimeter of the same suspension of tubercle bacilli and at the end of ten days there was in all of them a well-marked increase of thoracic dullness. Three animals were kept as controls whereas the remaining four received repeated injections of leucocytes.
Effect of Injected Leucocytes upon a Tuberculous Lesion.
Chart 7. Experiment 14; injection of leucocytes.

Chart 8. Experiment 15; control.

Chart 9. Experiment 16; control.

Chart 10. Experiment 17; control.
Effect of Injected Leucocytes upon a Tuberculous Lesion.

Experiment II.—Dog, wt. 5,650 grm. After inoculation the animal lost about 1,000 grm., the most marked loss in weight following the first two injections of leucocytes. After four weeks the animal regained and subsequently much exceeded its original weight. Thoracic dullness which had increased as the result of inoculation disappeared after the first leucocytic injection and did not reappear (Chart 4). The animal received into the right pleural cavity seven injections. On the twenty-fifth day of the disease a nodule appeared upon the chest wall caused doubtless by infection of the needle tract at the time of inoculation. Leucocytes were twice injected into the nodule which at one time measured 4 cm. across; it diminished in size and almost completely disappeared two weeks after its appearance. The nodule reappeared at the same site and after two weeks was 2 cm. across; four injections of leucocytes (1 c.c.) were not followed by any diminution of size and after attaining a diameter of 4 cm. the nodule broke and discharged during several weeks upon the surface. Healing with scar formation followed. The animal increased much in weight and became strong and active. It acquired the habit of bounding to the top of its cage and would jump continuously during several hours. Five months after inoculation it suddenly became sick and died two days later. At the time of death it was well nourished, weighing one thousand grams more than at the time of inoculation.

Autopsy.—Fat in the subcutaneous tissue and elsewhere is abundant. The pleural cavities contain no fluid. The mediastinum is delicate and membranous and very redundant so that it can be pouched far to the right or left; the membranes below the pericardium are delicate and exhibit the same redundancy. In these membranes and upon the surface of the diaphragm are small, little elevated, patches with reddish gray color; there is no tuberculous tissue and the pleural surfaces are smooth and glossy. The mediastinal lymphatic glands are slightly enlarged, measuring 1 cm. in length and are red and succulent.

The liver is apparently normal. The duodenum near the stomach for a distance of about 7 cm. is plum-colored and apparently in part gangrenous; the adjacent mesentery including the entire pancreas with the exception of a small part of the duodenal arm is infiltrated with blood; the adjacent lymphatic glands particularly those near the liver are enlarged and hemorrhagic. There is no fat-necrosis.

Experiment II.—Dog, wt. 5,530 grm. Following inoculation there was slight gradual increase of weight. Thoracic dullness steadily increased during two weeks and diminished immediately after the first injection of leucocytes. During the eleven days between the second and third injection there was increase of dullness which again fell to a level little above that before inoculation. Subsequent elevations above this level were in every instance the result of injection into one or both pleural cavities. In Chart 5 and in subsequent charts injection into both pleural cavities is indicated by two heavy lines side by side, the length of these lines representing the quantity of leucocytes employed. One week after inoculation a nodule appeared in the chest wall at the site of puncture; during ten weeks the nodule received ten injections of leucocytes (0.5 to 1 c.c.). During this time it increased to a maximum diameter of 4 cm. and gradually disappeared without discharging upon the surface. After the fourth intrapleural injection there was cough; an area of relative dullness 5 cm. across
with a peculiar hard resistent character on percussion appeared about the site of injection and persisted several days; cough disappeared. The animal five months after inoculation is well and weighs 1,120 grams more than before inoculation.

Experiment 13.—Dog, wt. 6,450 gm. After inoculation thoracic dullness gradually increased but its level rapidly fell immediately after the first injection of leucocytes (Chart 6); it rose to a high level after the second injection and absolute dullness appeared but it fell to normal after the third injection which was made into both pleural cavities. At this time the animal was very sick and there was cough and purulent discharge from the nose and eyes; body weight had diminished 1,700 gm. and the animal was very thin. After the fourth week weight steadily increased and evidences of bronchitis disappeared. After the effect of the fourth injection had disappeared there was (on the thirty-eighth day) an increase of thoracic dullness but subsequent elevations (see chart) occurred only as the immediate result of leucocytic injections.

Three weeks after inoculation induration appeared along the tract marked by the inoculating needle; this nodule received one injection of leucocytes (0.5 c.c.) and lying immediately below the skin broke upon the surface. Two subsequent injections were made but it persisted until the seventeenth week after inoculation, disappearing finally.

The animal is large and strong and having grown considerably, its weight is 4,500 grm. greater than before inoculation.

Experiment 14.—Dog, wt. 6,150 grm. After inoculation thoracic dullness rose quickly to a high level (Chart 7), but fell considerably immediately after the first injection of leucocytes. Body weight diminished rapidly and the animal became very thin. After the second leucocytic injection the level of dullness increased and absolute dullness appeared. Changes following the six subsequent injections, of which with three there was injection into both the right and left pleural cavities, were almost constant, namely, fall of the level of dullness, either immediately or after a preliminary increase, to a level below that at the time of injection and subsequently, after the effect of the injection had disappeared, an increase of dullness. Between succeeding injections the level of dullness became gradually lower and after the immediate effect of the ninth injection had subsided there was no elevation, subsequent increase of dullness occurring only as a sequence of leucocytic injection. After the fifth week of the disease the animal began to gain weight.

