

On the Recognition of the Individual by Hæmolytic Methods.
(Preliminary Communication.)

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(Communicated by Dr. C. J. Martin, F.R.S. Received May 5,—Read
June 16, 1910.)

(From the Hygienic Institute, Public Health Department, Cairo.)

The following is a brief account of some results obtained during the course of an investigation into the artificially produced hæmolytic isolysins of the ox.

The peculiar interest attaching to this class of bodies was first pointed out by Ehrlich and Morgenroth in their classical studies on hæmolysis, where they showed by the treatment of goats with large quantities of laked goats' blood, that hæmolytic isolysins were formed in the blood of the immunised animals. Their investigations were made with sera prepared in this way.

The isolysins so obtained were, however, somewhat weak: in the most favourable case 0·3 c.c. of serum being required to hæmolyse 1 c.c. of 5 per cent. suspension of red blood corpuscles.

It occurred to us that it might be interesting to examine the serum of the cattle used for the production of Rinderpest serum at the Serum Institute in Cairo, as these animals would appear to be under ideal conditions for the formation of isolysins. We had at our disposal about 100 Egyptian cattle immunised against Rinderpest by Kolle and Turner's method which consists in a preliminary simultaneous inoculation of a small quantity of virulent blood and a suitable quantity of serum, followed after a time, by a massive dose of 4 litres of virulent cattle blood given intramuscularly and repeated at intervals of about two months.

The results of the investigation of the blood of these animals are being published separately and need not be gone into in any detail here, but we may state that they entirely agree with the results obtained by Ehrlich and Morgenroth in the case of the isolysins for the goat. We were, however, fortunate in having at our disposal a large number of animals yielding powerful sera.

The fresh serum of the immune cattle when tested on normal ox corpuscles shows little or no hæmolytic action, but if a small quantity of fresh guinea-pig serum is added, hæmolysis takes place very rapidly.

It was thus found that the serum of almost all the immune cattle, in the presence of a suitable complement, was highly hæmolytic, the hæmolytic power naturally depending on the amount of blood which the animal had received and on the date on which it was bled.

The hæmolytic power of all the animals in the Institute was examined. The sera of 76 out of a total of 101 were found to be capable of causing the complete hæmolysis of 1 c.c. of a 5 per cent. suspension of the corpuscles of cattle imported from Cyprus, in one hour at 37° C., in as small an amount as one-hundredth of a cubic centimetre in the presence of a suitable amount of guinea-pig complement, and many sera hæmolysed at much smaller doses.

If the serum of one immune animal is tested on the corpuscles of a number of individual animals of the same species, a great variation is seen in its hæmolytic action on the various corpuscles; some being very highly susceptible, others less so, and others almost unaffected.*

If now the serum of a second immune animal is tested on the same series of corpuscles, we get again differences in the action on the various corpuscles, and again certain corpuscles are picked out as susceptible, while others are less affected.

The two sera, however, do not necessarily pick out the corpuscles of the same individuals, so that corpuscles which may be highly sensitive to one serum may be almost unaffected by another.

It is a curious fact that, so far as we have seen, the race of the animal appears to be more or less immaterial and any influence exerted by this is quite masked by the individual characteristics.

In no case was the serum of an immunised animal found to be hæmolytic for its own red blood corpuscles, so that we were dealing with an iso-, not an auto-lysin. This result agrees with what Ehrlich and Morgenroth found in the case of their goats.

The complement of the ox, as occurring in the fresh immune serum, even in the presence of a powerful hæmolytic isolysin, appears to be capable of effecting hæmolysis of ox corpuscles only to a very slight extent or not at all.

