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# THE THERMOSTABILE ANTICOMPLEMENTARY CONSTITUENTS OF THE BLOOD.

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The present article deals with the antihæmolytic principles of the blood, and especially of normal blood serum, which act against that form of hæmolysis caused by the amboceptor-complement complex.

The present state of our knowledge permits us to divide the antihæmolytic principles of normal blood into two classes. The principles of the first class are directed against certain definite hæmolysins—glucosides, mercuric chloride, agaricin—and certain hæmolytic toxins of micro-organic origin (bacterial toxins). The principles of the second class are directed against the more complex hæmolysins as they exist in normal and immune sera. Preliminary to the consideration of the immediate subject of this paper I will enumerate the factors, other than those which come into play in connection with the normal serum, which influence the process of hæmolysis.

The hæmolytic process can be altered and reduced in different ways. Temperatures above 50° C. render all complements with few exceptions [1,2] inactive. Divers micro-organisms,[3] organic tissues,[4] certain tissue constituents,[5] and the complex antigen-antibody to which belong the complement fixation of specific precipitates [6-12] all reduce or prevent hæmolysis by interfering under certain conditions with the union of complement and hæmolytic amboceptor. Bordet [13,14]

[1] Ehrlich and Morgenroth, Ueber Hämolysine, Zweite Mittheil., *Berl. Klin Woch.*, 1899, xxxvi, 481.

[2] Noguchi, On the heat lability of the complements of cold-blooded animals, *Centralbl. f. Bakteriologie*, 1903, xxxiv, 283.

[3] M. Wilde, Ueber die Absorption der Alexine durch abgetötete Bacterien, *Berl. klin. Woch.*, 1901, xxxviii, 878.

[4] V. Dungern, Beiträge zur Immunitätslehre, *Münch. med. Woch.*, 1900, xlvii, 677.

and Ehrlich and Morgenroth [15] describe anticomplements in the strict sense, the existence of which is denied by Moreschi, who accounts for the effect ascribed to them by the complement binding power of specific precipitates.

Ehrlich first described in relation to tetanolysin the antihæmolytic property of normal serum, and Kraus and Clairmont,[16] found this property to be exerted against many bacterial hæmolysins. Neisser and Wechsberg [17] observed the neutralization of staphylolysins by normal guinea-pig serum. A great advance was made by Ransom's [18] discovery of the antihæmolytic action of cholesterin upon saponin. Noguchi,[19] following upon Ransom's discovery, found that cholesterin prevented hæmolysis by agaricin and tetanolysin. These observations drew active attention to the part played by the lipoids of the blood in relation to hæmolysis. P. Th. Müller,[20] Kyes and Sachs,[21] Landsteiner and v. Eisler,[22] Pascucci,[23] studied the nature of the antihæmolytic bodies of normal serum. Müller confirmed the antihæmolytic property of cholesterin. Kyes and Sachs found that cholesterin has no antihæmolytic power against staphylolysin and v. Eisler [24] located the antistaphylolytic principle in the globulin fraction. Pribram [25] found that cholesterin is active against the Nasik vibriolysin; and E. Abderhalden and E. R. Le Count [26] attempted to locate the groups upon which depend the antihæmolytic properties of cholesterin, finding that the free hydroxyl group has a certain relation to these properties. Detre and Sellei [27] stated that lecithin is capable of preventing sublimate hæmolysis.

The investigations summarized indicate that the normal serum contains definite chemical substances which can prevent the hæmolysis caused by bacterial toxins, mineral chemicals, and certain vegetable principles of definite composition. Some differences of opinion exist regarding the nature of the inhibiting substances. Undoubtedly the lipoids of the serum as represented by cholesterin and lecithin possess this property. Apparently a similar property resides in the proteids of the serum. In regard to the latter the question which would at

[5] P. A. Levene and E. R. Baldwin, On the antihæmolytic action of some cell and tissue constituents, *Jour. of Med. Research*, 1904, xii, 205.

[6] Gengou, Sur les sensibilisatrice des serums actifs contre les substances albuminoides, *Ann. Inst. Pasteur*, 1902, xvi, 734.

[7] F. P. Gay, The fixation of alexines by specific serum percipitates, *Centralbl. f. Bakteriologie*, 1905, xxxix, 603.

[8] Moreschi, Zur Lehre von den Antikomplementen, *Berl. klin. Woch.*, 1905, xlii, 1181.

[9] Neisser and Sachs, Ein Verfahren zum forensischen Nachweise der Herkunft des Blutes, *Berl. klin. Woch.*, 1905, xlii, 1388.

[10] Pfeiffer and Moreschi, Ueber scheinbare Antikomple mentär- und Antiamboceptorwirkung precipitirender Sera im Thierkörper, *Berl. klin. Woch.*, 1906, xliii, 33.

[11] Liefmann, Ueber die Komplementablenkung bei Precipitationsvorgängen, *Berl. klin. Woch.*, 1906, xliii, 448.

[12] Muir and Martin, On the deviation of complement by a serum and its antiserum and its relations to the precipitin test, *Jour. of Hygiene*, 1906, vi, 265.

[13] Bordet, Les serums hémolytiques et leurs antitoxines, *Ann. Inst. Pasteur*, 1900, xiv, 257.

once arise is whether all the lipoids had been separated from the proteid fraction of the serum.

A second class of antilytic substances, present in normal serum, are the direct antagonists of complements and amboceptors. Camus and Gley [28] found that heating eel and other normal sera to 56° C. develops in them an antihæmolytic property which they regard as acting antagonistically to the lytic principles. Müller[29] observed this behavior of sera heated to 56° C. and, in addition, the antilytic property exerted by certain unheated sera. Ehrlich and Sachs [30] and Sachs [31, 32] found that at 60° C. but not at 50° C. dog serum becomes anticomplementary. According to their view the zymotoxic group, but not the haptophoric, is destroyed by the higher temperature whereby complementoid is produced. Heating does not, however, leave the haptophore group intact, since complementoid exerts weaker affinity for amboceptor than the original complement. Although dog serum is not rendered anticomplementary when heated to 49° to 50°C., yet it is rendered inactive. No adequate explanation of this inactivity has been advanced. Ox serum was also observed to become anticomplementary when heated to 60° C., but the existence of complementoid could not be demonstrated, because no union between its amboceptor and guinea-pig corpuscles occurs in the absence of normal complement (horse). Recently Bordet and Gay[33] have shown that other factors operate in this phenomenon. Guinea-pig corpuscles can really be sensitized by ox amboceptor, but when the excess of inactive ox serum is removed, horse complement does not exert a dissolving action on the corpuscles (although guinea-pig complement dissolves them promptly). Hence they assert that inactivated ox serum contains bodies which energize the power of horse serum. Neisser and Döring [34] observed in a case of uraemia that the serum, on heating to 56° C., became anticomplementary. Neisser and Friedemann [35] made a similar observation, and in addition they found that heating to 57° C.

