THE EFFECTS OF MERCAPTOETHANOL UPON FORM AND MOVEMENT OF AMOEBA PROTEUS [1]

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Although sol-gel transformations probably underlie the mechanism of amoeboid movement, there is disagreement as to whether the motive force essential for locomotion is initiated by a contraction of the plasmagel (Mast, 1926; Landau *et al.,* 1954) or through a contraction of non-Newtonian endoplasm (plasmasol) in the anterior portion of the cell (Allen, 1961). Several reports (Zimmerman *et al.,* 1958; Zimmerman, 1962b; Landau, 1959) suggest that the ATP system may, indeed, be directly associated with the sol-gel transformations responsible for amoeboid movement. However, the nature of the protein system through which the cell develops its motive force has not been completely elucidated.

Experiments employing mercaptoethanol, a compound having readily available source of −SH groups, have demonstrated that gelated structures within the cell, such as the mitotic apparatus (Mazia and Zimmerman, 1958), and the cortical plasmagel of cleaving eggs (Zimmerman, 1962a, 1964), are markedly altered by the addition of thiol compounds. Since it is generally believed that gelation reactions in amoebae are responsible for the maintenance of amoeboid form and locomotion, presumably mercaptoethanol, by interfering with the sol-gel reactions, should alter the structural characteristics of the plasmagel and thus have an effect upon pseudopodial form and activity.

It is well established that both temperature and pressure exert marked effects on the gelational state of cytoplasmic structures, and these effects can be evaluated quantitatively. The formation of gelated structures within cells appears to represent an endothermic reaction which is accompanied by a volume increase (Marsland, 1956). Thus, decreasing temperature and increasing pressure tend to weaken the plasmagel structure of amoeba by causing a shift in the sol-gel equilibria toward the sol state. In the present study, the effects of mercaptoethanol on the form of amoebae were determined under systematically varying conditions of pressure.

Materials and Methods

Culture methods. Actively growing cultures of *Amoeba proteus* were obtained from Dr. J. A. Dawson. The amoebae were cultured by the method of Brandwein (1935), modified by the elimination of agar. The cultures were grown at 18–20° C. in the dark, at pH 6.9.

[1] This work was supported in part by Grant GM 07157-03, 04, 05 from the Division of General Medical Sciences, United States Public Health Service.

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Pressure-temperature equipment. The pressure apparatus was patterned after one designed by Marsland (1950) with certain modifications. The microscope-pressure chamber permits cells to be observed at magnifications up to 600 × while being subjected to pressures up to 20,000 lbs./in.^{2}. Pressure is developed by means of an Aminco pressure pump at the rate of 5000 lbs./in.^{2}/ second. The pressure is released virtually instantaneously by means of a needle valve. The microscope-pressure chamber, as well as all glassware and test solutions, was housed in the temperature control chamber. The temperature chamber permits the temperature to be set at any level between −5° and 60° C., with a maximum internal variation of ± 0.2°. In the present experiments the temperature was kept constant at 20° C.

Immersion procedure. For each experiment about 50–100 actively streaming amoebae were washed with Brandwein solution and placed into mercaptoethanol so that the final dilution of mercaptoethanol was 10^{-1}–10^{-6} *M*. The amoebae were then transferred to a small lucite chamber and placed into the temperature-equilibrated pressure bomb. After a one-hour immersion in mercaptoethanol, the cells were subjected to pressure. During the 20-minute pressure treatment, the cells were kept under observation. The percentage of amoebae with some persisting pseudopodia, as compared with amoebae which were completely spherical, was established.

Chemicals. The 2-mercaptoethanol ($HSCH_2CH_2OH$) was obtained from Eastman Organic Chemicals, Rochester, N. Y. Fresh solutions of mercaptoethanol in Brandwein solution were made up daily and equilibrated at the desired temperature prior to use.

