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SOME OBSERVATIONS ON THE CONSTITUTION OF THE COMPLEMENTS OF DIFFERENT ANIMALS

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In the past a considerable amount of attention has been paid by serological workers to the complex characters of serum-complement and many attempts have been made to subject complement to more detailed biochemical analysis. Valuable information has thus been obtained regarding the biological action and properties of this important element of animal serum.

The study of complement has been principally based on its cytolytic effects towards red blood corpuscles sensitised with the homologous immune body, and the general tendency of research on this subject has been to elicit the complexity of constitution of complement and the numerous factors on which its action depends.

The fresh serum of the guinea-pig represents with ox corpuscles + rabbit versus ox immune body or cobra venom, one of the most active complements and it has therefore been commonly used for studies on complement action.

Attention was first drawn by Stephens (1) to the hemolytic effect of certain snake venoms along with fresh serum. In the case of guinea-pig's serum, which has a powerful activating effect, this characteristic property is annulled by heating the serum at 55°C. and corresponds as regards thermolability to the complementing action of serum with immune body. Observations made by Browning and Mackie (2) on the complementing action of serum with immune body in relation to its hemolytic effect with cobra venom showed that the venom-activating constituent of serum though similar in many characters to the complement which acted with immune body was not identical with the latter.

Ferrata (3) and later Brand (4) Liefman (5) Sachs (6) demonstrated that complement could be fractioned into two components neither of which exhibited any degree of complementing effect by itself, although together they reproduced the full hemolytic activity of the native serum. These two moieties were designated "end-piece" and "mid-piece" the latter combining directly with red blood corpuscles + immune body.

In Liefman's method carbon dioxide gas was passed through serum diluted with distilled water and this led to the precipitation of part of the globulins of the serum which represented the "mid-piece" while the "end-piece" consisted of the albumin and that portion of the globulin still remaining in solution. These fractions were carefully studied by Browning and Mackie in the case of guinea-pig's serum and it was found that the venom-activating constituent could also be fractioned into two similar components.

A "third component" of complement was described by Ritz (7) which had no end-piece or mid-piece properties and unlike these components was invariably stable at 57°C. This constituent was demonstrated by inactivating complement with venom and by the restoration of its activity on the addition of heated serum.

It was subsequently found (Browning and Mackie (8)) that complement could be fractioned into four different components by Liefman's method followed by precipitation of the proteins in different concentrations of ammonium sulphate. These four components were represented respectively by the albumin, pseudoglobulin from "end-piece," pseudoglobulin from "mid-piece" and euglobulin. None of these fractions corresponded to any of the previously described complement components "end-piece," "mid-piece" or "third component." These observations were carried out with guinea-pig's serum and the general results showed that the complement constituents were distributed over all the different proteins of the serum but appeared to be concentrated chiefly in the pseudoglobulin. Of the four components, three, including always the albumin, were generally necessary for full restoration of complement action and the albumin

fraction appeared to represent an essential constituent of the complement.

While it is doubtful if albumin, pseudoglobulin and euglobulin separated by ammonium sulphate constitute homogeneous protein entities (Martin and Chick (9)), there is strong evidence that they represent different complement constituents, and Liefman's method certainly elicits a striking difference between the two moieties of the pseudoglobulin separated by carbon dioxide. With a view to throwing further light on the structure of complement and especially the venom-activating constituent of serum, further experiments have been carried out with the sera of certain other animals.

The technique followed was that originally described in the Journal of Pathology and Bacteriology and the Zeitschrift für Immunitätsforschung (8).

The hemolytic systems used were (1) ox red blood corpuscles + immune body (rabbit versus ox), and (2) ox red blood corpuscles + cobra venom. The sera of man, rabbit, and horse were selected for comparison with guinea-pig's complement.

With ox corpuscles + immune body rabbit versus ox the average minimal hemolytic dose of these sera as shown by Muir (11) are: Guinea-pig, 0.01 cc.; rabbit, 0.1 cc.; man 0.11 cc.; horse, ∞ cc.; for 1 cc. of a 5 per cent suspension of blood.

