Table 2. Effect of somatotropin on protein synthesis in tissue culture.

Somatotropin		Controls tyrosine (μg/ml)
Dose (μg/ml)	Tyrosine (μg/ml)	
50	13.6	13.2
100	11.4	9.6
200	15.0	13.6
200	13.2	11.9
200	9.2	6.5
200	14.8	11.2
25*	14.6	12.6
50*	15.7	12.6
100*	14.9	12.6
200*	17.0	12.6
25†	11.2	10.2
50†	11.0	10.2
100†	12.0	10.2
200†	13.9	10.2

* These experiments were run simultaneously.
† These experiments were run simultaneously.

somatotropin acts directly on individual cells. The present observations indicate that somatotropin exerts a significant growth-promoting effect at the cellular level that is manifested by an increase in number of cells and in the rate of protein synthesis.

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References and Notes

1. We gratefully acknowledge the advice and assistance of C. Henry Kempe of the department of pediatrics, University of California School of Medicine, San Francisco, and of Hao Li of the Hormone Research Laboratory, University of California, Berkeley.
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5. This investigation was aided by research grant C-2155 from the National Cancer Institute of the National Institutes of Health, U.S. Public Health Service; American Cancer Society Institutional Grant 43; a grant from the E. C. Fleischner Memorial Fund and from the Edwards Fund by the Committee of Research, University of California, San Francisco.
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Oxygen Consumption in Ovulating Fragments of Ovaries of Rana pipiens

It has been reported previously (*1*) that two known inhibitors of the cytochrome oxidase system inhibit *in vitro* ovulation of fragments of ovaries of *Rana pipiens*. Since the specificity of these inhibitors (KCN and sodium azide) is neither perfectly established nor invariable, the following experiments were undertaken to study further the dependency of ovulation on oxygen by (i) observing the effect of anaerobic incubation on ovulation in isolated ovarian fragments, (ii) comparing the oxygen consumption of ovulating and nonovulating ovaries, and (iii) attempting to associate the inhibitory effect of cyanide on ovulation with concomitant inhibition of oxygen consumption.

Standard techniques of Warburg respirometry were used (*2*). A fragment of ovary containing 20 eggs was placed in each manometer flask. The total volume of fluid and tissue was kept at 4 ml throughout the respirometry. In all runs the system was allowed to equilibrate for ½ hour before readings were begun. In view of the potential danger of damage to the follicles from continuous shaking over the necessarily long incubation period of 24 hours, the manometers were shaken (80 cycles per minute) only during the 20-minute period immediately preceding a reading. For experiments involving anaerobic incubation, manometer flasks were gassed with either carbon dioxide (in which case the KOH was omitted) or nitrogen and allowed to equilibrate for ½ hour. Ovulation was stimulated by the addition of pituitary homogenate in a volume representing one-sixteenth of a triturated homoplastic anterior lobe.

Under anaerobic conditions, ovulation was not observed in 18 ovarian fragments taken from six frogs. In 36 comparable control fragments, taken from the same frogs but incubated aerobically, 27.3 percent of the available follicles extruded eggs.

In Fig. 1 the data from an experiment measuring the oxygen consumption of a number of ovulating and nonovulating ovarian fragments are compared. Respiration was minimal during the first 12 hours of incubation, for ovulation did not begin until 10 to 12 hours after the introduction of pituitary extract. In four experiments, utilizing 27 fragments from ovaries of four frogs, the average rate of oxygen consumption over the entire incubation was found to be 1.64 μlit/hr in nonovulating fragments and 2.46 μlit/hr in ovulating fragments. In two experiments, in which ovarian fragments that were exposed to pituitary extract failed to ovulate, the average oxygen consumption was 1.44 μlit/hr. Analysis of the distribution of differences of 24 pairs of oxygen-consumption values from four experiments indicates a statistical confidence level of $p < 0.01$.