A nodule which developed after two weeks at the site of inoculation attained a diameter of 2 cm.; after injection of 0.5 c.c. of leucocytes there was no increase of size. A second injection was made. The nodule diminished in size and during the sixth week of the disease was represented only by induration at its former site; a third injection was made in the neighborhood of the indurated tissue.

After the second intrapleural injection of leucocytes extensive emphysema of the subcutaneous tissue on the right side of the body made its appearance and disappeared after four days.

The animal is well and strong and weighs 400 grm. more than at the time of inoculation.
Effect of Injected Leucocytes upon a Tuberculous Lesion.

Control Experiments.

Experiment 15.—Dog, wt. 5,300 grm. Control. Thoracic dullness increased gradually during the first three weeks after inoculation and subsequently more rapidly. The animal became thin and died at the end of 35 days. The fall of dullness (and small amount of effusion found at autopsy) suggest that there was rapid absorption of fluid just before death (Chart 8).

Autopsy.—The right pleural cavity contains 25 c.c. of reddish serous fluid; the left cavity contains 5 c.c. Throughout the mediastinum are masses of hard caseous tissue, the largest being situated just above the diaphragm; similar masses occur in the subpericardial membranes on either side. Flat, gray white nodules occur upon the posterior surface of the right lung. The middle lobe of the right lung exhibits pneumonic consolidation. The subternal lymphatic glands are greatly enlarged and firmly caseous; enlarged hard glands are found near the pancreas and in the retroperitoneal tissue beside the adrenal glands.

Experiment 16.—Dog, wt. 4,950 grm. Control. Thoracic dullness gradually increased from the time of inoculation until death (Chart 9). At the end of about three weeks the animal was very thin and there was abundant purulent discharge from the nose and eyes; an ulcer formed upon the right cornea. Death occurred at the end of 36 days.

Autopsy.—The pleural cavities contain no fluid; the mediastinum is injected and contains small nodules. Above the diaphragm is a caseous mass about 1 cm. across. The subternal lymphatic glands are moderately enlarged and caseous. At the bifurcation of the trachea is a mass of caseous lymphatic glands which encircle and compress the right bronchus. The lungs contain numerous patches of pneumonic consolidation.

Experiment 17.—Dog, wt. 6,800 grm. Control. After inoculation of the animal, a stout pug, weight rapidly increased and continued much greater than before inoculation. Nevertheless relative dullness increased steadily and absolute dullness made its appearance, disappearing later (Chart 10). Almost immediately after inoculation a nodule appeared at the site of puncture. The nodule increased greatly in size, broke through the skin and discharged during several weeks. The mass below the skin diminished in size and finally disappeared, leaving a small scar. Absolute dullness disappeared at the end of five weeks, but abnormal relative dullness has persisted until the present time. The animal (at the end of five months) is very fat and apparently well, weighing 1,450 grm. more than before inoculation.

Of the animals which were inoculated as controls two died at the end of five weeks, a time corresponding to the time of death of the controls of Series A. The third animal used as control, a relatively large dog, was little affected by the pleural and subcutaneous tuberculosis with which it was infected, but, on the contrary, increased considerably in weight; nevertheless increased thoracic dullness did not return to normal.

Of four tuberculous animals injected with leucocytes one (Experiment 11) exhibited normal thoracic dullness after the third
week and a second (Experiment 12) after the sixth week. In two animals treated with leucocytes the disease was much more severe and there was great loss of body weight. One animal (Experiment 13) passed through a stage in which there were physical signs of fluid in considerable amount, and exhibited normal thoracic dullness only after the sixth week. In the remaining animal (Experiment 14) there was, after the effect of each injection had subsided, an increase of dullness, doubtless referable to the tuberculous process which was still active. Each injection during this period after a primary rise depressed thoracic dullness to a somewhat lower level so that after the seventh week there was no increase except as the result of injection of leucocytes.

In this series of experiments leucocytic injections were at first made at short intervals in relatively small quantity—approximately ten cubic centimeters. Even after thoracic dullness had returned to a level approaching that before inoculation with tuberculosis, leucocytic injections were continued. Since numerous examinations had shown that the tuberculous lesion was bilateral, injections were often made simultaneously into the two cavities.

**Series D.**—The effect upon thoracic dullness of tubercle bacilli and leucocytes injected simultaneously; the effect of leucocytes preserved during several days at low temperature.

Since the previous experiments have afforded evidence that leucocytes injected into the plural cavity already infected with tuberculosis retard the development of the lesion and tend to cause its disappearance, the possibility has suggested itself that injection of leucocytes, together with tubercle bacilli, might prevent the onset of tuberculosis. The clinical course of the disease in two animals immediately after injection of a mixture of ten cubic centimeters of leucocytes with half of a cubic centimeter of a suspension of tubercle bacilli gave some support to belief that the organism had been wholly destroyed; nevertheless at the end of two weeks such well-marked increase of thoracic dullness occurred that there was no doubt that tuberculosis had developed. The animals were subsequently used to test the efficiency of cells which had been preserved from twenty-four to forty-eight hours at a low temperature, several degrees above the freezing point. One of the animals which
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Chart 11. Experiment 18; inoculation with B. tuberculosis and leucocytes; injection of leucocytes.