EXPERIMENTS WITH RABBIT'S SERUM

Various specimens of rabbit's serum were fractioned by Liefman's carbon dioxide method into "end-piece" and "mid-piece" and the two moieties were further subdivided by the ammonium sulphate method.

The different globulins were then tested separately and in certain combination as regards their complementing action with immune body.

Table 1 demonstrates the results of one of these experiments; the pseudoglobulin from end-piece showed distinct activity which was increased to the standard of the native serum by the addition of the pseudoglobulin from mid-piece; the latter by itself

displayed no complementing properties. The addition of euglobulin to the mixture of pseudoglobulins did not further add to the hemolytic value. A mixture of the pseudoglobulin from end-piece and euglobulin also yielded a fully active complement but euglobulin along with pseudoglobulin from mid-piece was quite inactive.

Thus the complementing property of rabbit's serum is invariably resident in the globulins and distributed among them; but a mixture consisting of only two of these globulin fractions,

TABLE 1

RABBIT'S SERUM	LYSIS OF 0.5 CC. OX BLOOD SUSPENSION + 5 DOSES OF IMMUNE BODY				
	0.01 cc.	0.025 cc.	0.05 cc.	0.075 cc.	0.1 cc.
Native complement	tr.	dist.	c.	c.	c.
Albumin	0	0	0	0	0
Pseudoglobulin from end-piece	0	0	0	dist.	c.
Pseudoglobulin from mid-piece	0	0	0	0	0
Euglobulin	0	0	0	0	0
Pseudoglobulin from end-piece + pseudoglobulin from mid-piece	0	tr.	c.	c.	c.
Pseudoglobulin from end-piece + pseudoglobulin from mid-piece + euglobulin	0	dist.	c.	c.	c.
Pseudoglobulin from mid-piece + euglobulin	0	0	0	0	0
All four components	0	dist.	c.	c.	c.
Pseudoglobulin from end-piece + euglobulin	0	dist.	c.	c.	c.
Albumin + pseudoglobulin from mid-piece	0	0	0	0	0
Albumin + euglobulin	0	0	0	0	0

In this and in subsequent tables: tr. = trace; f.tr. = faint trace; dist. = distinct; mk. = marked; c. = complete; j.c. = just complete; al.c. = almost complete.

provided they do not both belong to the mid-piece, is sufficient to reconstitute the complement. In the case of rabbit's serum also certain constituents may be considered as interchangeable. It is to be noted that there is an actual qualitative differentiation of the pseudoglobulin of end-piece and that contained in the mid-piece fraction. This was also noted in the case of guinea-pig's serum. In contrast with guinea-pig's serum however the albumin fraction does not appear to contain any complement constituents.

In general, rabbit's serum has no activating effect with cobra venom and ox's corpuscles, though with immune body it shows marked complementing action.

TABLE 2A

RABBIT'S SERUM	LYSIS OF 0.5 CC. OF 5 PER CENT SUSPENSION OX BLOOD + 0.0005 GRAM COBRA VENOM						
	0.04 cc.	0.1 cc.	0.16 cc.	0.2 cc.	0.24 cc.	0.3 cc.	0.36 cc.
Fresh serum	0	0	0	0	0	0	0
Globulin precipitate by $(NH_4)_2SO_4$	0	0	tr.	dist.	mk.	al.c.	c.
Albumin separated by $(NH_4)_2SO_4$	0	0	0	0	0	0	0
Globulin ($(NH_4)_2SO_4$) + Albumin ($(NH_4)_2SO_4$)	0.2 cc. + 0.05 cc. = 0	0.24 cc. + 0.06 cc. = 0	0.3 cc. + 0.075 cc. = 0	0.36 cc. + 0.09 cc. = 0			

TABLE 2B

RABBIT'S SERUM	LYSIS OF 0.5 CC. OF 5 PER CENT OX BLOOD SUSPENSION + 5 DOSES IMMUNE BODY						
	0.01 cc.	0.025 cc.	0.05 cc.	0.075 cc.	0.1 cc.	0.2 cc.	0.3 cc.
Native serum	0	mk.	al.c.	c.	c.	c.	c.
Globulin $(NH_4)_2SO_4$ method		mk.	c.	c.	c.	c.	c.
Albumin $(NH_4)_2SO_4$ method	0	0	0	0	0	0	0
Globulin + albumin $(NH_4)_2SO_4$ method		mk.	c.	c.	c.	c.	c.
	LYSIS OF 0.5 CC. OF OX BLOOD SUSPENSION (NO IMMUNE BODY)						
Globulin $(NH_4)_2SO_4$	0.3 cc. = no lysis						
Native serum	0.3 cc. = no lysis						

It was found however that the globulins separated by half saturation with ammonium sulphate showed distinct complementing action with cobra venom.

