one-tenth* of the animal's blood being contained in the circuit.

That 0.8 per cent. of chloroform vapour in the inspired air produced paralysis of the heart under these circumstances in 16 min. 25 sec. must be due to the rapid rise in vapour tension of chloroform in the small and isolated quantity of circulating blood. This small quantity of blood cannot give its chloroform to the tissues and lymph. For the same reason the rapidity of recovery when chloroform administration was suspended in these experiments corresponds to the rapidity with which the heart is affected. This is seen in tracing (1). No sudden failure occurs from the action of chloroform on the isolated heart. The fall of blood pressure is gradual or steep according to the percentage of chloroform vapour in the air administered, but there were no abrupt variations in the rate or efficiency of the heart such as occur in an animal with an intact nervous system.

EXPERIMENTS WITH THE HEART ONCOMETER.

Twelve experiments were performed with the view to recording the changes in the volume of the heart produced by chloroform administration. The instruments used were air oncometers. Several sizes were necessary to accommodate different hearts. It consisted of a hollow metal vessel, larger than the heart for which it was used. The top was closed, with the exception of a small opening into which was let the tube for connexion with the tambour. The bottom was open, but constricted into a neck with a turned-out lip. The opening in the neck was about two-thirds the diameter of the vessel.

In applying the oncometer to the heart, the sternum, with about 3 inches of rib from each side, was removed, after the intercostal and internal mammary arteries had been secured. The pericardium was then opened wide enough to admit the neck of the oncometer. The heart was next raised into the vessel, and the pericardium drawn up firmly and secured around the neck of the oncometer by an elastic band. The heart was thus enclosed in an almost unyielding chamber, the interior of which was in communication with the tambour. Artificial respiration was, of course, necessary throughout. Simultaneous records of arterial blood pressure were taken.

The heart was found to undergo rapid dilatation when the air respired contained 3 per cent. chloroform vapour and upwards. Slower dilatation occurred when lower percentages were employed; at the same time marked diminution occurred in the contraction volume *pari passu* with the fall of blood pressure. Dilatation happened, also, when the vagi were cut. The diminution in the contraction volume tailed off to imperceptibility as the blood pressure reached zero. When the vagi were intact, however, inhibition often terminated the circulation suddenly, and both tracings simultaneously ceased.

These results correspond with those of MacWilliam.⁷

CONCLUSIONS REGARDING THE EFFECTS OF CHLOROFORM ON THE HEART ISOLATED FROM THE CENTRAL NERVOUS SYSTEM.

1. Chloroform has an immediate and progressively paralytic effect upon the heart muscle. There is no preliminary period of stimulation. There is no abrupt change in the rate or efficiency of the heart.

* Six attempts were made to determine this quantity. They gave widely varying results. One estimation gave as high as one-sixth and another as low as one-sixteenth of the total blood. The lowest value was obtained in an experiment in which the heart was vigorously contracting prior to the ligaturing off, and continued to beat well afterwards, driving the blood from the left to the right side through the coronaries only; the highest value when the heart was dilated and beating feebly before the last ligature was tied. These estimations were effected in the following manner: The morphinized dog was curarized. A standard was then made by diluting 1 c.cm. of the dog's blood with 99 c.cm. of distilled water. Artificial respiration was then commenced. The thorax was next opened by section along the sternum. The exposed aorta was tied close up to the left subclavian artery. The inferior vena cava and the azygos vein were next tied. The left subclavian was next tied, followed by the trunk of the right subclavian and carotids, simultaneously with the superior vena cava. The lungs and heart were then removed, and water run through from the inferior vena cava and out by the aorta until the washings came out colourless. The washings were then measured, and 10 c.cm. taken and diluted to match the colour of the standard. From the data thus obtained the amount of dog's blood in this portion of the circulation in relation to the total blood was readily calculated. The total blood was assumed to be one-thirteenth of the body weight.

2. Heart muscle is very sensitive to the poisonous effect of chloroform—a tension of chloroform in blood corresponding to 0.8 per cent. chloroform vapour in the air inhaled kills the isolated mammalian heart in sixteen minutes.

3. In the administration of chloroform by inhalation to the intact animal, the vapour tension of chloroform in the blood only slowly reaches that of the inspired air.

(To be continued.)

A CONTRIBUTION TO THE STUDY OF THE PRESENCE AND FORMATION OF AGGLUTININS IN THE BLOOD.