Results

Concentration series. Before investigating the effects of pressure on mercaptoethanol-treated amoebae, it was necessary to find a concentration of mercaptoethanol in which the amoebae would continue to display their normal form and mobility. Amoebae, at 20° C., were immersed into mercaptoethanol at various concentrations ranging from 10^{-1} to 10^{-5} *M*. At a concentration of 10^{-1} *M*, the amoebae reacted quickly to the drug. The pseudopodia retracted and protoplasmic streaming decreased. When the amoebae were placed in a concentration of 10^{-2} *M* about 50% retracted their pseudopodia. However, within a short time most of the amoebae immersed in 10^{-2} *M* mercaptoethanol developed short pseudopodia and streaming was observed. When the amoebae were immersed in mercaptoethanol solutions with concentrations ranging from 10^{-3} to 10^{-5} *M* there was no retraction of pseudopodia and amoeboid movement was not modified perceptibly.

After three hours' incubation in 10^{-1} *M* mercaptoethanol, many of the cells underwent cytolysis while the remaining amoebae retracted their pseudopodia and did not exhibit any protoplasmic activity. Following a three-hour immersion in 10^{-2} *M* mercaptoethanol, about 50–70% of the amoebae were stellate, with short pseudopodia and with a darkened central mass of cytoplasmic granules. Twenty-four hours later, surviving amoebae in 10^{-1} and 10^{-2} *M* mercaptoethanol were rounded and did not exhibit protoplasmic streaming, and a large percentage of the cells had undergone cytolysis. However, the amoebae in the more dilute mercaptoethanol concentration (10^{-3} to 10^{-5} *M*) for a period of 24 hours exhibited apparently normal amoeboid movement and active protoplasmic streaming.