The albumin was inactive and also inhibited the action of the globulin even in doses corresponding to one-fourth of the doses of globulin used.

These experiments clearly demonstrate that rabbit's serum, which had no power of producing hemolysis of venomised corpuscles, still contained venom activating constituents which were resident in the globulin fraction. These constituents were, like complement, thermolabile (at 55°C.). In the case of hemolysis with immune body, as already shown, the albumin exerted no inhibitory action (table 2 B).

The globulin fraction of rabbit's serum apparently represents the whole complement of the serum both for immune body and venom but in the case of venom the native serum is inactive in

TABLE 3

	LYSIS OF 0.5 CC. OF 5 PER CENT OX BLOOD SUSPENSION 0.0005 GRAM COBRA VENOM					
Guinea-pig's complement	0.0075 cc.	0.01 cc.	0.02 cc.	0.04 cc.	0.06 cc.	0.1 cc.
+	+	+	+	+	+	+
Rabbit's "end-piece"	0.015 cc. dist.	0.02 cc. mk.	0.04 cc. dist.	0.06 cc. tr.	0.12 cc. f.tr.	0.2 cc. 0
Guinea-pig's complement	0.0075 cc. j.c.	0.01 cc. c.	0.02 cc. c.	0.04 cc. c.		
Guinea-pig's complement	0.0075 cc.	0.01 cc.	0.0 cc.	0.04 cc.	0.06 cc.	0.1 cc.
+	+	+	+	+	+	+
Guinea-pig's "end-piece"	0.015 cc. c.	0.02 cc. c.	0.04 cc. c.	0.08 cc. c.	0.012 cc. c.	0.2 cc. c.

Controls
0.2 cc. guinea-pig's end-piece=0
0.2 cc. rabbit's end-piece=0

virtue of inhibition by the albumin. It was concluded that the deficiency of the whole serum in this respect was due to the albumin antagonizing or "masking" the activity of the globulin.

It was found also that rabbit's serum-albumin inhibited the action of guinea-pig's serum with venom. In the experiment shown (table 3), varying amounts of guinea-pig's complement mixed with quantities of rabbit's albumin (end-piece) representing respectively double these amounts of rabbit serum, were tested in series with 0.5 cc. of ox blood suspension + cobra venom. There was marked inhibition and a zone phenomenon was produced.

Corresponding mixtures of guinea-pig's complement with guinea-pig's end-piece show no inhibition of lysis. This experiment shows an interesting difference in the albumin fraction of these two sera in regard to cobra venom hemolysis.

EXPERIMENTS WITH HUMAN SERUM

Specimens of fresh human sera were also investigated in the light of the findings with rabbit's serum and it was found that

TABLE 4

HUMAN SERUM	LYSIS OF 0.5 CC. OF OX BLOOD SUSPENSION + 0.0005 GRAM COBRA VENOM						
	0.01 cc.	0.025 cc.	0.05 cc.	0.075 cc.	0.1 cc.	0.15 cc.	0.5 cc.
Fresh serum	0	0	0	0	0	0	0
Globulin precipitated by $(NH_4)_2SO_4$ method	0	0	tr.	dist.	mk.	j.c.	
Albumin precipitated by $(NH_4)_2SO_4$ method	0	0	0	0	0	0	
Albumin+Globulin $(NH_4)_2SO_4$ method	0	0	0	0	0	0	

HUMAN SERUM	LYSIS OF 0.5 CC. OF OX BLOOD SUSPENSION + 5 DOSES IMMUNE BODY					
	0.01 cc.	0.025 cc.	0.05 cc.	0.075 cc.	0.1 cc.	0.15 cc.
Fresh serum	mk.	j.c.	c.	c.	c.	c.
Globulin	dist.	v.mk.	c.	c.	c.	c.
Albumin	0	0	0	0	0	0
Albumin+globulin	mk.	al.c.	c.	c.	c.	c.

