CONTRIBUTION TO THE KNOWLEDGE OF THE ANTIENZYMATIC, LYTIC AND AGGLUTINATING EFFECTS OF BLOOD SERUM AND LYMPH

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CONTRIBUTION TO THE KNOWLEDGE OF THE ANTIENZYMATIC, LYTIC AND AGGLUTINATING EFFECTS OF BLOOD SERUM AND LYMPH

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I. Serum Diagnosis of Enzymes

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The studies made by Fermi [1], Pernossi [1], Hammarsten, Hahn [2], Röden [3], Hildebrant [4] and Morgenroth [5] reveal that blood serum has the ability to neutralize the effect of some enzymes. Fermi and Hahn conducted their experiments with digestive enzymes, while Röden and Morgenroth used rennin.

It can easily be understood that it was expected to use this peculiar behavior of serum for investigation of enzymes. Morgenroth [6] hopes to conduct experiments using the effects of serum to show that several active groups can be found in rennet. In a different manner, v. Dungern [7] attempted to utilize the antienzymatic effects of serum by immunizing animals with various microbes and showing that the resulting serum has a specific effect against the bacterial enzymes which he introduced. Therefore, in these experiments we are dealing with a kind of "serodiagnosis" of bacteria, using a roundabout route via their enzymes. It appears that a practical use of this behavior has not yet been found.

The experiments I conducted used serum as an ancillary means for distinguishing those animal enzymes which could not be differentiated in any other way. For the object of my investigation I chose tryptic enzyme (trypsin). I proceeded from the supposition that enzymes of the same name but from different species of animals could be characteristic for each species, as is the case for

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the active agents in their serum, the hemoglobins and certain other constituents of red blood cells, as well as for the cells of animals in general.

The perception of regularly occurring characteristics of very similar, initially indistinguishable substances in the different species was aided in part by chemical investigations, however, physiological and morphological deliberations led to the same conclusions. On the basis of experience gained with transplantation experiments and his investigations on the lens structure of the eye, Rabl [8] has recently pointed out the constancy of such differences found in homologous structures of different animal species.

In the case of enzymes, the most apparent method would have been to use /358 the sera of animals previously injected with enzymes. This sera would be similar to that which Morgenroth [9] produced by administering rennin to goats; a serum which was very active toward rennin.

Morgenroth himself has pointed out the difficulties which may be encountered in these experiments. In the case of rabbits, e.g., he did not succeed in rendering the serum active toward rennin. I experienced similar failures with the injection of large quantities of trypsin into rabbits; even after several injections there was no increase of the antitryptic effect.

I then used normal serum from different species of animals and let it act on pancreatin obtained from different species. With the difference of the sera and the similarity of the enzymes, we can, under these circumstances, expect a proportionality of the effect of sera A, B, C... on trypsins a, b, c...; with different sera and different enzymes, we may expect disproportionality. The experiment seemed to indicate differences in the enzymes, since combinations of sera and enzymes with non-proportional effects could be found.

Rabbit serum inhibited the digestion of gelatine by rat trypsin more than guinea pig serum, while, inversely, the same guinea pig serum had a stronger effect on canine trypsin. Experiments with rabbit serum and bovine serum and with rat tryrsin and bovine trypsin yielded analogous results. The fact that the strength of the antienzymatic effect varies in various individuals of one animal species, makes evaluation of these experiments difficult. However, in spite of this variance, there are certain constant differences in the sera of different species of animals.

Because of the complicated relationship of the interacting agents, it is desirable to examine the production of specific sera.

II. Occurrence of Antienzymatic Substances in the Body

In order to determine the origin of bactericidal substances in the blood serum, investigations were conducted with the purpose of detecting these substances in the tissues. Only in the case of polynuclear leukocytes were these investigations successful. This is also evident from the most recent studies [Moxter]. It seemed desirable to extend the investigations to those antienzymatic substances, whose behavior offers certain analogies to bactericidal substances, as e.g., by artificial intensification of their effect [v. Dungern: 10; and Morgenroth: 10].

Indications as to the reaction of trypsin with fresh organ pulp were made by Fermi and Pernossi [11]. They determined that freshly triturated organs (liver, spleer and muscle from guinea pigs) during a 24-hour digestion period, destroy, just as fresh blood, the effectiveness of a trypsin solution. This effect could be eliminated by heating the organs.

