THE ACTION OF SOME HEMOLYSIS ACCELERATORS UPON LIPID AND PROTEIN MONOLAYERS*

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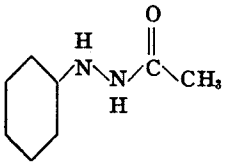
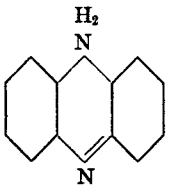
According to Ponder (1948) the membrane of the mammalian erythrocyte is an oriented lipoprotein monolayer, made up chiefly of stromatin, cephalin, and cholesterol. Hence, the action of hemolytic substances may possibly be due to their penetration of these lipoprotein films, with consequent changes in permeability, or actual disruption of the cell membrane.

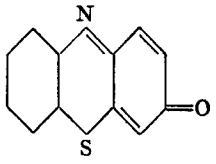
Lyons and Rideal (1929) and Schulman and Hughes (1935) investigated the phenomenon of "film penetration" and found increases in the surface pressure of monolayers when penetrating substances were injected beneath these monolayers. They concluded that both polar and hydrophobic groups take part in this type of interaction. Schulman and Rideal (1937) used the penetration technique to investigate the action of hemolytic agents upon artificial lipoprotein monolayers and found that the hemolytic activity paralleled the effects of these compounds upon the monolayers.



Acetylphenylhydrazine



9-Aminoacridine



Phenothiazone

Ponder (1926) first investigated the phenomenon of acceleration of hemolysis, in which a compound, not hemolytic in itself, is able to increase the action of a lysin. However, the effect of such accelerators upon monolayers has apparently not been investigated. Acetylphenylhydrazine, which produces a hemolytic anemia *in vivo*, has been shown by Collier (1947) to accelerate hemo-

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lysis *in vitro*; and in the present investigation the effect of this compound upon various lipid and protein monolayers has been studied. A few observations are also reported upon 9-aminoacridine and phenothiazone, which were shown to be accelerators by Collier (1945) and Collier and Allen (1942).

Methods

Surface pressure measurements were made with a Cenco hydrophil balance, a modification of the Langmuir trough. The area of the water surface before the mica barrier was about 700 sq. cm. and the fluid capacity was approximately 1500 ml. Film-forming substances, dissolved in appropriate solvents, were spread upon 0.04 M sodium phosphate buffer of pH 7.0 in the trough, using a micropipette. The hemolysis accelerators were dissolved in the buffer before the lipid or protein was spread as a monolayer, and the results were compared with those obtained when the film-forming substances were spread upon buffer alone. All experiments were performed at room temperature, 20–25°C.

Area and surface pressure measurements were made in what Bull (1947) has termed the "high pressure" region; *i.e.*, at pressures above 1 dyne per cm. Force-area curves were constructed from these measurements, and the limiting area was estimated by extrapolation to zero pressure of the linear portion of the curve. Where possible, the minimum compressibility coefficient, δ_m, was calculated by the method of Bull (1947).

RESULTS

Acetylphenylhydrazine.—Crystalline bovine plasma albumin (Armour Laboratories), dissolved in buffer, was spread upon buffer containing acetylphenylhydrazine (British Drug Houses) in concentrations from 0.0002 to 0.010 M. The resulting force-area curves are represented in Text-fig. 1 A, and the changes in limiting area are given in Table I. The minimum compressibility coefficient for the albumin on buffer alone was 0.0227 at 9 dynes/cm. and an area of 0.55 sq. m./mg. For albumin spread on 0.010 M acetylphenylhydrazine the corresponding δ_m was 0.0256 at 9 dynes/cm. and 0.615 sq. m./mg.

Cholesterol and plasma albumin were spread together from isopropanol-sodium acetate solution by the method of Ställberg and Teorell (1939). Text-fig. 1 B illustrates the effect of 0.001 M acetylphenylhydrazine upon such a film (cholesterol and albumin in a ratio of 1:4 by weight); the force-area curve was obtained 3 hours after spreading. The value of δ_m for the film on buffer alone was 0.0131 at 10 dynes/cm. and 0.62 sq. m./mg. For the film on acetylphenylhydrazine the value was 0.033 at 11 dynes/cm. and 0.67 sq. m./mg. Evidently there was an appreciable increase in compressibility.