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THE origin in the animal organism of substances antagonistic to microbes has for a long time attracted the serious attention of observers, and many have endeavoured to solve a question which from so many points of view is so intensely interesting. Although special researches with a view to localizing the origin and the formation of agglutinins in the animal organisms are numerous, observers unanimously recognize that of all organs of the body the blood is richest in agglutinins, though these peculiar substances are also present in the lymph, in the pericardial and pleuritic fluids, and even in oedematous exudations which contain but very few cellular elements.

PREVIOUS RESEARCHES.

Nevertheless, it is by no means clear in what organ or organs agglutinins originate. Achard and Bensaude, Arloing, Widal, and Sicard endeavoured to ascertain whether, before passing into the blood, agglutinins were formed in the inflammatory fluid found at the point where a given microbe had been inoculated. Their conclusions were either negative or uncertain. Achard found the agglutinating power of pleuritic serous fluid sometimes greater, occasionally smaller, and at times equal, to that of the serum. Paul Courmont thought that occasionally agglutinins might be formed even in serous membranes. Pfeiffer and Marx, however, were the first to seek systematically for the place of formation of agglutinins. They injected cholera vibrios into young rabbits, killed the animals at varying times after inoculation, and observed that agglutinins were formed early in the lymphoid organs, the medulla of the bones, the lymphatic glands, and more especially in the spleen. Nevertheless, this fact was not sufficient to warrant them in drawing definitive conclusions.

Later, van Emden studied the distribution of the agglutinins of the bacillus *aërogenes* in the animal organism. According to this author, agglutinins were formed in the spleen, in the medulla of bone (which is able to compensate the spleen when that organ is removed), in the lymphatic glands (which, indeed, were sometimes richer in agglutinins than the spleen), and in the liver. But these organs contained more agglutinins than the blood only very soon after the injection of specific microbes. Later on, the blood was richer in agglutinins than these organs. These results were not confirmed by Rath who, however, used the bacillus *typhosus* for his experiments.

In the course of his researches on the resorption of cells Metchnikoff observed several times that agglutinins appeared first in the peritoneal exudations, and in the blood several days afterwards. Later on, the peritoneal liquid became less active than the serum. Metchnikoff was inclined to attribute a preponderating share in the production of agglutinins to the cells found in the peritoneal inflammatory fluid.

Deutsch observed in his interesting researches on anti-typhoid bodies that in immunized animals the liver, the kidneys, and the suprarenal bodies contain traces of agglutinins merely; whereas varying quantities of agglutinins could be obtained from the lymphoid organs—spleen, medulla of bones, lymphatic glands—though these organs

never contained as much agglutinin as the serum. Strangely enough the lungs of non-immunized guinea-pigs were the only organs which in the majority of cases possessed agglutinating properties greater than those of the serum. But this action was not a specific one, and must be considered as quite independent of the action of the specific agglutinins of immunized animals.

Gengou, when studying the agglutination of an attenuated anthrax bacillus by organs and fluids of non-immunized guinea-pigs, found that the largest quantities of agglutinins were found in the blood, the organs showing but a feeble and inconstant agglutinating action. As extracts of leucocytes proved less active than blood and oedematous fluids, Gengou concluded that agglutinins were not to be considered as products of the cells of the animal organism.

Thus, as already stated, experimenters are unanimous in recognizing that agglutinins are constantly present in the blood, and that blood contains more agglutinins than do other organs of the animal organism. Almost all observers, however, noticed that the lymphoid and blood-forming organs contained a certain though varying amount of agglutinins.

Applying to agglutinins Deutsch's conclusions with regard to antibodies, one might conclude that there are no agglutinin-producing organs, but that there are parts of the body in which agglutinins are formed; and, further, that the one characteristic sign common to all these parts is a great wealth of leucocytes.

The researches recited above led us to consider whether the agglutinins were not formed in the formed constituents of the blood, and more specially in the red corpuscles.

We may mention here Nolf's researches. This author, with another object in view, disintegrated the red blood corpuscles of fowls, and injected their contents and their stromata into rabbits. The injection of the stromata of the corpuscles caused agglutinating properties to appear in the serum, the injection of the contents of the red corpuscles, on the other hand, being followed by the appearance of globulicidal properties in the blood. These experiments, however, give us no information regarding the possible agglutinating effect of the stromata themselves. Apart, also, from Gengou, whose researches on leucocytes have been quoted above, we do not know that any observer has endeavoured to study the formation of agglutinins in the formed constituents of the blood. In his beautiful work on immunity, Metchnikoff has pointed out that Gengou killed his animals at a time when their blood had well-marked agglutinating properties already, that is, when agglutinins might already have passed from the formed constituents into the

The question, therefore, of the possible origin of agglutinin in the formed constituents of the blood has hardly been touched upon. In order to work it out systematically we first ascertained the agglutinating power on several microbes of the white and red blood corpuscles of non-immunized animals. We then investigated the agglutinating effect of the same constituents of the blood of immunized animals.

