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ON THE ELECTRICAL CHARGE OF THE NATIVE PROTEINS AND THE AGGLUTININS.

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In a previous paper[2] it was shown that the particles of both toxin and antitoxin wandered under the influence of an electric current toward the cathode and that the reaction (acidity or alkalinity) of the solvent did not influence the direction of migration. Since Hardy[3] and Pauli[4] demonstrated that the proteins which they used were amphoteric, *i. e.*, that they pass toward the anode in an alkaline medium and to the cathode in an acid one, there has been a tendency to generalize by assuming that all proteins behave in this manner. If such were the case, we pointed out, it would follow from our experiments that toxin and antitoxin are not true proteins. At the same time, however, we mentioned that from the few experiments in which this question had been considered, the protein matter of the broth or serum seemed in every instance to travel with the toxin or antitoxin toward the cathode. Further experiments have confirmed this result. It was also shown that the protein of normal horse serum and of non-toxic broth travels toward the cathode. Hence our work offers as yet no evidence either for or against the view that toxin and antitoxin are non-protein in nature.

We maintain that the results which Hardy and Pauli obtained, working with denaturalized proteins, are in no wise applicable to the native proteins, but that these carry a distinct electrical charge and are not amphoteric. We are here in accord with Iscovesco[5] and his co-workers, who investigated the charge of colloids con-

[1] Assisted by a grant from the Rockefeller Institute for Medical Research.
[2] Field and Teague, *Journal of Exper. Med.*, 1907, viii, 86.
[3] *Jour. of Physiol.*, 1899, xxiv, 288.
[4] *Hofmeister's Beit.*, 1906, vii, 531.
[5] *Compt. rend. Soc. biol.*, 1906, lxi, 195, 355, 378, 470, 568.

tained in various body fluids. Their method consisted in treating the fluid with electro-negative (arsenic sulphide) and electro-positive (ferric hydrate) inorganic colloids and their conclusions were based upon the fact that colloids of opposite sign when brought together form precipitates. Thus they found that the peritoneal fluid of the horse contains only electro-positive colloids, while the pericardial fluid contains those of both signs; that blood plasma contains both positive and negative albumins with positive and negative globulins, whereas the serum contains only the positive globulin along with albumins of both signs; that the fluid of a tubercular abscess deprived of its leucocytes contains only electro-negative colloids; that the amniotic fluid contains both positive and negative albumins, but only negative globulins. From these experiments Iscovesco concludes that there are no colloids which do not bear a distinct electrostatic charge.

Since our method gave no indication of the presence of an electro-negative albumin in normal serum, we are inclined to believe that Iscovesco by his manipulations produced a change in sign of the charge carried by certain proteins and that all of his findings are therefore not applicable to the proteins originally present in the fluids he investigated.

In our previous work with tetanus toxin we investigated only the tetanospasmin and its antibody; we have since shown by testing the agar extracts for their lytic or antilytic action on horse cells that both tetanolysin and antitetanolysin travel toward the cathode under the influence of an electric current. Having determined the electrical charge of toxin and antitoxin, we next applied the same method to an investigation of the agglutinins.

The agar was divided into one centimeter lengths; the agglutinin was found to have traveled seven centimeters into the cathode agar, the anode agar remaining free of agglutinin. The first centimeter length was extracted with five cubic centimeters of water and this extract would still agglutinate at a dilution of 1 : 100.

The specific agglutinins investigated travel toward the cathode. These results are diametrically opposed to those of Biltz, Much and Siebert,[6] who are the only workers, so far as we know, who

[6] *Zeit. für diatet. und phyckal. Ther.*, 1905, viii, 19.

have investigated this subject. They passed a current through lacto-serum contained in a U-shaped tube for from one half to one hour, and found that the fluid around the anode agglutinated at 1:20, that around the cathode not at all, and that from the middle of the U-shaped tube at 1:8. Normally the serum agglutinated at 1:4. They state that after the passage of the current the fluid from around the anode was 1/10 normal acid. We would expect this amount of acid to agglutinate at approximately 1:20, since 1:200 represents about the flocking limit of hydrochloric acid for bacteria.

As stated in a previous article, we took special precautions to eliminate the products of electrolysis. However, to show conclusively that it was the specific agglutinin, and that alone, which was responsible for the agglutination in our experiments, the extracts were also tested against other bacilli than those which were agglutinated by the serum under investigation.

TABLE I.

STRENGTH OF ELECTRIC CURRENT 110 VOLTS; ½ TO 1 MILLI-AMPERE.

Serum agglutinating the typhoid bacillus at 1 : 2000. Current passed for six hours.

Organism.	Cathode Agar cm. Lengths.											Anode Agar.	
	1	2	3	4	5	6	7	8	9	10	11–20	1–10	11–20
B. typhosus.	+++	+++	+++	+++	++	++	+	o	o	o	o	o	o
B. coli.	o	o	o	o	o	o	o	o	o	o	o	o	o
Shiga's bacillus.	o	o	o	o	o	o	o	o	o	o	o	o	o
Para typhoid b.	o	o	o	o	o	o	o	o	o	o	o	o	o
Biuret react.	+	+	+	+	+	+	trace						

NOTE.—As one centimeter lengths of the anode agar showed no agglutinin in repeated experiments we have here tested extracts from ten centimeter lengths.

If the agglutination were due to the presence of products of electrolysis we would expect the other bacilli to be agglutinated as well as typhoid. Such, however, was not the case. Hence, we believe that we have shown conclusively that the agglutinins travel toward the cathode.

It has been shown by Bechhold[7] and Buxton, Schaeffer, and Teague[8] and others that bacteria move toward the anode under

[7] *Zeit. physik. Chem.*, 1904, xlviii, 385.
[8] *Ibid.*, 1906, lvii, 47.

the influence of an electric current, that is, they carry a negative charge.[9] Our findings with regard to the agglutinins is therefore especially interesting, since it shows that in the phenomenon of agglutination we have the combination of an electro-negative suspension with an electro-positive colloidal solution. Since ions of opposite sign are essential for a chemical reaction and colloids of opposite sign when brought together form precipitates, our results harmonize with both the chemical and the colloidal view of the phenomenon.

Bacteria which have been saturated with agglutinin and then washed in a number of changes of water until the wash water contains no more agglutinin were placed in the cell and after eight hours the agar was tested for agglutinin. A small amount was found in the cathode agar showing that under the influence of the electric current the agglutinin bacteria combination was disassociated and that the agglutinins passed to the cathode. Bacteria have been disassociated from agglutinins by other means,[10] but so far as we are aware, this is the first time that disassociation has been affected by means of the electric current.

CONCLUSIONS.

1. Tetanolysin and antitetanolysin travel toward the cathode under the influence of an electric current.

2. The specific agglutinins are electro-positive.

3. The proteid matter of serum is not amphoteric but travels toward the cathode whether its reaction be acid, neutral, or alkaline.

4. The bacteria-agglutinin combination may be disassociated by means of the electric current.

[9] Cernovodeanu and Henri (*Compt. rend. Soc. de Biol.*, 1906, lxi, 200) claim that dysentery bacilli travel toward the cathode but we have not found this to be the case.

[10] Quoted by Eisenberg in *Cent. f. Bakt*, 1906, xxxi, 540 are the following: Joos (if fresh bacilli are added to agglutinated bacilli, which have been previously washed free from serum, the former are agglutinated), Landsteiner and Jagio and Landsteiner and Reich (dissociation at high temperatures).