ditions of the atmosphere the introduction of seeding agents may profoundly alter the normal developmental sequence of a cloud system.

Widespread Modifications of Synoptic Weather Conditions Induced by Localized Silver Iodide Seeding

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In previous papers it was indicated that the introduction of a few hundred g of finely dispersed silver iodide into the atmosphere in New Mexico on July 21, 1949, gave rains averaging more than .1 inch over an area of 33,000 sq mi. The total rain that could be attributed to the seeding amounted to 780,000 acre-feet, corresponding to an average rainfall of .44 inch.

Preliminary studies indicated that the known seedings in Arizona and New Mexico may have led to unusually heavy rains in eastern Kansas a few days later at distances of 700–900 miles from the points of seeding.

Beginning early last winter, more extensive silver iodide seedings have been carried out in California, Arizona, and New Mexico by increasing numbers of experimenters. These seedings have been of two kinds: promiscuous seedings made under favorable synoptic conditions, and systematic seedings, made in accord with prearranged schedules not dependent upon the weather. Studies of rainfall for 6 months at the 160 Weather Bureau stations that report each day the 24-hour precipitation have indicated significantly high correlations between the times of rainfall and the times of the systematic seedings up to 2,000 miles downwind from the point of seeding.

During the winter and spring repeated seedings in the Southwest apparently gave, 5–6 days later, heavy widespread rains over areas of more than 600,000 sq mi extending ENE from the western boundaries of Louisiana, Arkansas, and Missouri. West of this line and south of central Colorado there was at this time unusually low rainfall. As summer approached, the area showing high correlation with the systematic seedings gradually receded nearer to the point of seeding, so that in July the average rainfall in New Mexico greatly exceeded previous records.

During the winter in the Southwest there is insufficient moisture and convection activity for the silver iodide to be carried into clouds where ice crystals could form. The prevailing winds therefore carry it over the Mississippi Valley where it meets moist air from the Gulf. The heat liberated by the condensation in the showers that are set up over wide areas greatly exceeds the heat delivered by the sun. Cyclogenesis leads to the influx of moist Gulf air in the east, with Northwest winds in the rear of the rain area. Thus drought in the Southwest and heavy rains in the Southeast may have resulted from changes in the synoptic weather conditions induced by seeding in the Southwest. During the summer, however, there is enough Gulf air in the Southwest to make both the promiscuous and the systematic seeding effective in giving rain in that region.

Technical Papers

The Hemolytic and Antihemolytic Activities of Various Centrifugally Separated Fractions of Adult and Fetal Liver Cells[1]

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We have recently reported (*16*) that saline extracts of fetal guinea pig livers possess a very high hemolytic activity, which can be demonstrated by incubating such preparations for 2–4 hr with a 1% suspension of washed red blood cells. The agent is not species-specific, since it will lyse not only the red cells from the same fetus but also those from the mother, as well as those from the rat. This hemolytic activity is due, for the most part, to a heat-sensitive factor and, to a lesser extent, to a heat-resistant substance. This observation, together with other considerations such as its sensitivity to certain enzyme poisons, suggests that the hemolytic agent is an enzyme, or may be produced by enzyme action. Adult liver extracts show very little lysis in 2–4 hr and then only with much higher concentrations (*16*).

It has long been known that hemolytic agents can be prepared from normal, as well as from pathological, tissues (*22, 1, 10, 11*). The pertinent literature bearing on the probable nature of the fetal autohemolytic agent has been briefly reviewed elsewhere (*16*). It is also known that the plasma or serum contains substances that possess the capacity to protect red cells from a variety of lytic agents (*12, 8, 21, 11*). There is, however, considerable uncertainty as to the chemical nature of both the hemolytic and inhibitor agents, and as to the mechanism of their actions. Since this paper does not, strictly, deal with these phases of the problem, reference is made to the excellent reviews of Ponder (*11, 12*) for a detailed consideration of these substances.