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[14] *Idem.* Bemerkung über die Antikomplemente, *Berl. klin. Woch.*, 1906, xliii, 17.

[15] Ehrlich and Morgenroth, Ueber Hämolysine, Vierte Mitth., *Berl. klin. Woch.*, 1900, xxxvii, 681.

[16] Kraus and Clairmont, Ueber Bacteriohämolysine und Antihämolysine *Wien. klin. Woch.*, 1901, xiv, 1016.

[17] Neisser and Wechsberg, Ueber das Staphylotoxin, *Zeitschr. f. Hygiene*, 1901, xxxvi, 299.

[18] Ransom, Saponin und sein Gegengift, *Deut. med. Woch.*, 1901, xxvii, 194.

[19] Noguchi, The antihæmolytic action of blood sera, milk, and cholesterin upon agaricin, saponin, and tetanolysin, *Centralbl. f. Bakteriologie*, 1900, xxxii, 377.

[20] P. Th. Müller, Geht das Tetanolysin mit den Proteiden des Serums und des Eiklares eine ungiftige Verbindung ein? *Centralbl. f. Bakteriologie*, 1903, xxxiv, 567.

[21] Kyes and Sachs, Zur Kenntniss der Cobragift activirenden Substanzen *Berl. klin. Woch.*, 1903, xl, 59, Footnote 3.

[22] Landsteiner and v. Eisler, Ueber Agglutinin-und Lysinwirkung, *Centralbl. f. Bakteriologie*, 1905, xxxix, 309.

[23] Pascucci, Die Zusammensetzung der Blutscheibenstromes und die Hæmilyse, *Hofmeister's Beiträge*, 1905, vi, 552.

inactivated the serum without destroying the anticomplementary action. They endeavored to account for the phenomenon by supposing the amboceptor of uraemic blood to be less heat stabile than normal, whereby, at 56° C., the cytophylic group is destroyed and the complementary one is left intact (amboceptoid) and capable of deviating complement. They thought to prove their view by pointing out that extraction of the heated serum by susceptible red corpuscles did not remove the inhibitory action of the serum. Unfortunately, they seem not to have tested the corpuscles for resistance. Laqueur[36] confirmed the work of these investigators and found, in addition, not only that similar conditions existed in the blood in other pathological states, but that they may be absent from uraemic blood. Senator[37] failed to find the phenomenon in classical examples of uraemia, and Bergmann and Keuthe[38] ascertained that it is irregular in its occurrence in uraemia and occurs in other pathological conditions. That rabbit serum may, after heating to 56° C., develop anticomplementary action is shown by certain experiments of Sachs[39] and Pfeiffer and Friedberger.[40] Muir and Browning[41] mention that guinea-pig serum, from which complement has been removed by means of sensitized corpuscular stromata, previously heated to 56° C. over night and washed, does not become anticomplementary upon heating to 55° C., because no complement exists to be changed into complementoid. Manwaring[42] describes a qualitative change in heated immune serum after digestion with corpuscles, but does not define accurately this alteration.

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[24] v. Eisler, Bedeutung der Lipoide für die antihämolytische Wirkung des Blutes, *Zeitschr. f. exper. Pathologie und Therapie*, 1906, iii, 296.

[25] Pribram, Ueber Bakteriohämotoxine (Lysine) und Antihämotoxine, Handbuch der path. Mikroorganismen, 1906, Ergänzungsband, i, 291.

[26] E. Abderhalden and E. R. Le Count, Die Beziehungen zwischen Cholesterin, Lecithin, and Cobragift, Tetanustoxin, Saponin und Solanin, *Zeitschr. f. exper. Pathologie und Therapie*, 1905, ii.

[27] Detre and Sellei, Hämolytische Wirkung des Sublimates, *Wien. klin. Woch.*, 1904, xvii, 1195, 1234.

[28] Camus and Gley, A propos de l'existence, dans un serum sanguin, d'une action antagoniste de l'action hémolytique, *Compt. rend. d. l. Soc. d. Biologie*, 1901, liii, 732.

[29] P. Th. Müller, Ueber die Antihämolysine normaler Sera, *Centralbl f. Bakteriologie*, 1901, xxix, 860.

[30] Ehrlich and Sachs, Ueber den Mechanismus der Amboceptorenwirkung, *Berl. klin. Woch.*, 1902, xxxix, 492.

[31] H. Sachs, Giebt es einheitliche Alexinwirkung? *Berl. klin. Woch.*, 1902, xxxix, 216.

[32] *Idem*, Ueber Komplementoide, *Centralbl. f. Bakteriologie*, 1905, xl, 125.

[33] Bordet and Gay, Sur les relations des sensibilisatrice avec l'alexine, *Ann. Inst. Pasteur*, 1906, xx, 467.

[34] Neisser and Döring, Zur Kenntnis der hämolytischen Eigenschafte des menschlichen Serums, *Berl. klin. Woch.*, 1901, xxxviii, 593.

[35] Neisser and Friedemann, Ueber Amboceptoidbildung in einem urämischen Serum, *Berl. klin. Woch.*, 1902, xxxix, 677.

The difference in the heat-effects, according as the temperatures to which the sera are exposed are about 50° C. or between 56° and 60° C., is remarkable. If we assume, as Ehrlich and Sachs have done, that complementoid is formed at 60°C., what are we to infer happens at 50° C.? If the anticomplementary effect is caused by destruction of the zymotoxic groups, what changes are produced at 50° C. leading to inactivation without developing any antagonistic activity? The view that the difference depends on the thermolability of the amboceptor is hypothetical, and the experiments adduced by Neisser and Friedemann to uphold it are unconvincing. I shall return to this point later in connection with my experiments which seem to me to afford a solution of the problem.

