Original Articles.

THE SERUM TEST FOR BLOOD.¹

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DURING the past ten years the immense amount of work which has been done upon the blood, in the study of various questions concerning antitoxins and immunity, has resulted in the discovery of the formation in the blood of various specific antibodies, such as the antitoxins, and also of certain specific precipitins, which will produce precipitates in different bacteriological products, in different kinds of milk and in different kinds of blood. The study of these bodies is still in its infancy, and offers a vast field for special investigations.

I need not go into the details of previous work done upon these bodies. The investigations of Krause, in 1897, first demonstrated the presence in the blood of the specific precipitins in the antisera of the cholera, plague and typhoid. Those of Bordet, in 1899, showed the formation in the blood of the rabbit of the specific antibodies which act upon milk. The antibodies formed acted only upon the special kind of milk with which the rabbit was treated. If the rabbit was injected with human milk, its serum precipitated the casein from human milk only; its serum would not precipitate the casein from cow's milk or goat's milk. Later, these results were confirmed and the investigations extended, so as to demonstrate the formation in the blood of antisera, which acted upon the different kinds of blood or blood serum. The first discovery of blood antisera was made by Tehistovitch in 1899, and his work has been confirmed and extended since then by Uhlenhuth, Wassermann and Schütze, Myers and Nuttall.

It has been found that rabbits are the animals which are best suited for this kind of experimentation. If a rabbit is prepared by injecting into the peritoneal cavity, several times, at intervals of a few days, about 10 cc. of any kind of blood or blood serum, there will be formed in the blood of that rabbit a peculiar body, called by Myers and Nuttall a specific *precipitin*, which will produce a precipitate, called by them the *precipitum*, in the diluted blood or blood serum of an animal of the same kind as the one with which the rabbit was prepared. It will not produce a precipitum in the diluted blood or blood serum of an animal of any other kind unless it be very closely allied generically, nor will it produce a precipitate with the dilute blood or blood serum of a normal rabbit. A rabbit may also be treated with any pathological fluid containing human blood serum, such as pleuritic exudation and, as Dr. Whitney has determined, hydrocele fluid, instead of blood serum, when the precipitin will be formed which will produce the precipitum in diluted human blood.

¹ Read before the Massachusetts Medico-Legal Society, Jan. 4, 1902.

Uhlenhuth, in February, 1901, prepared rabbits both with human blood and ox blood, producing in the rabbit human antiserum in one case and ox antiserum in the other. His comparative tests were made with the blood of man, ox, horse, donkey, pig, sheep, dog, cat, deer, fallow-deer, hare, guinea pig, rat, mouse, rabbit, chicken, goose, turkey and pigeon. No precipitum was formed in the diluted blood solution of any of these animals except the one with which the animal was prepared.

Uhlenhuth made the important discovery that the precipitate was produced in clear solutions made by treating dried blood stains with normal salt solution, just as well as in diluted fresh blood.

Wassermann and Schütze, also in February, 1901, found that it was not necessary to treat the rabbit with blood, but only with the blood serum. The hemolysins obtained by treating the rabbit with the red blood cells, and the agglutinins formed by the action by the hemolysins upon the red blood cells, have no importance from a medico-legal point of view, since the red blood cells may be completely destroyed in blood stains which have been exposed to various conditions. For this reaction it is necessary that some red blood cells should be in suspension. These authors made comparative experiments with the blood of man, horse, donkey, goat, cow, ox, sheep, pig, dog, cat, baboon, guinea pig, rabbit, mouse, rat, goose, duck, chicken, sparrow, eel, pike and tench. They found that the antiserum from the rabbit prepared with human blood reacted also with the blood of the baboon, but much more slowly and less strongly than it did with diluted human blood.

Stern, February, 1901, found also that the blood of the humanized rabbit reacted feebly with the diluted blood of three different kinds of monkeys, a species of *cercopithecus*, *macacus cynomolgus*, L. ("Java-Ape"), and the "Crown-Ape."

Nuttall, July, 1901, made comparative experiments with thirty-six different kinds of blood; the bloods being those of man, four species of monkey: *Cercopithecus campbelli*, Waterh., *cercopithecus patas*, west coast of Africa; *cercopithecus lalandii*, Is. Geoffr., South Africa; *macacus rhesus*, India; the rufous rat-kangaroo (*hyposiprymnus rufescens* [Gray], New South Wales), the capybara (*hydrochoerus capybara*, South America); the polecat (*mustela putorius*), suricate (*suricata tetradactyla*), South Africa; squirrel (*sciurus vulgaris*), guinea pig, tame and wild rabbit (*lepus cuniculus*), white rat, black rat (*mus rattus*), horse, ox, sheep, white-tailed gnu (*connochaetes gnu*), South Africa; gazelle (*gazella arabica*), deer (*gervus axis* Erxl, India); dog, cat, pig, bat (*plecotus auritus*), pigeon, chicken, pheasant, swan (*cygnus olor*), duck, chaffinch (*fringilla coelebs*), cross-bill (*nucifraga caryocatactes*), rook (*corvus frugilegus*), swallow (*hirundo urbica*), corn-crake (*crex pratensis*), frog (*rana temporaria*), newt (*molge cristata*), and snake (*tropidonotus natrix*). The