Chart 12. Experiment 19; inoculation with B. tuberculosis and leucocytes; injection of leucocytes preserved at low temperature.

Chart 13. Experiment 20; control.
had received tubercle bacilli and leucocytes was subsequently injected with leucocytes freshly obtained, whereas the second animal which had received the same mixture was injected with leucocytes which had been preserved in cold storage. In some instances the cells employed for corresponding injections in the two animals were identical, the animal in Experiment 19 receiving its injection one or two days after that of Experiment 18. Experiment 20, in which tubercle bacilli alone had been employed, served as control.

Experiment 18.—Dog, wt. 7,950 grm. The clinical course after inoculation with a mixture of tubercle bacilli and leucocytes is shown in Chart 11. The animal was given after the inoculating injection six injections of freshly obtained leucocytes and was killed at the end of 50 days; it was strong and active when killed.

Autopsy.—The pleural cavities contain no fluid. Several flat fibroid patches occur upon the surface of the lungs, but the pleural surfaces are almost free from evidence of tuberculosis. The subpericardial membranes and mediastinum in front of the heart are delicate save for the presence of a thin firm mass of tuberculous tissue about 7 mm. across. The mediastinum above the level of the heart is thickened and contains several indurated nodules. The substernal lymphatic glands are soft, moderately enlarged, and contain several caseous foci. In the lungs are miliary tubercles. The liver contains numerous small tubercles of which many have a delicate fibrous capsule. In the kidney are a few opaque tubercles.

Experiment 19.—Dog, wt. 6,700 grm. The clinical course after inoculation, identical with that of Experiment 18, is shown in Chart 12. A nodule appeared at the site of inoculation and after receiving four injections (0.5 to 1 c.c.) of leucocytes kept at low temperature almost completely disappeared, but although injected three times subsequently increased to a diameter of nearly 4 cm. The animal received into the pleural cavities six injections of leucocytes which had been preserved in cold storage during one or two days. The animal which was very weak was killed after 50 days for comparison with the preceding.

Autopsy.—The subcutaneous tissue is jaundiced. The pleural cavities each contain 200 c.c. of fluid. Upon the pleural surfaces are elevated plaques of tuberculous tissue. The mediastinum from the lymphatic glands at the base of the neck, which are enlarged to a diameter of 3 cm., to diaphragm is converted into a thick crinkled mass by partly caseous tissue. Similar masses of large size occupy the subpericardial membranes and are scattered over the right parietal pleura and surface of the diaphragm. In the substance of the right lung corresponding to the site of inoculation is a tuberculous mass; the bronchial lymphatic glands on the same side are much enlarged and tuberculous. The lungs contain tubercles. The liver is enlarged and beset with numerous large tubercles, occupying on section at least a third of the tissue.
Effect of Injected Leucocytes upon a Tuberculous Lesion.

Control Experiment.

Experiment 20.—Dog, wt. 8,050 grm. Control. The animal received 0.5 c.c. of the suspension of tubercle bacilli employed in the two preceding experiments mixed with 10 c.c. of 0.85 per cent. sodium chloride solution. The clinical course is shown in Chart 13. The animal was killed after 50 days.

Autopsy.—The right pleural cavity contains 2 c.c. of red turbid fluid; the left cavity contains 15 c.c. Upon the surfaces of the lungs and upon the diaphragm are numerous flat tubercles. The mediastinum and subpericardial membranes are thickened and beset with numerous tubercles. A hard mass of gray white tissue (1.2 cm. across) is situated in the right, a second (2 cm. across) in the left subpericardial membrane at its junction with the diaphragm; a third mass is situated in the mediastinum above the diaphragm. The substernal lymphatic glands are moderately enlarged, hard and almost wholly caseous. In the lungs are miliary tubercles. The liver contains numerous tubercles; in the kidney opaque tubercles often 1.5 cm. in diameter are fairly numerous.

In the animal of Experiment 20, used as control, relative and absolute thoracic dullness increased gradually after inoculation, but diminished suddenly after the thirty-fifth day; nevertheless relative dullness maintained a high level until the animal was killed. In the dog of Experiment 18, which received fresh leucocytes, the clinical course was identical with that illustrated by Experiments 11 to 14 of Series C, namely, depression of dullness, perhaps preceded by temporary increase, after each injection. Comparison of these two animals of the present series confirms the result of former experiments and affords clinical and anatomical evidence that the presence of artificially introduced leucocytes has retarded the development of the tuberculous lesion.