I proceeded in the following manner: before letting the pulverized /359

organs (liver, kidney. spleen) of guinea pigs react with trypsin, I washed them of the strongly antitryptic serum by repeated washing with NaCl solution (0.6%) and centrifugation. Under these circumstances, the antitryptic effect of the organs or of their extracts could not be clearly established. Extracts from polynuclear leukocytes were as ineffective as the organ extracts. However, I found an antitryptic effect in muscle serum, which was prepared in the following manner: A rabbit was bled to death and the blood vessels were then washed of blood by injecting large quantities of NaCl solution. A part of the muscle tissue was very finely diced and pressed in a handpress. The muscle serum that was thus obtained only impeded liquidation of the gelatine if an ample amount of a mixture of gelatine and trypsin was added*. Albumin from a chicken behaved in the same manner.

The question discussed is connected to the problem of the manner by which animal tissues are protected from the effect of the enzymes they contain.

Fermi [12] and Matthes [13] have recently formulated the thesis that living cells are completely resistant to enzymes, while Hahn [14] has pointed out the possible connection of this resistance to the antienzymatic characteristics of the serum. Thus, we are either dealing with humoral or with cellular immunity of the tissues to enzymes. Determination as to which of the two possibilities is applicable or whether both factors act together is not possible at this time since in the digestion experiments on living tissue of higher animals, the blood could not be excluded, and because, on the other hand, experiments on isolated cells (amoeba, mushroom, plant seeds, insect larvae) do not permit any con-

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*The resistance of the muscles to digestion may be related to this; also Matthes [loc. cit., p. 355] and the repetition of his experiment according to Claude Bernard.

clusion as to the behavior of the tissue of higher animals. I could not convince myself of the resistance of red blood cells, at least not if artificial tryptic digestion fluid was used. The presence of antienzymatic substances in the cells of liver, kidney or spleen cannot be proven with simple extraction.

III. Distribution of Bactericidal Substances in the Body Fluids

Information regarding the bactericidal effect of the lymph fluid does not always agree. I found, as did most other investigators, that lymph from the thoracic duct of dogs is able to kill bacteria. To attest such behavior, I used Vibrio cholerae. Similar to earlier experiments, the effects of the lymph were less strong than those of blood serum.

For comparison, I tested the effect of the duct lymph and lymph from the extremities of a dog. In order to collect this lymph in as pure a state as /360 possible, I used a method similar to the one described by Paschutin [16] and Emminghaus [17], i.e., I inserted cannulas into the lymph ducts of the dog's extremities. Concerning the bactericidal effect of the lymph in the peripheral areas, Hamburger [18] has obtained positive results. However, in a number of other studies we find indications of ineffectiveness of the lymph which collects upon the occurrence of edema in the peripheral parts of the body. Such experiments dealing with the ineffectiveness of cell-free edematous lymph have often been used to strengthen the relationship between the antibacterial effect of blood and its leukocyte content.

In my experiments, the lymph from dog extremities had a bactericidal effect on Vibrio cholerae. This characteristic could be tested by the inoculation of gelatine plates when compared with fresh serum heated to 60°C, and it could also be shown if the lymph was used to activate heated cholera serum, in

which I observed the alteration of the vibrions into small globules. Of course, the lymph contained a few polynuclear leukocytes.

The effectiveness of this lymph was considerably less pronounced than that of the duct lymph, and it could only be shown if small amounts of bacteria were used. This behavior could be attributed to the fact that either the lymph of different organs contains different quantities of bactericidal agents, or that the quantity of these agents increases during passage through the lymphatic glands. The second supposition, which would correspond to the relationship between lymphatic glands and leukocyte production, was tested by comparing the lymph before and after passage through the glands.

For this purpose, I inserted a cannula into one of the two large lymph vessels on the hind leg of a dog. The lymph of these vessels had not yet passed through a gland [Emminghaus]. A second cannula was inserted into the large lymph vessel of the thigh of the other hind leg. This lymph vessel can be found in the sheath of the great femoral vessels; it receives lymph from the lymph nodules of the popliteal fossa. In other experimental animals, I used only one hind leg by first collecting lymph from a central and then later from a peripheral area.

The differences found while testing the bactericidal effects of the samples were too negligible to warrant the assumption that the lymph glands do exert an influence.

In order to explain the pronounced difference which I observed between the lymph of the thoracic duct and that from peripheral lymph vessels, it is important to investigate the diffusion of substances from the inner organs into the thoracic duct.