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The question we are concerned with here is to account for the high activity in the fetus, compared with the relative absence of this phenomenon in the adult. Assuming—and the evidence strongly favors it (*16*)—that the lytic agent is an enzyme or is produced by one, we have a rather unusual situation of an enzyme that exhibits greater activity in the fetus than in the adult. The results of most comparative studies show that such activity in the fetus, particularly by systems concerned with the respiratory metabolism of the cell, is decidedly lower than in the adult (*6, 20, 15, 17, 18, 4*). Furthermore, on the basis of prevailing opinion that enzyme activity is a measure of enzyme concentration, one would be justified in concluding that the system concerned with this phenomenon exists in higher concentration in the fetus. On the other hand, if there actually is little or no difference in the enzyme concentration between the fetus and the adult, then it should be possible to account for the fetal-maternal differences in activity on the basis of regulatory or inhibitory substances that act either directly on the enzyme or on the products of the enzyme action.

The experiments reported here were carried out with the purpose of testing this hypothesis. The results demonstrate the presence *in the liver cell* of an inhibitor, as well as a hemolytic agent. In the adult these two substances exist in proportions (or possibly as a loosely combined complex) such that the activity of the enzyme or lytic agent is inhibited. A separation can be easily made by ultracentrifugation. It is found that the inhibitor substance is associated, at least insofar as the force required to separate it is concerned, with the mitochondrial fraction of the cell. The hemolytic agent, on the other hand, remains in the supernatant fluid after centrifugation at $100{,}000 \times g$. Further proof is obtained by recombining the various fractions in the proportions existing in the original extract, with the result that the adult preparation returns to its inactive state. Support is also offered for the thesis that the high activity of fetal liver extracts may be due to a lower concentration of the inhibitor component, and not to a greater concentration of the enzyme, or lytic agent.

Twelve guinea pig fetuses, ranging in size from 32 to 102 mm (crown-rump length), and 4 pregnant adults were used in this study. The mothers were killed by decapitation, and their blood collected in oxalated tubes. The livers from the fetuses and mothers were kept on dry ice as removed, until all had been collected and were ready for use. While still frozen, they were broken into small pieces to facilitate grinding, and weighed amounts were ground in a porcelain mortar and made up with 0.9% NaCl to a 20% suspension. These suspensions were then given a preliminary centrifugation at 2,500–3,000 rpm for 5 min, using a refrigerated International centrifuge. The sediment of this centrifugation, which consisted of liver debris, clumps of liver cells, and many red blood cells, was discarded, and the supernatant fluid was labeled either F_1 (fetal) or A_1 (adult) and was used for further fractionation as follows: 15.0 ml of F_1 and A_1 were centrifuged in a Spinco centrifuge at $25{,}000 \times g$ for 15 min. The resulting supernatant was removed for further fractionation, and the sediment was made up to 15.0 ml with saline, blended in an all-glass homogenizer (*14*), and labeled F_2 or A_2. The supernatant that was saved was then centrifuged for 1 hr at a force of $100{,}000 \times g$, and the resulting sediment was made up to 15.0 ml with saline, blended with a homogenizer, and labeled F_3 and A_3, respectively, while the supernatants of this centrifugation were labeled F_4 and A_4, respectively. All the above preparations were carried out in the cold, and the temperature in the Spinco[2] was maintained between 5° and 10° C.

The general procedures used to determine hemolytic activity have been described in a previous report (*16*). Color controls (that is, the entire hemolytic system but without the red blood cell suspension) were run and are necessary, particularly with Fractions F_1 and F_4. In those tests involving recombinations of the various fractions, the final volume of all tubes, including, of course, controls, was 7.2 ml, and 0.9% NaCl was used where necessary adjustments had to be made. Further to ensure constancy of conditions, the red blood cell suspension was always added last. In every experiment a complete test was made of all fetal and adult fractions simultaneously. During the preparation of the various fractions, all were stored in the cold until used the same day. However, we have found that, if stored at 0° C, they still retain the same activity for 2–3 days.

Fractions 1 (F_1 and A_1) contained cellular particles, nuclei, and occasional whole cells. Fractions 2 (F_2 and A_2) consisted principally of brownish particles, which gave a cloudy suspension and resembled what Hogeboom, Schneider, and Pallade (*7*) have described as mitochondria. The sediment that made up Fraction 3 (F_3 and A_3) came down as a small homogenous, pale-red gelatinous pellet and, in this respect, corresponded to what Claude (*3*) has described as the microsomes. Fraction 4 (F_4 and A_4), or the supernatant after $100{,}000 \times g$, was a clear, red fluid in the case of the adults and the younger fetuses, whereas in some of the older fetuses (80–100 mm) it contained some visible oil and fat droplets.