That the blood lipoids may antagonize hæmolysis by amboceptor-complements has also been shown. Landsteiner and v. Eisler[43] extracted lipoids from blood corpuscles and noted that they exerted an antihæmolytic action against serum hæmolysins. Later, these authors[44] found that blood serum contained similar bodies in smaller amounts than the corpuscles. They believed the action of the lipoids to be anti-amboceptoric, in the sense that they constitute the receptors, which in the intact corpuscles anchor the amboceptors. Bodies having the same action can be obtained from the stroma of corpuscles. Bang and Forssmann[45,46] extracted ox corpuscles with ether and obtained a mixture which, according to them, contains the hæmolysin or amboceptor-producing agent and antihæmolysin (complement-neutralizer). By treating the ethereal extract with aceton they obtained in solution the latter principle; the insoluble residue retained this amboceptor-producing body. This can be separated from phoshatides and cerebrosides by extraction with alcohol, ether, chloroform or cold benzol. Bang and Forssmann regard the amboceptor-fixing body (receptor in Ehrlich's sense) and amboceptor-producing substance as distinct from each other. Landsteiner and v. Eisler and Bang and Forssmann ob-

[36] Laquer, Zur Kenntnis urämischer Zustände, *Deut. med. Woch.*, 1901, xxvii, 744.

[37] H. Senator, Ueber die hämolytische Eigenschaft des Blutserums bei Urämie, *Berl. klin. Woch.*, 1904, xli, 181.

[38] Bergmann and Keuthe, Die Hemmung der Hämolyse durch inactivirte menschliche Sera, *Zeitschr. f. exper. Pathologie und Therapie*, 1906, iii, 255.

[39] H. Sachs, Ueber das Zusammenwirken normaler und immunisatorisch erzeugter Amboceptoren bei der Hämolyse, *Deut. med. Woch.*, 1905, xxxi, 705.

[40] Pfeiffer and Friedberger, Beitrag zur Lehre von den antagonistischen Serumfunktionen, *Centralbl. f. Bakteriologie*, 1906, xli, 223.

[41] Muir and Browning, On the properties of anti-immune bodies and complementoids, *Jour. of Hygiene*, 1906, vi, 1.

[42] Manwaring, The absorption of hemolytic amboceptor, *Jour. of Infect. Diseases*, 1905, ii, 485.

served that stromata of red corpuscles, freed of fat by solvents, are no longer able to fix amboceptor.

I wish, in this place, to point out that the anticomplementary body of Bang and Forssmann is, according to their view, of specific nature. It is questionable, indeed, whether their experiments wholly support this view (their experiments *l. c.* p. 261 can be interpreted so as to show the effect to be upon the amboceptor rather than upon the complement). This body is thermostable: boiling for four minutes does not destroy it, but longer boiling does. It is of some interest to learn that these authors look upon this body as fixing the complement to the corpuscles after it has been exposed for action by the union of corpuscles and amboceptors. Hæmolysis results, therefore, from union of amboceptor and complement separately with the corpuscles.

A review of the literature upon the inhibition of serum hæmolysis brings out from a large mass of hypothesis a few definite facts. It is established that many normal and pathological bloods contain certain constituents which possess the power to suppress the action of serum-complements. This anticomplementary action may be present in the native serum or it may manifest itself only after the serum has been heated to 56° C. or higher. It has been shown that by extraction with certain solvents red corpuscles and serum yield substances which exert antilytic action. Whether the antilytic action of fresh and heated sera depend wholly, or at all, upon these substances is not proven beyond doubt. It is not proven that any of these antilytic substances are merely modified complements and amboceptors, and the existence of complementoid and amboceptoid has not been established. Apparently there are differences of considerable magnitude between the influences of heat and chemicals upon the antilytic action of sera and these extractives. It may be an

[43] Landsteiner and v. Eisler, Ueber die Wirkungsweise hämolytischer Sera, *Wien. klin. Woch.*, 1904, xxvii, 676.

[44] *Idem*, Ueber Agglutinin- und Lysinwirkung, *Centralbl. f. Bakteriologie*, 1905, xxxix, 309.

[45] Bang and Forssmann, Untersuchung über die Hämolysinbildung, Vorl Mitth., *Centralbl. f. Bakteriologie*, 1905, xl, 150.

[46] *Idem*, Untersuchung über die Hämolysinbildung, *Hofmeister's Beiträge*, 1906, iii, 238.

accidental circumstance that the temperatures which destroy complement and amboceptor also remove this antilytic property from the serum. The influence of temperature upon combination and dissociation of serum constituents is probably very wide. The different effects produced at 50° C. are cases in point. Manwaring[47,48] found that normal goat serum, heated to 56° C. to 59°, acquired certain properties which he ascribed to a third serum component. The heated serum might be purely auxilytic, or purely antilytic, or again, auxilytic in certain amounts and antilytic in others, or, even as a fourth possibility, completely inactive. Sera of different normal animals heated under these conditions might differ widely in the action of the third components. No explanation was offered for these variable effects. Possibly this effect is brought about by liberation of certain hæmolytic lipoids from inactive combinations, and these may be powerful enough to overcome the antilytic action of the antilysins whose action we are considering. I have observed many instances of the auxilytic effect described by Manwaring, but sera from which the lipoids have been extracted by alcohol and ether do not develop, on heating, auxilytic properties.

### NORMAL SERUM AS ANTIHÆMOLYSIN.

To bring out the antihæmolytic action of normal serum it is customary to inactivate it at 56° C. Either the heated serum and active hæmolytic serum are first mixed and the blood corpuscles added later, or, the heated serum and complement are first brought together and at a later stage the inactive hæmolytic serum (amboceptor) is added. Fresh serum can be tested accurately for antilytic action only when it does not contain suitable complement.

Table I shows the normal sera of the dog and sheep, heated to 56° C., to become antihæmolytic, while the same sera in the fresh state are non-protective. On the other hand, fresh ox serum protects its corpuscles, but heating to 56° C., removes the protective property. Inactivated ox serum is, however,

[47] Manwaring, On auxilysins, *Jour. of Infect. Diseases*, 1906, iii, 225.

[48] *Idem*, The third serum component, *Jour. of Infect. Diseases*, 1906, iii, 645.