IV. The Chemical Behavior of Lysins, Agglutinins* and Antienzymes /361

Bovine serum was diluted six-fold by distilled water containing an ample amount of carbonic acid; the globulin precipitate was separated, and the liquid was divided into a small portion (A) which contained the globulin, and a greater portion (B) which was free of the precipitate (ratio of the liquid volumes = 1:4). NaCl solution (6%) was added to both portions until the salt content was about 0.6%. During this procedure the globulin precipitate dissolves, with only a faint turbidity remaining. Now (A) had a much stronger agglutinating effect on the red blood corpuscles of guinea pigs than the globulin-poor solution (B). The agglutinating effect of globulin can still be shown if the liquid volumes of the two solutions are kept the same. The effect can still be found in globulin solutions which have been rinsed with water several times, in the cold. A large part of the agglutinating substance passed into these globulin precipitates, which were produced either by dialyzation of bovine serum or by precipitating with ammonium sulfate. This result is related to the observations of Winterberg [19], who, like Widal and Sicard [20], found that most substances which precipitate globulin also precipitate the specific typhus agglutinins. Winterberg, who used different precipitating agents, found a difference in their ability to precipitate globulins and agglutinins, a relationship which must still be tested for normal agglutinins, probably the mother material for these

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*The serum of healthy humans not only has an agglutinating effect on animal blood corpuscles, but also on human blood corpuscles from different individuals. It remains to be decided whether this phenomenon is due to original individual differences or to the influence of injuries and possible bacterial infection. I observed this behavior as especially pronounced in the case of blood from severely ill patients. This phenomenon could be related to the dissolving capacity of serum for blood corpuscles in the case of various diseases, as it was described by Maragliano (10th Congress of Internal Medicine, 1892).

specific substances.

When both solutions (A) and (B*) were tested as to their reaction with trypsin, it was found that (A) did not have a stronger antitryptic effect than (B), from which it is evident that the antitryptic effect is for all practical purposes independent of the globulins. The globulins which were obtained by means of dialysis yielded similar results. If bovine serum is precipitated with an equal volume of ammonium sulfate solution, and after filtering, with crystallized ammonium sulfate, the solution of the globulin precipitate which was produced first, is ineffective toward trypsin, whereas the solution of the albumin precipitate has an antitryptic effect, before as well as after removal of the ammonium sulfate by means of dialysis.

If solutions (A) and (B) are tested as to their content of blood cell dissolving substances (red blood cells from guinea pigs), it is found that both solutions are rather ineffective. Buchner [21] proceeded in a similar man- /362
ner during his investigation of bactericidal substances and found that those solutions which contain primarily albumin have a stronger effect than those which contain globulin. As far as the globulicidal substances are concerned, he found that the primarily globulin-containing solution from a sodium sulfate precipitate of canine serum is able to dissolve blood cells. I, too, found this characteristic in the case of precipitates produced by carbonic acid using bovine serum, however, the effect of the concentrated solution was very small when compared to the original serum, therefore, the effect could merely be due to the substances which clung to the precipitate. With this experimental

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*Both solutions inhibit the effect of abrin on red blood corpuscles. When compared to the original serum, both solutions have a rather weak effect on the cleavage of hydrogen peroxide.

arrangement, most of the globulicidal effect is lost, regardless of whether the precipitation is caused by dilution and carbonic acid or by dialysis. The probable reason for this behavior is the destructive effect of water, which was pointed out by Buchner. Therefore, I conducted new experiments, where I again precipitated the globulins with carbonic acid, but where I did not dilute with water, using instead a solution of grape sugar (glucose, 5%). Using this method, it is possible to maintain the globulicidal effect, and it could now be shown that the globulin-rich liquid has no greater effect than the globulin-poor solution, in fact, in several cases the latter seemed to have a more pronounced effect. Accordingly the globulins would be rather unimportant for the dissolution of foreign blood corpuscles in the serum. The sensitizing substances of Ehrlich and Morgenroth [22] were found, in the case of the dialyzed bovine serum, in the still-dissolved portion but not in the precipitated globulin. If this solution was mixed with NaCl, it was not until it contained about 0.6% NaCl that it rendered the blood corpuscles of guinea pigs sensitive to the alexins of guinea pig serum, just as heated serum did in the experiments cited above.

Vienna, February 10, 1900.