serum of the rabbit, which was prepared with sheep's blood, gave a slight reaction with the blood of the gazelle and axis deer, and there was very slight clouding in the blood of the ox, squirrel and swan. The serum of the rabbit treated with ox blood gave a distinct reaction with the blood of the gazelle and axis deer, and slight cloudiness in the blood of the sheep, gnu, squirrel and swan. The serum of the rabbit prepared with human blood gave a slight reaction with the blood of the four kinds of monkeys tested. He found that it gave a very faint cloudiness in solutions of the blood of the horse, ox and sheep. The serum of the humanized rabbit gave positive reactions with diluted human serum, pleuritic exudation, both fresh and putrid, with blood which has undergone putrefaction for two months, with serum from a blister, and a slight reaction with both nasal and lachrymal secretions.

The method of humanizing a rabbit is to inject into the peritoneal cavity about 10 cc. of human blood serum at intervals of two or three days, until the rabbit has received six or eight injections. The details of this operation will be described to you by Dr. Whitney. The rabbit should be allowed to rest about a week after the last injection. The blood serum for performing the test may then be obtained either by killing the animal, by bleeding it from one of the large vessels, or, without killing the animal, by removing a little blood from one of the large veins of the ear. The blood thus collected should be placed in a cool place and allowed to coagulate. The serum which separates from the clot may be used for performing the test.

The blood to be tested should be prepared as follows: If it be fresh blood, it should be diluted about 1:100 with normal salt solution. Thus diluted it should have a light pink color, and this diluted solution, if not perfectly clear, should be allowed to settle until it is clear, and the clear supernatant fluid decanted into another test tube. If the blood to be tested is a dry blood stain, a little of it may be scraped off with the point of a knife on to a watch glass, if the stain be upon some hard surface so that the blood has not penetrated into the substance of the material. If the blood stain be upon cloth into which the blood has soaked, it is necessary to cut out a few threads and transfer them to a watch glass. These fragments of dried blood should then be treated with two or three drops of distilled water, until the soluble portion of the dried blood has been dissolved. The clear solution is then transferred carefully to a very narrow test tube, and to this should be added an equal volume of double normal salt solution. The clear fluid thus obtained, either by diluting fresh blood or by dissolving the blood serum from the dried blood stain, is then tested by adding to it a few drops of the serum obtained, as above described, from the humanized rabbit. If the solution contains human blood serum, there will occur an immediate cloudiness which gradually increases, so that

there is a distinct precipitation within one-half hour after the addition of the serum from the humanized rabbit. It is better to allow the anti-serum to flow down the side of the test tube, so as to form a separate layer under the solution to be tested, in the same way that we add nitric acid to urine in testing for albumin. In this case the cloudiness and precipitate may be seen very distinctly in the zone at the point of contact of the two fluids. The precipitate occurs best when the mixture is kept at a temperature of about 37° C.

It has also been found that the antiserum obtained from the humanized rabbit can be kept in dried form by soaking filter paper or blotting paper with it and allowing it to dry. In this way it is said to preserve its activity for forty-two days more or less (Dr. Nuttall). When it is desired to use this for a test, it may be dissolved from the filter paper by means of a little normal salt solution and filtered if necessary. This clear solution, added to the diluted blood, or solution from a dried blood stain, will give the cloudiness and precipitation the same as the original antiserum obtained from the humanized rabbit.

I have recently applied this serum test in a murder case, which is now being tried in New Hampshire. The test was applied separately to the following stains: One about one-quarter of an inch in diameter on the right elbow of a brown jacket; another about one-half inch in diameter on the lower left front of the same garment. One-half of the stain on the elbow was cut out and soaked with a few drops of distilled water. The material from the other stain was obtained by scraping the surface of one-half of the stain with a knife on to a watch glass, and the powder thus obtained was treated with a few drops of distilled water. As mentioned above, this distilled water solution was transferred to a small test tube and treated with an equal volume of a double normal salt solution. Four small spatters on one leg of a pair of overalls were scraped off with a knife and treated in a similar manner; also one stain, about one-fourth inch in diameter, on the other leg of the same garment. A few threads from two blood stains on the towel were cut out, and a solution made as above described. Also some clotted blood found on a stone which weighed about three pounds, this being the implement with which the murder was committed, the victim being killed by blows upon the head with the stone; a solution of this blood was made in a similar way. These solutions were placed in small test tubes side by side, and, for purposes of comparison, several other test tubes containing solutions of dried human blood, of that of a dog, ox, pig and sheep. All of these solutions being thus prepared, there was added to each one two or three drops of the test serum obtained from the humanized rabbit. A distinct precipitate occurred within one-half hour in the test tubes containing the fluid obtained from both stains on the jacket, those obtained from both legs of the overalls, from the stain on the towel, from the

blood on the stone, and in the test tube containing the solution of known human blood. No precipitate or cloudiness occurred in the test tubes containing the solution of blood from the dog, pig, ox or sheep.