## TABLE I.

THE ANTIHÆMOLYTIC POWER OF DOG, SHEEP, AND OX SERA WAS TESTED AGAINST THE ACTIVE ANTI-DOG (RABBIT), ANTI-SHEEP (GOAT) AND ANTI-OX (RABBIT) SERA. THE HÆMOLYTIC VALUES OF THESE IMMUNE SERA WERE, IN THE FRESH STATE, 0.05 C.C. FOR ANTI-DOG, 0.07 C.C. FOR ANTI-SHEEP AND 0.05 C.C. FOR ANTI-OX SERUM, UPON THEIR CORRESPONDING CORPUSCLES.

| Doses of the normal serum mixed with 0.2 of active immune serum. | Anti-dog serum 0.2 | | | | | | Anti-sheep serum 0.2 | | | | | | Anti-ox Serum 0.2 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Dog serum | | Sheep serum | | Ox serum | | Dog serum | | Sheep serum | | Ox serum | | Dog serum | | Sheep serum | | Ox serum | |
| | Fresh | 56° C | Fresh | 56° C | Fresh | 56° C | Fresh | 56° C | Fresh | 56° C | Fresh | 56° C | Fresh | 56° C | Fresh | 56° C | Fresh | 56° C |
| 1. | CH | none | CH | none | CH | none | CH | none | CH | none | almost CH | none | CH | none | slight | none | much | CH |
| 0.7 | " | " | " | trace | " | slight | " | none | " | " | " | slight | " | none | " | " | " | " |
| 0.5 | " | trace | " | much | " | almost | " | slight | " | " | " | " | " | slight | " | trace | CH | " |
| 0.3 | " | " | " | " | " | CH | " | moder- | " | trace | " | much | " | " | almost | slight | " | " |
| 0.2 | " | " | " | almost | " | " | " | ate | " | " | " | " | " | mod'te | CH | mod'te | " | " |
| | | | | CH | | CH | " | much | " | | " | " | " | | CH | | | |
| 0.1 | " | " | " | " | " | | " | " | " | | " | almost | " | much | " | much | " | " |
| 0.07 | " | much | " | " | " | " | | almost | | slight | | CH | " | almost | " | almost | " | " |
| 0.05 | " | " | " | CH | " | " | " | CH | " | mod'te | " | " | | CH | | CH | | |
| 0.03 | " | CH | " | " | " | " | " | " | " | much | " | CH | " | CH | " | CH | " | |
| 0 (control) | CH | CH | CH | CH | CH | CH | CH | CH | CH | CH | CH | CH | " | " | " | " | " | CH |
| | | | | | | | | | | | | | CH | CH | CH | CH | CH | |

protective for alien corpuscles. The heated sera are in general antihæmolytic for many kinds of corpuscles, against several, at least, different kinds of actively hæmolytic sera. Table II brings out this fact, and it also shows that heated dog serum neutralizes the action of fresh dog serum. There is, therefore, no indication of a specific antihæmolytic action of the heated sera.

TABLE II.

| Amount of heated normal serum added to 0.5 c.c. of fresh active dog serum | Sheep corpuscles | Ox corpuscles |
|---|---|---|
| Sheep serum (56° C) 1 c.c. | no hæmolysis | slight hæmolysis |
| Ox serum (56° C) 1 c.c. | moderate hæmolysis | moderate hæmolysis (in other instances none or complete). |
| Dog serum (56° C) 1 c.c. | moderate " | moderate hæmolysis. |
| 0.9% saline solution 1 c.c. | complete " | complete " |

NATURE OF ANTIHÆMOLYTIC ACTION.

It is necessary to decide whether the antagonistic action is directed against complement or amboceptor. A hæmolytic combination of inactivated anti-sheep serum (from goat) and guinea-pig complement was prepared. An amount of 0.1 cubic centimeter of the former and 0.01 cubic centimeter of the latter caused prompt lysis of the sheep corpuscles. Now, to one cubic centimeter of each of the inactivated normal sera 0.01 cubic centimeter guinea-pig complement was added, the mixture being kept at 37° C. for one hour. The sheep corpuscles were then added, and after another half hour, 0.1 cubic centimeter of inactive anti-sheep serum. Reading was made at the expiration of twenty hours; the result was as follows:

Inactivated normal sheep serum (56° C.) 1 c.c. = No hæmolysis.
" " ox " " 1 c.c. = " "
" " dog " " 1 c.c. = " "
Control (no normal serum) saline 1 c.c. = Complete hæmolysis.

The corpuscles were now separated from the different mixtures, resuspended in two cubic centimeters of 0.9 per cent. saline

solution, and 0.1 cubic centimeter guinea-pig complement added to each. The effect was as follows:

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Corpuscles treated with inactive sheep serum | | | | | | = | Marked hæmolysis | |
| " | " | " | " | ox | " | = | " | " |
| " | " | " | " | dog | " | = | Complete hæmolysis | |

To each of the separated fluids 0.4 cubic centimeter amboceptor for sheep corpuscles was added; no hæmolysis was produced by these mixtures showing no active complement to be present. Hence it is concluded that the antihæmolytic action is due to neutralization of complement.

It may be stated, in this place, that the corpuscles separated from the inactive sera do not undergo lysis as readily as the controls without serum. The latter underwent lysis with 0.01 to 0.02 cubic centimeter guinea-pig serum, while the former required 0.1 cubic centimeter, which amount produced incomplete lysis in some cases. This result might be taken to indicate that the action is anti-amboceptoric, a conclusion which later experiments disproved. Mere digestion of corpuscles in inactive sera raises the resistance to lysis of the corpuscles. The anti-complementary action of the heated sera is also brought out by the next experiments.

Anti-sheep amboceptor (goat) heated to 50° C. 0.4. c.c.
Dog serum heated to 70° 0.5 c.c.
Kept one hour at 37° C., after which were added sheep corpuscles and 0.02 c.c. guinea-pig serum. Result: Complete hæmolysis.
Guinea-pig serum 0.2 c.c.
Dog serum heated to 70° C. 0.5 c.c.
Kept one hour at 37° C., after which sheep corpuscles and finally anti-sheep amboceptor 0.4 c.c. were added. Result: No hæmolysis.

## INACTIVATION AND ANTILYSIN OF NORMAL SERA.

That temperatures approaching 60° C. rendered the majority of normal sera antihæmolytic, while somewhat lower temperatures (49° to 50° C.) merely inactivated them, has been established by Müller, Ehrlich and Sachs, Neisser and Friedemann, and others.[49] The changes in the serum through which an antilytic property is developed is considered by Ehrlich and Sachs to be

[49] *Op. cit.*

due to the formation of complementoid and by Neisser and Friedemann of amboceptoid. My own experiments have shown heated sera to be anticomplementary. The next experiments refer to the temperatures at which the antilysin develops.

