

Mice surviving for 96 hours or more were considered protected by the serum. The results of this test are given in Table I.

Twenty-seven mice were protected when injected with a mixture of one unit, or 0.005 cc of ketenized serum and pneumococci numbering from 450,000 to 4,500,000, whereas out of 15 mice injected with one unit of original antiserum and a similar number of organisms one died in 75 hours, another in 85 hours. Nine unprotected control mice died within 16 to 48 hours after injection with from 9 to 900 organisms.

The results of the present study indicate that the protective antibody of antipneumococcus horse serum is unchanged when treated with ketene, and strongly suggest that the ketenization of antisera might be very useful in serum therapy.

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Delayed Sedimentation in Antihemocyanin-Systems.

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In the course of some turbidimetric observations on precipitative systems, using the Evelyn photoelectric photometer, it became desirable to correlate the measurements with particulation and evenness of sedimentation as determined by direct inspection. Within a certain limited range of rather strong mixtures of *Limulus* hemocyanin and its antiserum it was found that sedimentation was enormously delayed, sometimes requiring several days for completion. The same kind of behavior was noted with the hemocyanin of *Fulgur carica* but not with crude horse serum and its comparatively weak antiserum. If the mixtures were agitated by convective currents, when the tubes were partly immersed in a waterbath, no zone of delayed sedimentation was observed. In weaker mixtures, even though they covered the same range of optimal flocculation, sedimentation proceeded also in the regular way.

One-milliliter volumes of mixtures of a constant amount of antiserum with decreasing quantities of antigen were pipetted into small tubes, 67 x 4.8 mm inside, which were arranged perpendicularly, protected from jar and agitation as far as possible, and kept at room-temperature (*ca* 23°C). In those tubes in which sedimentation was

markedly delayed, particulation first occurred in the topmost part of the column of fluid and gradually progressed downward.* Other mixtures particulated, if at all, evenly throughout the column and sedimentation occurred quickly without formation of the sharp upper boundary that characterized the other slowly settling aggregates.

This boundary was usually as clearly marked as that seen in the sedimentation of unagglutinated erythrocytes. The top layer of the settling aggregate gradually thickened and became more opaque while the finely web-like structure of the underlying column remained unchanged in density until the whole aggregate was deposited in the bottom of the tube. In many tubes a clot-like retraction of the aggregated structure occurred; narrow clear zones appeared along the sides of the tubes from which the aggregates had shrunk entirely or to which they remained attached at one or more points. Presumably these points of adhesion were rough or dirty spots on the glass. Occasionally the aggregated structure appeared to cling to a bubble on the surface or showed a funnel-shaped depression in the top. These irregularly occurring adhesions naturally made the sedimentative rates less orderly.

As an example of this peculiar delay in sedimentation, the reaction of *Fulgur* hemocyanin with its antiserum is given in Table I which shows the relation of the speed of settling to the composition of individual mixtures.

In numerous experiments the zone of slowest sedimentation has been rather near but always to the left of the tubes showing most rapid particulation. When the excess of antigen was considerable, although particulation was slow and incomplete, sedimentation occurred without the development of the columnar gel-structure under a clear supernate. With excessive antibody sedimentation was prompt. When the concentrations shown in the table were halved this type of delayed sedimentation was not observed. When the concentrations were doubled, more than a week was required for complete settling in some tubes and the range of the reaction was broadened. The phenomenon did not recur if the final deposits were thoroughly dispersed with a fine capillary pipette and again allowed to flock out.

The behavior of *Limulus* hemocyanin was more irregular. In

* Because these antigens are respiratory proteins it seemed conceivable that the presumably higher oxygen-tension at the top of the column might lead to earlier particulation. However, the use of freshly boiled salt solution as diluent and freshly cooled paraffin oil to overlay the mixtures thickly did not alter the result.

some ranges of concentration two prominent peaks of delayed sedimentation were seen with a trough of more rapid settling between them. This may plausibly be ascribed to the influence of mixed antigen-antibody systems—*Limulus* blood contains several molecular species differing in size and antigenic specificity.¹

The general appearance of this phenomenon closely resembles that diagrammed by Basset *et al.*⁴ in a recent report on the effect of high pressure upon specific flocculation of the tobacco-mosaic viral pro-

TABLE I.
Reactions of *Fulgur carica* hemocyanin with its antiserum.