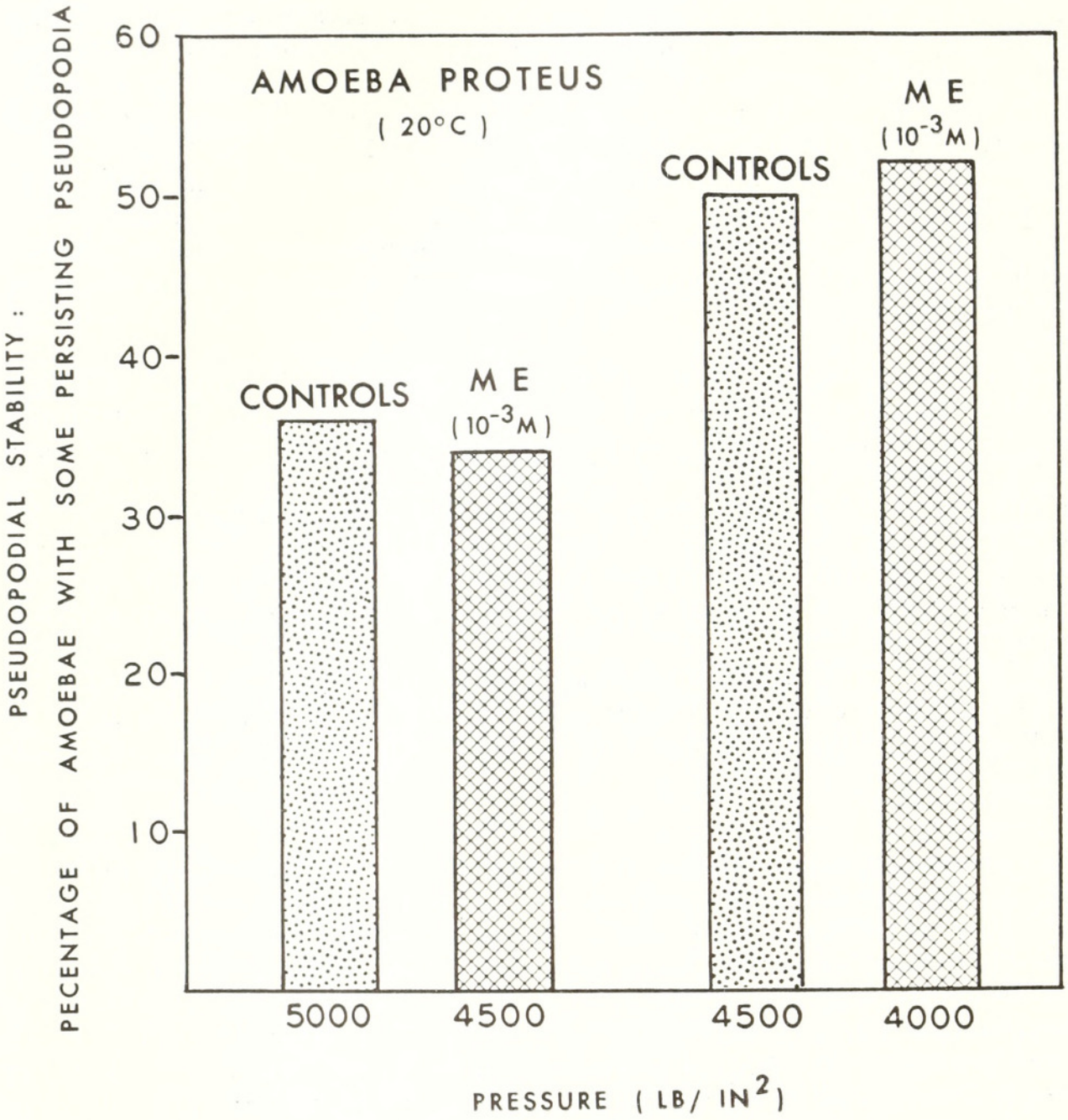

FIGURE 1. The effects of mercaptoethanol (10^{-3} *M*) on pseudopodial stability in *Amoeba proteus*. In each experiment the values give the percentage of amoebae with definitely persisting pseudopodia after a compression period of 20 minutes at the designated pressure.

Pressure studies. Previously it was reported (Marsland and Brown, 1936; Landau *et al.*, 1954) that the pseudopodia of amoebae become unstable and the amoebae round up into a spherical shape when they are subjected to high hydrostatic pressure. From pressure-centrifuge experiments it was established that the pressure level necessary to induce this rounding is a function of the relative strength of the plasmagel of the amoeba. Consequently, the rounding of the amoeba under pressure was employed as an index of plasmagel strength and the effects of mercaptoethanol were evaluated upon the pseudopodial stability.

Having established that amoebae exhibit apparently normal form and activity for a 24-hour period in 10^{-3} *M* mercaptoethanol, this concentration was

chosen for a series of pressure experiments. In each experiment about 50–100 amoebae were placed in 10^{-3} *M* solution of mercaptoethanol which was previously equilibrated to 20° C. After an incubation of one hour, the amoebae were subjected to a pressure of 4000–5000 lbs./in.^{2} for a period of 20 minutes. Prior to the application of pressure and during the pressure treatment the amoebae were kept under constant observation. Counts were made to determine the percentage of the specimens which lost all pseudopodia and became spherical as compared with

