though more quantitative growth yield experiments (*18*) are needed to define more precisely growth yields for MR-1, these values clearly indicate that $MnO_2$ functions as an electron acceptor for MR-1. A dissimilatory Fe(III)- and Mn(IV)-reducing bacterium, GS-15, was recently isolated by other investigators from the sediments of the Potomac River, Maryland (*19*); GS-15 can couple its growth to the reduction of Fe(III) or Mn(IV). GS-15 has not yet been classified taxonomically, but it is clearly different from MR-1 in that it is an obligate anaerobe.

Microbial Mn reduction has been reported in marine, freshwater, and terrestrial environments (*6–9, 20*), which implies that Mn could play an important role in carbon mineralization. The ability of MR-1 to grow by the use of manganese oxide as a terminal electron acceptor suggests that MR-1, and other bacteria with similar properties, may play a significant role in the cycling of Mn and in organic matter mineralization in anaerobic sediments that contain abundant Mn, such as in Oneida Lake, Green Bay, and Lake Michigan.


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12. Preliminary estimates of Mn(II) flux obtained with bottom flux chambers indicate a flux of 1 to 3 mmol $m^{-2}$ $day^{-1}$ (K. H. Nealson and M. Enzien, unpublished data). Given the observed experimental rates of Mn reduction by MR-1 (see text discussion of Fig. 1), approximately $1 \times 10^5$ to $5 \times 10^5$ MR-1 cells $ml^{-1}$ could account for the reduction rates observed in the summer of 1987.
13. LO medium consisted of Oneida Lake water with 10 m*M* Hepes [4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid] buffer (*p*H = 7.4), 0.01% Bacto-peptone, 0.02% Bacto-yeast extract (Difco Laboratories, Detroit, MI); after autoclaving, sodium acetate or succinate and sodium bicarbonate were added from sterile stock solutions to yield final concentrations of 15 m*M* and 2 m*M*, respectively. Manganese oxide ($\delta MnO_2$) was prepared (*3, 7*), ground to a fine powder with mortar and pestle, and sterilized by dry heat (200°C) for several hours. Before addition to the cultures, the $\delta MnO_2$ was suspended in *p*H 11 sterile water and sonicated to reduce particle size. Experiments were conducted under anaerobic conditions (10% hydrogen plus 90% nitrogen) with a Coy anaerobic chamber (Coy Laboratory Products, Ann Arbor, MI) (*3, 7*).
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15. Defined medium (*p*H = 7.4) consisted of 15 m*M* sodium succinate ($Na_2C_4H_4O_4$), 9.0 m*M* $(NH_4)_2SO_4$, 5.7 m*M* $K_2HPO_4$, 3.3 m*M* $KH_2PO_4$, 2.0 m*M* $NaHCO_3$, 1.01 m*M* $MgSO_4$, 0.485 m*M* $CaCl_2$, 67.2 μ*M* $Na_2EDTA$, 56.6 μ*M* $H_3BO_3$, 10.0 μ*M* NaCl, 5.4 μ*M* $FeSO_4$, 5.0 μ*M* $CoSO_4$, 5.0 μ*M* $Ni(NH_4)_2(SO_4)_2$, 3.87 μ*M* $Na_2MoO_4$, 1.5 μ*M* $Na_2SeO_4$, 1.26 μ*M* $MnSO_4$, 1.04 μ*M* $ZnSO_4$, 0.2 μ*M* $CuSO_4$, L-arginine HCl (20 μg $ml^{-1}$), L-glutamine (20 μg $ml^{-1}$), and DL-serine (40 μg $ml^{-1}$). This defined medium, when supplemented with alternative appropriate terminal electron acceptors (Table 1), was able to support the growth of MR-1.
16. The carbon sources tested were: arabinose, cellobiose, fructose, galactose, glucose, inositol, maltose, malonic acid, mannose, mannitol, melibiose, raffinose, rhamnose, sorbitol, sorbose, sucrose, xylitol, and xylose.
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# Potassium Salt Microinjection into *Xenopus* Oocytes Mimics Gonadotropin Treatment