The employment of leucocytes which have been preserved at a low temperature has not had an equally favorable result, but the experiment is indecisive, for autopsy has shown that the lung has been punctured at the time of inoculation. Tuberculosis of the lung and of the bronchial glands doubtless explains in part the rapid progress of the disease. Nevertheless the charted thoracic dullness shows that changes which follow injection of leucocytes kept at low temperature during several days may be identical with those caused by freshly obtained leucocytes. Injection on the fifteenth day of the disease was followed after an interval of several days by diminution of relative dullness and disappearance of absolute dullness referable to diminution of the fluid contents of the chest.
Series E.—The clinical and pathological changes following simultaneous injection of tubercle bacilli and leucocytes.—Injection of leucocytes together with tubercle bacilli in the preceding experiments has been followed by the reaction which occurs when leucocytes are injected into the normal pleural cavity (see Charts 11 and 12); there is accumulation of fluid which quickly disappears. Subsequent increase of dullness, which is the otherwise constant result of tuberculous pleurisy, is delayed. The same experiment has been repeated and, in order that the resulting changes may be compared by anatomical examination, the animals have been killed as soon as that which has received leucocytes has exhibited increase of thoracic dullness.

Experiment 21.—Dog, wt. 5,950 grm. A suspension of tubercle bacilli (0.5 c.c.) mixed with 10 c.c. of leucocytes which had been kept at a temperature slightly above freezing during three days was injected into the right pleural cavity. The level of thoracic dullness (Chart 14) rose abruptly, subsided, and remained normal during at least a week; it then rose and the animal was killed 16 days after inoculation.

Autopsy.—The right pleural cavity contains a small amount of turbid whitish fluid (not measured); the left cavity contains a somewhat greater quantity. The mediastinum is delicate and contains a few tubercles; the right and left subperi-
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cardial membranes are studded with tubercles. Just above the diaphragm is a small, firm, succulent mass. The mediastinal lymphatic glands are moderately enlarged, measuring 1.2 cm. The surfaces of the diaphragm and the parietal pleura are smooth. The liver contains small miliary tubercles.

**Experiment 22.**—Dog, wt. 6,900 grm. Control. A suspension of tubercle bacilli (0.5 c.c.) was diluted with physiological salt solution to 10 c.c. and injected into the right pleural cavity. Thoracic dullness (Chart 15) increased gradually during 12 days and then more rapidly. The animal was killed at the end of 16 days.

**Autopsy.**—The right pleural cavity contains 120 c.c. turbid, whitish, coagulable fluid; the left cavity, 95 c.c. Upon the surface of the diaphragm and upon the parietal pleura in greatest abundance on the right side are large, flat, gray white nodules. The mediastinum is thick and beset with small tubercles and partially caseous masses, the largest being just above the diaphragm; the subpericardial membranes on either side contain large hard masses of gray white tissue. The substernal lymphatic glands are considerably enlarged, measuring 1.7 cm. The liver contains small miliary tubercles.

The changes of thoracic dullness observed in Experiment 21 after inoculation with tubercle bacilli mixed with leucocytes are identical with those noted immediately after injection of the same mixture in two animals of the preceding series; in each series there has been the same contrast with the control. The persistence of normal thoracic dullness during a week after the preliminary rise and fall suggests that the development of the tuberculous process has been retarded. Twenty-four hours after the appearance of abnormal dullness in the animal which received leucocytes both animals of the present series were killed; effusion was abundant in the control but much less in the animal with leucocytes; tuberculous pleural nodules were numerous and massive in the former, but almost absent in the latter; the membranes within the thorax contained abundant tuberculous tissue in the one, but little in the other. The substernal lymphatic glands were much larger in the control.

The experiment furnishes additional evidence that leucocytes tend to check the development of a tuberculous lesion, even though they have been preserved during several days at a temperature a little above the freezing point.

**Series F.**—Anatomical changes found in animals with tuberculous pleurisy killed after repeated injections of leucocytes.

In the following series of experiments both control and animal treated by intrapleural injections of leucocytes were killed at the
same interval after inoculation in order to determine by anatomical examination the effect of the injected cells. The animals in the two following experiments were of unequal size, that which received injections being a small puppy; each received into the right pleural cavity half of a cubic centimeter of the same suspension of tubercle

Experiment 23.—Dog, wt. 4,150 gm. During the first two weeks after inoculation relative thoracic dullness over the infected cavity increased from 3 to 7.7 cm. Injection of leucocytes (16 c.c.) on the fourteenth day was followed by a fall of this level. Two subsequent injections caused diminution of the area of dullness which after the third week twice returned to normal but rose slightly after the effect of the injection had disappeared. Twenty-four hours before the animal was killed relative dullness measured 4.4 cm. and the animal received into the right pleura 9.5 c.c. leucocytes. Cough and purulent nasal discharge appeared during the third week of the disease and persisted until death. There was a nodule at the site of inoculation which received one injection of leucocytes; it did not increase in size but remained until death. The animal was killed after 32 days.

Autopsy.—The right pleural cavity contains 12 c.c. turbid blood-stained fluid; the left, 11 c.c. The parietal pleura is smooth save at one point corresponding to the site of inoculation where there is a group of small nodules; upon the opposing surface of the lung and extending into the substance in an area of fibrous tuberculous tissue. The bronchial lymphatic glands on the right side are enlarged and tuberculous. In the mediastinum and subpericardial membranes are tuberculous masses of considerable size. The substernal lymphatic glands are greatly enlarged and caseous. Tuberculous glands are found near the pancreas. The lungs contain patches of broncho-pneumonia and miliary tubercles.

