<table>
<thead>
<tr>
<th>項目</th>
<th>内容</th>
</tr>
</thead>
<tbody>
<tr>
<td>項目</td>
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<td>項目</td>
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<tr>
<td>項目</td>
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</tr>
</tbody>
</table>

（日本語の文書を表示）
Studies on the heterogenetic Antigen

The 2nd Report

By

Shiro FUJIMURA (藤村泰郎) and Makoto ITIBAKASE (一番⽥栄真)

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(Received January 29, 1941)

Introduction

Since Forssman's discovery of the fact that rabbits injected with suspensions of organs of guinea-pigs or a few other animals produce hemolysin capable of hemolyzing sheep red corpuscles, this fact has been confirmed by many investigators. The hemolysins generated by the injection of organs of such animals were called "heterogenetic antibodies" and the corresponding antigens "heterogenetic antigens," which were found to be present in the tissues of animals of many kinds, but absent in others. On the chemical nature of the heterogenetic antigens many investigations have been attempted. From the fact that the alcoholic extract of organs reacts with the corresponding antibodies, it was considered that the heterogenetic antigens have the lipid nature. One of the authors has experimented in the first place to determine the chemical nature of the substance which is contained in the alcoholic extracts and reacts with the corresponding antibodies, horse's, dog's and hen's organs, and its results have indicated that the active substance as the antibody detector is not of lipid nature but of protein nature. In the present research we experimented with lung, heart, kidney and liver of guinea-pigs in the same method as described in the preceding report.

Experimental

For the injections rabbits were used and in selecting the animals those were excluded in which the serum, prior to the injection, gave a strong hemolysis in a dilution of 1:25 in a condition of the testes.

Preparation of the material for injection and antibodies.

To one part of organ tissue, passed through a mincing machine, is added nine parts of physiological salt solution and the mixture is shaken well for several hours and then centrifuged. The rabbits, fed with vegetables, were injected with 3 cc. of such a supernatant liquid one or two times at weekly intervals. 5 to 7 days after the last injection, the blood was taken out and used for experiments. In general one injection was sufficient for producing a potent serum.
Test for hemolysis.

In a series of test tubes, 0.5 cc. of serum of rabbits immunized as above, in a dilution as in the table, 0.5 cc. of guinea-pig serum as complement and 0.5 cc. of 3 per cent. suspension of sheep red corpuscles were put in and well mixed. After a lapse of one hour at 37°C the degree of hemolysis was examined. The result is as follows. In the tables, cases where no hemolysis occurs are shown as (−), and complete hemolysis as (++) while at intermediate cases signs (+), (±) and (−) are used.

<table>
<thead>
<tr>
<th>No. of rabbit</th>
<th>Material injected</th>
<th>Degree of dilution of serum</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 : 100</td>
<td>1 : 200</td>
</tr>
<tr>
<td>94</td>
<td>Heart extract</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>93</td>
<td>&quot;</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>64</td>
<td>Lung extract</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>56</td>
<td>&quot;</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>10</td>
<td>Kidney extract</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>20</td>
<td>&quot;</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>35</td>
<td>Liver extract</td>
<td>++</td>
<td>++</td>
</tr>
</tbody>
</table>

Preparation of alcoholic extract of organs.

To one part of minced tissue, free from fat, was added nine parts of absolute alcohol and the suspension was kept at room temperature for several days with occasional shaking. The extract was then filtered and kept in a dark place. As an antibody detector in complement fixation reactions this solution was diluted with physiological salt solution to 30 times volume of it and in an interval from 20 minutes to 2 hours after the dilution it was used in experiments.

Complement fixation reaction.

The complement fixation reactions were performed according to Browning's method. The antiserum was inactivated by heating at 56°C for 30 minutes and diluted to ten times volume of it with salt solution. The antibody detector was used in an amount which just fails to prevent the hemolysis of sensitized ox red corpuscles containing 2 units of complement. As the complement guinea-pig serum was always used, and its amount which is sufficient to hemolyze 0.5 cc. of 3 per cent. suspension of ox red corpuscles under the presence of 5 units of hemolysin was taken as one unit. In a series of test tubes 0.5 cc. of the diluted antiserum, a fitting amount of antibody detector and increasing amount of complement as 2, 4, 6, 8, and 10 units were put in and well mixed. After the lapse of 1.5 hours at 37°C, 0.5 cc. of the sensitized ox red corpuscles suspension was added and incubated again for 1.25 hours. At the end of the time the degree of hemolysis was observed. Of course in each experiment the control tests only with detector and complement or antiserum and complement were performed.
The experiment indicates that the alcoholic extract of organ tissues contains a substance which reacts as antibody detector with the heterogenetic antigen in the presence of complement.

The fractionation of the alcoholic extract of organ tissues. The fractionation of the alcoholic extract of organ tissues was performed as follows. The extract was condensed in vacuum at a temperature not exceeding 40°C to a syrupy consistency under a slow current of carbon-dioxide gas. The residue was dissolved in a small amount of ether repeatedly. To the ethereal solution was added an excess of aceton and the precipitate thus obtained was collected. This precipitate was dissolved in a small amount of ether and precipitated again by the addition of aceton. The procedure was repeated three times. This fraction is phospholipin. The aceton soluble portions were combined and dried at a temperature of 40°C and dissolved in alcohol. Both fractions, ether insoluble and aceton soluble, were tested for an antibody-detecting property in the complement fixation reaction using the corresponding antibody and its result showed that they are entirely devoid of the property of the detector. The alcoholic solution of the phospholipin fraction was also tested concerning its property as an antibody detector against the heterogenetic antigen, where one part of it was diluted with nine parts of physiological salt solution to an emulsion and after 20 minutes was used for experiment. The result is as follows.

