hormone exerted a detectable influence on K^+ level of the diaphragm *in vitro*. Moreover, diaphragms which were K^+ depleted and could not take up K^+ during the incubation period, still responded to bovine growth hormone with an increase in amino acid transport. Thus it appears unlikely that the effects of hypophysectomy and growth hormone on amino acid transport by rat diaphragm are mediated through changes in muscle K^+ content.

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The Effect of Metrazol in Isolated Mammalian Cells.* (26623)

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In vitro studies in rat brain slices show that addition of Metrazol (pentamethylenetetrazole), inhibits 20-25% of the oxygen uptake(1). Studies of the mode of action of Metrazol in intact animals indicate that it stimulates oxygen consumption and causes symptoms akin to those of hypoxia(2). We decided to investigate the effect of Metrazol on a homogeneous cell population, propagated *in vitro*. Strain L mouse fibroblasts (3) were selected for the initial investigation because they are stable, sturdy, and can easily be grown in quantity.

Materials and methods. L strain, NCTC 929, mouse fibroblasts and all media were obtained from Microbiological Associates, Inc., Bethesda, Md. Metrazol was purchased from the Bilhuber-Knoll Corporation, Orange, N. J. Cells were maintained and incubated in Eagle's minimum essential medium (4) supplemented with 10% horse serum and a penicillin-streptomycin mixture. They were grown in monolayer cultures with three changes of medium a week. Harvested cells were introduced into a U-shaped chamber and grown in submerged aerated cultures with continuous inflow of fresh medium and outflow of spent broth. Agitation was sufficient to keep the cells dispersed and to prevent adhesion to the walls. Portions of the suspension were withdrawn at regular time intervals and viable cells were counted in a haemocytometer. Other portions of the suspension were transferred to Warbug vessels equipped with CO_2 traps for measurement of oxygen uptake.

Results. The normal oxygen uptake of strain L cells measured on portions of a suspension culture shaken in Warbug vessels is 0.25 μl per mg of cells per minute. Addition of Metrazol to the Warbug vessels brings about an immediate decrease in the oxygen uptake. Thirty minutes after addition of Metrazol (4×10^{-4} M) the rate of oxygen uptake decreases 94% (Fig. 1) and then remains constant for several hours. Comparison of the rates of oxygen consumption 30 min. after addition of various amounts of

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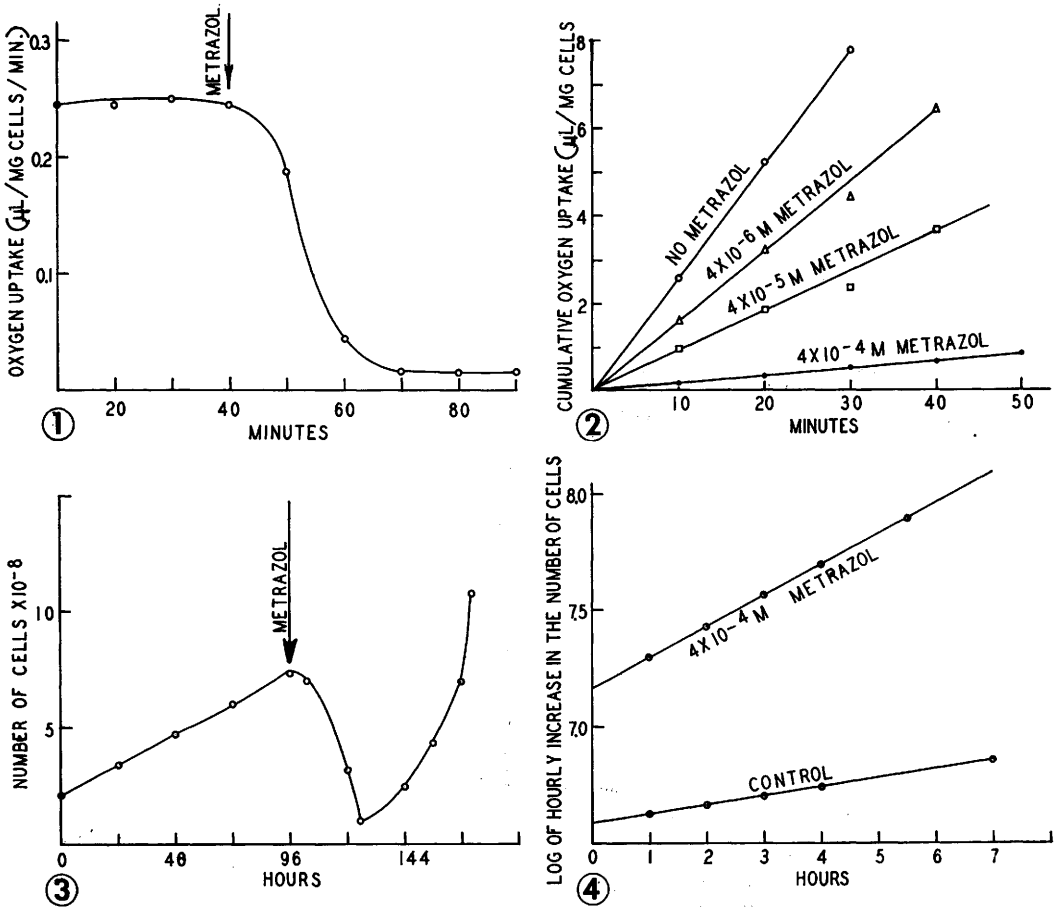

FIG. 1. Typical experiment showing decrease in Q_{O_2} after addition of Metrazol (4×10^{-4} M) to cell suspension in Warburg vessel.