Experiment 24.—Dog, wt. 7,000 gm. Control. Two weeks after inoculation relative dullness had increased from 3 to 8.6 cm. and absolute dullness had made its appearance; on the eighteenth day relative dullness was 12 cm. and absolute dullness 8.5 cm. These levels were maintained until the animal was killed 32 days after inoculation. A nodule appeared at the site of inoculation and broke upon the surface of the skin.

Autopsy.—The right pleural cavity contains 235 c.c., the left 184 c.c. of turbid pale yellow fluid. The pleural surfaces are intensely injected and the parietal pleura is thickly beset with flat tuberculous nodules which near the diaphragm are confluent. The mediastinum is occupied by a large mass of hard tuberculous tissue extending into the left subpericardial membrane. The substernal lymphatic glands are greatly enlarged and caseous; tuberculous glands occur near the pancreas. The lungs contain miliary tubercles.

Injection of leucocytes was followed by diminution of thoracic dullness already increased by tuberculous pleurisy but even before the animal was killed it was evident that the tuberculous process was still active, for slight increase of dullness had occurred just before death. Difference between injected and uninjected animals
was well marked in the pleural cavities; in the control there was fluid in great quantity, the pleural membranes were injected and raised tuberculous plaques were present in immense numbers upon the parietal pleura, whereas in the injected animal the pleural surfaces were smooth and the cavities contained very little fluid. Elsewhere, both in the control and in the injected animal, tuberculosis was advanced and widely disseminated. It is noteworthy that the lung had been punctured at the time of inoculation, so that, although as the subsequent course of the disease showed, the pleura had been infected, there was tuberculosis of the lung and of the bronchial lymphatic glands as well.

The following experiments are described to illustrate further the anatomical effect of injected leucocytes. They confirm those which have already been cited and furnish additional evidence that injected leucocytes influence in greatest degree tuberculosis in structures with which they come into immediate contact. One animal, used as control, received a suspension of tubercle bacilli (0.5 c.c.). The other animal received the same suspension (0.5 c.c.) mixed with leucocytes (10 c.c.); subsequently leucocytes were injected into one or both pleural cavities at intervals of about a week.

**Experiment 25.**—Puppy, wt. 5,650 grm. After simultaneous injection of leucocytes and tubercle bacilli there was no permanent increase of thoracic dullness until the sixth day when it had increased from 3.2 to 3.7 cm. Subsequent injections of leucocytes into one or both pleural cavities reduced this level and it remained at the normal level save as the immediate result of injection until death. There was bronchitis and the animal lost weight. The animal was killed after 33 days.

**Autopsy.**—The pleural cavities contain no fluid and there is no tuberculous tissue in the adjacent membranes. The mediastinum and subpericardial membranes are delicate and evidently greatly stretched so that they form redundant folds and can be pouched far to the right or left (Fig. 4). The parietal and visceral pleurae are smooth except for patches and shreds of soft reddish tissue which represent perhaps the site of tuberculous plaques. The substernal lymphatic glands are enormously enlarged, measuring 3.5 cm. in length; they are homogeneously caseous and surrounded by a fibrous capsule. The lungs contain an occasional milky tubercle; the liver contains innumerable tubercles.

**Experiment 26.**—Puppy, wt. 6,050 grm. Control. Thoracic dullness, measuring before inoculation 2.8 cm., increased continuously after inoculation; there was cough and purulent discharge from the nose after the third week. A tuberculous nodule formed at the site of inoculation. At the end of 32 days relative dullness had increased to 8.2 cm., absolute dullness to 4.6 cm. The animal was killed at the end of 33 days (24 hours before death 10 c.c. of leucocytes had been injected into the right pleural cavity).
Autopsy.—The right pleural cavity contains 170 c.c. of turbid fluid; the left, 125 c.c. of less turbid coagulable fluid. The parietal pleura is injected and rows of tuberculous plaques are situated between the ribs; similar plaques occur upon the diaphragm. The mediastinum above the diaphragm contains a tuberculous mass of great size extending into both subpericardial membranes which are thickened and studded with tubercles (Fig. 5). The mediastinum above this mass is thickened and beset with tubercles and larger tuberculous masses. The substernal lymphatic glands are enormously enlarged, 3.5 cm. in length and caseous. Tubercles are moderately numerous in the lungs and are present in enormous number in the liver.

In the animal which had received leucocytes the pleural membranes exhibited no evidence of tuberculosis whereas in the control there was pleural effusion and advanced tuberculosis of the pleura and mediastinum (compare Figs. 4 and 5). Nevertheless tubercle bacilli had found lodgment in the substernal lymphatic glands of the injected animal; tubercle bacilli perhaps disseminated from this focus had caused miliary tuberculosis of lungs and liver.
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In the following experiment repeated injection of leucocytes had little effect upon the development of tuberculosis.

Experiment 27.—Dog, wt. 4,850 grm. On the fifth day after simultaneous injection of tubercle bacilli (0.5 c.c.) and leucocytes thoracic dullness had increased to 4.3 cm., the original level being 2.5 cm. Injection of leucocytes repeatedly reduced thoracic dullness but failed to restore it to normal. Cough appeared and was accompanied by purulent nasal discharge, conjunctivitis and ulceration of the right cornea. The animal lost weight and became sick and weak. It was killed after 39 days; just before death relative dullness measured 5.5 cm., absolute dullness 2.8 cm.

