THE PRECIPITATING SERA.¹

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IF one injects several doses of serum from an eel into a rabbit, one easily observes that the serum taken from the rabbit possesses the curious property of producing a marked precipitate in serum from an eel.

This phenomenon was first observed by Tchistowitch,² and he has repeated his experiments with the serum of rabbits treated with horse's serum. Bordet,³ then Nolf⁴ and Leblanc, confirmed these results.

Bordet afterwards showed that if one injects cow's milk into the peritoneal cavity of rabbits one obtains from the latter animals a serum which, when added to cow's milk, "causes the formation in the mixture of granules which are at first fine, but which rapidly increase in size and form thick flocculi." The liquid afterwards separates into two parts. These experiments were subsequently repeated, and with the same results, by Wassermann and Schütze, and by Uhlenhuth.

Myers⁵ afterwards showed that by bleeding rabbits which have been systematically inoculated with the albumen of the fowl's egg, one obtains a serum which precipitates solutions of egg albumen. He also prepared a rabbit serum which precipitated solutions of Witte's peptone.

In the beginning of the year 1901, along with Professor Leclainche, I pointed out that the serum of rabbits which had received frequent injections of human albuminous urine precipitated such urine after the manner of a veritable chemical re-agent.⁶

Many other authors have obtained precipitating sera. Uhlenhuth by injecting human blood into the peritoneum of guinea-pigs prepared a serum which precipitated human blood.⁷ Wassermann and Schütze⁸ and Stern⁹ by an analogous procedure obtained the same results as Uhlenhuth.

Mertens¹⁰ and Zuelzer¹¹ subsequently confirmed the results obtained by Leclainche and Vallée with regard to sera precipitating albuminous urine.

C. G. Farnum,¹² by injecting rabbits with various sorts of semen, prepared sera which precipitated the particular semen used in the treatment.

¹ Translated from the "Revue Générale de Méd. Vét.," January 1903.

² Tchistowitch: Immunisation contre le serum d'anguille. "Annales de l'Institut Pasteur," 1899, p. 406.

³ Bordet: Le Mechanisme de l'agglutination. Id. 1899, p. 225.

⁴ Nolf: Contribution à l'étude des serums antihématiques. Id. 1900, p. 297.

⁵ Myers (Walter): Immunität gegen Proteid. "Centralblatt für Bakteriologie," T. XXVIII., p. 237.

⁶ Leclainche et Vallée: Sur les anti-corps albumineux. "C. R. de la Société de biologie," 1901, p. 51.

⁷ Uhlenhuth: "Deutsche medicinische Wochenschrift," 7th February 1901.

⁸ Wassermann and Schütze: "Berliner klinische Wochenschrift," 18th February 1901.

⁹ Stern: "Deutsche medic. Wochenschrift," 14th March 1901.

¹⁰ Mertens: Idem.

¹¹ Zuelzer. Id. 4th April.

¹² C. G. Farnum: "Transactions of the Chicago Pathol. Society," 9th December 1902.

Nedrigailov¹ and Mirto Domenico² by injecting with human ascitic liquid obtained sera which precipitated human albumen.

Finally, Hausner,³ Butza, Linossier and Lemoine,⁴ Minovici, Strube,⁵ Michaelis, Rostoski, Umber and Falloise,⁶ published interesting researches on the preparation and properties of precipitating sera.

This historical survey suffices to show that it is possible to obtain sera which precipitate, *in vitro*, albuminoid substances by the appropriate treatment of various animals with these substances. The precipitating property of these various sera is precisely the result of the treatment carried out. It is, in fact, easy to observe that the serum of normal animals which have not been treated in any way do not possess the power of precipitating solutions of the various albuminoid substances; one may thus formulate the following law:—

If on several occasions one inoculates into an animal A an albuminoid material (blood serum, semen, ascitic liquid) coming from an animal of a different species B, the serum of the animal A acquires the property of precipitating, *in vitro*, albuminoid solutions derived from the species B.⁷

Sera thus obtained have therefore been properly termed precipitating sera, and we shall see later the important practical purpose to which they may be applied.

The precipitating sera, whatever they may be, present one common character—viz., that their properties are not altered by exposure for one hour to a temperature of 55° C. That effect is produced only after heating to 70°. In this they are distinguished from the cytolytic, bacteriolytic, or toxic sera, which are very sensitive to heat. On the other hand, in this point they resembled the agglutinating, anti-toxic, or anti-infectious sera. It has been proposed to give to their active ingredient, which is precipitated by sulphate of magnesia, the name of coagulin, but this has been rejected in favour of the term precipitin, which is more exact, since the turbidity which is produced in albuminous solutions by the addition of the corresponding precipitating serum is the result of the precipitation of albumen and not of coagulation of that substance.