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**Gonadotropin stimulates protein synthesis and growth in ovarian oocytes. The hormone is also known to modify transfollicular $K^+$ fluxes and is now shown to cause increased intraoocytic $K^+$ activity ($a_K$). The hormone's effect on $a_K$ was duplicated by microinjecting $K^+$ salts into oocytes which were incubated in paraffin oil. This treatment mimicked the influence of gonadotropin on both the rate of protein synthesis and the synthesis of specific polypeptides. These findings suggest that gonadotropin-stimulated oocyte growth is attributable largely to the hormone's influence on transfollicular $K^+$ fluxes. They support the hypothesis that the $K^+$ flux and $a_K$ changes observed during cell activation are critical in causing subsequent increases in protein synthesis and growth.**


IN MANY VERTEBRATE SPECIES, OVARIan oocytes undergo cycles of quiescence and hormone-activated protein synthesis and growth (*1*, *2*). Quiescent *Xenopus*


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*laevis* oocytes incorporate [$^3$H]leucine into protein at a low or basal rate. Exposure of the oocyte in its follicle to gonadotropin stimulates [$^3$H]leucine incorporation (*3*) (Table 1). Hormone treatment also modifies transfollicular $K^+$ fluxes (*4*) and increases intraoocytic $K^+$ activity ($a_K$). In six experiments in which oocytes were treated with human chorionic gonadotropin (hCG), $a_K$ was raised from 7 to 24% above the quiescent or control level (Table 2). The hormone's effects are long-lasting; when induced in vivo by intraperitoneal hCG injection, both the increase in leucine incorporation and the increase in $a_K$ persist for at least 10 days (*5*).

Stage IV oocytes (*2*) contain 30 to 33 nmol of $K^+$ as determined by cryomicrodissection and atomic absorption spectroscopy (*6*). To mimic the hCG effect on $a_K$, it is necessary to raise the oocyte's $K^+$ content by 3 to 10 nmol. We did this by microinjecting concentrated KCl solutions in 10-nl quantities (~5% of oocyte volume). Data from five of these experiments are presented in Table 3and Fig. 1.

When a KCl solution was microinjected, leucine incorporation increased, indicating an increase in $a_K$ alone was sufficient to cause an increase in protein synthesis. (Experiments in which $K^+$ acetate and phosphate were microinjected gave similar results.) The dose response was linear, and regression lines with slopes (*S*) comparable to those in Fig. 1 were observed in every experiment.

In addition, the hCG-induced $a_K$ increase can quantitatively account for the hormone's stimulatory effect on incorporation. Table 3 shows that in experiments in which KCl injection increased $a_K$ to the same extent as hCG (Table 2), the increase in incorporation ($R^{K^+}/R^C$) was equal to the increase stimulated by hCG (Table 1). The regression lines in Fig. 1, which are based only on the data of control and KCl-injected cells, adequately describe the hCG-treated cells as well.

Our data show that hCG incubation and KCl microinjection have similar effects on protein synthetic rates, and suggest that they act at the same step in protein synthesis (*7*). If this is true, hCG and KCl should also have a similar influence on the rates of synthesis of the individual polypeptides. Newly synthesized polypeptides from control, hCG-, and KCl-stimulated oocytes were separated by two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) (Fig. 2, A through C). We made comparisons by gel superimposition with visual examination and by