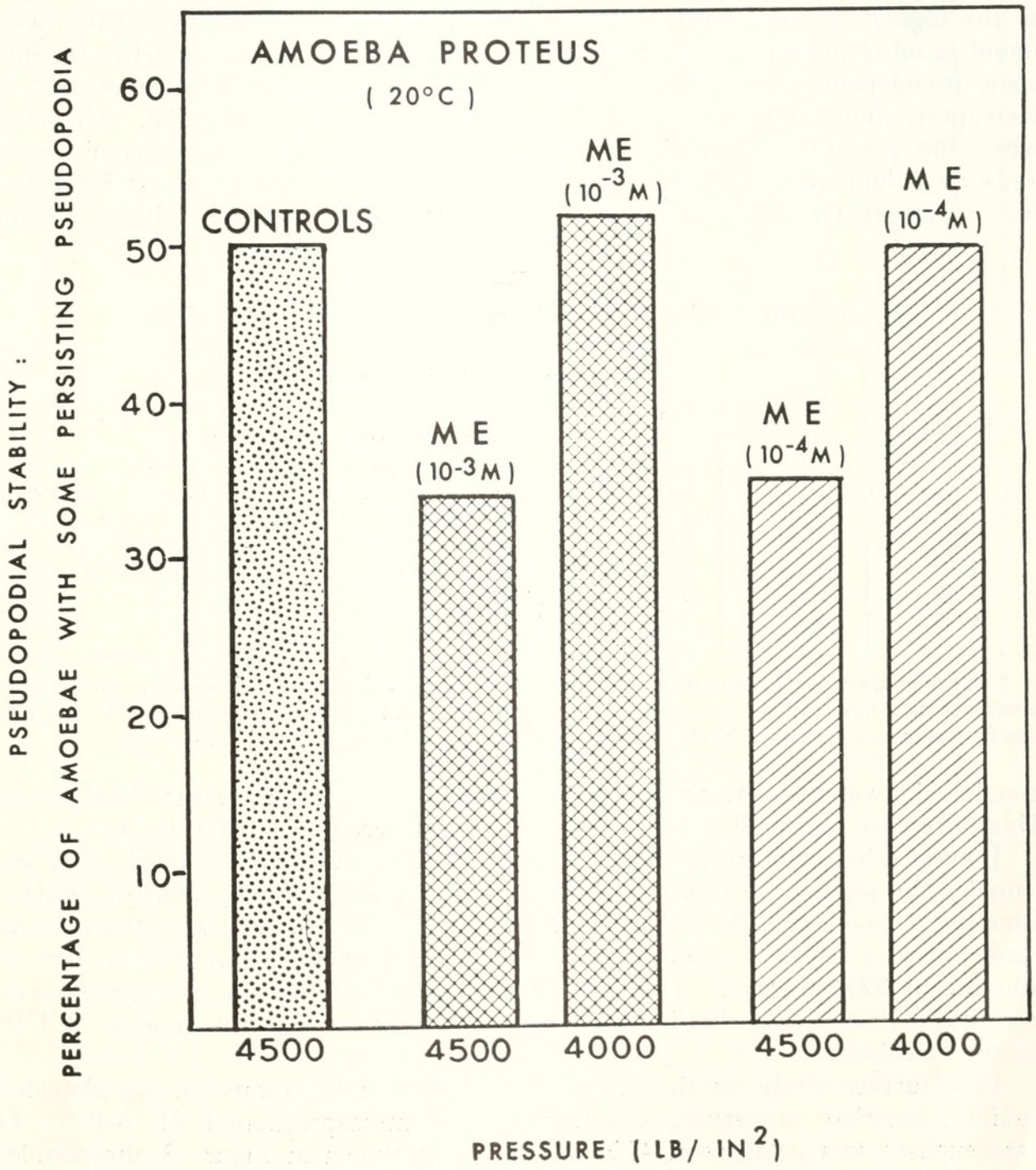

FIGURE 2. The comparative effects of 10^{-3} *M* mercaptoethanol and 10^{-4} *M* mercaptoethanol on pseudopodial stability. In each experiment the values show the percentage of amoebae with definitely persisting pseudopodia after a standard compression period of 20 minutes at 4500 lbs./in.^{2} and 4000 lbs./in.^{2}.

those which retained distinct vestiges of the pseudopodia. Following the pressure treatment the specimens were decompressed. Shortly after decompression, essentially normal form and streaming were evident.