Table III

<table>
<thead>
<tr>
<th>No. of rabbit</th>
<th>Antibody producer</th>
<th>Antibody detector</th>
<th>Unit of complement</th>
<th>Detector Antiserum</th>
</tr>
</thead>
<tbody>
<tr>
<td>94 Heart extract</td>
<td>Phospholipin fraction from heart</td>
<td>2 4 6 8 10 12 15</td>
<td>+ # # # # # # # #</td>
<td></td>
</tr>
<tr>
<td>93 &quot;</td>
<td>&quot;</td>
<td>- - - - - - - -</td>
<td># #</td>
<td></td>
</tr>
<tr>
<td>64 Lung extract</td>
<td>Phospholipin fraction from lung</td>
<td>- - - + # # # #</td>
<td># #</td>
<td></td>
</tr>
<tr>
<td>56 &quot;</td>
<td>&quot;</td>
<td>- - - - - - - -</td>
<td># #</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td></td>
<td>+ # # # # # # #</td>
<td># #</td>
<td></td>
</tr>
<tr>
<td>35 Liver extract</td>
<td>Liver alcoholic extract</td>
<td>+ # # # # # # #</td>
<td># #</td>
<td></td>
</tr>
</tbody>
</table>
From these results it can be seen that the property as an antibody detector against the heterogenetic antibodies of the alcoholic extract of organ tissues depends almost entirely upon the phospholipin fraction of it, and the other fraction lacks this property.

It is a well known fact that owing to the amphoteric property lipids may combine with various chemical substances and it is very difficult to prepare lipids free from other substances. In this respect lipid prepared only through extraction and precipitation by means of organic solvents, is far from being pure. The phospholipin above prepared is a yellowish mass and the determination of its contents of nitrogen and phosphor is performed by means of Kjeldahl's and Youngburg's method respectively and the molecular ratios of N:P are as follows.

Table IV

<table>
<thead>
<tr>
<th>Phospholipin prepared from</th>
<th>Heart</th>
<th>Lung</th>
<th>Kidney</th>
<th>Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>N:P</td>
<td>1.51:1</td>
<td>1.45:1</td>
<td>1.42:1</td>
<td>1.48:1</td>
</tr>
</tbody>
</table>

It indicates that the phospholipin thus obtained combines with another substance rich in nitrogen content.

We also examined whether in a purified state the phospholipin reacts as above or not. The purification of phospholipin was performed as follows. To the alcoholic solution of the crude phospholipin was added an excess of saturated alcoholic solution of cadmium chloride and the phospholipin was precipitated as a double-salt with cadmium. The precipitate was washed many times with absolute alcohol by centrifugation until nothing was extracted in the supernatant liquid. Then the double-salt was suspended in hot alcohol and decomposed by an addition of ammonium carbonate little by little to slight alkaline reaction and filtered hot. The precipitation by an addition of cadmium chloride was repeated once more and after washing with absolute alcohol, the precipitate of double-salt was recrystallized with acetic ester, and then decomposed with ammonium carbonate as described before. Finally the hot filtrate solution was condensed to syrup in vacuum at 40°C in a slow current of carbondioxide gas and dissolved in a small amount of ether. Then to the etheral solution was added an excess of aceton and the phospholipin was precipitated. The solution and precipitation were repeated three times and we obtained purified phospholipin. They are a white hygroscopic mass and their N:P ratios are as follows.
Studies on the heterogenic Antigen

Table V

<table>
<thead>
<tr>
<th>N : P</th>
<th>Heart</th>
<th>Lung</th>
<th>Kidney</th>
<th>Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.10 : 1</td>
<td>1.09 : 1</td>
<td>1.11 : 1</td>
<td>1.15 : 1</td>
</tr>
</tbody>
</table>

Using the alcoholic extract of the purified phospholipin thus obtained the complement fixation reactions were performed against heterogenic antibodies. The results are shown in the table.

Table VI

<table>
<thead>
<tr>
<th>No. of</th>
<th>Antibody producer</th>
<th>Antibody detector</th>
<th>Unit of complement</th>
<th>Detector Antiserum</th>
<th>Complement</th>
</tr>
</thead>
<tbody>
<tr>
<td>rabbit</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>94</td>
<td>Heart extract</td>
<td>Purified phospholipin from heart</td>
<td>2 4 6 8</td>
<td>+ + + + #</td>
<td># #</td>
</tr>
<tr>
<td>93</td>
<td>&quot;</td>
<td>&quot;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>64</td>
<td>Lung extract</td>
<td>Purified phospholipin from lung</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>65</td>
<td>&quot;</td>
<td>&quot;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Kidney extract</td>
<td>Purified phospholipin from kidney</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>&quot;</td>
<td>&quot;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>Liver extract</td>
<td>Purified phospholipin from liver</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Thus it is clear that the purified phospholipin lacks the character as an antibody detector, but it can not be decided at once that the property of the crude phospholipin to react with the corresponding heterogenic antibodies depends on their impurities, because the active lipid substance may be eliminated during der purification of the lipid. We tried therefore to decide whether the property of the crude phospholipin as antibody detector depends really on lipid or not, by hydrogenating the unsaturated lipid and separating the less water insoluble hydrogenated lipid from the water soluble impurities. The hydrogenated lipid is more hydrophobic than the original lipid and therefore the impurities contained in it are easily separable from the lipid.