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AGGLUTINATION PHENOMENA IN NORMAL HUMAN BLOOD

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AGGLUTINATION PHENOMENA IN NORMAL HUMAN BLOOD

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Some time ago, I observed and reported [1] that the blood serum of */1132 normal humans can sometimes clump the red blood cells of other healthy individuals. At that time, I was under the impression that the ability of the blood serum to clump foreign red cells was especially pronounced in the case of certain diseases, and thought that it could be connected with the strong lytic ability of pathological sera, as was observed earlier by Maragliano [2], since agglutination and lytic abilities often, if not always, undergo parallel changes. The reaction studies conducted by Maragliano would not be equated with the hemolytic reaction studies which are now so frequently conducted, because the addition of NaCl (and not heating) until a normal NaCl content is reached, eliminates the lytic ability of the sera. Maragliano himself distinguishes his observations from the phenomenon of Landois (hemolysis by serum foreign to the species), since in Maragliano's case the hemoglobin is not only dissolved but also destroyed. One significant difference between my observations and those of Maragliano is that in his case the serum only affects the blood cells taken from the same individual and that the reaction is only successful if conducted with pathologic blood. However, my observation pointed up clear differences between the blood serum and the blood cells of apparently completely healthy persons.

According to his descriptions and to his pictures, the observation of Shattock [3] belongs in this context, even though he only found the reaction in

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*/Numbers in the margin indicate pagination of the original foreign text.

cases with febrile diseases, not in normal blood. Shattock relates this reaction to increased coagulability and rouleau formation in the febrile blood.

According to Ehrlich and Morgenroth [4], the clumping of human blood /1133 by human serum, which will be discussed further, should be designated as isoagglutination. These two researchers described their experiments shortly after my publication appeared. They described experiments in which they succeeded, by means of homogeneous blood injection, in producing isolysins and isoagglutinins, i.e., sera which would act on cells of the same species. These very detailed experiments confirm the unexpected occurrence of clearly distinguishable differences in the bloods of one species of animal, partly because of the different circumstances in the testing of the individual experimental animals.

In the study by Ehrlich and Morgenroth, the phenomenon of isolysis (isohemolysis) is subjected to a more detailed discussion from the viewpoint of Ehrlich's theory.

Since the publication of the reports made by Shattock and myself, a number of investigators have studied the behavior of isoagglutination in humans. The evaluation of those studies* which consider this reaction to be specific for a certain disease, is valueless as is apparent by the fact that this reaction occurs among healthy persons. Other studies even record observations on intensity and frequency of the reaction in cases of disease.

In various forms of anemia, Donath [5] found the phenomenon more frequently than in healthy persons, but not every time. Ascoli [6] observed the phenomenon in healthy persons, but noted more intensity in diseased ones. Eisenberg conducted observations on both healthy and diseased persons. As other authors,

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*For other references, see article by Eisenberg: Wiener klinische Wochenschrift No. 42, 1901.

he found that the reaction is frequent in cases of disease, and constitutes rather an exception in healthy individuals. This result is not in agreement with my findings*.

Since, in the above-mentioned publications I gave only brief descriptions, I will indicate below the results of some recently conducted experiments. The tables are quite simple. Approximately equal quantities of serum and red blood cell suspension (about 5%) were mixed in NaCl solution (0.6%) and observed in a suspended drop preparation or in test tubes (the plus sign designates agglutination).

TABLE I. CONCERNING THE BLOOD OF SIX APPARENTLY HEALTHY MALES.

Serum from:	Blood cells from:					
	Dr. St.	Dr. Plecn.	Dr. Sturl.	Dr. Erdh.	Zar.	Landst.
Dr. St.	-	+	+	+	+	-
Dr. Plecn.	-	-	+	+	-	-
Dr. Sturl.	-	+	-	-	+	-
Dr. Erdh.	-	+	-	-	+	-
Zar.	-	-	+	+	-	-
Landst.	-	+	+	+	+	-

A fourth, similar table, concerning the sera of Table II, combined with the blood cells of Table I, and certain other tested sera, e.g., from one case

*Although Eisenberg attacks the results of my work, and simultaneously confirms them as far as the blood of the patients is concerned, he mentions my work only in the bibliography but does not refer to it at all in the text.

TABLE II. CONCERNING THE BLOOD OF SIX APPARENTLY HEALTHY PUERPERAE.

Serum from:	Blood cells from:					
	Seil.	Linsm.	Lust.	Mittelb.	Tomsch.	Graupn.
Seil.	-	-	+	-	-	+
Linsm.	+	-	+	+	+	+
Lust.	+	-	-	+	+	-
Mittelb.	-	-	+	-	-	+
Tomsch.	-	-	+	-	-	+
Graupn.	+	-	-	+	+	-

TABLE III. CONCERNING THE BLOOD OF FIVE PUERPERAE AND SIX PLACENTAE (BLOOD FROM THE UMBILICAL CORD).

