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

bloods of eight species of *Canidae*, but not with other bloods. "Anti-ox" and "antisheep" serums have continued to give reactions similar to those already reported; that is, they act upon the bloods of other true ruminants, to a greater degree upon the bloods of more closely allied species. I shall presently refer to certain slight reactions observed in other bloods.

At a recent meeting of the Cambridge Philosophical Society I reported briefly upon the results obtained with antisera for the bloods of the pig, fowl, alligator, turtle, frog, lobster, and for the egg-albumen of the fowl. I have since succeeded in obtaining antisera for the bloods of the ostrich and bear, and am preparing still other antisera, upon the action of which I shall be able to report in due course.

One of the two "antipig" serums has proved to be especially powerful. It yielded a voluminous precipitum even in high dilutions of pig's blood. On the other hand, it produced a marked clouding in a number of other mammalian bloods, especially when the tubes were allowed to stand for some time. The bloods in which these cloudings, or slight reactions, took place were those of man, several species of monkey, bear, dog, raccoon, cat, coati, genet, stoat, rat, mouse, antelope, deer. Faint clouding was, moreover, produced after half an hour or after up to two hours in the bloods of bats (six species), three species of Edentates, and eight species of Australian marsupials. It is especially interesting to note that the blood of the porpoise immediately gave a slight clouding upon the addition of antipig serum, a well-marked though slight deposit being formed within twenty-four hours.

The more general action of this particular antipig serum on other mammalian bloods may at first appear to contradict what has hitherto been claimed with regard to the relatively specific character of these antisera; but this is actually not the case. I have already noted elsewhere the occasional occurrence of clouding in non-homologous bloods upon the addition of an antiserum. This is frequently noted if a time limit is not put upon the test; but it may take place immediately with what we have termed "powerful" antiserum for want of a better word. In the absence of any standards it has been impossible hitherto to define the various grades of power possessed by antisera. I have shown that a relatively weak antihuman serum will produce a precipitum in the blood of man and the anthropoid apes, less in that of the *Cercopitheciidae*, but none in that of the *Haplorhinae*, etc. A more powerful antiserum will include the *Haplorhinae*, but confines itself to the group. A still more powerful antiserum will extend out beyond the group, and may include other mammals, the reaction in all cases being very much slighter and progressing very much more slowly than with homologous or closely-allied bloods. The occasional action of non-homologous antisera upon bloods has been noted by other observers, but its significance has not been appreciated. I find that a powerful antiserum for any mammalian blood will produce a varying amount of clouding in all mammalian bloods. I have referred to this as the "mammalian reaction." In some cases this mammalian reaction begins to appear long after a well-marked precipitum is formed in a homologous blood dilution, and frequently it does not lead to any deposit, or but to a trace of deposit, after twenty-four to forty-eight hours.

These slight reactions would never be mistaken for a full reaction, such as a homologous blood gives. I think I am justified in thus speaking of a mammalian reaction, for the reason that I have not observed an antimammalian serum to produce even the slightest clouding in avian, reptilian, batrachian, and crustacean bloods.² There appear, then, to be different properties in blood, a common mammalian property, besides properties pertaining to groups and species. By the method of measuring the reaction which I describe below, I expect that we shall be able to define these degrees of reaction by figures.

"Antifowl" and "antiostrich" serum have been found to act to a greater or less degree on all bird bloods tested, but not on any non-avian bloods. The first has been tested on 510 bloods, of which 153 were avian, the second on over 100 bloods. "Antiegg" serum (very powerful) only produced a marked reaction with egg-white of the fowl, a feeble reaction only being obtained with fowl's blood, more or less clouding

in some avian bloods. It had no action on any non-avian bloods with the exception of the bloods of *Alligator sinensis*, *A. mississippiensis*, *Chelone midas*, and *Testudo ibera*, in the first and last of which it produced marked clouding, producing faint clouding in the other two. It is perhaps premature to draw any conclusion from this interesting observation; it is, however, very suggestive in view of the reptilian origin of birds. It is not impossible that a more powerful antiserum for avian blood will also bring about this slight, possibly "reptilian-avian" reaction.