PREPARATION OF PRECIPITATING SERA.

As we have already seen, it is an easy matter to obtain precipitating sera. It is interesting, however, in view of the practical importance which attaches to the preparation of these sera, to consider the methods by which one may very rapidly obtain a serum as active as possible.

According to the observations of some authors, the choice of the animal which is to furnish the serum appears to be a matter of great importance, for, as Bordet discovered, the production of an active

¹ Nedrigailov: "Vratsch," 1901, p. 857.

² Mirto (Domenico): "Reforma Medica," 1901, p. 857.

³ Hausner: "Nevrologitchesski Vestnek," 1901, p. 845.

⁴ Linossier and Lemoine: "C. R. Société de biologie," 1902, p. 85, 276, 320, 369, 415.

⁵ Strube: "Deutsche medic. Wochenschrift," 12th June 1902.

⁶ Falloise: Contribution à l'étude des serums precipitants. "Annales de l'Institut Pasteur," 1902, p. 833.

⁷ Kowavski ("Deutsche medic. Wochenschrift," 1901) has obtained sera which precipitate vegetable albumens.

serum seems to be directly related to the strength of the impression produced by the inoculations in the experimental animal.

A priori it is therefore advisable to choose for the preparation of a given precipitating serum an animal belonging to a species as different as possible from the one which will furnish the albuminoid material used in the treatment.

For example, in order to obtain a serum that will precipitate the albumens of the sheep, one will treat with these substances a rabbit or a dog in preference to a goat. Uhlenhuth considered the rabbit the best subject for the production of precipitating sera of various sorts, but Hausner preferred the dog for that purpose.

The choice of the animal to produce the serum having been made, what is the best substance to employ for the inoculations?

The works of Bordet, Tchistowitch, and Nolf have shown that the red corpuscles of the blood, when inoculated into animals, do not play any rôle in the formation of the precipitating substances. On the contrary, the essential rôle in this production devolves on the plasma or on the serum. It is, therefore, preferable to use in the preparation of the animals blood serum rather than blood itself.

When the object is to prepare serum which will precipitate albuminoid materials of animal origin, it is easy to obtain large quantities of serum by bleeding; but when one wishes to obtain serum that will precipitate human albuminoids, since it is rather difficult to procure human blood by repeated bleeding, one may utilise ascitic liquid or pleural effusion, or even blood from the placenta, or that which has been taken from the dead body.

These different organic products contain a mixture of various albuminoid materials, which are, as yet, badly known. Among these substances there are some which are readily precipitated by saturation with sulphate of magnesia; these are the so-called globulins. Others, on the contrary, remain in solution; these are the serins. Nolf¹ has proved that the precipitating property appears sooner in animals treated by injections of globulin than in those treated with serin.

There is, however, no inconvenience in employing ordinary serum, and since it is simpler to treat animals in that way it does not appear to us to have any interest to use, with this object, albumens which have been purified or separated from blood serum by precipitation with sulphate of magnesia or ammonia.

Apart from the choice of the animal and of the substance with which it is to be inoculated, the value of a precipitating serum depends on the quality of the substance inoculated, the place at which the injections are made, and the duration of the treatment. Intravenous and intra-peritoneal inoculations give a more active serum than subcutaneous inoculations.

On account of its simplicity, intra-peritoneal inoculation of tepid serum is to be preferred. In our own hands this has given the best results. Moreover, whatever be the method of inoculation adopted, it is a good plan to raise the sera intended for treatment to a temperature of 55° in order to destroy their toxicity.

The precipitating power of a serum furnished by a given animal increases in proportion to the length of the treatment. Thus, from

¹ Nolf, loc. cit.

rabbits treated with human blood every day for six days, Stern obtained a serum which was active in the strength of 1 per 1000. After several months of treatment this serum acted in the proportion of 1 in 50,000. Moreover, it is important to repeat injections of the albuminoid substances chosen as often as possible. Provided the health of the subject permits, the inoculations ought to be repeated every two days at least.

The serum may be collected from a treated animal the day after the last injection. However, it is better to wait five or six days, and always to bleed the animals in the fasting state. One thus obtains a serum which is absolutely limpid and devoid of bacteria, and which therefore keeps well when it is collected with aseptic precautions.