Autopsy.—The right pleural cavity contains 5.5 c.c. of almost clear fluid; the left cavity contains 10 c.c. of very turbid yellowish fluid. Upon the parietal pleura and diaphragm is an occasional tuberculous plaque. The mediastinum from subternal glands which are greatly enlarged and caseous to diaphragm is occupied by yellowish white tissue which is partly caseous and partly fibrous. Almost the entire right lung is consolidated by mottled red and gray patches of broncho-pneumonia; the left lung contains similar patches. The liver contains tubercles; lymphatic glands near the pylorus are enlarged and caseous.

Experiment 28.—Dog, wt. 4,400 grm. Control. Thoracic dullness (normal relative dullness measured 3.2 cm.) increased continuously after inoculation with 0.5 c.c. suspension of B. tuberculosis mixed with 10 c.c. salt solution; a nodule appeared at the site of inoculation. There was cough which disappeared. The animal was killed at the end of 39 days; before death relative dullness measured 8.5 cm. and absolute dullness 4.6 cm.

Autopsy.—The right pleural cavity contains 39 c.c. of opaque fluid; the left cavity, 28 c.c. The parietal pleura is intensely injected and upon its surface are flat inconspicuous tuberculous nodules. In the mediastinum above the diaphragm extending far into the subpericardial membranes on each side is a very large firm tuberculous mass of pearly white color. The subternal lymphatic glands are greatly enlarged and continuous with a large tuberculous mass in the mediastinum situated immediately below them. The lungs contain a few scattered tubercles; tubercles are numerous in the liver.

Series G.—Experiments with a more virulent tubercle bacillus.—The foregoing experiments have been performed with a strain of tubercle bacillus of moderate virulence; this organism killed guinea-pigs in three or four weeks after intraperitoneal inoculation, but failed to kill rabbits when injected into the peritoneal cavity. In the following experiments a much more virulent organism which killed rabbits five weeks after intraperitoneal inoculation was used. Its virulence was well illustrated by the effect on dogs—death occurred with considerable rapidity, and instead of the gray-white sarcoma-like masses containing foci of caseation new formed tissue, which exhibited almost homogeneous caseation, was the result of fatal inoculation.
EXPERIMENT 29.—Dog, wt. 6,050 grm. Dullness over the right side of the chest increased during the week following inoculation and was reduced by the injection of leucocytes (Chart 16). The second injection produced no decrease, but the third caused material fall of the levels of relative and absolute dullness. Subsequent injections were not equally favorable. A nodule appeared in the chest wall at the site of inoculation and was injected four times with leucocytes (0.5 c.c.); it increased in size measuring 4.5 cm. across, and then diminished slightly. The animal became thin and weak and died after 39 days.

Autopsy.—The right pleural cavity contains 60 c.c. of serous slightly turbid fluid; the left cavity contains 35 c.c. of less turbid fluid. The pleural membranes are intensely injected. The mediastinum contains a flat mass of greenish caseous material (Fig. 6); a second mass just above the diaphragm extends into the left subpericardial membrane; the corresponding membrane on the right side is uniformly thickened by partly caseous tissue. The mediastinal lymphatic glands are enlarged and caseous. Distributed over the pericardial diaphragm and pulmonary surfaces are plaques of yellow tissue. The chest wall (at the site of inoculation) contains an ill-defined area of thickening and caseation and opposite in the substance of the lung is a round nodule of tuberculous tissue; the bronchial lymphatic glands on the right side are tuberculous. The lungs contain a few small miliary tubercles. The liver is enlarged and jaundiced and contains tubercles in immense number.

EXPERIMENT 30.—Dog, wt. 7,500 grm. Control. After inoculation thoracic dullness increased gradually (Chart 17); a small nodule formed in the skin at the site of inoculation. The animal died at the end of 47 days.

Autopsy.—The right pleural cavity contains 195 c.c. of yellow opaque fluid; the left, 150 c.c. The pleural membranes are injected more markedly on the right side; on the parietal pleura of the right side are flat yellow tubercles. The entire mediastinum is occupied by a greenish-yellow somewhat soft caseous mass merging into the greatly enlarged caseous substernal lymphatic glands. Both subpericardial membranes are greatly injected and contain large caseous masses (Fig. 7). Opposite the site of inoculation is a small caseous nodule 0.5 cm. across and extending about 1.5 mm. into the substance of the lung. The lungs contain no tubercles; in the liver are numerous miliary tubercles.

Tuberculosis was almost equally advanced in the two animals, although in that which received leucocytes tuberculous masses in the mediastinum and elsewhere were smaller. Wide dissemination in the injected animal is referable in part to inoculation of the lung. In the following experiment both injected animals and control were killed at the end of the same period and neither gave evidence that the lung substance had been entered by the tubercle bacilli with which they had been inoculated.

EXPERIMENT 31.—Dog, wt. 5,250 grm. A gradual increase of dullness followed inoculation (Chart 18); the first injection caused a rapid increase of dullness followed by decrease maintained until the next injection. This injection was followed by an increase which did not subside. There was accumu-
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Chart 16. Experiment 29; injection of leucocytes.

Chart 17. Experiment 30; control.

Chart 18. Experiment 31; injection of leucocytes.