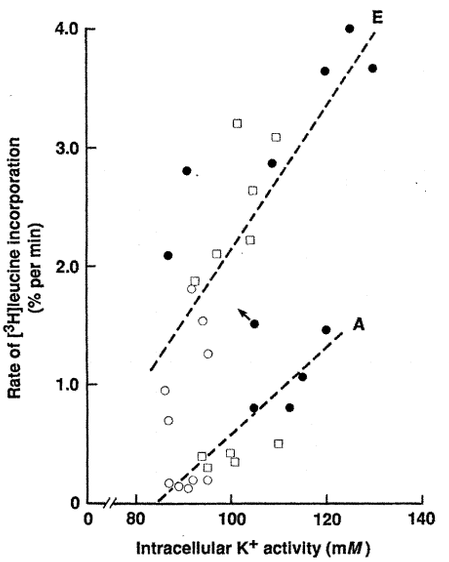


**Fig. 1.** Relation of $a_K$ to leucine incorporation rates in KCl-injected and uninjected oocytes and in oocytes stimulated with hCG. Procedure was as described in Table 3. Control oocytes (open circles) and KCl-microinjected oocytes (closed circles) were preincubated in OR2 (5 hours). Hormone-stimulated oocytes (squares) were preincubated in OR2 containing hCG (Table 1). Regression lines were determined by the method of least squares and represent control and KCl-injected oocytes only. (The arrow indicates that the data point belongs to experiment E.) Line slopes have the dimensions: % [$^3$H]leucine incorporated/min/meq $a_K$; for experiment A, $S$ = 0.39; for experiment E, $S$ = 0.60 (other measured variables for experiments A and E are found in Table 3). When $a_K$ increased 10 meq, leucine incorporation almost doubled in experiment A and increased by 40% in experiment E. Relative increase was determined by both $S$ and $R^C$.

**Table 1.** Gonadotropic hormone (hCG) stimulation of [$^3$H]leucine incorporation in *Xenopus* oocytes. Dumont (*2*) stage IV oocytes were isolated from *Xenopus laevis* that had not ovulated for at least 6 months. After incubation of oocytes (5 hours) in OR2 [a physiological saline solution (*15*)] or OR2 containing hCG (50 U/ml), we transferred them to paraffin oil (*6*, *16*) and microinjected 10 nl of [$^3$H]leucine [~1 p*M*; ~5% of the cellular pool (*17*)]. After a labeling period of 5 to 10 min, individual oocytes were frozen in Freon 12/liquid nitrogen and were stored in liquid nitrogen until analysis. Oocytes from a different animal were used in each experiment. To determine leucine incorporation, a frozen oocyte was placed in 10 ml of 0.5*N* perchloric acid (PCA) at 60°C and was extracted for 60 min to remove unincorporated labeled amino acid; the oocytes were then rinsed with PCA and extracted for 30 min. (Analysis of oocytes denuded of their follicular layer showed that the layer contributes <3% of the newly synthesized protein.) The PCA-insoluble material was then dissolved in 0.9 ml (0.1*N*) of NaOH at room temperature and neutralized with 1*N* HCl. The digested sample and a portion of each wash solution were analyzed with a scintillation spectrometer (Packard Tri-Carb model 4640). Data are given as $\bar{x} \pm$ SE; $n$, number of cells. $R^C$, rate in control cells; $R^G$, rate in gonadotropin-treated oocytes. $R^C$ and $R^G$ were determined by dividing the PCA-insoluble radioactivity by the total radioactivity recovered (~$10^5$ cpm per cell), giving the fraction of the injected [$^3$H]leucine incorporated during the labeling period (%/min). We found rates to be independent of labeling time if the fraction of the microinjected [$^3$H]leucine incorporated was kept below 30%. This condition applies to all data presented.

| Experiment | Incorporation rate ($n$) | | $R^G/R^C$ |
|---|---|---|---|
| | $R^C$ | $R^G$ | |
| A | 0.17 ± 0.01 (5) | 0.39 ± 0.03 (5)* | 2.29 |
| B | 0.27 ± 0.02 (6) | 0.55 ± 0.04 (8)* | 2.04 |
| C | 0.35 ± 0.06 (5) | 0.51 ± 0.07 (9) | 1.46 |
| D | 1.25 ± 0.25 (5) | 3.15 ± 0.44 (6)** | 2.52 |

*$R^G$ was significantly greater (by $t$ test) than $R^C$, $P < 0.001$. **$R^G$ was significantly greater than $R^C$, $P < 0.01$.