"Antialligator" (*A. mississippiensis*) serum tested on over 500 bloods only produced a reaction with its homologous blood, and that of *A. sinensis* a faint clouding in the blood of the green turtle (*Chelone midas*).

"Antiturtle" (*Chelone midas*) serum produced a reaction with its homologous serum, a marked clouding with that of *Testudo ibera*, a faint clouding with that of *A. mississippiensis*. Both this and antialligator serum produced no effect whatever on any non-reptilian bloods.

"Antilobster" (*Homarus vulgaris*) serum, also tested on over 500 bloods, only produced a precipitum in dilutions of lobster serum. It, however, produced marked clouding in the blood of the crayfish, and in that of five species of crab. This antiserum produced no effect whatever on 503 non-crustacean bloods.

"Antifrog" (*Rana temporaria*) serum formed a precipitum with its homologous blood, and with that of *Rana tigrina*. Some toad bloods which have been collected on filter paper, having proved insoluble in salt solution, have not yet been tested. This antiserum produced no effect on 508 non-batrachian bloods.

I continue to receive numerous blood samples from all parts of the world, being aided at present by the kind co-operation of some forty gentlemen at home and abroad. On a later occasion I shall thank them personally in print, but in the meantime I must express my great appreciation of their efforts on behalf of this research. In most cases the blood samples are sent to me dried upon filter paper, as directed in a circular letter which I have been sending out during the past year, this being the most convenient method I have found for obtaining a supply of a large variety of bloods. Dilutions made from these dried bloods give approximately accurate results, and serve very well for purposes of preliminary study by way of surveying the field for future work.

MEASUREMENT OF DEGREE OF REACTION.

In earlier papers I have dwelt upon differences in the degree of reactions obtained with different antisera, noting in several instances that the reaction is less and less marked as we come to testing the bloods of more distantly-related animals. It occurred to me at an early date that a method might be devised for the quantitative determination of the amount of precipitum. I believe that I have discovered a very simple method of doing this, and it therefore follows that we actually appear to possess a means of measurement for the degree of blood relationship. To work by this method exact dilutions of fresh fluid serums are used to which accurately measured quantities of antiserum are added.

Method for the Measurement of the Degree of Reaction.

Dilutions of the serums to be tested quantitatively are made with 0.6 per cent. normal salt solution to the degree desired—namely, 1:100, 1:200, etc. The solutions must be perfectly clear and free from suspended matter. Accurately-measured quantities—usually 0.5 c.cm. of the dilution—are transferred into a series of clean dry test tubes, having a capacity of about 1 c.cm. and a lumen of about 5 mm. The dilution is allowed to run into the tubes along a fine, freshly-drawn, sealed capillary tube in order to avoid the formation of bubbles or of overflow in filling the tubes. Each tube is supplied with a sealed capillary, which is left in place after the introduction of the dilution, subsequently serving a similar purpose when adding the antiserum. An accurately-measured quantity of antiserum is now added to the dilution, in the proportion of 20:1 to 200:1 or more of the blood in dilution. After the antiserum has been added to the series of tubes, the capillaries are removed and the mixture of dilution and antiserum is effected. This can be done very satisfactorily by applying the tip of the clean finger to the mouth of the tube and shaking it up and down. By using the fingers of both hands in rotation eight tubes can be shaken in succession, after which the hands must be cleansed before proceeding to the next set of tubes. This is only mentioned because it saves time. With a little practice it will be found easy to bring about complete mixture without the formation of troublesome air bubbles, and if the fluid is shaken down to the bottom of the tube before removing the finger the loss of fluid entailed by contact with the finger is practically negligible, the finger-tip remaining apparently dry after some practice. The mixture is now

left standing at a desired temperature, the rate of precipitum formation, etc., being noted.