Chart 19. Experiment 32; control.
Fig. 6. Experiment 39; animal injected with leucocytes.

Fig. 7. Experiment 39; control.

Fig. 8. Experiment 31; animal injected with leucocytes.

Fig. 9. Experiment 32; control.
Effect of Injected Leucocytes upon a Tuberculous Lesion.

loration of a large pleural effusion and subsequent injections exhibited only occasional tendency to depress its level (fifth and seventh injection). A nodule formed in the chest wall during the third week after inoculation, increased in size and broke upon the surface; it was injected three times with leucocytes. During the seventh week of the disease a swelling appeared over the lower bone of the right hind leg and soon breaking upon the surface formed an ulcer with undermined edges about which there was redness of the skin. Leucocytes similar to those previously employed were applied to the wound and injected beneath its edges; a sterile dressing was put about the leg. Two days later when the bandage was removed swelling and redness had almost disappeared and the ulcer was clean and dry but exhibited no tendency to heal. The animal became thin and very weak and died 55 days after inoculation.

Autopsy.—The right pleural cavity contains 33 c.c. of deep yellow fluid; the left cavity, 14 c.c. of reddish fluid. The pleura of the diaphragm and chest wall is roughened by a thin irregularly distributed layer of fibrin and on both parietal and pulmonary pleura occur a few small tuberculous plaques. The mediastinum is thickened and contains small masses of friable caseous tissue from 1 to 3 mm. in thickness (Fig. 8). Similar thin plaques of caseous material with rough surface of somewhat eroded appearance occupy the subpericardial membranes. The substernal lymphatic glands are hard and caseous, 1.7 cm. in length. The lungs contain no tubercles recognizable by microscopic examination. The liver is enlarged and contains tubercles. The spleen is large and soft, but exhibits no tubercles. Lymphatic glands in contact with the pancreas are enlarged and caseous; the mesenteric glands show the same alteration.

Experiment 32.—Dog, wt. 9,050 grm. Control. After inoculation thoracic dullness increased during three weeks (Chart 19), decreased and again increased; a nodule formed at the site of inoculation. The animal became thin and weak and conjunctival jaundice was present before it was killed, when almost moribund, 55 days after inoculation.

Autopsy.—The right pleural cavity contains 104 c.c. of almost clear, amber yellow fluid; the left pleural cavity, 116 c.c. The pleura is slightly thickened and upon the parietal and visceral surfaces, particularly on the right side, are numerous flat nodules. The mediastinum is replaced by a great mass of fairly soft, greenish yellow, in great part caseous tissue extending the whole length of the sternum and projecting outward into each subpericardial membrane (Fig. 9). The substernal lymphatic glands are greatly enlarged and caseous, measuring 2.3 cm. in length.

The lung on the surface and in its substance contains an immense number of tubercles. The liver is jaundiced and contains innumerable tubercles. The spleen is enlarged and contains thickly scattered opaque tubercles. Tuberculous glands of great size occur near the pancreas.

These experiments, in which a virulent tubercle bacillus was used, were undertaken under unfavorable conditions; dogs of approximately equal size were not obtainable and the dogs used as controls were much larger (9,050 and 7,500 grm.) than those which were injected (6,050 and 5,250 grm.). Fall of the level of thoracic
dullness frequently followed injection of leucocytes, but was less constant than in preceding experiments in which the less virulent microorganism was employed. It is noteworthy that for two injections in one animal (third and fourth injections in Experiment 29) and for one injection in the other (fourth in Experiment 31) cells were used which were obtained four and five days after injection of turpentine and were almost entirely necrotic. Nevertheless, although the injections failed to prolong life, the anatomical condition observed at autopsy showed that they had exerted an influence upon the development of the tuberculous lesion similar to that which was evident when somewhat less virulent microorganisms had been inoculated.

The quantity of fluid found at autopsy in the pleural cavities of the control animals has been far greater than that of the injected animals. The diagrams which show the size and distribution of tuberculous tissue indicate that the lesion has been more advanced in the controls, which contain throughout the mediastinum large masses of caseous tissue merging into one another (see Figs. 7 and 9). In the injected animals (see Figs. 6 and 8), especially in Experiment 31, tuberculous masses are less extensive and are of smaller size. In Experiment 30 the lung has been punctured and infected at the time of inoculation, so that there is no opportunity of comparing the effect of injections upon general dissemination of tuberculosis, but between Experiment 31 and its control, Experiment 32, in which the animals lived the same length of time, comparison is possible; tuberculosis in the lungs, liver and spleen was more advanced in the control than in the injected animal.

The almost constant effect of leucocytes injected into the pleural cavity of an animal from a week to ten days after intrapleural inoculation with tubercle bacilli is a fall of the level of thoracic dullness, elevated by the presence of effusion or of newly-formed tuberculous tissue. This fact is especially noteworthy because leucocytes in similar amount injected into the normal pleural cavity cause a rapid but temporary accumulation of fluid and increase of dullness over the dependent part of the chest. The first injection of leucocytes into eleven tuberculous animals was followed in seven instances by a fall of dullness recognizable twenty-four hours later;
in two experiments there was no depression of the level of dullness until forty-eight hours later. In only two animals did a reaction occur resembling that of the normal animal, namely, rise of dullness following injection with subsequent fall; in one instance this level finally fell below the level at time of injection, but in one instance it remained above the previous level.