**Table 2.** Increase of $a_K$ in *Xenopus* oocytes, induced by hCG. Oocyte isolation and incubation were as described in Table 1. The $a_K$ was measured by simultaneously impaling the oocyte under paraffin oil with $K^+$-selective and KCl-reference electrodes. Preliminary experiments showed (i) that $a_K$ measured in OR2 and paraffin oil were indistinguishable and (ii) that the $a_K$ of cells injected with 10 nl of a leucine solution was unchanged from that of uninjected control cells. Microelectrode fabrication and calibration, and the electrometer used were as described (*18*). $K^+$-selective microelectrodes had tip resistances of $10^9$ to $10^{10}$ ohms and were calibrated before and after every impalement. We obtained linear logarithmic responses with a Nernstian slope of 56 ± 0.4 mV per decade [range = 50 to 61 mV at room temperature (21° to 23°C)]. Reference microelectrodes (filled with 0.5*M* KCl) had tip resistances of 15 to 30 megohms. The $K^+$:$Na^+$ selectivity of the ion-selective microelectrodes was 91:1. Oocyte $Na^+$ activity is <10 meq, and therefore we needed no correction for $Na^+$ interference. Data are given as means ± SE; $n$, number of cells. $a_K^C$, $K^+$ activity in control oocytes; $a_K^G$, $K^+$ activity after gonadotropin treatment.

| Experiment | $K^+$ activity in m*M* ($n$) | | |
|---|---|---|---|
| | $a_K^C$ | $a_K^G$ | $a_K^G/a_K^C$ |
| A | 84 ± 2 (5) | 90 ± 3 (9) | 1.07 |
| B | 86 ± 1 (4) | 104 ± 2 (4)* | 1.21 |
| C | 86 ± 6 (5) | 107 ± 3 (6)** | 1.24 |
| D | 88 ± 2 (4) | 104 ± 3 (4)** | 1.18 |
| E | 90 ± 2 (5) | 105 ± 3 (6)** | 1.17 |
| F | 91 ± 1 (5) | 99 ± 2 (6)** | 1.09 |

*$a_K^G$ was significantly greater ($t$ test) than $a_K^C$, $P < 0.001$.
**$a_K^G$ was significantly greater than $a_K^C$, $P < 0.01$.

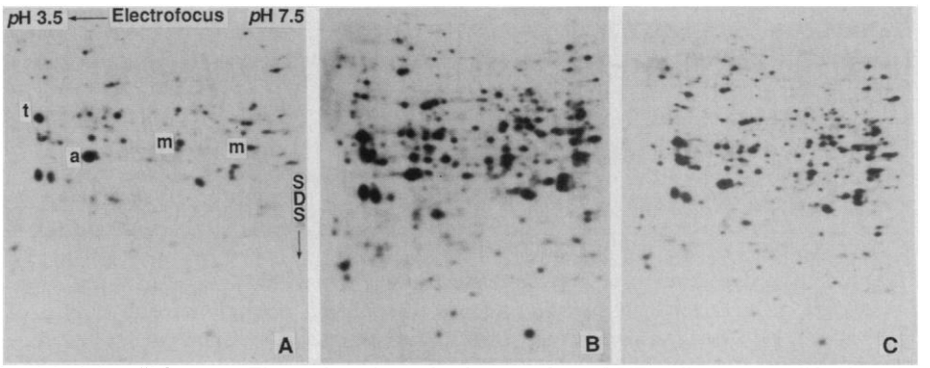


**Fig. 2.** Autoradiographs of 2D-gel slabs showing the newly synthesized polypeptides of control, hCG-stimulated, and KCl-injected oocytes. (**A**) Control oocyte (untreated with hCG and uninjected with KCl), $R_m^C$ ~0.12% per minute; (**B**) hCG-stimulated oocyte, $R_m^G$ ~0.28% per minute; (**C**) KCl-injected oocyte, $R_m^{K+}$ ~0.33% per minute. Folliculate oocytes were incubated in OR2 or OR2 + hCG (50 U/ml) for 5 hours and transferred to paraffin oil. Those oocytes incubated in OR2 alone were divided into groups, one of which was microinjected with KCl (5 n*M*). After 1.5 hours, all oocytes were labeled with [$^{35}$S]methionine (4 μCi per cell) for 2 hours and then frozen in Freon 12/liquid nitrogen. We removed follicular tissue by cryomicrodissection and weighed the still frozen oocyte (*6*). Some cells were used to determine rates of [$^{35}$S]methionine incorporation ($R_m^C$, $R_m^G$, $R_m^{K+}$) by the methods described for [$^3$H]leucine (Table 1). Those cells to be separated by 2D-PAGE were dissolved in a lysis buffer, with volume adjusted to normalize for differences in cell weight. Electrophoresis was by the procedure of O'Farrell (*19*) as modified by Paine (*20*). Gels were processed for fluorography (*21*) with a 66-hour exposure period. Actin (a), tubulin (t), and c-*myc* (m) polypeptides were identified by the immunoblot technique (*22*).