The method of hydrogenation.

About 2 gm. of platinum-black was suspended in 150 cc. of 2 per cent. alcoholic crude phospholipin solution and the mixture was passed through at 60° C with hydrogen gas, which was purified by bubbling through saturated mercuric chloride solution, 2 per cent. potassium permanganate solution and 5 per cent. sodium hydroxide solution in succession. After the disappearance of the yellowish colour of the alcoholic solution, the hydrogen gas was passed for one hour more, and then the mixture was left for the sedimentation of the platinum-black at 60° C for about 30 minutes, and filtered hot. The filtrate freed from platinum-black was colourless and the white precipitate of hydrolecithin separated out as cooling proceeded. After the removal of the separated hydrolecithin, the filtrate was hydrogenated once more as above
and filtered hot. The alcoholic filtrate was evaporated in vacuum at 40° C and well mixed with the hydrogenated lipid precipitate obtained in the first hydrogenation and dried. The hydrogenated impure lipid thus obtained is a white powder.

The fractionation of the hydrogenated lipid into both fractions of water soluble or chloroform soluble.

The hydrogenated impure lipid obtained as above was dissolved in chloroform and extracted with water at pH 2 several times by adding a small amount of alcohol. The chloroform fraction was evaporated dry in vacuum and recrystallized with acetic ester. The water fraction was washed many times with chloroform, neutralized and evaporated dry in vacuum. The water soluble fraction showed Biuret, Millon’s and Hopkins-Cole’s reactions. Molisch’s carbohydrate reaction was negative. Using the chloroform soluble fraction and the water soluble fraction obtained as above as antibody detector, the complement fixation reactions were performed against the heterogenetic antibodies. The experiment gives the following results.

Table VII

<table>
<thead>
<tr>
<th>No. of rabbit</th>
<th>Antibody producer</th>
<th>Antibody detector</th>
<th>Unit of complement</th>
<th>Detector + Complement</th>
<th>Antiserum + Complement</th>
</tr>
</thead>
<tbody>
<tr>
<td>64</td>
<td>Lung extract</td>
<td>Chloroform fraction of lung</td>
<td>2 4 6 8</td>
<td># # # #</td>
<td>#</td>
</tr>
<tr>
<td>56</td>
<td>&quot;</td>
<td>&quot;</td>
<td># # # #</td>
<td>#</td>
<td>#</td>
</tr>
<tr>
<td>10</td>
<td>Kidney extract</td>
<td>Chloroform fraction of kidney</td>
<td># # # #</td>
<td>#</td>
<td>#</td>
</tr>
<tr>
<td>20</td>
<td>&quot;</td>
<td>&quot;</td>
<td># # # #</td>
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<td>#</td>
</tr>
</tbody>
</table>

Table VIII

<table>
<thead>
<tr>
<th>No. of rabbit</th>
<th>Antibody producer</th>
<th>Antibody detector</th>
<th>Unit of complement</th>
<th>Detector + Complement</th>
<th>Antiserum + Complement</th>
</tr>
</thead>
<tbody>
<tr>
<td>64</td>
<td>Lung extract</td>
<td>Water fraction of lung</td>
<td>-- -- # #</td>
<td># #</td>
<td>#</td>
</tr>
<tr>
<td>56</td>
<td>&quot;</td>
<td>&quot;</td>
<td>-- -- # #</td>
<td>#</td>
<td>#</td>
</tr>
<tr>
<td>10</td>
<td>Kidney extract</td>
<td>Water fraction of kidney</td>
<td>-- + # #</td>
<td>#</td>
<td>#</td>
</tr>
<tr>
<td>20</td>
<td>&quot;</td>
<td>&quot;</td>
<td>-- -- # #</td>
<td>#</td>
<td>#</td>
</tr>
</tbody>
</table>

From these results it can be said that the character of the crude phospholipin fraction as an antibody detector against the heterogenetic antibodies depends on its impurities which by the hydrogenation of the lipid become separable from it and soluble in a water layer.

The serological relation between various heterogenetic antigens. In this section we examined whether the active substance as an antibody detector contained in various organ tissues reacts commonly against the heterogenetic antibodies or not. For this purpose the organ tissues of other animals belonging to the “guinea-pig type” like dog, horse or hen were also used,
and complement fixation reactions were performed with each other. The results of them are shown in the following tables.

**Table IX**

(1) **Antibody producer:** Water extract of guinea-pig heart.  
**Antibody detector:** Alcoholic extract of various tissues.

<table>
<thead>
<tr>
<th>No. of rabbit</th>
<th>Antibody detector</th>
<th>Unit of complement</th>
<th>Detector</th>
<th>Antiserum</th>
</tr>
</thead>
<tbody>
<tr>
<td>94</td>
<td>Guinea-pig lung</td>
<td>2 4 6 8 10 12 15</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>93</td>
<td></td>
<td></td>
<td>#</td>
<td>#</td>
</tr>
<tr>
<td>94</td>
<td>Guinea-pig kidney</td>
<td></td>
<td>#</td>
<td>#</td>
</tr>
<tr>
<td>93</td>
<td></td>
<td></td>
<td>#</td>
<td>#</td>
</tr>
</tbody>
</table>

(2) **Antibody producer:** Water extract of guinea-pig lung.  
**Antibody detector:** Alcoholic extract of various tissues.