Normal sheep, dog, and ox sera were tested against anti-sheep, anti-dog, and anti-ox sera. The anti-sera were prepared in rabbits. The anti-sera were inactivated at 50° C. and reactivated with guinea-pig serum. The titres of the anti-sera were about equal; 0.1 cubic centimeter anti-sera and 0.02 cubic centimeter guinea-pig complement was needed for complete hæmolysis. The normal sera and complement were first mixed and after the usual interval amboceptor and corpuscles were added. Table III shows that none of the normal sera except that of the ox acting on its own cells possesses in the fresh state any antilytic action; these sera heated to 50° C. develop sometimes antilytic properties, while a temperature of 60° C. brings out, almost uniformly, this activity. The absence in normal sera of antilytic power may be due to a masking of antagonistic action by interaction of suitable complements and amboceptors, but judging from the effect of the temperature of 50° C. it is more probable that the antilytic principle is present in the sera but is prevented from reacting with complements. If we turn now to a consideration of the action of normal sera in these several states upon a normal serum hæmolysin we shall see a considerable difference of effects. The antilytic action of sheep, ox, and dog sera was studied upon normal dog serum, the complete hæmolytic power of which was for ox corpuscles 0.35 cubic centimeter and for sheep corpuscles 0.25 cubic centimenter. Table IV exhibits the results and is to be compared with Table III.

The different effects brought out in Tables III and IV are considerable. Fresh normal serum of sheep and ox protect their own and each other's corpuscles from lysis by dog serum, but they are able only after heating to 56° C. or higher to protect these corpuscles from lysis by immune sera. In what does this distinction exist? Are the lysins of normal and immune sera distinct and do the normal antilysins act upon one set of lysins only, or are they identical, and do differences in power introduce

a new factor? The fact that heating the normal sera abolishes the differences in effect seems to point to a similarity in nature of the antilysins acting upon normal and immune sera. But in this case the antilysins must exist in different states in

TABLE III.

| Immune amboceptor +Complement. | Sheep serum 0.8 c.c. | | | | Dog serum 0.8 c.c. | | | | Ox serum 0.8 c.c | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Fresh | 50° C | 60° C | No serum | Fresh | 50° C | 60° C | No serum | Fresh | 50° C | 60° C | No serum |
| Anti-sheep amboceptor 0.1 guinea pig complement 0.02 | CH | moderate | none | CH | CH | CH | trace | CH | CH | CH | moderate | CH |
| Anti-dog amboceptor 0.2 guinea pig complement 0.02 | CH | CH | trace | CH | CH | much | none | CH | CH | CH | moderate | CH |
| Anti-ox amboceptor 0.1 guinea pig complement 0.05 | CH | much | slight | CH | CH | CH | none | CH | slight | moderate | CH* | CH |

*Slightly quicker than in absence of serum.

TABLE IV

| Dog serum and corpuscles. | Sheep serum 1.0 c.c. | | | | Ox serum 1.0 c.c. | | | | Dog serum 1.0 c.c. | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Fresh | 50°C | 60C° | No serum | Fresh | 50°C | 60°C | No serum | Fresh | 50°C | 60°C | No serum |
| Dog serum 0.25 vers. Sheep corpuscles | none | none | none | CH | trace | trace | trace | CH | CH | CH | none | CH |
| Dog serum 0.35 vers. Ox corpuscles | none | none | none | CH | none | trace | CH | CH | CH | CH | trace | CH |

the sera through which the two different actions are produced. I conceive that the antilysin exists in combination with other bodies in normal sera, and that part of it is easily extracted from this combination. Another and larger part, possibly, is liberated when the serum is heated to 50° C. All is set free when the temperature is raised to 60° C. The quantity which is readily yielded suffices to neutralize the complement which reacts with the amboceptors of normal sera, but is insufficient to neutralize the complement which reacts with the amboceptors of immune sera. At 50° C. a sufficiency of the antilysin is liberated to neutralize all the complement in the native sera, hence this inactivation; but as it rarely happens that a quantity in excess of this is liberated, sera heated to this temperature tend not to be antilytic. Since at 60° C. all antilysin is rendered available,

the antihæmolytic action is exhibited at its height. Perhaps the spontaneous disappearance of complement is caused by slow liberation of the antilysin in the shed blood.

Another possible explanation should be considered. The complement-antilysin is reversible. In the case of normal serum the degree of reversibility is small; but the large number of amboceptors contained in immune sera may through mass action possess the power of splitting the loose compound and appropriating the complement.

It is desirable to study more clearly the neutralizing action of heated dog serum upon fresh dog serum-hæmolysis of sheep and ox corpuscles. Samples of dog serum were heated respectively to 50°, 56°, 70°, and 90° C. In the case of the last two specimens the serum was diluted with double the volume of 0.9 per cent. salt solution. In the tests which follow guinea-pig complement was employed. The heated serum and complement were first mixed and kept for one hour at 37° C.; the corpuscles were next added, and after another half hour the normal and immune sera were introduced. The total volume was in each test three cubic centimeters, and the final reading was made at the end of two hours standing at 37° C. Table V gives the results, which may be expressed as follows: At 50° C. dog serum does not develop the antagonistic properties; at 56° and 70° C. the serum is rendered highly antagonistic—anticomplementary against the several combinations employed. Heated to 90° C. the serum loses the antihæmolytic action. Since serum complements and amboceptors are destroyed by temperatures of 70° C. the antilytic action here described cannot be explained by supposing the production of complementoid or amboceptoid. The disappearance of anticomplementary action in serum heated to 90° C. may, possibly, be explained by supposing (1) destruction, (2) the formation of a new fixed compound of antilysin and serum constituent, (3) the masking of anticomplementary by auxilytic action. Since, as will be shown later in this paper, I have succeeded in separating in an active state the anticomplementary substance, and since it shows, in this form, marked thermostability, the first supposition may be dismissed. To decide between the last

two possibilities is less easy, but the following facts may be stated: Sera heated to from 80° to 90° C. becomes auxilytic; but, when deprived of lipoids, heating to these temperatures does not bring out this property. The progress of hæmolysis when serum heated to 90° C. is present is slow as compared with the controls, a result that may be accounted for by supposing the interaction of two opposing forces, anticomplementary and auxilytic, which are not absolutely balanced.

Inasmuch as normal dog serum acquires when heated to 56°, or higher, antagonistic properties against the same serum in the fresh state, it is difficult to account for the phenomenon on the basis of complementoid or amboceptoid production.