Before entering into the main question, however, we deemed it necessary to ascertain whether the liquid cultures of a given microbe had or had not agglutinating properties for microbes of the same species.

METHODS AND OBJECTS OF PRESENT RESEARCH.

In order to measure the agglutinating action of a given liquid we chose as a standard the minimum dilution which still gave rise, in two hours time and in each field of the microscope, to the formation of at least four to six microbial agglomerations. Emulsions in distilled water of agar culture twenty hours old were prepared and allowed to rest for a few minutes in order to allow any small floculi to settle. As a rule three platinum loops were diluted with 10 c.cm. of distilled water, the emulsion holding just enough bacilli to render the liquid slightly opalescent. At first we used hanging drop preparations and dilutions in tubes. As far as possible the tubes were of the same size, and the dilutions were made in such a way that all the tubes contained about the same number of drops. Later on, however, we noticed that microbes agglutinated much more easily in hanging-drop preparations than in tubes, and after this we used the former only, the preparations being kept at the ordinary temperature of the laboratory.

Agglutinating Properties of Cultures freed from Microbes.

Malvoz was, we believe, the first who, studying the anthrax vaccine, recognized the agglutinating power of the liquid in which the bacillus anthracis had proliferated after the liquid had been freed from microbes by centrifugal force. More lately, Macfadyen and Rowland have found that the juice from the bodies of typhoid bacilli, when very weakly diluted, markedly agglutinated the typhoid bacilli.

Our experiments were made with cultures of bacillus pyocyaneus and typhosus on various media. The following table gives the main results of our experiments:

SERIES I.

Age of Culture.	Name of Microbe.	Agglutinating Power.	Medium and Preparation of Liquid Tested.
5 days ...	B. pyocyaneus	1 in 2	Bouillon culture filtered.
15 " ...	"	1 " 10	" " glycerinated peptone water and centrifugalized.
17 " ...	"	1 " 40	Bouillon centrifugalized culture.
30 " ...	"	1 " 20	" culture filtered.
30 " ...	"	1 " 20	" " dialyzed through collodion.
53 " ...	"	1 " 20	Culture in urine filtered.
3 months	"	1 " 10	Bouillon culture centrifugalized.
20 days ...	B. typhosus	1 " 5	" " " "
35 " ...	"	1 " 10	" " " "
49 " ...	"	1 " 20	Bouillon culture filtered."

It will be noticed that in all experiments the agglutinating effects were comparatively feeble but distinct. The culture possessing the most marked agglutinating powers was one of the bacillus pyocyaneus on pepto-glycerinated water which had grown for 17 days. This result may possibly have been due to the viscosity of the liquid or to the comparative ease with which glycerine dissolves most soluble ferments, and therefore possibly also agglutinins. The origin of these substances in cultures we have to leave unexplained, and we are content to point out that they exist.

The mode of sterilization of the culture does not appear to have any special effect on the quantities of agglutinating substances present. Filtered cultures have the same agglutinating power as centrifugalized or dialyzed cultures, provided all be equally old. The age of the culture and the constitution of the medium appear more specially to influence the quantity of agglutinins present.

Experiment also shows that agglutinins pass through collodion sacs (though they do so with difficulty), and further that they dialyze almost entirely if the dialysis be prolonged. This is opposed to Gengou's assertion; he, however, allowed the cultures to dialyze for 24 hours only.

Agglutinating Properties of the formed Constituents of the Blood of Non-immunized Animals.

The question which we endeavoured to solve was whether the formed constituents of the blood of non-immunized animals contained agglutinins or not. An animal (rabbit or guinea-pig) was bled with aseptic precautions, and the blood thus obtained divided into two parts. The first was left to clot and the serum allowed to separate at a low temperature; the second was defibrinated and centrifugalized. The supernatant liquid of the second part was decanted, and the corpuscles remaining at the bottom of the tube were washed with physiological salt solution (0.65 per cent.), until the wash water exhibited no trace of agglutinating properties. As a rule, two or three washings sufficed for this. Then twice or thrice as much distilled water as there was blood in the original liquid was added to the washed corpuscles. Care was taken to add the water gradually and in small quantities, the tube being forcibly shaken meanwhile in order to dissociate the corpuscular agglomerations which had formed during centrifugalization. Whenever necessary a platinum loop—or, better, a small pipette—was used for the latter purpose. Nevertheless, there always remained a few floculi which could not be broken up. The corpuscles having been thus emptied of their contents as far as possible, the stromata were separated from the liquid by a fresh centrifugalization.