If we imagine that the formation of these hæmolytic isolysins in the ox is protective, and that it is an attempt on the part of the organism to effect the solution and removal of the red blood corpuscles which have been introduced

* In this connection we have just seen a paper in the latest number of the 'Muenchener Medizinische Wochenschrift' (April 5, 1910) by v. Dungern and Hirschfeld, who, working with agglutinins, have, by a somewhat similar method, shown that it is possible to divide up the animals of one species into classes, according to their agglutinating reactions.

into the body, it would appear improbable that the organism should, so to speak, go to the trouble of elaborating a hæmolytic amboceptor which is practically useless for the only complement with which it is likely to come into contact, and it would seem probable that a suitable complement is really available somewhere in the body although not obviously present to any extent in the serum.

With a view to seeing if this is really the case, a normal Cyprus bull was injected intravenously with one litre of the mixed serum of ten immune cattle. This serum, although only 24 hours old, showed no action on ox corpuscles "in vitro" if no foreign complement was added; but in the presence of fresh guinea-pig serum was very powerfully hæmolytic. (0.01 c.c. being sufficient to hæmolyse 1 c.c. of 5 per cent. suspension of ox corpuscles.) A few hours after the injection, the urine was very darkly hæmoglobin stained, showing that a suitable complement had been forthcoming.

On testing the serum of the animal a few days later, it was found that, although it had now no hæmolytic action on its own corpuscles, it was distinctly hæmolytic for the corpuscles of many other individuals. It was thus possible to "exhaust" the immune serum for one particular corpuscle and to leave it still hæmolytic for many others.

This method of exhaustion "in vivo" was then replaced by exhaustion "in vitro." The technique being as follows:—

The immune serum was mixed with an equal volume of the washed corpuscles with which it was desired to exhaust the serum; the mixture kept at 37° C. for an hour, centrifuged, and the serum again treated in the same way with the washed corpuscles and the process repeated a third time. It was then found that the serum had lost all traces of hæmolytic power for the corpuscles in question.

By means of this method the sera of different immune cattle were now exhausted with the corpuscles of various individuals of the same species and the hæmolytic power of these sera after such treatment was studied on the corpuscles of different individuals.

It was found that if an immune serum is exhausted with corpuscles of an individual (A), it remains hæmolytic for the corpuscles of many other individuals, but loses its hæmolytic power for the corpuscles of some other individuals as well as for those of (A).

If now a second immune serum is exhausted with the same corpuscles (A), its action on the various corpuscles is not exactly parallel to that of the first serum, and often shows very marked differences.

This result is to be expected, as it was shown by Ehrlich and Morgenroth, that two goats each injected with similar doses of the same goat's blood at the

same times, gave quite different isolysins. In fact the isolysins formed depend upon two distinct factors:—

- (a) The individuality of the injected corpuscles.
- (b) The individuality of the animal into which they are injected.

When we consider the enormous number of variations possible in each of these factors, we see the almost unlimited possibilities in the resulting sera.

In view of the above it should be possible by taking a mixture of a sufficiently large number of immune sera and exhausting this with the corpuscles of one individual, to obtain a serum which is specific for the corpuscles of this one individual; *i.e.* which has no hæmolytic action on these corpuscles, but hæmolyses those of all other individuals of the same species. To test this, a mixture was made of the sera of between 60 and 70 immune Egyptian cattle. This mixture was then exhausted with the corpuscles of a normal Cyprus bull and then tested on the washed corpuscles of 20 immune Egyptian cattle, two normal Cyprus cattle, and the above mentioned Cyprus bull with whose corpuscles the mixture had been exhausted. For the test equal parts were taken of—

- (a) The exhausted serum;
- (b) A 5 per cent. suspension of the washed red blood corpuscles;
- (c) A one-tenth dilution of fresh guinea-pig serum in normal saline.

The tubes were kept at 37° C. for one hour and then left over-night in the ice-safe; after which the results were read off.

It was then found that complete hæmolysis had occurred in all the tubes, with the exception of the control tube containing corpuscles of the Cyprus bull with which the serum was exhausted. The “exhausted” serum was thus able to pick out, quite sharply, the corpuscles of one individual from those of 22 others. Following up these results, a second and more extensive test was made. In this case the same immune serum was used; it was, however, exhausted with the corpuscles of another normal bull.