As shown in Figure 1, at a pressure of 4500 lbs./in.^{2}, 50% of the control specimens retained some persisting pseudopodia following 20 minutes of pressure. However, when the amoebae were immersed in 10^{-3} *M* mercaptoethanol, only 34% of the specimens retained pseudopodia. When the pressure was reduced to 4000 lbs./in.^{2}, the pseudopodial stability was comparable to that of the controls at the higher pressure level. Similar pseudopodial stability characteristics were found at other pressures. At 5000 lbs./in.^{2}, 35% of the control amoebae retained some pseudopodia after 20 minutes of pressure, but in the presence of 10^{-3} *M* mercaptoethanol, only 27% of the cells retained their pseudopodia. However, when the pressure was lowered to 4500 lbs./in.^{2}, the mercaptoethanol-treated amoebae exhibited stability values equivalent to those of the controls at 5000 lbs./in.^{2}. Consistently the percentage of mercaptoethanol amoebae with persisting

TABLE I

Pressure-induced solation of Amoeba proteus *in mercaptoethanol solutions*

Pressure lbs./in.^{2}	Percentage of fully rounded quiescent specimens					
	Controls	Mercaptoethanol concentration				
		$10^{-2}M$	$10^{-3}M$	$10^{-4}M$	$10^{-5}M$	$10^{-6}M$
5000	64 (409)	78 (27)	73 (48)	—	—	—
4500	50 (1827)	68 (258)	66 (301)	65 (107)	66 (134)	45 (150)
4250	38 (100)	51 (72)	61 (358)	—	—	—
4000	28 (71)	59 (64)	48 (79)	50 (54)	—	—

* In each experiment, the percentage of completely rounded quiescent specimens, without any persisting pseudopodia, was determined after a standardized compression period of 20 minutes. The figures in parentheses indicate the total numbers of specimens observed in each case.

pseudopodia was less than that of the control amoebae at each pressure level tested. This difference in stability indicates a pressure differential of 500 lbs./in.^{2}.

Pseudopodial stability in 10^{-3} and 10^{-4} *M* mercaptoethanol proved to be essentially the same. As is shown in Figure 2, 34–35% of the amoebae retained some pseudopodia at 4500 lbs./in.^{2} in both 10^{-3} and 10^{-4} *M* mercaptoethanol solutions, whereas 50–52% retained pseudopodia when immersed in 10^{-3} or 10^{-4} *M* solutions at 4000 lbs./in.^{2}. This latter degree of stability for the mercaptoethanol-treated amoebae at 4000 lbs./in.^{2} was equivalent to that of control amoebae at 4500 lbs./in.^{2} (Table I).

In a further study on the effect of varying concentrations on pseudopodial stability, amoebae in varying concentrations of mercaptoethanol (10^{-2}–10^{-6} *M*) were exposed to a pressure of 4500 lbs./in.^{2}. As shown in Figure 3, the pseudopodial stability of amoebae in 10^{-2}–10^{-5} *M* solutions was distinctly less than that of the controls. At a concentration of 10^{-6} *M*, however, pseudopodial stability was similar to that of the controls. In fact, a small increase in stability was indicated.