Control studies were carried out on (i) oxygen consumption of pituitary extract in the absence of fragments of ovary, (ii) oxygen consumption of freshly ovulated eggs in the absence of ovarian tissue, (iii) the effect on respiration of inhibiting maturation of ovulated eggs by colchicine, and (iv) the contribution to over-all oxygen consumption made by contaminant bacteria. The increased oxygen consumption observed in ovulating ovaries could not be shown to be owing to any of these factors.

The addition of potassium cyanide ($10^{-4}M$) to flasks containing fragments of ovary completely inhibited oxygen consumption in all flasks and completely inhibited ovulation in the flasks that contained pituitary extract. Upon introduction of methylene blue from a second side arm, which brought the concentration in the medium bathing the fragments to $10^{-5}M$, the rate of oxygen consumption returned to approximately normal and the fragments subsequently ovulated. Although ovulation in fragments so treated was not completely normal, it did reach an average of 62.5 percent of that seen in control fragments exposed to methylene blue but not to potassium cyanide.

It appears that the follicular mechanisms which respond to pituitary stimulation producing the discharge of ova are aerobic and utilize molecular oxygen through the cytochrome oxidase system; this constitutes presumptive evidence of energy expenditure in ovulation. These data are consistent with the hypothesis

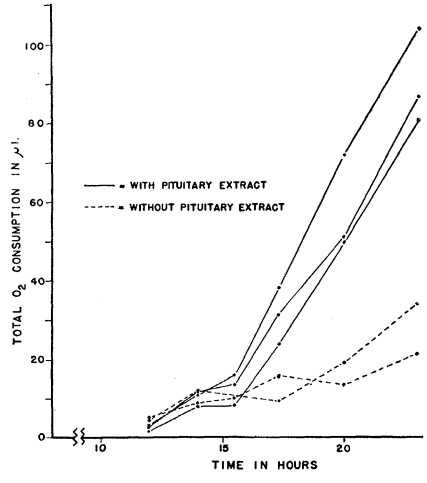

Fig. 1. Oxygen consumption in ovulating and nonovulating fragments. Each curve represents the course of oxygen consumption of a separate 20-egg fragment. Final ovulation of the fragments represented by the top three curves was 83 percent; by the bottom two curves, 0 percent.

that follicular smooth muscle cells (found only in Amphibia) are necessary for the extrusion of ova. However, the observations that $10^{-4}M$ KCN does not inhibit the normal contractions of intact, excised ovaries and that autonomic blocking agents, antihistamine, and smooth muscle depressants do not inhibit pituitary-induced ovulation suggest that the energy-consuming process is not muscular contraction. Ovulation seems more likely to be related to proteolysis of the stalk membrane or to more obscure processes supporting increased intrafollicular pressure.

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Effect of Gibberellin on Germination of Lettuce Seed

The gibberellins are a group of plant regulators that were discovered nearly 20 years ago by Japanese scientists (*1*), and lately these compounds have attracted the attention of plant physiologists in many countries (*2*). The gibberellins which are known chemically are produced by strains of the fungus *Fusarium moniliforme* (Sheld.) Snyder and Hansen emend., the asexual stage of *Gibberella fujikuroi* (Saw.) Wr. Materials with identical physiological activity have been shown to occur also in flowering plants (*3*). The gibberellin effect that was observed first and has been studied in a wide variety of plants is promotion of stem elongation. More recently, however, it was found that the gibberellins also have profound morphogenetic effects; they induce bolting and flower formation in cold-requiring plants and in long-day plants under temperature and light conditions which usually do not permit flowering (*4*).

The finding that gibberellin can "replace" light (long days) in flower induction of long-day plants led to our study of the effects of gibberellin on the germination of light-requiring seeds, since the action spectra of the light control of flowering and of seed germination are similar (*5*). The purpose of this report is to summarize the findings that have been obtained to date (*6, 7*).