Antigen-N, γ *	31	24.8	21.7	12.4	8.7	7.4	5.3	4.4
Time of first particulation, min.	>180	?	41	25	21	21	23	28.5
Depth (mm) of supernate†								
after 1 hr‡		0.5	0.5	1	1.5	2	4s	s
1.5 "		1.5	1.5	2	3.5	8	32s	S
2 "		2	2	4	14	24	S	
3 "		4.5	4.5	18.5	30	35.5		
3.5 "	s	5	6	23.5	33.5	38		
4.5 "	s	5	10	29	36	S		
6.5 "	s	8	23	34	39			
29 "	S	18	S	S	S			
In supernate of ten-fold stronger mixtures:								
Antigen, γ §	52	20	2	0	0	0	0	0
Antibody	0	0	0	0	0	0	0	Trace
Antibody-N in precipitate, γ	811	1110	1071¶	951	853	806	685	636
Ratio Ab N/Ag N**	3.14	4.9	5.8	7.7	9.8	10.9	12.9	14.4

*Contained in 0.5 ml and mixed with 0.5 ml of antiserum 846₃ diluted 1:10.

†The distance of the upper boundary of the settling aggregate from the meniscus.

‡That is, 1 hr after particulation first occurred in the series.

§Estimated by speed of particulation with a standardized serum.³

||Tested with 1.5 γ of antigen-N.

¶This mixture contained 186 γ of antigen, not 217.

s = sedimenting with poorly defined boundary or none.

S = completely sedimented.

** These ratios are surprisingly higher than were anticipated from results with the hemocyanin of the closely related *Fulgur canaliculatus*.¹ An explanation was sought in the possibility that the *carica* preparation, stored in glass for 2 years, might have dissociated into smaller components and thus offer a larger surface with which more antibody could combine. Components about the size of the molecule of hemoglobin would be expected, judging from the magnitude of the observed ratios.² However, no specifically reactive material in the diluted *carica* hemocyanin could be made to pass through a membrane with an APD of 14 $m\mu$, which did permit the passage of hemoglobin.

1 Hooker, S. B., and Boyd, W. C., *J. Immunol.*, 1936, **30**, 33.

2 Boyd, W. C., and Hooker, S. B., *J. Gen. Physiol.*, 1934, **17**, 341.

3 Hooker, S. B., and Boyd, W. C., *J. Gen. Physiol.*, 1935, **19**, 373.

4 Basset, J., Gratia, A., Macheboeuf, M., and Manil, P., *Proc. Soc. Exp. Biol. and Med.*, 1938, **38**, 248.

tein. Inasmuch as this protein and these hemocyanins are of huge molecular size it may be that the formation of slowly settling aggregates is a peculiarity of macromolecular systems. However, the sera I have used were unusually powerful (846_s contained over 14 mg of antibody-protein per ml) and other sizes of antigenic molecules must be investigated under comparable conditions before such an assumption would be justified.

Summary. An unusual zone of greatly delayed sedimentation was observed in titrations of the hemocyanins of *Limulus polyphemus* and *Fulgur carica* with their precipitins. The extent of the zone was influenced by both the relative and the absolute concentration of the two specific reactants.

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Spontaneous Agranulocytosis in the Cat.*

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During the course of making some routine blood counts on cats one animal was found with only 350 white blood cells per mm³ and no neutrophils in the peripheral blood. A portion of the liver of this animal was ground up with Alundum in Locke's solution. After settling, the supernatant fluid was used for injection into 5 additional cats. This resulted in the development of the same blood picture in 2 of these 5 cats. Following this a similar preparation was made from the liver of Cat 36 and 6 additional cats injected with this material, part of them receiving the material intraperitoneally and a part subcutaneously. This process was continued as shown in Chart 1 until 13 transmissions were accomplished.

Following injection a typical animal has a period of about 5 days when it appears normal. Then, it is frequently noted that food is left in the cage and the temperature is usually found to be elevated (39° to 41°C by rectum). The animal becomes slightly listless and, after a day or two (*i. e.*, the 6th to the 8th day) may not care to stand on its feet. There is no nasal or eye discharge except in a rare

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