TABLE V.

| | Normal dog serum 0.5 c.c. each. | | | | Control no serum. |
|---|---|---|---|---|---|
| | 50° C—30″ | 56° C—30″ | 70° C—15″ | 90° C—15″ | |
| Anti-sheep amboceptor (goat) (50° C.) 0.4 guinea pig complement 0.02 | CH | none | none | CH slow | CH |
| Anti-sheep amboceptor (rabbit) (50° C.) 0.1 guinea pig complement 0.01 | CH | none | none | CH slow | CH |
| Anti-ox amboceptor (rabbit) (50° C.) 0.1 guinea pig complement 0.02 | CH | none | none | CH slow | CH |
| Normal dog amboceptor (50° C.) 0.5 guinea pig complement 0.4 guinea pig corpuscles. | CH | slight | none | CH slow | CH |
| Normal dog amboceptor (50° C.) 0.5 guinea pig complement 0.4 Sheep corpuscles. | CH | trace | slight | CH slow | CH |
| Normal dog serum 0 25 sheep corpuscles. | CH | trace | trace | CH slow | CH |

ABSORPTION OF THE ANTILYSIN BY BLOOD CORPUSCLES.

Since heated serum interferes with hæmolysis when brought into fluid relation with the hæmolytic serum, the question arises whether this action is exerted only against the serum hæmolysin or

whether it influences the corpuscles themselves. The experiments already related show, quite conclusively, that the thermostable antilysin is anticomplementary in effect. It is to be assumed that, under the condition of experiments given, the complement and antilysin combine to form, as far as hæmolysis is concerned, an inert compound. These effects do not indicate whether in the reactions the corpuscles behave in an entirely passive manner. The next experiments to be described show that under certain conditions the corpuscles may take up the antilysin, through the appropriation of which they show an increased resistance to hæmolytic substances.

If blood corpuscles are suspended in serum heated from 56° to 70° C., at 37° C. for several, at room temperatures for eighteen, hours, separated and washed free from serum, they will be found to have become altered in susceptibility to serum hæmolysis. Tables V, VI, VII, and VIII give the results of three such experiments. Thoroughly washed sheep corpuscles, one cubic centimeter, were mixed with four cubic centimeters of the several heated normal sera and kept at 37° C. for six hours. The separated corpuscles were washed with 0.9 per cent. salt solution, thirty cubic centimeters being used at each washing. The corpuscles were then subjected to the action of anti-sheep serum (inactive) and guinea-pig complement (Table VI), or fresh rabbit serum (Table VII), or fresh dog serum (Table VIII).

TABLE VI.

| Anti-sheep amboceptor (50°C.) 0.1 c.c. | Normal sera used for digesting sheep corpuscles. | | | |
|---|---|---|---|---|
| Quantity of complement | Rabbit No. 11 (60° C.) | Ox serum (60° C.) | Dog serum (60° C.) | Control untreated corpuscles. |
| 0.15 c.c. | much H | CH | moderate H | CH |
| 0.1 " | none | CH | none | CH |
| 0.05 " | none | much H | none | CH |
| 0.03 " | none | slight | none | almost CH |
| 0.02 " | none | none | none | much H |

TABLE VII.

| Fresh rabbit serum | Normal sera used for digesting sheep corpuscles. | | | |
|---|---|---|---|---|
| | Rabbit No. 12 (60° C.) | Ox serum (60° C.) | Dog serum (60° C.) | Control untreated corpuscles |
| 0.5 c.c. | none | CH | CH | CH |
| 0.3 " | none | much | much | CH |
| 0.2 " | none | moderate | moderate | almost CH |
| 0.15 " | none | slight | slight | moderate |
| 0.1 " | none | trace | trace | slight |

TABLE VIII.

| Fresh dog serum | Normal sera used for digesting sheep corpuscles. | | | |
|---|---|---|---|---|
| | Rabbit No. 13 (60° C.) | Rabbit No. 14 (60° C.) | Ox serum (60° C.) | Control untreated corpuscles. |
| 0.2 c.c. | CH | CH | CH | CH |
| 0.1 " | moderate | much | CH | CH |
| 0.07 " | none | none | slight | CH |
| 0.05 " | none | none | slight | much |
| 0.04 " | none | none | trace | slight |
| 0.03 " | none | none | none | trace |
| 0.02 " | none | none | none | none |

TABLE IX.

| | Temperatures to which the normal dog serum was heated before being employed for digesting corpuscles from the same blood. | | | | | |
|---|---|---|---|---|---|---|
| | Fresh | 50° C | 56° C | 70° C | 90° C | Untreated corpuscles |
| Anti-dog amboceptor 0.05 c.c. guinea pig complement 0.02 c.c. | CH | CH | slight H | trace H | slight H. | CH |

TABLE X.

| Amount of Serum | Normal dog serum (56° C.) | | Normal rabbit serum (56° C.) | |
|---|---|---|---|---|
| | Digested | Not digested | Digested | Not digested |
| 0.5 c.c. | CH | none | CH | none |
| 0.3 " | " | " | " | none |
| 0.2 " | " | " | " | trace |
| 0.1 " | " | trace | " | slight |
| 0.05 " | " | almost CH | " | almost CH |
| 0.03 " | " | CH | " | CH |
| 0.02 " | " | " | " | " |
| Control, no serum | CH | CH | CH | CH |

TABLE XI.

| Amount of digested serums. | Dog serum 56° C. | | | Sheep serum 56° C. | | | Ox serum 56° C. | | |
|---|---|---|---|---|---|---|---|---|---|
| | Rabbit cells | Ox cells | No digestion | Rabbit cells | Ox cells | No digestion | Rabbit cells | Ox cells | No digestion |
| 0.4 c.c. | slight | CH | none | CH | CH | none | much | CH | none |
| 0.3 c.c. | moderate | CH | none | CH | CH | trace | almost H | CH | trace |
| 0.2 c.c. | much | CH | none | CH | CH | slight | CH | CH | slight |
| 0.1 c.c. | CH | CH | trace | CH | CH | much | CH | CH | CH |

The tables show that sheep corpuscles treated with several heated sera have their resistance to serum hæmolysis increased and that the effect is overcome by increasing the quantity of complement in the mixture (Table VII) or the quantity of the normal hæmolytic sera (Tables VI and VIII). In these tests the heated sera were foreign to the corpuscles; the next tests were made with serum and corpuscles from the same animal. Table IX shows that if dog corpuscles are digested in heated dog serum they acquire the power of resisting a combination of amboceptor and complement which completely hæmolyzes untreated corpuscles, and, also, that this property of protecting the corpuscles is developed by a temperature of 56° C. but not of 50° C. A second experiment with serum and corpuscles shows that at 56° C. the property is likewise developed in this serum for its own corpuscles.