The stromata of the corpuscles, after one or two washings, were then allowed to macerate at 37° C. in distilled water or various saline solutions, such as sodium chloride (10 per cent.), magnesium sulphate (5 per cent.), or sodium sulphate (5 per cent.) for a period varying from forty-eight hours to eight days. The amount of liquid in which the stromata macerated was equal to or double the primitive volume of the blood. The macerations were then centrifugalized and the liquid examined for agglutinating properties, and always compared with the pure original solution.

The agglutinating properties of the following liquid were examined: (1) The serum obtained by allowing the blood to coagulate at a low temperature; (2) the serum obtained by centrifugalization; (3) the contents of blood corpuscles; and (4) the macerated stromata. For brevity sake we omitted to indicate in the following table the salts used for macerating the stromata, as the results were identical with all the salts.

SERIES II.

No. of Animal.	Microbes Used.	Agglutinating Power of Serum.	Agglutinating Power of Centrifugalized Serum.	Extract of Blood Corpuscles.	Macerated Stromata.
Rabbit, No. 3 ...	B. typhosus	1 in 40	1 in 40	o	o
	B. coli	20	20	o	o
	B. pyocyaneus	70	70	o	o
Rabbit, No. 4 ...	B. typhosus	1 in 40	1 in 40	1 in 5	o
	B. coli	25	25	o	o
	B. pyocyaneus	100	100	o	o
Rabbit, No. 5 ...	B. typhosus	1 in 25	1 in 25	o	o
	B. coli	10	10	o	o
	B. pyocyaneus	40	40	o	o
Rabbit, No. 6 ...	B. typhosus	1 in 25	1 in 25	o	o
	B. coli	30	30	o	o
	B. pyocyaneus	40	40	o	o
Rabbit, No. 7 ...	B. typhosus	1 in 10	1 in 10	o	o
	B. coli	25	25	o	o
	B. pyocyaneus	20(?)	50	o	o
Rabbit, No. 8 ...	B. typhosus	1 in 20	1 in 20	o	o
	B. coli	15	15	o	o
	B. pyocyaneus	80	80	o	o
Rabbit, No. 9 ...	B. typhosus	1 in 25	1 in 25	1 in 3	o
	B. coli	10	10	o	o
	B. pyocyaneus	70	70	o	o
Rabbit, No. 10 ...	B. typhosus	1 in 10	1 in 10	o	o
	B. coli	10	10	o	o
	B. pyocyaneus	50	50	o	o
Guinea-pig, No. 1	B. typhosus	1 in 10	1 in 10	o	o
	B. coli	5	5	o	o
	B. pyocyaneus	20(?)	50	o	o
Guinea-pig, No. 2	B. typhosus	1 in 20	1 in 20	o	o
	B. coli	30	30	o	o
	B. pyocyaneus	—	—	o	o

It was clearly useless to proceed further with these investigations, as the 10 observations tabulated above uniformly showed that the agglutinating properties of the contents of the blood corpuscles and the macerated stromata were practically *nil*. We have also studied the agglutinating properties of stromata dissolved in a solution of 1 per cent. caustic soda, but the experiments were unsatisfactory and not clear owing to the effects of the caustic soda itself.

The agglutinating effect of the serum of some animals (especially big rabbits) on the bacillus typhosus is as high as 1 in 40. Similar facts have been observed by Koeller for the normal serum of men and dogs. Needless to say, this is of some practical importance in serum diagnosis. It will be noticed also that the bacillus pyocyaneus always agglutinated very easily.

Agglutinating Properties of the Formed Constituents of Immunized Animals.

We followed the same methods as for non-immunized animals. The formation of specific agglutinins was obtained by the injection into the peritoneum of 1 or 2 c.cm. of a culture sterilized by chloroform or, more often, by heat (100° C. during a quarter of an hour). Sometimes one injection only was made, sometimes several injections at different intervals, the blood being taken at varying dates after the injection. Our experiments were made with the bacillus typhosus and the bacillus pyocyaneus.

SERIES III.