All our experiments have been performed with seeds of lettuce (*Lactuca sativa* L., variety Grand Rapids). Several types of light effects are known for lettuce seed. Some lots yield low germination in the dark, and germination may be increased by a brief exposure to light; they may be said to have a "primary" light requirement. Other lots exhibit equal or nearly equal germination percentages in light and in darkness, but a "secondary" light requirement can be induced in at least two different ways: (i) if such seeds are imbibed and stored at a temperature of 35°C, their percentage germination in darkness is greatly reduced and can be restored to the original level or higher by a brief exposure to light (*5*); (ii) the presence of an osmotically active material in the medium reduces germination in the dark in direct proportion to the osmotic pressure of the solution, and this "dark-osmotic inhibition" is also released by a small quantity of light (*7*). It is likely that the basic mechanism of these light actions is the same, red light having the greatest effect in promoting germination of lettuce seed, and its effect being reversible by subsequent irradiation with far red light.

We have worked with two lots of seeds, one (seed lot A) possessing the primary type of light requirement and the other (seed lot B) acquiring the secondary type during pretreatment with high temperature or in the presence of an osmotically active material such as mannitol in solution (*8*). The gibberellin preparation used in these experiments consisted of a mixture of gibberellin A_1 (gibberellin A) and gibberellin A_3 (gibberellic acid) and will henceforth be called "gibberellin" (*9*).

Table 1 shows that the primary light requirement of seed lot A is bypassed by the addition of gibberellin. In the presence of 100 mg of gibberellin per liter, germination in darkness is as high as germination after a brief exposure to red light. An identical result has been reported by Lona (*10*) for light-requiring seeds of a wild species of lettuce, *Lactuca scariola* L.

Table 2 shows that, when gibberellin is present during pretreatment of seed lot B with high temperature, no secondary light requirement becomes apparent. As is shown in Table 3, gibberellin also promotes germination in darkness after a dependency on red light has been established by pretreatment with high temperature; thus, it removes the secondary light requirement formed by the pretreatment. Table 4 shows that gibberellin also reduces or negates dark-osmotic inhibition when it is given simultaneously with the inhibitory solution or when it is supplied as a pretreatment.

Thus, in lettuce seed, gibberellin apparently can substitute for red light in all cases examined in which such light has a promotive effect on germination; it "replaces" the primary light requirement that is typical of certain seed lots, and it prevents or releases the secondary light requirement that can be created in other lots. Whether these effects are based on a common mechanism, and how they are related to the effect of red light, will have to be the subject of fur-

Table 1. Effect of gibberellin on germination in the dark of lettuce seed possessing a natural ("primary") requirement of red light for germination (seed lot A).

Treatment	Germination (%)
Water	24
Water; 3 min of red light	70
Gibberellin (100 mg/lit)	70

Table 2. Effect of gibberellin supply during treatment with high temperature on subsequent germination of lettuce seed in darkness at 21°C (seed lot B).

Treatment (5 days at 36°C)	Germination (%)
Water	20
Water; 10 min of red light following high-temperature period	94
Gibberellin (50 mg/lit)	92

Table 3. Effect of gibberellin supply after pretreatment with heat on germination of lettuce seed in darkness at 21°C (seed lot B). All seeds were given 5 days at 36°C on water and were completely dried following the pretreatment with heat.

Treatment	Germination (%)
Reimbibed on water	26
Reimbibed on water; then 10 min of red light	94
Reimbibed on gibberellin (50 mg/lit)	45
Reimbibed on gibberellin (100 mg/lit)	68

Table 4. Effect of gibberellin on dark-osmotic inhibition of lettuce seed (seed lot B); 0.15M mannitol was used.

Pretreatment (6 hr)	Solution	Gibberellin (mg/lit)	Germination (%)
None	Water	0	82
None	Mannitol	0	22
None	Mannitol	35	61
Water	Mannitol	0	23
Gibberellin (50 mg/lit)	Mannitol	0	87