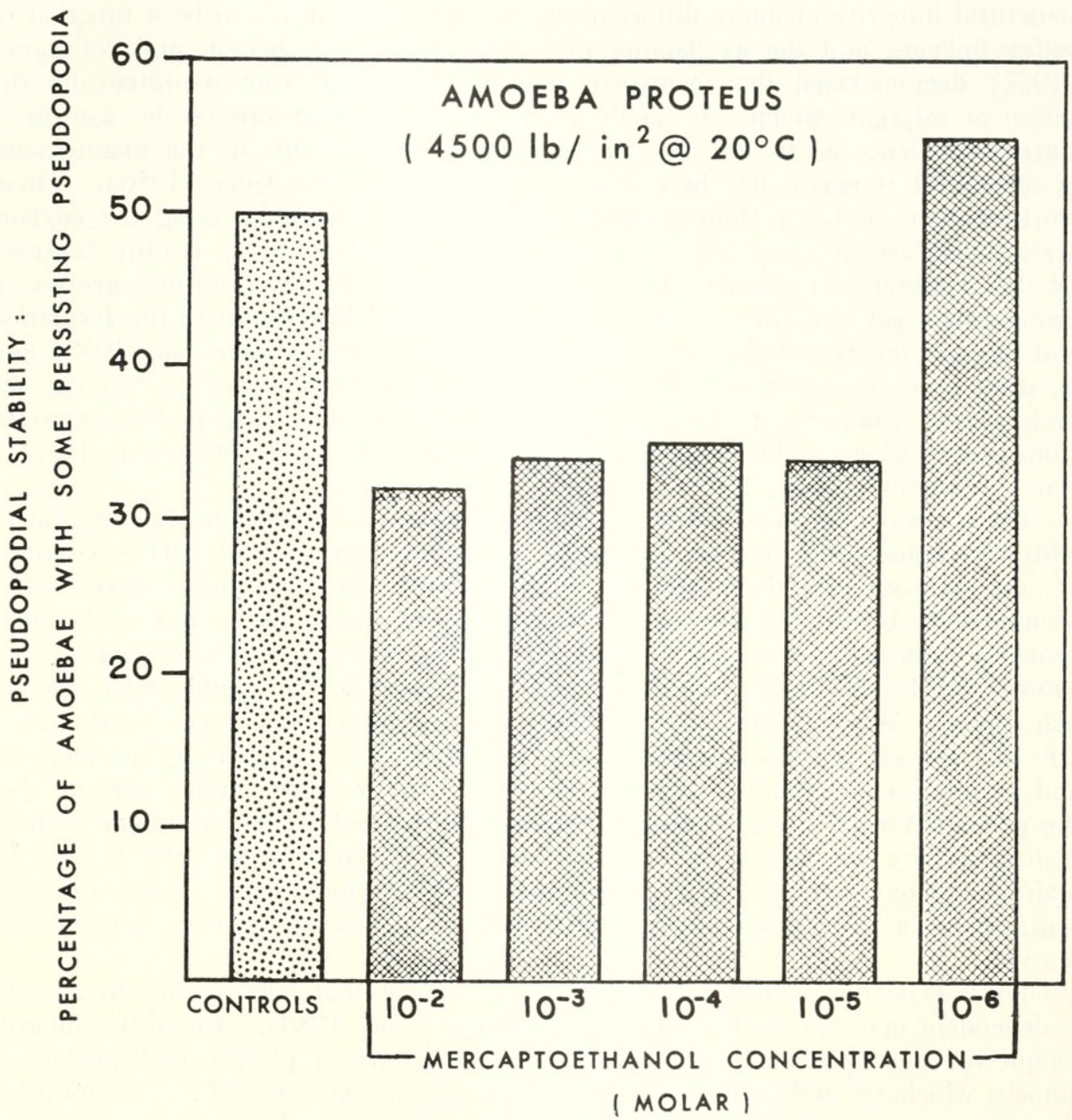

FIGURE 3. The effects of varying concentrations of mercaptoethanol on pseudopodial stability. In each experiment not less than 100–150 specimens were used and each value represents the percentage of non-rounded specimnes with some definitely persisting pseudopodia, after a 20-minute exposure to a pressure of 4500 lbs./in.^{2}, at 20° C.

DISCUSSION

In general, the results support the hypothesis that a proper balance of thiol and disulfide groups may be essential for the formation and maintenance of gelated structures in the cell. In the amoeba, the results support the supposition that high concentrations of −SH groups interfere with the establishment of labile protein linkages, modifying protoplasmic gelation reactions, and ultimately inhibiting amoeboid movement (see, also, Mazia and Zimmerman, 1958).