Number of Animal.	Microbe Used.	Agglutinating Power of Serum before Injection.	Number of Injections.	Date of last Injection.	Serum Obtained by Clotting.	Serum Obtained by Centrifugalization.	Extract of Blood Corpuscles.	Macerated Stromata.
Rabbit No. 1	B. typhosus	1 in 10	1	24 hours	1 in 10	1 in 10	o	o
" 2	"	25	1	48 "	30	30	o	o
" 3	"	20	2	48 "	30	30	1 in 5	o
" 4	"	15	1	3 days	40	40	o	o
" 5	"	10	1	6 "	80	80	o	o
" 6	"	20	1	13 "	120	120	1 in 2	o
" 7	"	25	2	17 "	120	120	o	o
" 8	"	40	3	19 "	100	100	o	o
" 9	B. pyocyan.	50	1	3 "	90	90	o	o
" 10	"	70	1	5 "	120	120	o	o
" 11	"	80	2	12 "	250	250	o	o
" 12	"	40	3	23 "	150	150	o	o

It follows clearly from these experiments that the formed constituents of the blood of immunized animals contain no agglutinins at any time during immunization. It is true that the extracts from red corpuscles sometimes showed slight traces of agglutinins, but this was probably due to the fact that during the process of washing, some of the clumps of red corpuscles were not entirely disintegrated.

We add now some details of some of our experiments.

Rabbit No. 3.—On July 31st a rabbit was inoculated intraperitoneally with 1 c.cm. of typhoid culture heated at 100° C. during a quarter of an hour. The serum of this rabbit, when experimented on before inoculation, agglutinated the typhoid bacillus when diluted 20 times. On August 10th the animal received another injection of 2 c.cm. of the same culture. The animal was killed on the 12th. The blood was divided into two parts; the first was allowed to stand at a low temperature; the other (5 c.cm.) was defibrinated and centrifugalized. The serum of this latter was then decanted, and the red corpuscles washed twice with salt solution and centrifugalized. The wash water contained no agglutinins. We now added to the centrifugalized corpuscles 10 c.cm. of distilled water, in small quantities at a time, taking care to shake the tube forcibly after each addition of water, and the larger part of the flocculi further dissociated by means of a pipette. The stromata, washed and centrifugalized at two different intervals, were then left to macerate in distilled water for forty-eight hours at a temperature of 37° C. The serum obtained by clotting and that obtained after defibrination and centrifugalization agglutinated at the end of two hours in a dilution of 1 in 30. In each preparation one saw several small groups of 7 to 10 bacilli or more, several of the groups being still motile; free bacilli were still numerous. The contents of the blood corpuscles gave only very few agglutinated heaps, when 5 parts of extract were added to 1 part of emulsion of bacillus typhosus. The stromata gave no agglutination whatever. The slight effect of the extract of the blood corpuscles was probably due to the fact that they could not be washed thoroughly as they could not be dissociated.

Rabbit No. 6.—On September 10th this rabbit was inoculated in the peritoneum with 1 c.cm. of a culture of typhoid bacillus sterilized by chloroform. The agglutinating power of the serum before inoculation was 1 in 20. Thirteen days after the inoculation, on September 23rd, the animal was killed and its blood submitted to the same treatment as the blood of the preceding rabbit. The stromata from 2 c.cm. of blood were left to macerate at 37° C. in distilled water during seven days, but several small clots were still present, and these could not be dissociated. The serums still showed feeble agglutination in a dilution of 1 in 120. The extracts of red blood corpuscles showed very feeble agglutinating power in a dilution of 1 in 2.

Rabbit No. 8.—On July 31st this rabbit received intraperitoneally 1 c.cm. of a bouillon culture of typhoid bacillus killed by heat. On August 10th this rabbit received a second injection of 1.75 c.cm. of the same culture, and a third injection of 2 c.cm. on the 16th. On September 4th, nineteen days after the last injection, the animal was killed and the blood treated in the way described above. The blood corpuscles from 4 c.cm. of blood were dissolved in 12 c.cm. of water. The stromata washed twice were left to macerate in a 5 per cent. solution of sulphate of magnesia at 37° C. during forty-eight hours. The two serums still showed feeble agglutination in a dilution of 1 in 100; the extract of blood corpuscles and stromata showed no agglutinative action whatever.