**Table 3.** Increases in [$^3$H]leucine incorporation rate caused by KCl microinjection. Procedures were as described except that after transfer from OR2 to paraffin oil, oocytes were microinjected with KCl solutions to increase $a_K$ and then incubated for 1.5 hours (to allow $a_K$ to restabilize) before being labeled with [$^3$H]leucine. Microinjection of 100-m*M* KCl, which does not change $a_K^C$, had no effect on incorporation rates. Control cells were not KCl-injected. Data are given as means ± SE; *n*, number of cells. $a_K^{K+}/a_K^C$, relative increase in $a_K$; $R^{K+}/R^C$, the relative increase in [$^3$H]leucine incorporation. Incorporation was significantly greater by *t* test in KCl-injected oocytes ($R^{K+}$) than in controls ($R^C$) in every experiment; (for A, C, and D, $P < 0.01$; for B and E, $P < 0.05$).

| Experiment | $R^C$ | | $a_K^C$ (m*M*) | KCl injected | | $a_K^{K+}/a_K^C$ | $R^{K+}/R^C$ |
|---|---|---|---|---|---|---|---|
| | %/min | *n* | | nmol | *n* | | |
| A | 0.17 ± 0.01 | 5 | 91 ± 1 | 4–8 | 4 | 1.25 | 6.1 |
| B | 0.31 ± 0.08 | 5 | 87 ± 4 | 8 | 5 | 1.16 | 2.7 |
| C | 0.32 ± 0.04 | 5 | 90 ± 3 | 3–6 | 4 | 1.26 | 3.1 |
| D | 0.59 ± 0.11 | 4 | 90 ± 2 | 6–9 | 5 | 1.17 | 2.6 |
| E | 1.25 ± 0.25 | 5 | 90 ± 2 | 8 | 7 | 1.12 | 1.8 |

computer-assisted analysis, using a system consisting of an Eikonix flat-bed scanner, a Comtal image analyzer, a VAX 11/750 computer, and appropriate software (*8*). We found that every polypeptide that was detected on gels of control oocytes was present in greater abundance on gels of the hCG- and KCl-stimulated oocytes. Furthermore, although the degree to which synthesis was increased differed for different polypeptides, the increase in synthesis of a given polypeptide (relative to the others) was similar, whether the increase was induced by hCG incubation or KCl microinjection (*9*).

We often found polypeptides that were not visible on gels of control oocytes on the gels of hCG- and KCl-stimulated oocytes, usually as the least prominent spots. However, autoradiographs of control oocytes that were overexposed, and others made of heavily loaded gels, displayed all of these polypeptides, showing that they are in fact synthesized in control oocytes but were not seen because of their low rates of synthesis.

We conclude that hCG, an activator of growth in immature oocytes, increases cellular $a_K$ and that this increase can account for the hormone's short-term stimulatory effect on protein synthesis. It is not clear whether somatic cell growth factors (*10*) also act to stimulate protein synthesis by increasing $a_K$. Suggestive $K^+$ flux and concentration changes have been reported (*11*). Increases in $a_K$ of the appropriate magnitude do occur in somatic cells during other types of growth activation. When WRL-10A cells are released from contact inhibition, $a_K$ increases from a growth-arrested value of 89 m*M* to a growth-stimulated value of 116 m*M* (*12*). Similarly, when mouse liver is induced to regenerate (by partial hepatectomy), $a_K$ increases from 94 ± 6 m*M* to 124 ± 4 m*M* (*13*). Also, during much of the cell cycle, $a_K$ and protein synthetic rates vary in parallel (*14*). It seems likely that $K^+$ regulation of protein synthesis in oocytes may be important for understanding growth control in other cells as well.

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