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Estimation of the Third Component (C'3) of Complement.*

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The third component of complement (C'3) has been defined in terms of its role in a hemolytic system. It is also known as the component which is inactivated by cobra venom or by yeast. In an attempt to discover which fraction of yeast inactivated C'3, Pillemer and Ecker[1] succeeded in isolating an insoluble carbohydrate from yeast which destroys or removes the activity of C'3 in amounts vastly smaller than the whole yeast or the zymin preparation of Whitehead, Gordon and Wormall.[2] For the sake of simplicity this insoluble carbohydrate will henceforth be called *zymosan.*† It was suggested that the inactivation of complement by yeast was due to the adsorption of C'3 on this carbohydrate. In a subsequent paper Ecker, Jones and Kuehn[3] noted that in the presence of 10% NaCl zymin did not inactivate C'3. Likewise, it has since been found that 10% NaCl suppresses the adsorption of C'3 by zymosan.

In the light of these facts it was therefore proposed to study the adsorbate with regard to the uptake of nitrogen. A stock suspension of the zymosan was made by placing it in saline (0.9%) in the proportion of 1 mg per ml. This lot of carbohydrate contained 3.14% N. Five ml of this zymosan suspension was added to each of a series of tubes, centrifugalized, and the supernates discarded. Varying amounts of pooled guinea pig serum were then added (in duplicate) to tubes containing the zymosan. Making use of the fact that the zymosan did not inactivate C'3 in the presence of 10% NaCl, duplicate tubes containing enough solid NaCl to make 10% were run parallel to each given quantity of serum. Rubber stoppers were inserted into the tubes which were then thoroughly shaken and incubated in a water bath at 37°C for one hour. Shaking was repeated every 10 minutes during the incubation period. Subsequently all tubes were centrifugalized and the precipitates washed 3 times with cold saline (0.9%) before their quantitative transfer to micro-Kjeldahl flasks. The supernatant serum was retested in one- and 2-unit aliquots for any residual hemolytic activity by the method of Ecker and Pillemer.[4] The sera containing 10% NaCl were restored to isotonicity by dilution with distilled water previous to titration. Table I shows that the serum in the tubes with 10% NaCl was not inactivated by the zymosan. The amount of zymosan chosen was sufficient to inactivate the serum in all quantities except the highest, namely, 8 ml. At this volume about 20% of the original activity was retained. When the N contained in the zymosan is subtracted from that of the entire adsorbate and expressed per ml of serum, a constant amount of N is not obtained. However, when the N contained in the parallel tubes with 10% NaCl is subtracted from that of the total N obtained from the untreated serum, an approximately constant figure is obtained.

The data further show that as the volume of serum is increased per constant amount of zymosan, the total amount of N taken up per ml of serum decreases rapidly to a critical level, at which point C'3 begins to remain in the supernate. Table I shows (third column from right) that more than C'3 is removed

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1 Pillemer, L., and Ecker, E. E., *J. Biol. Chem.*, 1941, **137**, 141.

2 Whitehead, H. R., Gordon, J., and Wormall, A., *Biochem. J.*, 1925, **19**, 618.

† This name for the insoluble carbohydrate was chosen to indicate its source from yeast and its general carbohydrate character.

3 Ecker, E. E. Jones, C. B., and Kuehn, A. O., *J. Immunol.*, 1941, **40**, 81.

4 Ecker, E. E., and Pillemer, L., *Annals N. Y. Acad. Sci.*, 1942, **43**, 63.

TABLE I.
Nitrogen Determination* of the Zymosan Adsorbate.

Zymosan, mg	Guinea pig serum, ml	NaCl 10%	% activity remaining, Units 1	% activity remaining, Units 2	Total N, mg	Total N less Zymosan N, Per given vol. serum, mg	Total N less Zymosan N, Per ml serum, mg	Total N less NaCl blank—"C'3 N", Per given vol. serum, mg	Total N less NaCl blank—"C'3 N", Per ml serum, mg
5	1	0	0	0	.200	.043	.043	.025	.025
5	1	+	90	100	.175	.018	.018		
5	2	0	0	0	.220	.063	.032	.037	.019
5	2	+	90	100	.183	.026	.013		
5	4	0	0	0	.261	.104	.026	.081	.020
5	4	+	95	100	.180	.023	.006		
5	8	0	20	40	.328	.171	.021	.147	.018
5	8	+	95	100	.181	.024	.003		
5	0	0			.157				

*Average of duplicate.

from the serum when an excessive amount of zymosan is used. For instance, using 1 ml of serum with 5 mg of the zymosan a total of 0.043 mg N is taken up, but when 4 ml is employed with the same amount of zymosan, the N uptake decreases to 0.026 mg per ml of serum. A volume of 8 ml exceeds the capacity of the zymosan to remove all the C'3, and some of the third component remains free; hence the C'3 N comprises a little more than 0.021 mg per ml. This value compares favorably with those of the last column in which it is found that although C'3 is suppressed in the presence of 10% NaCl, some other material is taken up. The assumption is made in the last column that this additional N can be used as a blank to subtract from the total N adding from untreated serum; the difference is presumably "C'3 N." Nonetheless, it must be stressed that an average of about 0.02 mg N per ml represents only a *maximum* value for C'3, because an excess of the zymosan removes other material from serum besides C'3. This phenomenon was also noted in the case of zymin when an excess was used.[3]

Human serum was studied in the same manner as guinea pig serum, and similar values were found. In addition, attempts at varying experimental conditions in the case of human serum prior to the determination of N gave some interesting results. It was observed that when the same serum sample was treated with constant amounts of the zymosan that the total amount of N adsorbed after the first treatment was the same regardless of the volume of serum employed (2 to 8 ml). Two percent NaCl was found to be sufficient to suppress the inactivation of C'3 by the zymosan. The total N "adding" from heated serum (56° for 30 minutes) was not consistent. Human serum diluted as much as 1:5 was inactivated by constant amounts of the zymosan and as much N was added as from the original volume of undiluted serum. Previous treatment of the zymosan with 0.1 N NaOH and heating in a boiling water bath for 30 minutes practically destroyed its C'3 inactivating capacity. Furthermore, a preliminary study now going on indicates that acidification of the serum to a pH of 5.4 inhibits the removal of C'3 provided a minimum amount of the zymosan is employed. Experiments on elution of C'3 also are in progress.