Table 1 summarizes the degree of hemolysis produced by various quantities of the above fractions. Fetal Fraction F_1 exhibits a very high hemolytic activity, which is usually complete within 1 hr when 1.0 or 0.5 ml of the preparation is used. On the other hand, 1.0 ml of adult fraction A_1 shows only a trace of hemolysis after 3 hr incubation. The fetal mitochondria (F_2) and microsomes (F_3) also show considerable hemolysis, but not to the degree found with Fraction F_1, whereas the corresponding adult fractions show no activity at all within the 3-hr period.

The findings with Fraction 4 of the adult are striking. Whereas all other fractions of this animal were inactive, the activity with Fraction 4 was equal to, and in some experiments slightly greater than, that of the fetal Fraction 4. With 1.0 ml or 0.5 ml of this fraction, hemolysis usually was complete within 1 hr.

[2] We are indebted to Carlton Schwerdt, of the Poliomyelitis Research Center, Department of Epidemiology, The Johns Hopkins University, for use of the Spinco centrifuge.

TABLE 1

Fraction	Milliliters used			
	1.0	0.5	0.25	0.10
F_1	+++	+++	+++	++
A_1	±	0	0	0
F_2	++	+	±	0
A_2	0	0	0	0
F_3	++	+	±	0
A_3	0	0	0	0
F_4	+++	+++	++	±
A_4	+++	+++	+++	±

F_1 and A_1 = whole homogenate of fetal and adult liver, respectively (see text); F_2 and A_2 = mitochondria fraction; F_3 and A_3 = microsome fraction; F_4 and A_4 = supernate after 100,000 × g for 1 hr. 1.0 ml of a 1% suspension of a 3 times washed maternal red blood cells was used. Incubation time, 2½ hr; temperature, 38° C. +++ = complete hemolysis; 0 = none. Final volume, 2.0 ml.

Now, since A_1 was inactive and A_4 was very active, it appeared that somewhere during the preparation of A_4 an inhibitor was removed. If this was the case and no serious change had occurred in its properties during the procedures, it should be possible to restore the inactive state of A_1 by the simple process of recombining the various fractions in the original proportions. Table 2

TABLE 2

A_4	F_4	A_3	F_3	A_2	F_2	0.9% saline	RBC	Hemolysis
0.2	—	—	—	—	—	6.0	1.0	+++
.2	—	0.2	—	—	—	5.8	1.0	++
.2	—	—	—	0.2	—	5.8	1.0	0
.2	—	0.2	—	0.2	—	5.6	1.0	0
.2	—	—	3.0	—	—	3.0	1.0	+++
.2	—	—	—	—	3.0	3.0	1.0	++
0.2	—	—	3.0	—	3.0	—	1.0	++
—	0.2	—	—	—	—	6.0	1.0	+++
—	.2	0.2	—	—	—	5.8	1.0	+++
—	.2	—	—	0.2	—	5.8	1.0	±
—	.2	0.9	—	0.9	—	4.2	1.0	0
—	.2	—	3.0	—	—	3.0	1.0	+++
—	.2	—	—	—	3.0	3.0	1.0	+++
—	0.2	—	0.9	—	0.9	4.2	1.0	+++

Incubation time, 3 hr; temperature, 38° C; final volume, 7.2 ml. Figures represent ml used; other symbols as in Table 1.

summarizes such experiments, and it can be seen that recombining A_4 with A_3 and A_2 restores the inactive state found in A_1. The results point to the A_2 fraction (mitochondria) as the one containing the greatest inhibitory activity.

If the fetal-maternal activity differences found in Fraction 1 are due to differences in inhibitor concentrations, then fetal Fractions F_2 and F_3 should not be as effective as adult Fractions A_2 and A_3 in inhibiting the hemolytic activity of either Fractions F_4 or A_4. The results in Table 2 show that such is the case.

There is also some evidence indicating that other tissues, such as the adult brain or plasma, contain a factor that will inhibit the activity of the hemolytic agent. At present, we do not know whether the inhibitor (or inhibitors) in plasma is similar to the factor in the liver cells. The plasma factor is heat-stable. The hemolytic agent found in F_4 and A_4 is destroyed in 1 min by heating in boiling water. Ten ml of F_1 was dialyzed for 24 hr in the cold against an equal volume of 0.9% saline. There is no indication that this substance can pass through the cellophane membrane. These results are summarized in Table 3.