The evidences which were obtained of the absorption by corpuscles of some substance present in heated serum which interferes with serum hæmolysis would have led naturally to the ascertainment of a corresponding disappearance of the substance from the sera in which the corpuscles were digested. It happened that this phenomenon was observed before the increase in resistance of the treated corpuscles was discovered and the observation led to the discovery of the protection of corpuscles. Table X shows that heated dog and rabbit sera, digested with corpuscles, lose their antihæmolytic property. Two cubic centimeters of dog and rabbit sera were heated to 56° C. and digested with one cubic centimeter of highly condensed (equal to five cubic centimeters original blood) washed sheep corpuscles at

20° C. for eighteen hours. The sera separated from the corpuscles were tested along with the heated sera in which no corpuscles had been placed. Anti-sheep amboceptor (inactive) 0.1 cubic centimeter and guinea-pig complement 0.1 cubic centimeter were used as the hæmolytic combination. Similar experiments to this did not always give uniform results, due doubtless to difference in degree of extractions of the antilytic bodies.

The similarity of the influence of the sheep corpuscles upon the heated rabbit and dog sera in removing antilytic bodies may be taken to indicate that the removal of the antilysin from the sera is not an act of elective absorption, and hence that these antilysins are probably not specific bodies. The experiment summarized in Table XI proves conclusively that any kind of corpuscles can deprive the heated sera of the protective property. Normal dog, sheep, and ox sera were heated to 56° C. and two cubic centimeters of each were treated with one cubic centimeter of condensed rabbit corpuscles. Heated dog and sheep sera were also treated with ox corpuscles. The separated sera were tested for antihæmolytic power.

### EXTRACTION OF ANTILYSIN FROM SERUM AND CORPUSCLES.

The next step was to obtain, if possible, this thermostabile anticomplementary substance in a state of greater concentration. The earlier work of Landsteiner and v. Eisler, and Bang and Forssman, and my own observations indicated that the body probably occurred among the lipoid constituents of the blood. It was, therefore, among these that I sought it. The method which I employed does not differ essentially from the extraction method of Bang and Forssmann. I employed the separated serum and corpuscles for my work. Two hundred cubic centimeters of dog and sheep blood were shaken separately with 400 cubic centimeters of ether in a shaking apparatus, and the ethereal extracts separated and dried. These were now extracted with boiling benzol and the residue discarded. The benzol extract was dried and extracted, in turn, with aceton in the cold. Two fractions were obtained: the insoluble fraction consisting chiefly

of lecithin and other phosphatids, and the soluble fraction consisting of cholesterin, fatty acids, neutral fats, and probably other less defined bodies. While the reaction of the insoluble fraction was neutral, the reaction of the soluble fraction was acid. The fractions deprived of aceton were emulsified in forty cubic centimeters each of saline solution. The insoluble fraction exercised no protective or anticomplementary action; hence it was discarded. The soluble fraction, on the other hand, exerted powerful protective activities that were not reduced by filtration through compressed filter paper which removed suspended particles. For the sake of brevity I will speak from now on of this saline extract as "protectin," the effects of which I proceeded to compare with those of the heated sera.

ANTICOMPLEMENTARY AND NON-SPECIFIC ACTIONS OF PROTECTIN.

The next step was, therefore, to expose susceptible blood corpuscles to the action of a hæmolytic combination in the presence of "protectin" from sera and cells and to compare the effects with suitable controls. For the experiments sheep corpuscles, anti-sheep serum (goat, inactivated at 50° C.) 0.2 cubic centimeter and guinea-pig complement 0.1 cubic centimeter were employed. Tables XII, XIII, and XIV show the results, which may be stated as follows: Protectin prevents hæmolysis of sheep corpuscles quite as completely as do heated sera and agrees with the heated sera in failing to exhibit evidences of specific activity. I now proceeded to ascertain whether the action was upon the complement of the hæmolytic combination or upon some other constituent. Table XV gives the results obtained and is taken to indicate action upon the complement of the hæmolytic complex. An identical result was obtained with sheep protectin.

ABSORPTION OF PROTECTIN BY CORPUSCLES.

Since up to this point the protectin solution acted in a manner which was not to be distinguished from the heated sera, a test of the power of corpuscles to absorb the active agent from the

solution was next made. Table XVI shows that in this respect the protectin solution is comparable with the heated sera.

### EXTRACTION OF PROTECTIN FROM DRIED AND HEATED SERA AND CORPUSCLES.

There was available for preparing the protectin a large quantity of dried sera and corpuscles. These products had been collected, dried, and subjected for thirty minutes to a temperature of 150° C. I had at my disposal the sera and corpuscles of dog, ox, and horse which, after the treatment just mentioned, had been on hand for nearly two years. The several products were powdered and extracted in a shaker with ether (forty-eight hours), after which the further steps were identical with those already given. I was thus enabled to obtain relatively large quantities of the protectin fraction which was tested against various normal and immune sera with the results already given. By means of many tests I came to the conclusion that both blood serum and corpuscles, of all the mammals studied by me, contain a body which has the property of protecting corpuscles of different mammals from the effects of serum hæmolysins, and that this body is highly stable and very resistent to heat.

TABLE XII.

| Amount of protectin in c.c. | Source of protectin | | | |
|---|---|---|---|---|
| | Dog | | Sheep | |
| | Serum-protectin | cell-protectin | Serum-protectin | Cell-protectin |
| 0.5 | none | none | none | none |
| 0.4 | | | " | |
| 0.3 | | | trace | |
| 0.2 | trace | | slight | trace |
| 0.15 | " | | " | " |
| 0.1 | slight | | much | slight |
| 0.07 | moderate | trace | almost CH | " |
| 0.05 | almost CH | slight | CH | almost CH |
| 0.03 | CH | " | | CH |
| 0.02 | | almost CH | | |
| 0 (control) | | CH | | |

TABLE XIII.

Sheep corpuscles were to be protected from fresh active immune serum (goat).

| Amount of anti-sheep serum | Control (no protectin) | Sheep cell-protectin 0.2 c.c. |
|---|---|---|
| 0.3 c.c. | CH | trace |
| 0.2 " | | |
| 0.15 " | | |
| 0.1 " | | |
| 0.07 " | much H | none |
| 0.05 " | slight | |
| 0.04 " | trace | |
| 0.03 " | none | |

TABLE XIV.