When one interrupts the injections of the albuminoid substance into the animal intended to furnish the serum, the precipitating power of the serum falls; and, according to Steube, two months after cessation of the treatment, rabbits' serum which was originally active in the strength of 1 per 1000 has entirely lost its precipitating power. Moreover, the same author has observed that when the treatment of such animals is resumed they soon furnish a serum that is much more active than that which was obtained after the first period of treatment.¹

Precipitating sera which have been collected pure and kept cool and protected from light will retain their precipitating properties for several weeks. Rapid desiccation of the sera at a low temperature *in vacuo* preserves their properties for a much longer time.

RESEARCHES REGARDING PRECIPITATING POWER.

In order to put in evidence the existence of precipitating power in a serum prepared in the manner described above, it suffices to add to a given volume of the albuminous solution employed in the treatment an equal volume of the prepared serum. As the precipitated albumen which forms is soluble in excess of the precipitable solution, it is better to increase the proportion of the precipitating serum. If one employs albuminous dilutions it is always better to make these dilutions in physiological salt solution (.8 per cent.). It is important to utilise in such researches only liquids that are very limpid.

When one endeavours to dissolve organic albuminous substances, such as blood, semen, or sputum, in physiological salt solution, one always obtains a turbid mixture. It is well to clarify this by repeated filtration through paper before submitting it to the action of precipitating serum.

Immediately after the substances have been mixed the original limpidity of the two liquids appears to persist, but after some minutes the liquid becomes opalescent or milky, and gradually loses its limpidity. After some hours the limpidity resolves itself into a more or less abundant flocculent precipitate. In every case the reaction is extremely distinct, and it is always obtainable when a given serum is mixed with an albuminous solution of the same animal origin as that

¹ When one employs rabbits in order to produce serum, and the treatment has been brought to a termination, it is better to sacrifice them by extracting all the blood possible from the carotid.

which was employed in the treatment of the animal furnishing this serum.

The temperature does not seem to exercise any marked influence on the reaction. According to Linossier and Lemoine,¹ the flocculent deposit is not readily obtained at 0°, and the reaction is most rapid at about 35°. I have always operated at the temperature of the laboratory (15° to 20°), and in every case I have obtained excellent results. It is best to operate with a neutral or slight alkaline medium.

The delicacy of the reaction is altogether remarkable. Uhlenhuth,² for example, prepared a serum which precipitated the albumen of cows' milk, and found that a few drops of it added to a dilution of 1 per 100,000 was active, whereas, according to the same author, the chemical reactions were not discernible in weaker solutions than 1 per 1000. Hausner³ obtained for the precipitation of human blood a serum so active that when a dose of $\frac{1}{2}$ cc. was added it produced a distinct precipitate in the liquid obtained by diluting a drop of blood in 15 cc. of water. Linossier and Lemoine⁴ prepared a serum capable of revealing the presence of albumen in a dilution of 1 cc. of serum in five litres of water, and which could thus put in evidence amounts of albumen less than the hundredth part of a milligramme. On many occasions, by employing the anti-albuminous serum described by Leclainche and myself, I have been able to detect in human urine traces of albumen which were not recognisable by the chemical tests.

SPECIFICITY OF PRECIPITATING SERA.

The specific nature of the reaction furnished by precipitating sera on different albumens found in the same animal species, and on albuminoids furnished by subjects belonging to different species, is deserving of study.

With regard to the specificity of sera towards different albuminoid substances of the same species, opinions are divided. Thus, Nolf, Leblanc, and Michaelis prepared sera which precipitated blood serum and solutions of globulin, but not solutions of serin; while Linossier, Lemoine, Rostoski, and Falloise prepare animals by injecting them with globulin or serin. Uhlenhuth showed that the active precipitant of human serum is equally active in precipitating the other albuminous liquids of the organism, such as semen, purulent sputum, etc.

If for a moment we admit that it is possible to distinguish different human albumens by the employment of precipitating sera, a more profound examination of the matter has shown that in some cases one cannot count on such a result; and we agree with the authors who consider that the precipitating sera cannot be employed to distinguish between the albumens of the same animal species.

The results furnished by the precipitating sera are much more precise when the object is to distinguish between the albumens from animals belonging to different species.

Almost all the authors who have studied the precipitating sera ad-

¹ Linossier and Lemoine. *Loc. cit.*

² Uhlenhuth. "Deutsche medic. Wochenschr." 15 November, 1900.

³ Hausner. *Loc. cit.*

⁴ Linossier et Lemoine. "C.R. Soc. de biologie." 1902, p. 87.

mit with Tchistowitch, Bordet, and Uhlenhuth that these reactions are specific. In other words, the serum of a rabbit treated by repeated injections of blood or serum from the human subject precipitates only dilutions of human blood and serum, to the exclusion of serum from any of the lower animals. Leclainche and I have shown that the serum of rabbits inoculated systematically with human albuminous human urine precipitates only human urine, and leaves absolutely limpid albuminous urine from the various domesticated animals.