TABLE 3

No. ml		Hemolysis
0.2	F_4 control	+++
1.0	F_4 heated*	0
0.2	F_4 + 1.0 ml fresh plasma	0
0.2	F_4 + 1.0 ml heated plasma†	0
0.2	F_4 + 1.0 ml of 20% adult brain suspension	0
1.0	20% brain suspension	0
1.0	F_1 dialysate (see text)	0
1.0	dialyzed F_1 (see text)	+++
0.2	A_4 control	+++
1.0	A_4 heated*	0
0.2	A_4 + 1.0 ml fresh plasma	0
0.2	A_4 + 1.0 ml heated plasma†	0
0.2	A_4 + 1.0 ml 20% adult brain suspension	0

Incubation time, 3 hr; temperature, 38° C. Plasma from maternal guinea pig oxalated blood. Maternal brain suspension centrifuged at 2,500 rpm and supernatant used. * Heated over boiling water for 1 min. † Heated over boiling water for 5 min. Final vol of all tubes, 3.0 ml.

Most studies to date show that the various centrifugally separated cellular components, when recombined, act synergistically for a given activity. For instance, it can be seen from the data of Le Page and Schneider (*9*) that the glycolytic activity of recombined fractions of the cell is greater than the sum of the activity of the separate parts. According to Borsook and his colleagues (*2*), this is also the case for the uptake of labeled glycine, and we have observed a similar synergistic behavior for O_2 consumption between various cellular components (*19*). No such synergistic behavior is found for the phenomenon now being reported. The hemolytic activity shows an inhibitory type of regulation between substances associated with the mitochondria and the hemolytic enzyme or agent found in the supernatant fluid.

Of greater interest to us is the substantial evidence from these experiments that differences in the rate of enzymatic activity, as found between fetus and adult, are not necessarily due to differences in enzyme concentrations and, in this case, can be accounted for by the presence or absence of inhibitory regulating mechanisms. It remains to be determined to what degree low activity of other enzyme systems in the fetus may be due to similar regulating mechanisms.

The implications of these findings on the mechanism of blood destruction in normal states, as well as in certain pathological hemolytic conditions, are evident. Here is further evidence for a mechanism of erythrocyte destruction involving a lytic process. Furthermore, it appears that the lytic agent can be held in check by a mechanism residing *in the cell*, as well as by the plasma. In the past, most of the emphasis concerning inhibitors

or accelerators of lysins has been directed to the nature and variations of the antihemolytic substances in the plasma, and practically no attention has been given to the capacity of the tissues in this activity. This study suggests that inhibitory agents in the cell may play a very important role in the occurrence of hemolysis *in vivo*. It is possible that the slow hemolytic activity produced by slices of normal human tissues (*10, 11*) and the faster activity found in extracts of certain tumor cells (*22, 5*) may be accounted for on the basis of the effective inhibitor concentration.

The precise manner of the mechanism of interaction of these substances remains to be determined, and several possibilities suggest themselves for further study: (1) an inhibitor-lysin complex may normally exist, which may be acted on by a heat-labile enzyme to release a heat-stable lytic agent (*13, 16*); (2) by enzyme action a lytic agent is produced which, in turn, can be inactivated by the inhibitor; (3) the lytic agent is an enzyme which can act directly on the red cell membrane, but the inhibitor behaves as a preferred competitive substrate; (4) the inhibitor may, in some manner, increase the resistance of the red cells against hemolysis.

Ponder (*11*) has suggested that, under conditions where the production of a lysin may remain constant, the effective concentration of the lysin may be altered by the addition of accelerators or inhibitors, "... the net effect of which is an inhibitory one." The demonstration here of lysins and inhibitors within the cell and of the consequences of differences in the effective concentration of the latter lends support to that idea.