Rabbit No. 13.—This animal received in the peritoneum 1 c.cm. of culture of bacillus pyocyaneus heated at 100° C. during a quarter of an hour. Ten days afterwards the animal received 1.75 c.cm. of the same culture, and six days afterwards a third injection of 2 c.cm. The animal was killed twenty-three days afterwards, and its blood treated in the manner described above. The blood corpuscles from 3 c.cm. of blood were dissolved in 6 c.cm. of distilled water, and the stromata macerated in 3 c.cm. of sodium sulphate solution (5 per cent.) at 37° C. during six days. The serums still showed fairly marked agglutinating action in a dilution of 1 in 150. The aggregations of bacilli were rather voluminous though motile. The other liquids showed no agglutinating action whatever.

Agglutinating Properties of Polynuclear Leucocytes of Non-immunized Animals.

We obtained a large number of leucocytes by injecting gluten-casein into animals, thus following the method of Buchner. The mode of preparing the solution to be injected was Gengou's, with this difference, that we used pure and

commercial gluten-casein. A 10 per cent. solution was made in $\frac{1}{2}$ per cent. solution of potash heated to 100° C. After half an hour, the mixture was placed on the water bath at 55° C. for two to three hours, and then sterilized for a quarter of an hour at 100° C. on two consecutive days. Twenty-four hours after injecting this liquid into the pleural cavities of rabbits, one or two cubic centimetres of inflammatory liquid were found in the pleura. This varied somewhat in clearness, viscosity, and thickness, and contained a certain quantity of fibrin, and polynuclear leucocytes in enormous number.

This inflammatory fluid was pipetted off aseptically from the pleura and centrifugalized. The leucocytes at the bottom of the tube were then washed two or three times with physiological salt solution, and, after being centrifugalized once more, suspended in a quantity of bouillon equal in amount to the primitive exuded fluid. This emulsion was frozen according to Buchner's method by plunging it in a mixture of salt and sodium chloride. After staying half an hour in this mixture, the fluid was quickly removed to an incubator at 37° C., so far as to facilitate the breaking up of the leucocytes. After remaining for twelve hours in the incubator, the mixture was centrifugalized, and the liquid, which had become more or less ropy and tenacious, decanted off. It was then examined and compared with the serum of the same animal.

Whenever possible, we also tested the centrifugalized liquid of the pleural inflammatory fluids for agglutinins. Unfortunately, the inflammatory fluid sometimes contained such a quantity of fibrin that it coagulated *en masse* immediately after removal from the pleura. When this occurred the leucocytes were repeatedly washed until the mucin-like coagulum was completely dissolved and the last traces of it disappeared. We also always examined the last washing in order to make sure that it had no agglutinating power.

SERIES IV.

RABBIT A.—*Pleuritic Inflammatory Fluid very Viscid; Coagulates en Masse on Removal. Repeated Washings. Deposit composed almost Exclusively of Polynuclear Leucocytes.*

Microbe Tested.	Agglutinating Power of Serum.	Agglutinating Power of Leucocyte Extract.
<i>B. typhosus</i>	1 in 20	1 in 30
<i>B. coli</i>	10	10
<i>B. pyocyaneus</i>	50	15

RABBIT B.—*Pleuritic Inflammatory Fluid Abundant. Ratio of Polynuclear Leucocytes 70 per cent. Several Lymphocytes Present.*

Microbe Tested.	Agglutinating Power of Serum.	Agglutinating Power of Leucocyte Extract.	Agglutinating Power of Pleural Liquid.
<i>B. typhosus</i>	1 in 20	1 in 20	1 in 15
<i>B. coli</i>	15	15	5
<i>B. pyocyaneus</i>	80	10	20

RABBIT C.—*Very Liquid Inflammatory Fluid, Poor in Cells. Ratio of Polynuclear Leucocytes 62 per cent., several Mononuclear Lymphocytes and various other Cells present.*

Microbe Tested.	Agglutinating Power of Serum.	Agglutinating Power of Leucocyte Extract.	Agglutinating Power of Pleural Liquid.
<i>B. typhosus</i>	1 in 25	1 in 15	1 in 2
<i>B. coli</i>	15	10	2
<i>B. pyocyaneus</i>	70	10	35

RABBIT D.—*Inflammatory Fluid Rich in Cells, composed almost entirely of Polynuclear Leucocytes.*

Microbe Tested.	Agglutinating Power of Serum.	Agglutinating Power of Leucocyte Extract.	Agglutinating Power of Pleural Liquid.
<i>B. typhosus</i>	1 in 10	1 in 20	1 in 5
<i>B. coli</i>	—	—	—
<i>B. pyocyaneus</i>	1 in 100	1 in 15	15