There is strong evidence that −SH groups of constituent protein molecules may play an important role in the assembly of macromolecular complexes. The

structural integrity of many different systems has been shown to be a function of sulfur linkages and the availability of −SH groups. Amberson and colleagues (1957) demonstrated that Δ-myosin may be separated from Δ-protein by the action of salyrgan which, presumably, acts on the thiol groups of the Δ-protein. Further evidence as to the importance of sulfhydryl groups in the maintenance of structural integrity has been reported by Madsen and Cori (1956). These workers showed that p-chloromercuribenzoate is capable of breaking the enzyme α-phosphorylase into smaller fractions, and that this reaction is readily reversed by the addition of cysteine. The importance of the balance of thiol groups in protoplasmic gel structure has also been demonstrated in relation to the formation and the maintenance of the mitotic apparatus (Mazia and Zimmerman, 1958) and in studies on the structural characteristics in cortical plasmagel of cleaving sea urchin eggs (Zimmerman, 1962a, 1964). Contractility of thread models prepared from fertilized sea urchin eggs is also accompanied by −SH changes in the contractile protein (Sakai, 1962).

The decrease of pseudopodial stability in *Amoeba proteus* following treatment with mercaptoethanol may be explained as an interference with SH⇌SS interactions, necessary for the formation of the gel structure. At high concentrations of mercaptoethanol (*e.g.*, 0.1 *M*), the pseudopodia were not maintained. This probably indicates a lowering of plasmagel strength, since pressure studies have shown that below certain values for plasmagel rigidity, the pseudopodia are not maintained. When lower concentrations of mercaptoethanol were used (10^{-2}–10^{-5} *M*) the gel formed was apparently rigid enough to support the pseudopodia and permit pseudopodial formation. However, the pressure studies indicate that the gel structure is not as strong as that of the control cells. Although pseudopodial stability was lower with mercaptoethanol treatment (10^{-2}–10^{-5} *M*), it remained approximately the same within this concentration range. When the concentration was decreased further, however, pseudopodial stability returned to normal.

There is strong evidence to support the concept that contractility in amoeba is dependent upon an ATP system (Zimmerman *et al.*, 1958). Recently, Simard-Duquesne and Couillard (1962a, 1962b) have prepared glycerinated models of amoeba which are activated in the presence of magnesium and ATP. In addition, they have prepared ATP-ase from amoeba and this resembles myosin ATP-ase. Further support for the assumption that the plasmagel layer of the amoeba represents an ATP-sensitive contractile system can be found in the recent work of Sells *et al.* (1961) and Zimmerman (1962b). These investigators demonstrated ATP-ase activity on the surface of the amoeba. Abe (1963) has reported that protoplasmic streaming in amoeba may be reversibly blocked with p-chloromercuribenzoate, which probably interferes with SS⇌SH interactions in the cell. These studies suggest, therefore, that physiological activity in the thiol-sensitive plasmagel structure of *Amoeba proteus* may be dependent upon the energy transactions of the ATP system.

The author wishes to express his thanks to Professor Douglas Marsland for his helpful criticism in the preparation of the manuscript. The author acknowledges with gratitude the technical assistance of Miss Sondra C. Corff and Mrs. Regina Schuel.

Summary

The effects on *Amoeba proteus* of mercaptoethanol solutions of varying concentrations were studied. In 10^{-1} *M* mercaptoethanol the amoebae lose their pseudopodia and after several hours undergo cytolysis. Amoebae immersed in lesser concentrations (10^{-3} to 10^{-5} *M*) maintain an apparently normal form and normal movement. The effects of mercaptoethanol on the stability of the pseudopodia, as indicated by their resistance to the solational action of high pressure, showed a distinct loss of pseudopodial stability. The decreased stability of the pseudopodia induced by these mercaptoethanol solutions indicated a pressure differential of 500 lbs./in.^{2} (at 20° C.), in comparison with the pseudopodial stability of control amoebae. This loss of stability in the mercaptoethanol-treated amoebae is interpreted as a weakening of the plasmagel structure of the amoeba. The experiments also suggest that the sol-gel equilibrium in amoeba is a thiol-sensitive system and that interference with this system inhibits protoplasmic gelation and reduces pseudopodial stability.