After twenty-four hours have elapsed, the precipitum has become deposited upon the bottom of the tube. Should any adhere to the walls of the vessel, it may be dislodged by gently tapping or rotating the tube upon its vertical axis. The particles thus displaced soon sink to the bottom of the tube, provided it does not contain too much serum, which through its viscosity impeded their gravitation. The clear supernatant fluid is now pipetted off by means of a conveniently bent bulb capillary pipette, the tip of which is allowed to slide down along the inner wall of the test tube, the precipitum not being disturbed, the clear fluid being aspirated into the bulb as the tip of the pipette slides down a little beneath the receding surface of the column of fluid in the tube. The bulk of precipitum and clear fluid left is so small that it can now be readily drawn up into the capillaries.

The capillaries used for measuring the precipitum volumetrically are made of fairly thick glass, are about 12 cm. long, and have a lumen of about 1 mm. The ends should be cut off square by means of a diamond, this being essential, as it is thus possible to practically remove all the fluid and precipitum in the bottom of the small test tube. The end of the capillary is placed against the bottom of the tube, and the deposit and fluid are well mixed by being drawn backwards and forwards into the capillary. It is imperative that bubbles should be avoided. The fluid is now drawn up a little way into the capillary, and the end of this is drawn out and sealed in a very small flame. The sealed end should be blunt to avoid possibility of breakage; the other end of the tube, which can now be cut down to a convenient length, is left open. After a number of capillaries have been thus prepared, they are placed vertically in a cool place. After twenty-four hours the height of the column of precipitum is marked upon the capillary with a glass pencil. After forty-eight to seventy-two hours it will be seen that the column of precipitum remains constant. The amount of precipitum may now be measured.

The accompanying figure shows a little apparatus which I have made in the laboratory, and have found very convenient for making these measurements of the amount of precipitum. A fairly large thermometer tube (A) is used which is graduated in tenths of a c.cm., the calibre being chosen so that a fine steel scale of 10 cm. (E) subdivided into 0.5 mm. corresponds to the length of column of fluid measuring 0.1 c.cm. in the tube. The tube is filled with pure water up to the graduation mark, the mouth being wiped dry with filter paper. The tube is fixed in a spring clip which is attached to the wooden rod (D) so that it pivots in a vertical plane, thus permitting the tube to rest in a horizontal position or at any desired angle. The rod (D) slides horizontally between the rods C and C', which are nailed to a solid block of wood beneath. Two small brass plates attached to rod C prevent rod D from being displaced. The steel cm. measure is attached to a metal support, which is screwed to the block beneath. By a lucky chance I happened to have an old "peep sight" from a sporting rifle which served admirably for carrying the metal scale, as it can be inclined at any angle and be screwed up or down. By pivoting the scale on a screw passing through the eyehole of the sight, the scale can be placed and retained at any angle on its support, thus making it possible to closely apply the fine scale to the thermometer tube in any position desired.

The height of the volume of precipitum having been marked upon the capillary tube, the latter is cut square by means of a diamond at the lower meniscus, a small quantity of air having been left in the lower end of the tube when it was sealed. The contents of the tube are blown out, the tube is drained as far as possible by blowing out the moisture it contains on to filter paper, or it can be washed out and dried. The mouth of the capillary is now brought in contact with the end of the thermometer tube, which has been slightly raised at one end (A), and fluid is allowed to pass into the capillary up to the pencil mark previously noted. The amount of fluid which has passed into the capillary is now read off on the scale. The thermometer tube is moved by means of the handle (F) so as to prevent expansion of the fluid through the heat of the hand. Where it is desired to know the actual quantity of precipitum, it is necessary to note the temperature and calculate accordingly. This may be disregarded when a simple comparison is being made with different quantities of precipitum in a given series, the measurements being made in rapid succession under similar outward conditions. If it is desired to make simply comparative measurements a diamond scratch on any suitable tube is sufficient, only the measurements on the steel scale being recorded.

I am at present engaged, with the aid of Mr. T. Strangeways-Pigg, in making measurements of the degree of reaction, etc., by means of this method, and we shall very shortly report upon our results, which are certainly striking. We hope to succeed in standardizing our antiserum by this method.