<table>
<thead>
<tr>
<th>No. of rabbit</th>
<th>Antibody detector</th>
<th>Unit of complement</th>
<th>Detector</th>
<th>Antiserum</th>
</tr>
</thead>
<tbody>
<tr>
<td>64</td>
<td>Guinea-pig heart</td>
<td>2 4 6 8 10 12 15</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>56</td>
<td></td>
<td></td>
<td>#</td>
<td>#</td>
</tr>
<tr>
<td>64</td>
<td>Guinea-pig kidney</td>
<td></td>
<td>#</td>
<td>#</td>
</tr>
<tr>
<td>56</td>
<td></td>
<td></td>
<td>#</td>
<td>#</td>
</tr>
</tbody>
</table>

(3) **Antibody producer:** Water extract of guinea-pig kidney.  
**Antibody detector:** Alcoholic extract or crude lipid of various tissues.

<table>
<thead>
<tr>
<th>No. of rabbit</th>
<th>Antibody detector</th>
<th>Unit of complement</th>
<th>Detector</th>
<th>Antiserum</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>Guinea-pig heart</td>
<td>2 4 6 8 10 12 15</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>20</td>
<td></td>
<td></td>
<td>#</td>
<td>#</td>
</tr>
<tr>
<td>10</td>
<td>Guinea-pig lung</td>
<td></td>
<td>#</td>
<td>#</td>
</tr>
<tr>
<td>20</td>
<td></td>
<td></td>
<td>#</td>
<td>#</td>
</tr>
</tbody>
</table>

(4) **Antibody producer:** Water extract of guinea-pig heart.  
**Antibody detector:** Crude phospholipin from various tissues.

<table>
<thead>
<tr>
<th>No. of rabbit</th>
<th>Antibody detector</th>
<th>Unit of complement</th>
<th>Detector</th>
<th>Antiserum</th>
</tr>
</thead>
<tbody>
<tr>
<td>94</td>
<td>Guinea-pig lung</td>
<td>2 4 6 8 10 12 15</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>93</td>
<td></td>
<td></td>
<td>#</td>
<td>#</td>
</tr>
<tr>
<td>94</td>
<td>Guinea-pig kidney</td>
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<td>#</td>
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</tr>
<tr>
<td>93</td>
<td></td>
<td></td>
<td>#</td>
<td>#</td>
</tr>
</tbody>
</table>

(5) **Antibody producer:** Water extract of guinea-pig lung.  
**Antibody detector:** Crude phospholipin from various tissues.

<table>
<thead>
<tr>
<th>No. of rabbit</th>
<th>Antibody detector</th>
<th>Unit of complement</th>
<th>Detector</th>
<th>Antiserum</th>
</tr>
</thead>
<tbody>
<tr>
<td>64</td>
<td>Guinea-pig detector</td>
<td>2 4 6 8 10 12 15</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>56</td>
<td></td>
<td></td>
<td>#</td>
<td>#</td>
</tr>
<tr>
<td>64</td>
<td>Guinea-pig kidney</td>
<td></td>
<td>#</td>
<td>#</td>
</tr>
<tr>
<td>56</td>
<td></td>
<td></td>
<td>#</td>
<td>#</td>
</tr>
</tbody>
</table>
(6) Antibody producer: Water extract of guinea-pig kidney.  
Antibody detector: Crude phospholipin from various tissues.

<table>
<thead>
<tr>
<th>No. of rabbit</th>
<th>Antibody detector</th>
<th>Unit of complement</th>
<th>Detector</th>
<th>Antiserum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2 4 6 8 10 12 15</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Guinea-pig lung</td>
<td>- - - - - - - -</td>
<td></td>
<td># # # #</td>
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<tr>
<td>20</td>
<td></td>
<td>- - - - - - - -</td>
<td></td>
<td># # # #</td>
</tr>
<tr>
<td>10</td>
<td>Guinea-pig heart</td>
<td>- - - - + - - - -</td>
<td></td>
<td># # # #</td>
</tr>
<tr>
<td>20</td>
<td></td>
<td>- - - - + - - - -</td>
<td></td>
<td># # # #</td>
</tr>
</tbody>
</table>

(7) Antibody producer: Water extract of guinea-pig heart.  
Antibody detector: Water fraction of hydrolipid from various tissues.

<table>
<thead>
<tr>
<th>No. of rabbit</th>
<th>Antibody detector</th>
<th>Unit of complement</th>
<th>Detector</th>
<th>Antiserum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2 4 6 8 10 12 15</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>94</td>
<td>Guinea-pig lung</td>
<td>- - - - # # # # #</td>
<td></td>
<td># # # #</td>
</tr>
<tr>
<td>63</td>
<td></td>
<td>- - + # # # # # #</td>
<td></td>
<td># # # #</td>
</tr>
<tr>
<td>94</td>
<td>Guinea-pig kidney</td>
<td>- - - - + # # # #</td>
<td></td>
<td># # # #</td>
</tr>
<tr>
<td>93</td>
<td></td>
<td>- - - - # # # # #</td>
<td></td>
<td># # # #</td>
</tr>
</tbody>
</table>

(8) Antibody producer: Water extract of guinea-pig kidney.  
Antibody detector: Water soluble fraction of various tissues.