In the case of cholesterol-albumin films, the effect of changes in pH was determined, and it was found that the increase in limiting area due to 0.001 M acetylphenylhydrazine was virtually independent of the pH:—

pH	Increase in limiting area
	sq. m./mg.
3.00	0.09
5.71	0.11
7.03	0.11

Gliadin was prepared from wheat gluten and this was dissolved together with cholesterol in 70 per cent ethanol (gliadin and cholesterol in a ratio of 1:4 by weight). This was spread upon 0.001 M acetylphenylhydrazine, and Text-fig. 1 C shows that there is a great increase in surface pressure, especially at the lower pressures.

TABLE I

The Effect of Hemolysis Accelerators upon the Limiting Area of Monolayers of Plasma Albumin

0.04 M phosphate buffer, pH 7.0.

Accelerator	Concentration	Limiting area	Increase in limiting area
	M × 10^4	*sq. m./mg.*	*sq. m./mg.*
None	—	0.67	—
Acetylphenylhydrazine	2.0	0.67	0
	6.0	0.67	0
	8.0	0.71	0.04
	10.0	0.75	0.08
	100	0.75	0.08
9-Aminoacridine	1.0	0.67	0
	2.0	0.74	0.07
	10.0	0.74	0.07
Phenothiazone	0.1	0.70	0.03
	0.4	0.77	0.10
	3.2	0.84	0.17

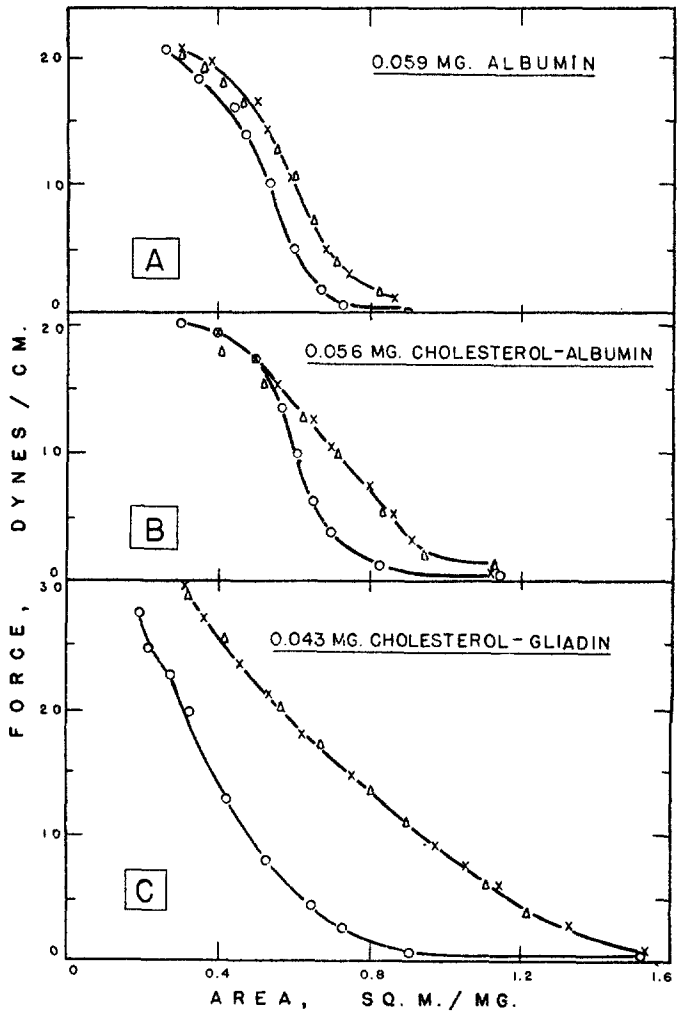
Films of cholesterol alone, cephalin alone (product of Wilson Laboratories), 1:1 cephalin-cholesterol, and 1:4 cephalin-albumin were found to be virtually unaffected by 0.001 M acetylphenylhydrazine.