Controls

0.5 cc. of suspension (no immune body nor venom)+human serum 0.5 cc.=no lysis.

0.5 cc. of suspension (no immune body nor venom)+globulin 0.2 cc.=no lysis.

the globulin fraction was actively hemolytic in the presence of venom even when the native serum had no action. Sera were fractioned into globulin and albumin by the ammonium sulphate method and the fractions were tested with immune body and with venom; it was observed that the globulin displayed practically the full complementing action of the serum for immune body.

In the case of venom the action of the globulin was inhibited by the albumin. Table 4 shows the results. In the experiment quoted the mimimum dose of human complement with immune body was relatively small, about 0.025 cc. for 0.5 cc. of the sensitised suspension. Observations were also made as to the power of the albumin of human serum to inhibit the hemolytic action of guinea-pig's serum for venomised corpuscles. For convenience end-piece of human serum separated by carbon-dioxide was employed and it was found to exert a marked inhibiting effect (table 5).

TABLE 5

	LYSIS OF 0.5 CC. OF OX BLOOD SUSPENSION + 0.0005 GRAM COBRA VENOM					
	0.005 cc.	0.01 cc.	0.02 cc.	0.04 cc.	0.06 cc.	0.1 cc.
Guinea-pig's serum.....	j.c.	c.	c.	c.	c.	c.
Human "end-piece".....	0	0	0	0	0	0
Guinea-pig's serum.....	0.005 cc.	0.01 cc.	0.02 cc.	0.04 cc.	0.06 cc.	0.1 cc.
+	+	+	+	+	+	+
Human "end-piece".....	0.005 cc.	0.01 cc.	0.02 cc.	0.04 cc.	0.06 cc.	0.1 cc.
	mk.	mk.	dist.	dist.	dist.	tr.
Guinea-pig's serum.....	0.005 cc.	0.01 cc.	0.02 cc.	0.04 cc.	0.06 cc.	0.1 cc.
+	+	+	+	+	+	+
Human "end-piece".....	0.01 cc.	0.02 cc.	0.04 cc.	0.08 cc.	0.12 cc.	0.2 cc.
	mk.	tr.	0	0	0	0

EXPERIMENTS WITH HORSE'S SERUM

Certain experiments with horse serum yielded very interesting results. As regards the hemolysis of ox corpuscles in the presence of venom, horse serum often exhibits a powerful activating effect and this property is partially retained even when the serum is heated to a temperature of 56°C.

It is apparent therefore that this activating power is not entirely due to serum constituents of complement nature.

Kyes (10) assumed that venom activation by serum was entirely due to the lecithin present in the serum and that the lipoid existed in combination with the serum proteins. In the

case of horse serum he suggested that the lecithin was very lightly bound and was therefore available in the fresh serum.

Horse serum has no complementing power for ox's corpuscles along with immune body (see Muir (11)).

The globulin and albumin were separated by the ammonium sulphate method and tested with venom and immune body.

TABLE 6

HORSE SERUM	LYSIS OF 0.5 CC. OF 5 PER CENT OX BLOOD SUSPENSION													
	+ 0.0005 gram cobra venom							+ 5 doses of immune body						
	0.01 cc.	0.025 cc.	0.15 cc.	0.1 cc.	0.02 cc.	0.3 cc.	0.5 cc.	0.01 cc.	0.025 cc.	0.05 cc.	0.1 cc.	0.2 cc.	0.3 cc.	0.5 cc.
Fresh serum	j.c.	c.	c.	c.	c.	c.	c.	0	0	0	0	0	0	0
Globulin $(NH_4)_2SO_4$ method	tr.	j.c.	c.	c.	c.	c.	c.	0	0	0	0	0	0	0
Albumin $(NH_4)_2SO_4$ method	dist.	c.	c.	c.	c.	c.	c.	0	0	0	0	0	0	0
Globulin+albumin $(NH_4)_2SO_4$ method	j.c.	c.	c.	c.	c.	c.	c.	0	0	0	0	0	0	0

HORSE SERUM	+ 0.0005 gram cobra venum (70°C.)					
	0.01 cc.	0.025 cc.	0.05 cc.	0.1 cc.	0.2 cc.	0.3 cc.
Globulin $(NH_4)_2SO_4$ method	0	0	0	0	ftr	
Albumin $(NH_4)_2SO_4$ method	dist.	c.	c.	c.	c.	
Globulin+albumin $(NH_4)_2SO_4$ method	dist.	c.	c.	c.	c.	