<table>
<thead>
<tr>
<th>No. of rabbit</th>
<th>Antibody detector</th>
<th>Unit of complement</th>
<th>Detector</th>
<th>Antiserum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2 4 6 8 10 12 15</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Guinea-pig lung</td>
<td>- - - - + - - - -</td>
<td></td>
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<td></td>
<td>- + # # # # # # #</td>
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<td># # # #</td>
</tr>
</tbody>
</table>

(9) Antibody producer: Water extract of guinea-pig lung.  
Antibody detector: Water soluble fraction of various tissues.

<table>
<thead>
<tr>
<th>No. of rabbit</th>
<th>Antibody detector</th>
<th>Unit of complement</th>
<th>Detector</th>
<th>Antiserum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2 4 6 8 10 12 15</td>
<td>+</td>
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</tr>
<tr>
<td>64</td>
<td>Guinea-pig kidney</td>
<td>- - - - # # # # #</td>
<td></td>
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<tr>
<td>56</td>
<td></td>
<td>- - # # # # # # #</td>
<td></td>
<td># # # #</td>
</tr>
</tbody>
</table>

(10) Antibody detector: Alcoholic extract of various tissues of guinea-pig.

<table>
<thead>
<tr>
<th>No. of rabbit</th>
<th>Antibody producer</th>
<th>Antibody detector</th>
<th>Unit of complement</th>
<th>Detector</th>
<th>Antiserum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>2 4 6 8 10 12 15</td>
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</tr>
<tr>
<td>17</td>
<td>Dog heart</td>
<td>Heart alcoholic</td>
<td>- - - - - - - -</td>
<td># #</td>
<td># #</td>
</tr>
<tr>
<td>13</td>
<td>Dog heart</td>
<td></td>
<td>- - - - - - - -</td>
<td># #</td>
<td># #</td>
</tr>
<tr>
<td>99</td>
<td>Dog kidney</td>
<td></td>
<td>- - # # # # # # #</td>
<td># #</td>
<td># #</td>
</tr>
<tr>
<td>85</td>
<td>Hen heart</td>
<td></td>
<td>- - - - - - - -</td>
<td># #</td>
<td># #</td>
</tr>
<tr>
<td>64</td>
<td>Mouse muscle</td>
<td></td>
<td>- - - - + # # # #</td>
<td># #</td>
<td># #</td>
</tr>
<tr>
<td>31</td>
<td>Sheep red</td>
<td>corpse</td>
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<td># #</td>
</tr>
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<td></td>
<td></td>
<td>- - - - - - - -</td>
<td># #</td>
<td># #</td>
</tr>
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<tr>
<td>13</td>
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<td></td>
<td>- - - - - - - -</td>
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<td># #</td>
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<td>Antibody detector</td>
<td>Unit of complement</td>
<td>Detector</td>
<td>Antiserum</td>
</tr>
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<td>-------------------</td>
<td>----------</td>
<td>-----------</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2     4     6     8     10      12      15</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
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<td>Kidney alcoholic extract</td>
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<td></td>
</tr>
<tr>
<td>85</td>
<td>Hen heart</td>
<td></td>
<td>-    -    -    -    -    -    -    -    #  #  #  #</td>
<td></td>
<td></td>
</tr>
<tr>
<td>64</td>
<td>Mouse muscle</td>
<td></td>
<td>-    -    -    -    -    -    +    #  #  #  #  #  #</td>
<td></td>
<td></td>
</tr>
<tr>
<td>31</td>
<td>Sheep red corpuscle</td>
<td></td>
<td>-    -    -    -    -    -    -    -    -    #  #  #  #</td>
<td></td>
<td></td>
</tr>
<tr>
<td>32</td>
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<td>-    -    -    -    -    -    -    -    -    -    -    -</td>
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</tr>
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</table>

(11) Antibody detector: Alcoholic extract of various tissues of guinea-pig.

<table>
<thead>
<tr>
<th>No. of rabbit</th>
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<th>Antibody detector</th>
<th>Unit of complement</th>
<th>Detector</th>
<th>Antiserum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>2     4     6     8     10      12      15</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>17</td>
<td>Dog heart</td>
<td>Liver alcoholic extract</td>
<td>-    -    -    +     #  #  #  #  #  #  #  #  #</td>
<td></td>
<td></td>
</tr>
<tr>
<td>99</td>
<td>Dog kidney</td>
<td></td>
<td>-    -    -    -    -    -    -    -    -    -    -    -</td>
<td></td>
<td></td>
</tr>
<tr>
<td>85</td>
<td>Hen heart</td>
<td></td>
<td>-    -    -    +    #  #  #  #  #  #  #  #  #</td>
<td></td>
<td></td>
</tr>
<tr>
<td>31</td>
<td>Sheep red corpuscle</td>
<td></td>
<td>-    -    -    -    -    -    -    -    -    -    -    -</td>
<td></td>
<td></td>
</tr>
<tr>
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<td>Dog heart</td>
<td>Lung alcoholic extract</td>
<td>-    -    -    -    -    -    -    -    -    -    -    -</td>
<td></td>
<td></td>
</tr>
<tr>
<td>99</td>
<td>Dog kidney</td>
<td></td>
<td>-    -    -    -    -    -    -    -    -    -    -    -</td>
<td></td>
<td></td>
</tr>
<tr>
<td>85</td>
<td>Hen heart</td>
<td></td>
<td>-    -    -    -    -    -    -    -    -    -    -    -</td>
<td></td>
<td></td>
</tr>
<tr>
<td>31</td>
<td>Sheep red corpuscle</td>
<td></td>
<td>-    -    -    -    -    -    -    -    -    -    -    -</td>
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<td></td>
</tr>
</tbody>
</table>

(12) Antibody detector: Crude phospholipin from guinea-pig kidney.