This exhausted serum was then tested on the corpuscles of 110 different cattle (3 Soudan, 34 Cyprus and 73 Egyptian).

In this test again all the tubes showed complete hæmolysis with the exception of the one containing the corpuscles for which the serum had been “exhausted.” This control tube showed no trace of hæmolysis.

A number of other tests have been made by exhausting the serum with the corpuscles of various individuals, and the general rule has so far always held except in one case. The serum exhausted with the corpuscles of a cow was

found to have lost its hæmolytic action, not only for the corpuscles of the cow, but also for those of its calf.

It is worth noting, however, that the serum exhausted with the corpuscles of the calf was specific for the calf.

Taking advantage of the fact that the isolysins for the ox act very powerfully on the corpuscles of the goat, we have made a series of experiments to test the utility of the method in investigating the corpuscles of closely related individuals. The method works well and is giving very interesting results.

These results show that the red blood cells of different individuals of the same species are not only not identical, but that they are characterised by a definite individuality and can be distinguished from the red blood cells of any other individual.

How far this is part of a general law affecting the other cells of the body is at present under investigation.

We are of the opinion that the method of investigation by means of exhausted polyvalent immune sera will prove most valuable in biological research not only as a means of identifying the cells of the individual, but in the investigation of the laws of heredity and many other important problems.

Conclusions.

1. The immunisation of the ox with the red blood corpuscles of other oxen gives rise to the formation of a hæmolytic amboceptor in the blood of the immunised animals.

2. The amboceptor so formed is an *isolysin* but not an *autolysin*.

3. The race of the animal appears to have very little influence on the resulting hæmolysins.

4. The serum of an animal so treated acts very differently on the red blood corpuscles of different individual oxen.

5. The sera of different individuals similarly immunised differ from one another in their action on the corpuscles of different individuals.

6. If the serum of a single immunised animal be "exhausted" with excess of the corpuscles of one other individual, the serum loses its power of hæmolysing the corpuscles of this individual, while retaining the power of hæmolysing the corpuscles of many, but not all, other individuals.

7. If, however, a polyvalent serum be made by mixing the sera of a large number of immunised animals, and this serum is exhausted with the corpuscles of any one individual, the serum entirely loses its power of hæmolysing the corpuscles of this individual, but remains strongly hæmolytic

for all other individuals not closely related to the individual whose corpuscles were employed for the exhaustion of the serum.

(N.B.—It is possible that exceptions may be found, but these have not yet been met with except in the case of close blood-relations.)

8. The red blood corpuscles of any individual are thus characterised by a definite individuality of their own, and can be distinguished from those of any other individual of the same species.

Experimental Researches on Vegetable Assimilation and Respiration. VI.—Some Experiments on Assimilation in the Open Air.

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(Communicated by Dr. F. F. Blackman, F.R.S. Received March 1,—
Read June 16, 1910.)

CONTENTS.

	PAGE
Section I.—Introduction	421
II.—On the Rate of Assimilation of Carbon Dioxide by Leaves of <i>Helianthus annuus</i> in Bright Sunshine	422
i. Experiments... ..	422
ii. Discussion of Results	430
iii. Brown and Escomb's Experiments with <i>H. annuus</i> ...	433
III.—Experiments with <i>Catalpa bignonioides</i>	435
IV.—On the Rate of Assimilation in Nature, and on the Occur- rence of Translocation during the day	439
V.—Summary	447

Section I.—INTRODUCTION.

In investigating assimilation under natural conditions, gasometric methods, which involve enclosing leaves, are unsuitable. The only method which is free from this objection is the half-leaf dry-weight method introduced by Sachs.* Unfortunately, owing to the overlooking of certain grave errors to which this method is liable, most of the earlier work is of uncertain value. Having, however, made a full investigation into the sources and magnitude of these errors, an account of which was published in an earlier paper,† I have

* 'Arbeit. d. Bot. Inst. in Würzburg,' III, 1883, p. 19.

† 'Roy. Soc. Proc.,' 1909, B, vol. 82, p. 1.