Sheep corpuscles were to be protected from fresh normal dog serum.

| Amount of dog serum | Control (no protectin) | Sheep cell-protectin 0.4 c.c. | Dog cell-protectin 0.4 c.c. |
|---|---|---|---|
| 1.0 c.c. | | moderate | slight |
| 0.7 " | | slight | " |
| 0.5 " | | | |
| 0.5 " | CH | | |
| 0.3 " | | | |
| 0.2 " | | | |
| 0.1 " | | none | none |
| 0.07 " | | | |
| 0.05 " | slight | | |

TABLE XV.

1) Dog serum-protectin 0.3 c.c. + g. p. complement 0.1, 1 hour at 37° C. + anti-sheep amboceptor 0.2 c.c. + corpuscles = no H.
2) Dog serum-protectin 0.3 c.c. + g. p. complement 0.2 c.c., 1 hour at 37° C. + anti-sheep amboceptor 0.2 c.c. + corpuscles = CH.
3) Dog serum-protectin 0.3 + g. p. complement 0.1 c.c., 1 hour at 37° C. + anti-sheep amboceptor 0.4 c.c. + corpuscles = no H.
4) No protectin (control) + g. p. complement 0.1 c.c., 1 hour at 37° C. + anti-sheep amboceptor 0.2 c.c. + corpuscles = CH.

TABLE XVI.

1) Sheep corpuscles 0.5 c.c. + sheep cell-protectin 4.0 c.c. kept 4 hours at 37° C., after which the corpuscles were separated from the fluid and washed in 0.9% saline solution.
This will be called "sheep cell-protectin treatment."
2) Sheep corpuscles 0.5 c.c. + dog cell-protectin 4.0 c.c. kept 4 hours at 37° C.,

after which the corpuscles were separated from the fluid and washed in 0.9% saline solution.

This will be called "dog cell-protectin treatment."

The hæmolytic complex was: anti-sheep amboceptor (50° C.) 0.1 c.c. and guinea pig complement 0.02 c.c.

Observation: 30 minutes at 37° C.

Result: "Sheep cell-protectin treatment" plus hæmolyser give no hæmolysis.
"Dog cell-protectin treatment" plus hæmolyser give slight hæmolysis.
Untreated sheep corpuscles plus hæmolyser give complete hæmolysis.

This condition of thermostability is one of its most interesting features, and brings it, at first sight, into contrast with the thermostabile, so-called protective principles of normal serum

TABLE XVII.

| Temperature | Duration of heating | Color | Reaction | Antihæmolytic power |
|---|---|---|---|---|
| 100° C | 1 min. | water clear | acid | no H |
| | 5 min. | " | " | |
| | 15 min. | " | " | " |
| | 30 min. | " | " | " |
| | 60 min. | " | " | " |
| | 90 min. | " | " | trace H |
| | 120 min. | trace yellow | neutral | slight H |
| 120° C | 10 min. | light yellow | " | " |
| 135° C | 10 min. | light amber | alkaline | moderate H |
| 150° C | 10 min. | amber | " | " |
| unheated | | | acid | no H |

Remarks: To raise the temperature from 100° C. to 120° C. 40 minutes, from 120° to 135° C. 10 minutes, and from 135° to 150° C. 10 minutes are required. To cool the antoclave from 150° to 100° C. it took about 60 minutes.

The question presents itself whether bodies which exhibit such marked differences in properties can possibly be identified with each other. My own views, already outlined on pages 732 and 736, make it possible for me to conceive readily that they are identical. It will be unnecessary to repeat here what I have already stated regarding my reason for thinking that loss of protective property of serum when heated to 90° C. is not due to destruction of the protective body, but to development of auxilytic compounds or recombination of the former body.

My next experiment was to ascertain the effects of high temperatures upon the protectin solution. Dog corpuscle protectin in 0.9 per cent. salt solution was placed in tubes, which were

hermetically sealed. Table XVII shows the temperatures to which the tubes were heated and the alteration in physical appearances which resulted. The antihæmolytic power of the solutions was tested against anti-sheep serum (50° C.) 0.2 cubic centimeter, guinea-pig complement 0.05 cubic centimeter, and sheep corpuscles. The controls give complete hæmolysis. The quantity of the protectin used in each tube was 0.2 cubic centimeter. From the table it will be seen that the solution is highly resistant to heat, but that a diminution in activity, associated with changes in reaction, occurs at the higher temperatures.

## SUMMARY.

Blood serum contains normally certain principles which inhibit serum hæmolysis by interfering with the action of complement. In the case of most sera the anticomplementary action appears only after heating to 56° C. or higher.

The inhibiting action of this principle is directed against alien as well as native complements, and is non-specific in character.

It would appear as if the inactivation of serum at 50° C. or thereabouts were due to a partial liberation of the antilytic principle which just about suffices to neutralize the action of the native complement. As the temperature of the serum is raised, up to a certain point, more and more of the antilysin is set free until the serum comes to contain an overplus, in excess of the quantity neutralizing its own complement, which is capable of interfering with the action of additional native or alien complements.

Serum heated to 90° C. is less antilytic than serum heated to 70° C., either because the antilysin has entered into new, more stable compounds which prevent its action, or, as is more probable, because of the liberation from the serum of a second group of principles in themselves hæmolytic directly, or indirectly by increasing the power of serum hæmolysins (auxilysins). This latter action tends to mask and to suppress the inhibitory influences of the antilysin.

The antilysin is removed from serum by digestion with many

kinds of blood corpuscles which, apparently, absorb the principle. While, by reason of this treatment, the serum is deprived of its inhibitory power, the corpuscles have acquired greater resistance to serum hæmolysins.

By treating blood serum and corpuscles with ether the antilysin can be extracted. The ethereal extract, freed from lecithin and certain related bodies, contains the antilysin in a concentrated but not in a pure form, which can now be taken up in saline solution in which it dissolves.

The saline solution of the antilysin, which for convenience has been denominated "protectin," behaves in all respects like the native antilytic sera except that it is uninfluenced by temperatures of 90° C. or even higher temperatures. Protectin inhibits serum hæmolysis directly by neutralizing complement and indirectly after absorption by corpuscles by increasing their resistance. Hence it is highly probable that the antilytic principle of heated serum and protectin extracted from serum and corpuscles are the same substance.

Thermostability is one of the characteristic properties of protectin. Serum and corpuscles, first dried, may be heated to 150° C. without losing the protecting principle, and the principle persists in such heated products for at least two years. Protectin in solution is unaffected by a temperature of 100° C. maintained for one hour, and suffers only slight reduction in activity at the expiration of two hours; while temperatures ranging from 135° to 150° C. bring about marked reduction in protective power. As these alterations are produced by high temperatures the reaction of the solution changes from acid, through neutral, to alkaline.