References

1. BERGENHEM, B., and FAHRAEUS, R. *Z. ges. exp. Med.*, 1936, **97**, 555.
2. BORSOOK, H., *et al.* *J. biol. Chem.*, 1950, **184**, 529.
3. CLAUDE, A. *J. exp. Med.*, 1946, **84**, 61.
4. FLEXNER, L. B., and FLEXNER, J. B. *J. cellular comp. Physiol.*, 1946, **27**, 35.
5. GROSS, L. *Proc. Soc. Exp. Biol. Med.*, 1947, **65**, 292.
6. HIMWICH, H. E., and FAZEKAS, J. F. *Am. J. Physiol.*, 1941, **132**, 293.
7. HOGEBOOM, G. H., SCHNEIDER, W. C., and PALLADE, G. E. *J. biol. Chem.*, 1948, **172**, 619.
8. LEE, J. S., and TSAI, C. *Quart. J. exp. Physiol.*, 1942, **31**, 271, 281.
9. LE PAGE, G. A., and SCHNEIDER, W. C. *J. biol. Chem.*, 1948, **176**, 1021.
10. MAEGRAITH, B. G., MARTIN, N. H., and FINDLAY, G. M. *Brit. J. exp. Path.*, 1942, **24**, 58.
11. PONDER, E. *J. gen. Physiol.*, 1944, **27**, 483.
12. ———. *Hemolysis and related phenomena.* New York: Grune & Stratton, 1948.
13. ———. Personal communication.
14. POTTER, V. R., and ELVEHJEM, G. A. *J. biol. Chem.*, 1936, **114**, 495.
15. POTTER, V. R., SCHNEIDER, W. C., and LIEBL, G. J. *Cancer Research*, 1945, **5**, 21.
16. TYLER, D. B. *Proc. Soc. Exp. Biol. Med.*, 1949, **72**, 491.
17. ———. *Arch. Biochem.*, 1950, **25**, 221.
18. ———. *J. biol. Chem.*, 1950, **184**, 711.
19. ———. Unpublished observations.
20. TYLER, D. B., and VAN HARREVELD, A. *Am. J. Physiol.*, 1942, **136**, 600.
21. TSAI, C., and LEE, J. S. *Chinese J. Physiol.*, 1941, **16**, 165.
22. WEIL, R. *J. med. Research*, 1907, **16**, 287.

Methanol Precipitation of Influenza Virus

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Sometime subsequent to the report by Cox, Van der Scheer, Aiston, and Bohnel (*1*) on purification and concentration of influenza virus in fresh chorioallantoic (CA) fluid by methanol precipitation, difficulties were encountered in obtaining good yields. As indicated by chicken red cell agglutination (CCA) titers (*2*), the yields obtained from various lots of infected fluids ranged from 25 to 79% instead of the 95–110% recorded in the original publication (*1*). The cause of this decreased yield is still unknown, but it may have been associated with variations in the components of allantoic fluid induced by seasonal, dietary, or other factors inherent in the laying flocks from which the egg supply was drawn.

No improvement in the yield was produced by varying the pH, alcohol concentration, time, and temperature of elution. Tests performed at various steps of the process indicated that the virus was being precipitated but was not being eluted.

Excellent recoveries of virus again were obtained when the concentration of the eluting phosphate buffer solution, pH 7.0, was changed from 0.1 *M* to 0.5 *M*. Further trials led to the adoption of 0.3 *M* phosphate buffer solution as the preferred eluant for influenza virus. Shown in Table 1 are some representative data obtained with two strains of influenza virus.

TABLE 1

Lee Strain—fresh allantoic fluid				PR8 Strain—fresh allantoic fluid			
Methanol, %	Phosphate buffer pH 7, *M*	CCA titer	Yield, %	Methanol, %	Phosphate buffer pH 7, *M*	CCA titer	Yield, %
31	0.1	73	60	23	0.1	131	69
31	0.3	114	94	23	0.3	189	100
Original CA fluid		121		Original CA fluid		189	

Satisfactory yields of virus were never obtained when 0.1 *M* phosphate buffer solution, pH 7.0, was used as the eluant for viral precipitates obtained from CA fluids that had been preserved with merthiolate 1: 5,000 and formalin 1: 10,000. As the data in Table 2 indicate, however, the use of 0.3 *M* phosphate buffer solution gave satisfactory yields of virus even from such preserved fluids.

The elution of methanol-precipitated influenza virus with 0.3 *M* phosphate buffer solution, pH 7.0, has been uniformly successful, regardless of uncontrolled variables