<table>
<thead>
<tr>
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<th>Unit of complement</th>
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<th>Antiserum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2     4     6     8     10      12      15</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>17</td>
<td>Dog heart</td>
<td>-    -    -    -    -    -    -    -    -    -    -    -</td>
<td></td>
<td></td>
</tr>
<tr>
<td>99</td>
<td>Dog kidney</td>
<td>-    -    -    -    -    -    +    #  #  #  #  #  #  #  #</td>
<td></td>
<td></td>
</tr>
<tr>
<td>85</td>
<td>Hen heart</td>
<td>-    -    -    -    -    -    -    -    -    -    -    -</td>
<td></td>
<td></td>
</tr>
<tr>
<td>64</td>
<td>Mouse muscle</td>
<td>-    -    -    -    -    -    -    -    -    -    -    -</td>
<td></td>
<td></td>
</tr>
<tr>
<td>31</td>
<td>Sheep red corpuscle</td>
<td></td>
<td>-    -    -    -    -    -    -    -    -    -    -    -</td>
<td></td>
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</tbody>
</table>

(13) Antibody detector: Purified phospholipin from guinea-pig kidney.
(14) Antibody detector: Crude phospholipin from guinea-pig heart.

<table>
<thead>
<tr>
<th>No. of rabbit</th>
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<th>Detector</th>
<th>Antiserum</th>
</tr>
</thead>
<tbody>
<tr>
<td>17</td>
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<td></td>
<td>- - - - -</td>
<td>+</td>
</tr>
<tr>
<td>99</td>
<td>Dog kidney</td>
<td></td>
<td>- -</td>
<td>+</td>
</tr>
<tr>
<td>38</td>
<td>Hen heart</td>
<td></td>
<td>- - - - -</td>
<td>+</td>
</tr>
<tr>
<td>64</td>
<td>Mouse muscle</td>
<td></td>
<td>- - -</td>
<td>+</td>
</tr>
<tr>
<td>31</td>
<td>Sheep red corpuscle</td>
<td></td>
<td>- - - - -</td>
<td>+</td>
</tr>
</tbody>
</table>

(15) Antibody detector: Purified phospholipin from guinea-pig heart.

<table>
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<th>Unit of complement</th>
<th>Detector</th>
<th>Antiserum</th>
</tr>
</thead>
<tbody>
<tr>
<td>17</td>
<td>Dog heart</td>
<td></td>
<td># # # #</td>
<td>#</td>
</tr>
<tr>
<td>99</td>
<td>Dog kidney</td>
<td></td>
<td># # #</td>
<td># # # #</td>
</tr>
<tr>
<td>38</td>
<td>Hen heart</td>
<td></td>
<td># # #</td>
<td>#</td>
</tr>
<tr>
<td>64</td>
<td>Mouse muscle</td>
<td></td>
<td># # #</td>
<td>#</td>
</tr>
<tr>
<td>31</td>
<td>Sheep red corpuscle</td>
<td></td>
<td># # #</td>
<td>#</td>
</tr>
</tbody>
</table>

(16) Antibody detector: Crude phospholipin from guinea-pig lung.

<table>
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<th>Unit of complement</th>
<th>Detector</th>
<th>Antiserum</th>
</tr>
</thead>
<tbody>
<tr>
<td>17</td>
<td>Dog heart</td>
<td></td>
<td># # # #</td>
<td></td>
</tr>
<tr>
<td>99</td>
<td>Dog kidney</td>
<td></td>
<td># # # #</td>
<td></td>
</tr>
<tr>
<td>38</td>
<td>Hen heart</td>
<td></td>
<td># # # #</td>
<td></td>
</tr>
<tr>
<td>64</td>
<td>Mouse muscle</td>
<td></td>
<td># # #</td>
<td></td>
</tr>
<tr>
<td>31</td>
<td>Sheep red corpuscle</td>
<td></td>
<td># # #</td>
<td></td>
</tr>
</tbody>
</table>

(17) Antibody detector: Purified phospholipin from guinea-pig lung.

<table>
<thead>
<tr>
<th>No. of rabbit</th>
<th>Antibody detector</th>
<th>Unit of complement</th>
<th>Detector</th>
<th>Antiserum</th>
</tr>
</thead>
<tbody>
<tr>
<td>17</td>
<td>Dog heart</td>
<td></td>
<td># # # #</td>
<td></td>
</tr>
<tr>
<td>99</td>
<td>Dog kidney</td>
<td></td>
<td># #</td>
<td></td>
</tr>
<tr>
<td>38</td>
<td>Hen heart</td>
<td></td>
<td># # #</td>
<td></td>
</tr>
<tr>
<td>64</td>
<td>Mouse muscle</td>
<td></td>
<td># # #</td>
<td></td>
</tr>
<tr>
<td>31</td>
<td>Sheep red corpuscle</td>
<td></td>
<td># # #</td>
<td></td>
</tr>
</tbody>
</table>

(18) Antibody detector: Purified phospholipin from guinea-pig liver.