I have described my method thus in detail, so as to give

others an early opportunity of putting it to the test. We have so far obtained what appear to be remarkable results. If we count the amount of precipitum, measuring about 0.03 c.cm., obtained from adding 0.1 c.cm. of an antiserum to 0.5 c.cm. of its homologous dilution (1 to 100 or 1 to 200) as 100, then we obtain figures somewhat as follows:

Antisheep Serum.					Antipig Serum.				
Sheep ...	...	...	...	100	Pig ...	...	...	...	100
Ox ...	...	...	...	80	Horse ...	...	...	...	16
Antelope ...	...	...	...	50	Hog deer ...	...	...	...	14
Hog Deer ...	...	...	...	47	Cat ...	...	...	...	14
Reindeer ...	...	...	...	30	Dog ...	...	...	...	13
Pig ...	...	...	...	20	Sheep ...	...	...	...	13
Horse ...	...	...	...	16	Wallaby ...	...	...	...	5
Cat ...	...	...	...	12					
Dog ...	...	...	...	7					
Wallaby ...	...	...	...	5					

In both series we observe the occurrence of what I have termed the mammalian reaction, both antisera being very powerful. I do not wish these numbers to be taken as final, nevertheless they very definitely show that the results which I have published, based upon cruder methods of experimentation, are essentially correct. To obtain a constant it will be necessary to make repeated tests with the bloods of each species and with different antisera of one kind, making the tests with different dilutions and different proportions of antiserum. I am inclined to believe that with care we shall perhaps be able to "measure species" by this method, for it appears from the above results that there are measurable differences in the reactions obtained from related bloods, in other words, determinable degrees of blood relationship which we may be able to formulate.

NOTES.

¹ BRITISH MEDICAL JOURNAL, May 11th, 1901, vol. i, p. 1141, September 1st, vol. ii, p. 669; *Journal of Hygiene*, July 1st, 1901, vol. i, pp. 367-387; *Proc. Roy. Soc.*, vol. LXX, pp. 150-153 (paper read November 21st); *Journ. Trop. Med.*, December 16th, 1901, pp. 405-408; *American Naturalist*, vol. XXXV, pp. 927-932; *Proc. Phil. Soc.*, Cambridge meeting of January 20th, 1902. ² In 2 instances out of 510 tests a faint clouding was observed to take place in a dilution of avian blood, tested by means of an antimammalian serum and vice versa. In both cases the blood samples had been collected with other bloods. In one case I received samples of avian and mammalian bloods collected on the same day by a sportsman, and I have no doubt but that the filter-paper strips came in contact with each other in a moist state, a possibility against which I have sounded a warning in my circular letter. As I was the first to show, these reactions come off, even in mixtures of several kinds of blood. Such accidents can be prevented by the use of paraffined envelopes, which I have sent out with my blood-collecting "outfit."

THE DIAGNOSTIC VALUE OF THE VARIATIONS IN THE LEUCOCYTES AND OTHER BLOOD CHANGES

IN TYPHOID AND MALARIAL REMITTENT FEVERS RESPECTIVELY.

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DURING the last few years much attention has been paid to the blood changes in the specific fevers, and the great diagnostic value, especially of the variations in the numbers, of both the total and individual leucocytes has been fully recognized. At the same time the discovery of the serum test for typhoid and Malta fevers and the great progress in our knowledge of malarial parasites has placed in our hands valuable aids in the differentiation of the still far too little known tropical fevers.*

Recently, through the kindness of the medical officers in charge of the different Calcutta hospitals, I have been able to study the blood changes in some fifty cases of continued and

* More than four years ago I made arrangements for applying these modern methods to the study of fevers in a large military station in India, but at a day's notice was whisked off to a frontier expedition, and until a few months ago was prevented by the exigencies of military and special duty from pursuing this interesting field of study, a fact which I only mention because too little allowance is made for the difficulties and interruptions of scientific work in India by those who criticize the paucity of results obtained in this country.