Controls: No immune body nor venom.

Globulin { 0.25 cc. = no lysis.
0.5 cc. = no lysis.

Fresh serum: 0.5 cc. = no lysis.

It was found that both the globulin and albumin individually displayed marked activating powers and the combination of the two fractions represented the full hemolytic power of the serum, of course only by a process of summation of effects (table 6).

To ascertain whether the serum lecithin played some part in producing lysis along with venom, these two fractions both

together and separately were tested with ox corpuscles + cobra venom which had been heated to 70°C. for one-half hour according to the method of Morgenroth and Kaya (12), who showed that with heated venom, lecithin is actively hemolytic while complement is inactive.

It was found on carrying out these experiments that the globulin fraction was quite inert with heated venom while the albumin was as active as with fresh venom (table 6); the lecithin nature of the activating constituents of the albumin fraction was thus demonstrated.

While therefore lecithin bodies play some part in the activating effect of horse's serum, other constituents probably of complement nature are equally concerned. It is of interest also to note that the lecithin substance should be associated with the albumin fraction. The other activating elements are contained in the globulin.

DISCUSSION AND CONCLUSIONS

These experiments elicit striking differences in the constitution of the complements of different animals apart from their relative activity with hemolytic immune body and venom.

In the case of human and rabbit's serum acting on ox red blood corpuscles + immune body or venom, the complement is entirely associated with the globulins of the serum while in the case of guinea-pig's serum which represents with these hemolytic systems a much more powerful complement, the albumin fraction is also an essential constituent of the complement.

Whether the potency of a complement depends on the presence of constituents associated with the serum albumin is a matter for further investigation.

In the case of human and rabbit's sera, however, acting with venom, the effect of the globulin is "masked" in the whole serum by the albumin while in the case of guinea-pig's serum the albumin also contributes to the full action of the serum along with the globulin.

It has also been shown how the albumin of human and rabbit's serum may inhibit the action of guinea-pig's serum globulin.

In the case of horse's serum the activating effect with venom is due not only to a complement body represented by the globulin but also to the lecithin contained in the albumin fraction.

REFERENCES

(1) STEPHENS, J. W. W.: Jour. Path. and Bacter., 1900, **6**, 273.
(2) BROWNING, C. H., AND MACKIE, T. J.: Jour. Path. and Bacter., 1912, **17**, 120; Biochem. Zeitsch., 1912, **43**, 229; Zeitsch. f. Immunitäts., Orig., 1913, **17**, 1.
(3) FERRATA, A.: Berl. klin. Woch., 1907, **44**, 366.
(4) BRAND, E.: Berl. klin. Woch., 1907, **44**, 1075.
(5) LIEFMANN, H.: Münch. med. Woch., 1909, **56**, 2097.
(6) SACHS, H.: Handbuch d. Tech. & Meth. d. Immunitäts., 1909, **2**, 969.
(7) OMOROKOW, L.: Zeitsch. f. Immunitäts., Orig., 1911, **10**, 285.
(8) BROWNING, C. H., AND MACKIE, T. J.: Zeitsch. f. Immunitäts., Orig., 1914, **21**, 422.
(9) CHICK, H., AND MARTIN, C. J.: Biochem. Jour., 1913, **7**, 380.
(10) KYES, P.: Jour. of Infect. Dis., 1910, **7**, 181.
EHRLICH, P.: Studies on Immunity, 2d ed., 1910, p. 291.
(11) MUIR, R.: Jour. Path. & Bacter., 1912, **16**, 523.
(12) MORGENROTH, J., AND KAYA, R.: Biochem. Zeitsch., 1908, **8**, 378; Biochem. Zeitsch., 1910, **25**, 88.