This specificity is very generally admitted. It has been observed, however, that a serum prepared to precipitate the albumens of a given species also precipitates, but to a slighter degree, the albumens from a species nearly related in the zoological scale. Thus, Myers has shown that the serum which precipitates albumen of the egg of the common fowl also precipitates that of the duck, and Uhlenhuth observed that serum active for fowl's serum also precipitated that of the pigeon. Again, Stern found that the precipitant active for human serum also acts on the serum of certain monkeys. However, all these authors recognised that a serum very active on human blood, for example, does not precipitate the serum of any of the domesticated animals, and that is the opinion generally accepted.

Some months ago MM. Linossier and Lemoine¹ observed "that the same precipitin may act on a very large number of different sera."

It is well to notice that these authors in their experiments added to not less than ten volumes of precipitating serum one volume of serum from different animals. These, however, are very special technical conditions, and the results change completely when, as I have often done, one mixes equal volumes of the precipitating serum and of the substance to be precipitated. When one works in this way the reactions are invariably specific.

APPLICATIONS OF THE PRECIPITATING SERA.

From what has just been said it is easy to foresee the useful practical applications to which the precipitating sera may be put.

When used in certain conditions they constitute excellent reagents to indicate the species which has furnished a given organic substance. Uhlenhuth was the first to propose to use precipitating sera for the diagnosis of blood stains, which are so important in legal medicine, and he made known the method in which this test ought to be carried out. It suffices to dissolve the blood stain which has to be examined, present on an article of clothing or an instrument, in a solution of .8 per cent. of chloride of sodium, and then to filter several times until an absolutely limpid liquid is obtained. To this solution one adds some drops of serum from a rabbit or guinea-pig treated with human blood. If the suspected stain is one of human blood a precipitate appears in the mixture, but if it remains limpid the blood must have come from some of the lower animals, and the precise species may be easily determined by testing the solution with sera which precipitate the albumens of the different animal species. According to Uhlenhuth the reaction may even be obtained with human blood which has been allowed to putrefy for several months.

¹ Linossier et Lemoine. *Loc. cit.*

This method also gives excellent results in the determination of spots of semen.

If one operates with sera of which the activity has been previously determined, and takes care to prepare control tubes, one may accept without reserve and with every confidence the indications afforded by the experiment.

Various authors, notably Wassermann, Schütze, Nedrigarlov, and Ziemke, have confirmed the value of Uhlenhuth's method, and Minovici declares it to be superior to those previously employed in legal medicine for the diagnosis of blood stains.

The sera that precipitate milk, which were first studied by Bordet, have been put to an interesting practical application by A. Schütze,¹ who showed that the serum of rabbits inoculated with cow's milk precipitates only the albumens of such milk, to the exclusion of all others. In the same way, rabbits treated with the milk of the goat furnish serum which precipitates only goat's milk. These sera may thus be utilised as very useful tests by cheese-makers who employ in their industry the milk of ewes. In view of the higher commercial value of the milk of the ewe, it is frequently adulterated with the less valuable milk of the cow. In the future it will therefore be possible, by testing the milk with different precipitating sera, to discover these frauds, and to determine the nature of the milk introduced into the mixture.

The precipitating sera have received still another valuable application in differentiating different kinds of flesh. This particularly important application will be made the subject of a special study.

Some very interesting biological facts have been brought to light by these precipitating sera. For example, Uhlenhuth has by this method proved that the albumen of the egg of the fowl is different from the blood serum of the fowl, and Kowarski² has been able to distinguish between the different vegetable albumens and to separate them from those of animal origin.

The precipitating sera are not only interesting from the scientific point of view, but also furnish valuable methods of investigation to chemists, and to those employed in legal medicine. Their application to the differentiation of samples of milk and of butcher-meat has appeared to us to justify the preparation of this critical review.

A PECULIAR STAINING REACTION OF THE BLOOD OF ANIMALS DEAD OF ANTHRAX.

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It will hardly be denied by anyone acquainted with the facts that at the present time the majority of ordinary practitioners, whether human or veterinary, do not habitually have recourse to bacteriological methods in the diagnosis of disease, even in those cases in which such methods are alone capable of conducting to a certain conclusion. This is notably the case with regard to anthrax. In this country the evisceration of the carcase of an animal suspected to have

¹ Zeits. für Hygiene, 1901, p. 5.

² Deutsche Med. Wochen, 1901, p. 442.