<table>
<thead>
<tr>
<th>No. of rabbit</th>
<th>Antibody detector</th>
<th>Unit of complement</th>
<th>Detector</th>
<th>Antiserum</th>
</tr>
</thead>
<tbody>
<tr>
<td>17</td>
<td>Dog heart</td>
<td></td>
<td># # # #</td>
<td></td>
</tr>
<tr>
<td>99</td>
<td>Dog kidney</td>
<td></td>
<td># #</td>
<td></td>
</tr>
<tr>
<td>38</td>
<td>Hen heart</td>
<td></td>
<td># # #</td>
<td></td>
</tr>
<tr>
<td>64</td>
<td>Mouse muscle</td>
<td></td>
<td># # #</td>
<td></td>
</tr>
<tr>
<td>31</td>
<td>Sheep red corpuscle</td>
<td></td>
<td># # #</td>
<td></td>
</tr>
</tbody>
</table>
From these results it is evident that the active substances contained in organ tissues of animals of "guinea-pig type" react with each other.

The serological relation between heterogenetic antigen and stroma of sheep red corpuscles.

It is a well known fact that the injections of stroma of red corpuscles into animals generate hemolysins against the corresponding red corpuscles. We then examined the serological relation between stroma of sheep red corpuscles and heterogenetic antibodies by complement fixation reaction. The stroma was prepared as follows. Defibrinated sheep red corpuscles, filtered through cotton wool for removal of leucocytes, were mixed with same amount of physiological salt solution. The erythrocytes were separated by centrifugation from the mother-liquid and washed with salt solution three times. The erythrocytes thus obtained were hemolyzed by an addition of five times volume of distilled water. Through this solution carbondioxide gas bubbled for 15 minutes and stroma was precipitated. The precipitate was washed with water repeatedly until nothing was extracted in the water layer. Then to the suspension of the stroma natrium hydroxide was added to a slight alkaline reaction, when a uniform suspension of stroma was obtained. Through this solution a current of carbondioxide gas was passed again and the stroma was precipitated and after washing with water it was dried in vacuum at a temperature not exceeding 40°C.

<table>
<thead>
<tr>
<th>No. of rabbit</th>
<th>Antibody producer</th>
<th>Antibody detector</th>
<th>Unit of complement</th>
<th>Detector</th>
<th>Antiserum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>94</td>
<td>Heart extract</td>
<td>Stroma</td>
<td>- - - - - - - -</td>
<td>#</td>
<td>#</td>
</tr>
<tr>
<td>93</td>
<td>Lung extract</td>
<td>&quot;</td>
<td>- - - - - +</td>
<td>#</td>
<td>#</td>
</tr>
<tr>
<td>64</td>
<td>Lung extract</td>
<td>&quot;</td>
<td>- - - - - +</td>
<td>#</td>
<td>#</td>
</tr>
<tr>
<td>56</td>
<td>Kidney extract</td>
<td>&quot;</td>
<td>- - - - - +</td>
<td>#</td>
<td>#</td>
</tr>
<tr>
<td>10</td>
<td>Kidney extract</td>
<td>&quot;</td>
<td>- - - - - +</td>
<td>#</td>
<td>#</td>
</tr>
<tr>
<td>20</td>
<td>&quot;</td>
<td>&quot;</td>
<td>- - - - - +</td>
<td>#</td>
<td>#</td>
</tr>
</tbody>
</table>

The control tests using the stroma of red corpuscles of ox or rabbit as an antibody detector were performed, and from the results we knew that hardly any complement was fixed by them. Thus it is clear that in the stroma of sheep red corpuscles there exists a substance which reacts serologically with the heterogenetic antibodies. Moreover we tried to decide what is the chemical nature of this active substance, and separated the stroma of sheep red corpuscles into two fractions of lipid and protein nature. For this purpose an irradiation of stroma in the presence of hematoporphyrin and an extraction with ether and petroleum ether were attempted as described as follows. Into 200 cc. of 1 per cent. suspension of sheep stroma hematoporphyrin was added in a concentration of 0.1 per cent. After standing for one hour in a dark place the mixture was irradiated within a Petri's dish for three hours with two 1000 watts electric lamps at a dis-
tance of 30 cm. avoiding the heat effect of the lamps by cooling with an electric fan. The irradiated stroma suspension was made pH 3 by an addition of hydrochloric acid and mixed well with same volume of alcohol. To the mixture the same amount of ether and petroleum ether was added and shaken well. The ether portion was removed and the water fraction was treated with ether and petroleum ether repeatedly until nothing was extracted in the ether layer. The water soluble fraction was then neutralized and sodium chloride was added in the concentration of 0.1 per cent. The ether fractions were combined and evaporated to dry in vacuum at 40°C in a slow current of carbondioxide gas. The dried material was dissolved in a mixture of one part of alcohol, three parts of ether and three parts of petroleum ether, filtered and then evaporated. The chloroform solution of the residue was washed with water many times in order to remove the mixed hematoporphyrin and then the chloroform was evaporated and the residue was dissolved in ether. The insoluble substance was removed and the ether was evaporated. The residue was again dissolved in petroleum ether, filtered and dried in vacuum. The water soluble fraction shows the colour and precipitation tests of protein. Using the water soluble fraction or ether soluble fraction as an antibody detector, complement fixation reactions were performed against the heterogenetic antibodies. The results are indicated in the following tables.