LITERATURE CITED

Abe, S., 1963. The effect of *p*-chloromercuribenzoate on amoeboid movement, flagellar movement and gliding movement. *Biol. Bull.*, **124**: 107–114.

Allen, R. D., 1961. A new theory of ameboid movement and protoplasmic streaming. *Exp. Cell Res., Suppl.*, **8**: 17–31.

Amberson, W. R., J. I. White, H. B. Bensusan, S. Himmelfarb and B. E. Blankenhorn, 1957. Δ protein, a new fibrous protein of skeletal muscle: preparation. *Amer. J. Physiol.*, **188**: 205–226.

Brandwein, P. F., 1935. The culturing of fresh water protozoa and other small invertebrates. *Amer. Natural.*, **69**: 628–632.

Landau, J. V., 1959. Sol-gel transformations in amoebae. *Ann. N. Y. Acad. Sci.*, **78**: 487–500.

Landau, J. V., A. M. Zimmerman and D. A. Marsland, 1954. Temperature-pressure experiments on *Amoeba proteus;* plasmagel structure in relation to form and movement. *J. Cell. Comp. Physiol.*, **44**: 211–232.

Madsen, N. B., and C. F. Cori, 1956. The interaction of muscle phosphorylase with *p*-chloromercuribenzoate. *J. Biol. Chem.*, **223**: 1055–1065.

Marsland, D. A., 1950. The mechanisms of cell division; temperature-pressure experiments on the cleaving eggs of *Arbacia punctulata*. *J. Cell. Comp. Physiol.*, **36**: 205–227.

Marsland, D. A., 1956. Protoplasmic contractility in relation to gel structure. *Int. Rev. Cytol.*, **5**: 199–227.

Marsland, D. A., and D. E. S. Brown, 1936. Amoeboid movement at high hydrostatic pressure. *J. Cell. Comp. Physiol.*, **8**: 167–178.

Mast, S. O., 1926. Structure, movement, locomotion and stimulation in amoeba. *J. Morph.*, **41**: 347–425.

Mazia, D., and A. M. Zimmerman, 1958. SH compounds in mitosis. II. The effect of mercaptoethanol on the structure of the mitotic apparatus in sea urchin eggs. *Exp. Cell Res.*, **15**: 138–153.

Sakai, H., 1962. Studies on sulfhydryl groups during cell division of sea urchin egg. V. Change in contractility of the thread model in relation to cell division. *J. Gen. Physiol.*, **45**: 427–438.

Sells, B. H., N. Six and J. Brachet, 1961. The influence of the nucleus upon adenosine triphosphatase activity in *Amoeba proteus*. *Exp. Cell Res.*, **22**: 246–256.

Simard-Duquesne, N., and P. Couillard, 1962a. Ameboid movement. I. Reactivation of glycerinated models of *Amoeba proteus* with adenosinetriphosphate. *Exp. Cell Res.*, **28**: 85–91.

SIMARD-DUQUESNE, N., AND P. COUILLARD, 1962b. Ameboid movement. II. Research of contractile proteins in *Amoeba proteus*. *Exp. Cell Res.*, **28**: 92–98.

ZIMMERMAN, A. M., 1962a. The effects of mercaptoethanol on cleaving eggs of *Arbacia punctulata*. *Biol. Bull.*, **123**: 518–519.

ZIMMERMAN, A. M., 1962b. Action of ATP on amoeba. *J. Cell. Comp. Physiol.*, **60**: 271–280.

ZIMMERMAN, A. M., 1964. Effects of mercaptoethanol on the furrowing capacity of *Arbacia* eggs. *Biol. Bull.*, **127**: 345–352.

ZIMMERMAN, A. M., J. V. LANDAU AND D. MARSLAND, 1958. The effects of adenosine triphosphate and dinitro-o-cresol upon the form and movement of *Amoeba proteus*. A pressure study. *Exp. Cell Res.*, **15**: 484–495.