9-Aminoacridine.—Plasma albumin was spread upon buffer containing 9-aminoacridine hydrochloride[1] at concentrations of 10^{-4}, 2×10^{-4}, and 10^{-3} M. The increases in limiting area of the protein film are recorded in Table I. The lowest concentration had no effect upon the film area, while the higher concentrations increased the limiting area by about 10 per cent.

[1] Kindly supplied by Dr. G. J. Martin of the National Drug Co., Philadelphia.

Films of cholesterol were unaffected by 9-aminoacridine.

Phenothiazone.—Phenothiazone was prepared from phenothiazine by the method of Pummerer and Gassner (1913) and was then recrystallized from



TEXT-FIG. 1. The effect of 10^{-3} M acetylphenylhydrazine in 0.04 M phosphate buffer pH 7.0, upon the force-area curves of monolayers of albumin (A), 1:4 cholesterol-albumin (B), and 1:4 cholesterol-gliadin (C).

○, film on buffer alone, × △, film on acetylphenylhydrazine.

ethanol. When it was dissolved in buffer it was found that the dye contained a film-forming impurity, which could be removed only by repeated sweeping

of the surface of the solution in the trough, until further sweeping caused no deflection of the barrier.

Plasma albumin was spread upon buffer containing phenothiazone in concentrations of 1.0×10^{-5}, 4.2×10^{-5}, and 3.2×10^{-4} M. The increases in limiting area of the protein film thus measured are given in Table I.

A film of 1:4 cholesterol-albumin (dissolved in isopropanol-sodium acetate) was spread upon a 4.2×10^{-4} M solution of the dye, and the increase in limiting area was found to be 0.080 sq. m./mg. Films of cholesterol alone, spread upon solutions of phenothiazone at 10^{-4} M concentration, were unaffected.

DISCUSSION

The accelerators of hemolysis which have been investigated are relatively simple compounds, not surface-active in themselves. Yet they have a strong effect in causing the expansion of albumin monolayers, while not affecting cholesterol monolayers. Presumably these compounds are bound to the protein molecules through their polar groups and also through the hydrophobic ring structures. A curious observation is that both acetylphenylhydrazine and 9-aminoacridine produce their maximum effects upon plasma albumin films at very low concentrations (10^{-3} and 2×10^{-4} M respectively), and at higher concentrations appear to have no greater effect.

In their acceleration of hemolysis of erythrocytes, it can only be surmised that these substances attack the protein component of the erythrocyte membrane, causing alterations in permeability which enhance the action of a hemolytic agent.

SUMMARY

The effect of three accelerators of hemolysis—acetylphenylhydrazine, 9-aminoacridine, and phenothiazone—upon protein and lipid monolayers has been examined. These compounds, in low concentration, cause marked expansion of monolayers of plasma albumin, but have no apparent effect upon cholesterol films. It is suggested that these accelerators may affect the protein component of the erythrocyte membrane, thus enhancing the action of hemolytic agents.

REFERENCES

Bull, H. B., 1947, *Advances Protein Chem.*, **3**, 95.

Collier, H. B., 1945, *J. Gen. Physiol.*, **29**, 57.

Collier, H. B., 1947, *Fed. Proc.*, **6**, 245.

Collier, H. B., and Allen, D. E., 1942, *Canad. J. Research*, sect. D, **20**, 283.

Lyons, C. G., and Rideal, E. K., 1929, *Proc. Roy. Soc. London, Series A*, **124**, 333.

Ponder, E., 1926, *Proc. Roy. Soc. London, Series B*, **99**, 461.

Ponder, E., 1948, Hemolysis and Related Phenomena, New York, Grune and Stratton.

Pummerer, R., and Gassner, S., 1913, *Ber. chem. Ges.*, **46,** 2310.
Schulman, J. H., and Hughes, A. H., 1935, *Biochem. J.*, **29,** 1243.
Schulman, J. H., and Rideal, E. K., 1937, *Proc. Roy. Soc. London, Series B,* **122,** 46.
Ställberg, S., and Teorell, T., 1939, *Tr. Faraday Soc.*, **35,** 1413.