RABBIT E.—*Viscid Inflammatory Fluid Containing a large amount of Fibrin, the Liquid Coagulating en Masse. Very Rich in Cells. Several Washings. Ratio of Polynuclear Leucocytes about 90 per cent.*

Microbe Tested.	Agglutinating Power of Serum.	Agglutinating Power of Leucocyte Extract.	Agglutinating Power of Pleural Liquid.
<i>B. typhosus</i>	1 in 10	1 in 20	1 in 5
<i>B. coli</i>	25	20	2
<i>B. pyocyaneus</i>	80	10	25

Whenever, therefore, the pleural exudation contained a large proportion of leucocytes, the cellular extract had an agglutinating effect on the bacillus typhosus and bacillus coli equal to or greater than the serum. We may presume, therefore, that the substances normally present which agglutinate these two microbes are possibly formed, but certainly found most abundantly, in the polynuclear leucocytes. But the case is different with the bacillus pyocyaneus, for the agglutinating power of the cellular extract on this microbe has always shown itself to be inferior to that of the serum. There is no reason, therefore, to suppose at present that for this microbe, the leucocytes are the source of the agglutinins present in the blood of non-immunized animals.

We venture to think, therefore, that each agglutinin present in the serum of non-immunized animals is a specific one, and that they need not necessarily have the same origin.

Agglutinating Properties of the Polynuclear Leucocytes of Immunized Animals.

Each animal received only one peritoneal injection of 2 c.cm. of a culture sterilized by heat, a quantity which is usually found to be sufficient to produce the formation of agglutinins. The introduction of gluten-casein into the pleurae of rabbits was made at varying intervals after the inoculation of the dead cultures. The following table gives the results of our experiments:

SERIES V.

Rabbits.	Microbe used.	Agglutinating Power of Serum before Inoculation.	Number of Days after Inoculation.	Agglutinating Power of Serum.	Agglutinating Power of Leucocyte Extract.	Agglutinating Power of Pleural Liquid.
No. 1	<i>B. typhosus</i>	1 in 25	24 hours	1 in 30	1 in 15	1 in 50
" 2	"	15	24 "	20	20	—
" 3	"	20	28 "	20	60	1 in 10
" 4	"	15	48 "	60	120	—
" 5	"	10	48 "	28	50	—
" 6	"	15	3 days	25	80	1 in 15
" 7	"	20	5 "	80	80	10
" 8	"	20	9 "	40	80	—
" 9	"	15	10 "	80	90	1 in 15
" 10	"	12	15 "	70	40	—
" 11	"	20	17 "	120	20	1 in 20
" 12	"	20	19 "	110	25	30
" 13	<i>B. pyocyaneus</i>	50	48 hours	70	140	25
" 14	"	70	3 days	100	150	—
" 15	"	40	5 "	130	250	1 in 80
" 16	"	50	13 "	300	90	80

We may draw from these experiments the conclusion, that the quantity of agglutinins increases the living leucocytes and especially polynuclear leucocytes before it does in the serum, and that, therefore, the specific agglutinins are probably formed in the leucocytes of animals immunized against particular microbes, just as frequently the agglutinins of non-immunized animals are probably often formed in them. The increased amount of agglutinins in these cells begins to show itself between the thirtieth and forty-eighth hours, but varies somewhat in different animals. Between the tenth and fifteenth day the amount of agglutinins present in the leucocytes begins to diminish; but it increases in the serum; and though no doubt the increase of the agglutinating action of the serum may be noticed already on the third day, it is relatively small, and, in any case, is far inferior to the increase in the leucocytic extract.

It is therefore not astonishing that Gengou found but a feeble agglutinating power in the leucocytic extracts he experimented with, for in several animals he raised the agglutinating property by repeated injections; thus the agglutinins had time to pass into the serum long before he examined the leucocytic extract.

The next question is whether this agglutination power which is so markedly developed in leucocytes after the inoculation of a specific microbe is really a specific increase or simply an increase of some pre-existing substance acting on all microbes without distinction.

If we measure the agglutinating value of the serums and leucocytic extracts of animals immunized against one particular microbe for other microbes, we find that at first all microbes are agglutinated in an increased degree by the leucocytic extracts. But this effect lasts a short time only, namely for three or four days; then the quantity of non-specific agglutinins falls suddenly to the normal level, and the agglutinating power on the microbe inoculated, alone persists during a fairly long time. The following table explains our meaning more clearly. The microbe inoculated was always the typhoid bacillus.