Table XI

<table>
<thead>
<tr>
<th>No. of rabbit</th>
<th>Antibody producer</th>
<th>Antibody detector</th>
<th>Unit of complement</th>
<th>Detector</th>
<th>Antiserum</th>
</tr>
</thead>
<tbody>
<tr>
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<td>Ether fraction</td>
<td>+</td>
<td>#</td>
<td>#</td>
<td>#</td>
</tr>
<tr>
<td>93</td>
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<td>+</td>
<td>#</td>
<td>#</td>
<td>#</td>
</tr>
<tr>
<td>64 Lung extract</td>
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<td>#</td>
<td>#</td>
<td>#</td>
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<td>20</td>
<td>&quot;</td>
<td>+</td>
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</table>

Table XII

<table>
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<tr>
<th>No. of rabbit</th>
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<th>Antibody detector</th>
<th>Unit of complement</th>
<th>Detector</th>
<th>Antiserum</th>
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</thead>
<tbody>
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<td>-</td>
<td>-</td>
<td>+</td>
<td>#</td>
</tr>
<tr>
<td>93</td>
<td>&quot;</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>#</td>
</tr>
<tr>
<td>64 Lung extract</td>
<td>&quot;</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>#</td>
</tr>
<tr>
<td>56</td>
<td>&quot;</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>#</td>
</tr>
<tr>
<td>10 Kidney extract</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>#</td>
</tr>
<tr>
<td>20</td>
<td>&quot;</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>#</td>
</tr>
<tr>
<td>35 Liver extract</td>
<td>&quot;</td>
<td>+</td>
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</tr>
</tbody>
</table>

It is obvious from these experiments that the substance contained in
Studies on the heterogenetic Antigen

the stroma of sheep red corpuscles and reacting with the heterogenetic antibodies, is not of lipid but of protein nature.

Also serological tests with antisheep red corpuscles serum and alcoholic extract of organ tissues of guinea-pig as an antibody detector were performed. The antiserum was obtained by immunizing rabbits with injection of washed sheep red corpuscles in the usual manner, and in this experiment antiserum, whose hemolysin titer was over 1000, was used.

As can be seen from these tables, crude phospholipin has a property of an antibody detector also against antisheep red corpuscles serum, and it is not due to the pure lipid but to its impurities combined with it.

<table>
<thead>
<tr>
<th>No. of rabbit</th>
<th>Antibody producer</th>
<th>Antibody detector</th>
<th>Unit of complement</th>
<th>Detector</th>
<th>Antiserum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td>+ Complement</td>
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<td></td>
<td></td>
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| Table XIII |

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<tr>
<th>No. of rabbit</th>
<th>Antibody producer</th>
<th>Antibody detector</th>
<th>Unit of complement</th>
<th>Detector</th>
<th>Antiserum</th>
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<tbody>
<tr>
<td></td>
<td>Sheep red corpuscle</td>
<td>Purified lipid from heart</td>
<td>2 4 6 8</td>
<td>+ Complement</td>
<td>+ Complement</td>
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| Table XIV |

<table>
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<th>No. of rabbit</th>
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<th>Unit of complement</th>
<th>Detector</th>
<th>Antiserum</th>
</tr>
</thead>
<tbody>
<tr>
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<td>Water fraction of hydrolipid from kidney</td>
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<td>+ Complement</td>
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</tbody>
</table>

| Table XV |

As can be seen from these tables, crude phospholipin has a property of an antibody detector also against antisheep red corpuscles serum, and it is not due to the pure lipid but to its impurities combined with it.
Summary

I. The alcoholic extracts of various organ tissues of guinea-pigs possess a property as an antibody detector against the heterogenetic antibodies in the complement fixation reaction. This property of the tissue extract does not depend on the lipid itself contained in it, but perhaps on the substance of protein nature which combines with the lipid and becomes separable from it by hydrogenation of the lipid.

II. The alcoholic extracts of various organ tissues of guinea-pigs also react serologically with antiserum obtained by immunizing rabbits with sheep red corpuscles as usual. In this case the antibody detecting property of the alcoholic extract is due to the impurities and not to the pure lipid.

III. The stroma of the sheep red corpuscles fixes complements very markedly in the presence of heterogenetic antibodies, and the stroma of ox or rabbit red corpuscles has no detecting property. When the stroma of sheep red corpuscles are separated into lipid and protein fractions, the former lacks the property as a detector and only the latter possesses the antibody detecting character.

IV. The active substances contained in the alcoholic tissue extracts of guinea-pig react not only with the corresponding heterogenetic antibodies but also with other heterogenetic antibodies reciprocall, and there seems to exist a serologically common substance in sheep red corpuscles and in organ tissues of "guinea-pig type."

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References

Doerr and Pick (1913): Biochem. Z. Bd. 50.
Meyer (1921): Biochem. Z. Bd. 72.