Serum from:	Blood cells from:					
	Trautm.	Linsm.	Seil.	Freib.	Graupn.	Mittelb.
Lust.	+	+	-	-	-	+
Tomsch.	-	-	+	-	-	-
Mittelb.	-	-	+	-	-	-
Seil.	-	-	+	-	-	-
Linsm.	+	+	+	-	-	+

of hemophilia and one case of purpura, showed perfectly corresponding regularities and could therefore be omitted. During the investigation of ten other normal persons (using 42 combinations) similar conditions were found.

These experiments show that my data did not have to be corrected. All of the 22 investigated sera of healthy adults yielded this reaction. The result would obviously have been different if I had not used a number of different blood corpuscles for testing purposes.

Halban [7], Ascoli and, most recently, Eisenberg, have already pointed out the variable resistance of the blood cells to this reaction. This difference can also be seen from the above tables. Moreover, the behavior of the 22 investigated blood samples showed a curious regularity. If we do not take into account the few blood serum investigations on fetal placenta blood which did not show agglutination (Halban also found that fetal blood seldom has an agglutinating effect), the sera in most cases could be separated into three groups:

In a number of cases (group A), the serum reacts with the cells of another group (group B), but not with those of group A; these A cells are acted upon in the same manner by serum B. In the third group (C), the serum agglutinates the corpuscles of A and B, while the red cells of group C are not acted upon by sera from A and B.

According to the customary terminology, one can say that in these cases there must be at least two kinds of agglutinins present, the one in A, the other in B, and both together in C. Naturally, the corpuscles must be considered insensitive to the agglutinins which are present in the same serum.

It cannot be denied that a postulate on the occurrence of a few different agglutinins in the investigated cases sounds quite strange, even though the experiments on isolysins made by Ehrlich and Morgenroth yielded similar results. It would be more satisfactory if continued observations would lead to a different interpretation.

However, the investigations indicate that attention should be paid to these

regularities in pathological cases.

Eisenberg attributes the formation of agglutinins to the resorption of constituents of the red blood corpuscles. This idea is not new, since Halban and Ascoli have already presented it as a possible explanation. I did not mention this explanation at an earlier time, as I had not succeeded in inducing autoagglutination in animals by injection of their own dissolved red blood corpuscles.

I do not believe Ehrlich has reported any positive results in this area, however, Ascoli does have positive, but not constant results. Halban points to the difficulties with the mentioned postulate. For, according to this postulate, the formation of naturally occurring hemagglutinins as well as the normal agglutinins which act on bacteria, would require two different explanations.

Furthermore, my experiments show that the different sera do not have /1134 identical effects with regard to agglutination. If it is believed that the sera owe their agglutinating ability to a kind of auto-immunization caused by cell constituent resorption, one must still assume the existence of individual differences, in order to interpret different sera. The blood corpuscles do indeed show a differing behavior, even already in the fetal blood (see Table III). If we assume the differences of the sera and of blood corpuscles, it is just as easy (or difficult) to understand agglutination within one species, as agglutination by a serum foreign to the species. However, the above-mentioned explanation cannot be excluded, moreover, if the still-unrefuted experiments of Ascoli are correct, it would be hard to circumvent. It would have to be assumed that the physiological decomposition of the cells of body tissue was the generative source of active serum components.

In order to eliminate the opinion that past pathological processes are of any significance, I would consider the experiments on the blood of infants and

of animals to be of some importance. Halban's experiments also contradict the existence of such a relationship.

The kind of agglutination described can also be caused by serum which has been dried and then immediately dissolved; I succeeded in producing this kind of agglutination with the solution of a dried drop of blood, which had been kept for 14 days on a piece of linen cloth. Thus the reaction may possibly be used in some cases for the identification, or better, for the recognition of unidentified blood samples, e.g., for forensic purposes, unless rapid variations of the agglutinating ability should be found, which would prevent this application. However, the 6 sera of Table I showed the same behavior when the second sample was taken, as the samples taken nine days earlier*.

Finally, it should be mentioned that the described observations permit the explanation of varying consequences resulting from therapeutical blood transfusions.

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*Dr. Richter and I plan to investigate the reliability of this method.

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