Injections after the first exhibited a similar almost constant tendency to reduce the level of thoracic dullness; a preliminary elevation followed within two or three days by a fall below the level at the time of injection was frequently observed.

The relation of depression of thoracic dullness to diminution of pleural effusion is well shown by the amount of fluid present at autopsy in the chest of injected and uninjected animals. The following figures represent the quantity of fluid present in the chest of such animals dead or killed at approximately equal intervals after inoculation.

<table>
<thead>
<tr>
<th>Injected Animal</th>
<th>Control</th>
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<tbody>
<tr>
<td>Number</td>
<td>Right</td>
</tr>
<tr>
<td>Experiment 18</td>
<td>23</td>
</tr>
<tr>
<td>23</td>
<td>0 c.c.</td>
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<tr>
<td>25</td>
<td>12</td>
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<tr>
<td>27</td>
<td>0</td>
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<tr>
<td>29</td>
<td>33</td>
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<td>31</td>
<td>2</td>
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In one series of experiments (Series B) the animal which had received injections contained more effusion than the control; in this instance chronic non-tuberculous pleurisy not apparently referable to the tubercle bacillus had been caused by frequently repeated and very large injections of leucocytes. The experiment was performed at the beginning of the present study and accurate measurement of the quantity of fluid was not made.

Diminution of abnormal thoracic dullness caused by repeated injection of leucocytes into the tuberculous pleural cavity is doubtless due to diminution of tuberculous tissue in and about the mediastinum as well as to diminution of fluid. Evidence of the disappearance of such tissue is obtainable only by autopsy and in a number

*In this experiment leucocytes were injected into the pleural cavity twenty-four hours before death.
of instances comparison has been made between injected and uninjected animals dead or killed at the same interval after inoculation with the same suspension of tubercle bacilli. The attempt has been made to illustrate the effect of leucocytes by the accompanying table in which parallel injected and control animals are compared.

It is noteworthy that in two instances in which the difference between injected and uninjected animals has been comparatively slight (Experiments 23 and 29) the inoculated tubercle bacilli have entered in part the substance of the lung, for opposite the site of injection there have been tuberculous masses within the organ and the bronchial lymphatic glands on the same side have been enlarged and tuberculous. In these cases examination during life has demonstrated the existence of tuberculous pleurisy, but coexisting pulmonary tuberculosis has doubtless formed a second focus from which dissemination might occur, so that the disease perhaps has been less readily influenced by the injected leucocytes.

The influence of injected cells has been found most evident where it may be assumed that the injected material has been in contact with tuberculous tissue. The pleural membranes exhibit in most marked degree the effect of the injected leucocytes. In injected animals the flat plaques of tuberculous tissue which occur in the controls upon the parietal pleura and diaphragm are almost or wholly absent; nodules and larger masses of newly formed tissue in the mediastinum have been much smaller after injections and the membrane has had in Experiments II (not included in the table) and in Experiment 25 the delicate translucent appearance observed in the normal animal. Tuberculous masses situated in the subpericardial membranes on either side are similarly retarded in their development.

The long diameter of the substernal lymphatic glands is given in the table as a crude measure of tuberculosis in these organs. Comparison of injected and uninjected animals killed after the same interval shows that these tuberculous glands have usually attained a greater size in the uninjected than in the injected animals, but this difference is not constant. Cells doubtless influence with greater difficulty these more remote structures and in Experiment 25 the pleural cavities of the injected animal were normal, but the substernal lymphatic glands were greatly enlarged.
The late changes which may occur as the result of injection of leucocytes in large quantity are well illustrated by Experiment 9. The control contained in the mediastinum and adjacent membranes large succulent masses of tuberculous tissue, whereas in corresponding situation in the injected animal there was scar-like fibrous tissue; the substernal lymphatic glands of the control were large, hard and tuberculous, whereas those of the injected animal were only moderately enlarged and did not exhibit evidence of tuberculosis.

Complete restoration to normal is better illustrated by Experiment II of Series C; the pleural surfaces are smooth and the mediastinal and subpericardial membranes, though stretched and redundant, are delicate and transparent. Tags of soft reddish tissue represent apparently the site of tuberculous nodules. The condition of the pleural cavities and adjacent membranes is identical in Experiment 25.

Evidence obtained from animals killed at varying intervals after inoculation does not afford evidence that injection of leucocytes increases or diminishes dissemination of tuberculosis. Both in controls and in injected animals the liver is invariably the seat of miliary tubercles, which may be numerous ten days after inoculation. Widespread distribution occurred in the injected animal of Experiment 9 and in the control, Experiment 32, but in each instance was absent in the animal used for comparison. Recovery of animals shows that disappearance of the local lesion tends to limit dissemination of bacteria.

The mechanism of the process by which leucocytes, obtained by use of a sterile irritant, inhibit the development of a tuberculous lesion has not been considered. It is noteworthy that the cells injected consist of polymuclear and in equally great number of mononuclear cells. The histology of tuberculosis in the dog presents features of interest and has exhibited alterations apparently as the result of leucocytic injection. Experiments which have been cited have suggested that leucocytes which are living can alone accomplish the changes which have been described. These details require special study.