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Effect of Metrazol upon Membrane Potential and Rate of Locomotion in *Amoeba proteus.** (28936)

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Since the initial measurement of the membrane potential in amoeba(1), numerous reports upon such values in protozoa, and relations to environmental conditions and cellular behavior have appeared. Cation involvement in the membrane potential in paramecium has been indicated(2), and changes in potential have been related to function of locomotor organelles(3,4). More recently a theory has been proposed linking changing membrane potentials to amoeboid locomotion (5). The central stimulant, Metrazol (pentamethylenetetrazol) has been shown to alter membrane permeability(6,7) in such a manner that membrane potentials might be expected to change. The stability of Metrazol makes it a desirable test substance for cellular testing. Amoeba were exposed to that substance while cell membrane potentials were determined and cellular behavior was closely observed.

Materials and methods. Amoeba proteus were cultured in Chalkley's solution and fed *Tetrahymena pyriformis* and *Chilomonas paramecium.* Amoeba were transferred every 14 days to fresh medium and cells from cultures 7 to 12 days old were used in all experiments.

Glass capillary electrodes of 0.5 to 1.0 μ inner tip diameter were employed. They were filled with 2.7 M KCl, and coupled through Ag-AgCl electrodes to a Keithley 603 Electrometer.

Amoebae were placed in Chalkley's solution on a glass slide in a special leucite chamber with side injection ports. A cover glass and mineral oil seal prevented water evaporation. Cells were continuously observed under 100 $\times$ magnification during electrode penetration, membrane potential measurement and Metrazol addition.

Amoeboid locomotion was evaluated by de-

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TABLE I. Effect of 1.75×10^{-4}M Metrazol and Salts Solution Addition Upon Membrane Potential and Rate of Locomotion in Single *Amoeba proteus*. Standard deviation indicated in parentheses.

Test (20 cells)	Normal	Metrazol	Change	% change
Membrane Potential (mV)	−40.7 (±10.1)	−29.1 (± 9.5)	+11.8	+30
Locomotion Rate (μ^3/sec)	1820 (±101)	4080 (±255)	+3260	+56
Control (4 cells)		Chalkley's solution		
Membrane Potential (mV)	−35.2 (±10.5)	−33.2 (± 8.9)	+2.5	+ 5
Locomotion Rate (μ^3/sec)	2140 (±36)	1870 (±49)	−270	−13

termining the time required for a pseudopod to be extended a distance one-half its diameter. Protoplasmic extension was calculated as a hemisphere and finally expressed as volume per unit time. In this way locomotion could be compared in instances of pseudopodia varying considerably in diameter. The rate of extension is related to the central streaming plasmasol.

Test materials were added by side ports on the leucite cell chamber. A volume of 0.05 ml of Chalkley's solution containing sufficient Metrazol was added to give a total of 0.2 ml of the final desired concentration within the chamber.

Results. In all instances the membrane potential existed with a relative negativity at the inner surface. Initial tests were conducted upon 7 cells in which the normal membrane potential was first determined, and then the potential determined as the cells were exposed to 1.75×10^{-4} M Metrazol. The average normal membrane potential was −51.0 mV; in Metrazol the average membrane potential was −37.4 mV. In another 20 cells, Metrazol addition caused pseudopodal extension to increase from a normal average of 1560 to 3130 μ^3 per second.

Subsequent studies were conducted to determine the effect of Metrazol upon both these characteristics in a single cell. A single amoeba was selected and placed in the chamber. The normal membrane potential and rate of locomotion were determined and then the changes noted following addition of Metrazol to the surrounding medium. Twenty such determinations were made, the results of which are shown in Table I. It was found that Metrazol decreased the potential of the membrane from a normal average of −40.8 mV to −29.1 mV, a 30% change. At the same time the rate of locomotion increased from a normal average of 1820 μ^3 per second to 4080 μ^3 per second, a 56% change.

Although the Metrazol solutions were added slowly, the possibility that current flows within the chamber might have altered the cellular conditions was also investigated. After determining the normal membrane potential and locomotion rate, a volume of Chalkley's solution equal to the volume of Metrazol solution normally used, was introduced in the chamber in the usual manner. Membrane potentials and locomotion rates were then noted. Such tests were conducted on 4 individual cells. Results are indicated in Table I. It was found that the membrane potential decreased an average of 5%, while the rate of locomotion was decreased 13%.

Additional tests were carried out using the entire procedure upon a single cell. The normal membrane potential and rate of locomotion were determined. The salts solution was introduced into the chamber and results noted. The results of 2 such tests are shown in Table II. It was found that neither the membrane potential nor rate of locomotion were appreciably altered until the addition of Metrazol.

Discussion. While pH and potassium ion have been shown to affect the rate of locomotion in amoeba(8), these were not variables in the present study. The salts solution containing the protozoa remained at pH 6.2 regardless of altered conditions in a test. Since a flow of bathing solutions did not ef-

TABLE II. Effect of Salts Solution and 1.75×10^{-4} M Metrazol Addition upon Membrane Potential and Locomotion in Single *Amoeba proteus*.

	Condition	Membrane potential	Rate of locomotion
Cell #1	Normal	−42 mV	1960 μ^3/sec
	Control	−41	2010
	Metrazol	−23	3100
Cell #2	Normal	−34 mV	1010 μ^3/sec
	Control	−32	1090
	Metrazol	−24	1890

fect a great change in cell behavior, it must be concluded that the addition of Metrazol was the cause of altered membrane potential and locomotion.

The mechanism of Metrazol has not been established, although membrane involvement in potassium transport has been indicated(6, 7). Protozoan membrane potentials are reduced by increased extracellular potassium(2, 3,4,5). The reduced potential is probably related to altered concentration gradient effects as seen in other cells. It may be that in altering membrane permeability to potassium the Metrazol has caused the observed change in the membrane potential. However, this is speculation beyond the studies described and would require particular testing.

It is interesting to relate our findings to those of Bingley and Thompson(5), who report a lower membrane potential (less negativity) at the forward-moving pseudopod than at the cell's posterior region. We have not confirmed their results, but do find that the decreased membrane potential is accompanied by an increase in pseudopodal extension. The mechanism of Metrazol's action upon amoeba is not clear, but it does not seem related to generally accepted attitudes of membrane potential maintenance or alteration.

Summary. While under constant observation, the membrane potential and rate of locomotion of *Amoeba proteus* have been determined under normal conditions and in the presence of 1.75×10^{-4} M Metrazol. The membrane potential was changed from a normal average of −40.7 to −29.1 mV, and the rate of pseudopodal extension increased from a normal average of 1820 to 4080 μ^3 per second. The possible significance of these changes are considered in relation to other reports upon Metrazol action and protozoan behavior.

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Excretion of C^{14}-Bilirubin in Newborn Guinea Pigs.* (28937)

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Reduced glucuronide formation in neonatal liver has been well documented(1,2,3), but there is no direct information as to what extent this interferes with bilirubin excretion in bile. Moreover, recent studies(4) have suggested that in addition to impaired glucuronidation, fetal liver also exhibits a reduced excretory function. In the present investigation the capacity of newborn liver to conjugate and excrete near-physiologic loads of bile pigments was assayed with C^{14}-bilirubin.

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