FIG. 2. Oxygen uptake in presence of varying concentrations of Metrazol. Measurements began 30 min. after addition of drug to suspensions of cells in Warburg vessels.

FIG. 3. Initial decrease and eventual increase in number of cells in perfused culture after introduction of 4×10^{-4} M Metrazol.

FIG. 4. Growth rate of perfused culture 72 hours after addition of Metrazol.

Metrazol fails to reveal a threshold concentration below which there is no inhibition of oxygen uptake. Low concentrations of Metrazol continue to inhibit although the inhibition is reduced (Fig. 2).

Inhibition of oxygen consumption proves to be reversible, however, in perfused cultures. At first, addition of Metrazol at the level of 4×10^{-4} M, to a perfusion culture causes a sharp drop in the number of cells. This is followed by a reversal after 30 hours (Fig. 3). After the reversal, the rate of growth increases rapidly. As the log phase of growth is reached the generation time decreases to an unprecedented 7.5 hours (Fig. 4, Table I). The average generation time under similar conditions but in the absence of Metrazol is 13 times longer. Oxygen utilization increases also but only three-fold. The net effect of these changes is to reduce the

TABLE I. Metabolism of Perfused Culture Maintained for a Week in Presence of Metrazol.

	Control	Metrazol
Q_{O_2} (μl/mg cells/hr)	15.5 ± 0.6	46.1 ± 1.9
Generation time	94.6 ± 0.4	7.5 ± 0.8

Q_{O_2} measured in Warburg vessels on aliquots withdrawn from perfusion chamber. Generation time (No. of hours required for doubling cell population) calculated from counts of viable cells at hourly intervals.

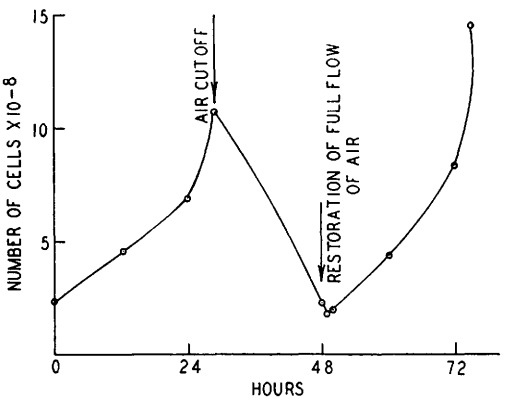

FIG. 5. Decrease in number of cells caused by 20% decrease in air flow through "Metrazol" culture and rebound effect after restoration of normal air flow.

consumption of oxygen per cell. This renders the culture extremely sensitive to small variations in oxygen supply. Decreasing the flow of air through the culture causes an immediate drop in the number of cells. Restoration of the full flow of air restores the rapid proliferation of cells (Fig. 5).

Discussion. Short-term tissue culture experiments indicate that Metrazol inhibits cell growth and thereby reduces oxygen uptake. This is in agreement with the findings of Kajdi and Williams in brain slices(1). However, in experiments of longer duration, which are possible with cell cultures but not with tissue slices, Metrazol stimulates rapid oxygen uptake and a more rapid growth of those cells which survive the initial inhibition. Since oxygen uptake does not keep pace with cell growth, the oxygen tension within the cells must be lower in cells growing in the presence of Metrazol than in control cultures. Possibly, the cell metabolism changes under the influence of Metrazol along the lines suggested by Warburg, who believes that when the respiration of a cell is gradually inhibited, the surviving cell compensates for the loss of energy by increasing the rate of fermentation(5). Lactic acid should accumulate in such a case, and indeed, Gourdjan *et al.* reported an accumulation of lactic acid in the brain during Metrazol-induced seizures(2). In a series of experiments with *S. cerevisiae,* which are capable of growing under either aerobic or anaerobic conditions, we found that the metabolic pattern of aerated yeast grown in the presence of Metrazol resembles the pattern of control cultures grown in the absence of oxygen (unpublished observations).

It is unlikely that adaptation of the cells to Metrazol involves synthesis of a detoxifying system capable of disposing of Metrazol. Since fresh Metrazol is continuously fed into the culture chamber, its removal would require too large an expenditure of energy. Synthesis of an active barrier preventing the penetration of Metrazol into the cytoplasm would also require a large expenditure of energy. On the other hand, it is possible that no adaptation but selection took place in our cultures and that cells which were originally resistant to Metrazol were the only ones to survive. In either case, whether through adaptation or selection, the average surviving cell differs considerably from the average prototype; it multiplies faster than the control cell but utilizes less oxygen during growth.

Summary. In short-term experiments, oxygen uptake by mouse fibroblast cells is inhibited by the addition of the convulsant Metrazol. After 30 hours of incubation in suspension culture, the effect of Metrazol is reversed. Both oxygen uptake and cell growth are enhanced. Cells grown in the presence of the drug multiply 13 times faster and use up 3 times as much oxygen as control cells.

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