No. of Animal Used.	Microbes Tested.	No. of Days after Inoculation.	Agglutinating Power of Serum.	Agglutinating Power of Leucocyte Extract.	Agglutinating Power of Pleural Liquid.
Rabbit No. 3 ...	B. typhosus	48 hours	20	60	10
	B. coli		10	10 (?)	10
	B. pyocyaneus		50	70	15
Rabbit No. 5 ...	B. typhosus	48 hours	20	50	—
	B. coli		15	30	—
	B. pyocyaneus		110	40	—
Rabbit No. 7 ...	B. typhosus	5 days	80	80	10
	B. coli		20	60	2
	B. pyocyaneus		100	50	20
Rabbit No. 8 ...	B. typhosus	9 days	40	80	—
	B. coli		20	20	—
	B. pyocyaneus		40	10	—
Rabbit No. 10 ...	B. typhosus	15 days	70	40	—
	B. coli		15	15	—
	B. pyocyaneus		50	20	—

Comparing the agglutinating power of the leucocytic extract of non-immunized animals with that of the animals in the above table, we find that the extract of the former agglutinates only at most in the proportion of 1 in 20, whereas the extract of the latter possesses two or three times the power even for microbes other than those used for inoculation. This increased agglutinating power, however, lasts but for a short time. It will be noticed also that the agglutinating effect of the leucocytic extract on the bacillus pyocyaneus, though increased (compare the other tables), still remained low, and always much below the agglutinating power of the serum.

CONCLUSIONS.

From all the facts recited above we draw the following conclusions:

1. The cultures of a microbe freed from that microbe by filtration, dialysis, or centrifugalization have a distinct, though feeble, agglutinating effect on that particular microbe. The age of the culture and the constitution of the medium

are important factors in determining the quantity of agglutinins present in such cultures.

2. The red blood corpuscles of non-immunized and immunized animals contain no trace of agglutinins.

3. On the other hand, the polynuclear leucocytes of non-immunized animals always possess an agglutinating power greater than, or more rarely equal to, that of the serum. They may therefore be rightly considered as the producers, or at any rate the carriers, of the agglutinins.

4. In immunized animals the specific agglutinins appear in the polynuclear leucocytes, and are therefore probably formed in them. The quantity of agglutinins begins to increase thirty to forty-eight hours after the injection, and goes on increasing up to the tenth day or thereabout. They then passed into the serum, the agglutinating power of which increases correspondingly.

5. The formation of specific agglutinins in polynuclear leucocytes and in the serum is preceded and accompanied during the first three or four days after the inoculation of a given microbe by an increase of agglutinins for other microbes. This latter increase is of short duration and stops suddenly, whereas the increase of specific agglutinins persists for a much longer time.

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PROGRESS REPORT UPON THE BIOLOGICAL TEST FOR BLOOD AS APPLIED TO OVER 500 BLOODS FROM VARIOUS SOURCES,

TOGETHER WITH A PRELIMINARY NOTE UPON A METHOD FOR MEASURING THE DEGREE OF REACTION.

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IN view of the extended character of the investigation I am engaged in, I deem it advisable to present a brief summary of the main results obtained up to the present, reserving a detailed publication for a future occasion. I have already reported from time to time upon some of my results, and I refer the reader to my other papers for further information.¹

RESULTS OF BIOLOGICAL TESTS.

I have now tested some 46 bloods of apes and monkeys by means of "antihuman" serum, the results being in accord with those previously published, that is, the bloods of the *Simiidae* give a precipitum apparently equal in quantity to that of human blood, the bloods of *Cercopithecidae* give less precipitum, whilst the least precipitum is given by bloods of *Hapalidae* and *Cebidae*. The bloods of three species of lemur have given a negative result. By means of the volumetric method of measuring the different degrees of reaction, to which I shall presently refer, I hope to be able shortly to give the different degrees of reaction in figures, not only for these bloods, but also for others tested by different antisera.

Dr. Grünbaum of Liverpool having kindly sent me some "antichimpanzee" serum which he had prepared, I have been able to test a series of human, ape, and monkey bloods with it. The results coincide with those obtained with antihuman serum upon the same bloods. Some antigorilla serum which was also sent me by Dr. Grünbaum was unfortunately too weak to give any results which are worth recording.

"Antihorse" serum has been tested with negative result on 499 bloods, only the blood of the horse and donkey reacting. "Antidog" serum has given a precipitum with the