NOTES ON THE PRODUCTION OF THE TEST SERUM IN RABBITS.¹

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As a rule, with moderate care there is little danger of infection of the rabbits by the injection. The abdomen should be shaved and the skin thoroughly scrubbed with strong alcohol. The rabbit is held out straight by an assistant taking his ears in one hand and his hind legs in the other. The skin is pinched up with the left hand and the needle introduced by a steady thrust with the right. The only chance is that the point may not be inserted into the peritoneal cavity, but under the loose skin when liability of an abscess forming seems to be greater. Such has been the experience in two of our cases. After withdrawing the needle the abdomen should be again scrubbed with alcohol.

Any syringe that can hold 10 cc. and can be properly sterilized is all that is required. The one we have used is a glass antitoxin syringe with a long, relatively large needle.

The serum which can be most readily procured is hydrocele fluid (in any large hospital several are tapped every week, and each one gives about 200 to 250 cc. of fluid). Ascitic and pleuritic fluid are not quite so common, but the amount is so much larger that it can be used for more rabbits.

Serum can be kept for a long time if collected in a sterilized bottle under aseptic precautions, and a little chloroform poured in and allowed to remain at the bottom of the flask. The mouth can be plugged with sterilized cotton.

These sera have the advantage over those obtained from blood, that they are perfectly clear and do not have to be separated from the blood clot and corpuscles. Human blood serum is most readily obtained by squeezing a freshly delivered placenta and then allowing the serum to separate by clotting. The centrifuge is often needed to clear the serum of any stray corpuscles. About 6 to 8 cc. can be thus obtained from each placenta.

As to the relative advantage of the serum from one source or another, our experiments, as yet, are not conclusive. Krause says that hydrocele fluid does not develop as strong a reaction in the rabbit as the serum obtained directly from the blood. On the other hand, Uhlenhuth says there is great difference in the susceptibility of rabbits.

In our experience in two parallel series, the hydrocele rabbit gave the better reaction in one and the blood serum rabbit in the other. Further

experiments are necessary before this can be regarded as settled.

Is there any indication by which it can be known beforehand whether a rabbit will yield a strong antiserum? The only indication which we have on this point is that in both of those which gave the best reaction, the blood did not coagulate so quickly as in those which gave a poorer reaction. Whether this is a condition which is developed by the formation of the antiserum, or whether it pre-existed, we have no knowledge. If it pre-existed and is more than a coincidence it might be used in selecting the animals for injection.

In two cases a single large dose (100 cc.) of hydrocele fluid was injected at once, but no reaction was obtained either the next day or at the end of a week. So that in this way at least the process cannot be shortened.

It was thought that the antiserum might possibly be secreted by the kidneys, and the urine of a highly sensitized rabbit was collected for this purpose. But no characteristic reaction was obtained.

While the operations and reactions are so simple that they can be performed by anyone, still they require care and exactness, and considerable previous practice should be obtained before one would be qualified to testify in a capital case.

In every case of death by violence, where murder is suspected, and there is a possibility of a blood stain having to be examined, a strip of filter paper should be soaked in the blood of the individual at the autopsy. This should be sent to the expert who makes the examination of the suspected blood stain in order that any doubt as to this individual's blood giving a serum reaction can be set at rest.

NOTES ON X-LIGHT.

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VACUUM TUBE REGULATORS FOR X-LIGHT TUBES.

The transformer type.—A chemical capable of yielding a vapor on heating, reabsorbing it again on cooling, is placed on a suitable shaped receptacle, usually a cylinder of glass one centimetre in diameter, which is connected with the x-light tube. A wire is laid from each pole of the generator to a coil of fine wire surrounding the cylinder containing the chemical. The secondary of this coil is a few turns of coarse wire, within or without the cylinder. In either case, the arrangement forms a stepdown transformer, in which the high potential current used to excite the x-light tube is transformed into a heating current, the effect of which is to liberate vapor from the chemical. The duration of the heat depends upon the length of time the current passes in the fine wire of the transformer, being determined by the length of the variable spark gap in the circuit containing the fine wire coil. The action is auto-

¹ Read before the Massachusetts Medico-Legal Society, Jan. 4, 1902.