alga as well as other photosynthetic material, is a prominent feature of illuminated suspensions; the spin label does not affect the amplitude or kinetic response to light of this "cell signal."

The rate of growth of cells grown in medium containing $10^{-6}M$ spin label in light or dark is the same as that of control cultures; the appearance and motility of cells is unaffected. The cells harvested from such cultures display no nitroxide signal, nor does the used growth medium. However, when spin label is added to these cells, some is immediately taken up. The first scan in the dark shows a signal smaller than that predicted on the basis of concentration. However, when the suspension is illuminated, the signal does not decrease but, on the contrary, increases by about 50 percent in 3 or 4 minutes. It retains an increased amplitude for as long as the light is on (up to 30 minutes) except for a decrease accompanied by a slight broadening. The total number of spins, however, remains nearly constant. These effects are fully reversible when the light is turned off and again on. That cells washed thoroughly (four times with 20 cell volumes of buffer-KCl) behave precisely as described above (Fig. 4) indicates a firm binding of the label. It does not matter whether the culture has been grown in light or in darkness.

The general aspect of the signals from cells grown in the light in spin label differs in one way from that of signals from cells grown in the dark or without spin label; in the former they develop a large manganese signal over a few hours. This finding indicates the release of manganous ion (bound manganese is not detected by EPR). In the latter, development of the manganese signal is not observed.

On the basis of our observations we can only suggest that the loss of spin label is due to reduction, that is

$$\rangle N - O \cdot e \rightarrow \rangle N - OH$$

where e is an electron. The reductant may be plastoquinone, whose abundance in the cell would approximate the amount of spin label signal which disappears; that is, one molecule per ten chlorophyll molecules (*5*). Plastoquinone in the dark-adapted cell is primarily in the oxidized state. Electron flow initiated by light converts it to a semiquinone, which can conceivably reduce the spin label molecule. Support for this hypothesis is provided by results of studies on an oxygen mutant which has the normal amount of plastoquinone, but which does not reduce it in the light (*6*). The spin label signal is attenuated by these cells, but at a rate that is even slower in the light than in the dark. Functioning of system I withdraws electrons from the quinone pool.

The spin label incorporated into cells grown in its presence loses its paramagnetism. It is probably bound at the site where it is reduced, since the light-induced attenuation of the signal no longer occurs. The increase in the signal upon illumination may be due to the evolution of oxygen (*7*). Broadening of the signal indicates a slight light-induced steric hindrance of the spin. The results of others (*1*) indicate that a change in protein conformation is involved.

ELLEN C. WEAVER
HYANGJU P. CHON
Biophysics Laboratory, Stanford University, Stanford, California

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3. DCMU is 3(3,4-dichlorophenyl)-1, 1-dimethyl urea.
4. Formerly called R (rapid) signal by one of these authors (E.C.W.); it now appears to be a property of System I; L. N. M. Duysens and J. Amesz, *Biochem. Biophys. Acta* **64**, 243 (1962); hence the return to the designation Signal I originated by B. Commoner in *Light and Life*, W. D. McElroy and B. Glass, Eds. (Johns Hopkins Univ. Press, Baltimore, 1961), pp. 356–77.
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6. An oxygen mutant is one which has little or no Hill reaction with benzoquinone, but in which System I is intact. N. I. Bishop has demonstrated the differences in oxido-reduction of the plastoquinone (personal communication).
7. We attribute the bubbles which form in the cuvette to oxygen, but have not directly demonstrated that it is the gas observed.
8. The study was supported by AEC contract 326-12. We thank H. McConnell and H. Weaver for stimulating discussions.

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Rat Mammary Gland RNA: Incorporation of C^{14}-Formate and Effect of Hormones and 7,12-Dimethylbenz[a]anthracene

Abstract. *In female rats, the incorporation of sodium C^{14}-formate into mammary gland RNA decreases immediately after a single feeding of 20 milligrams of 7,12-dimethylbenz[a]anthracene. In males, there is a gradual increase in the incorporation. In castrated rats, the decrease or increase of C^{14}-formate incorporation is dependent on the presence of estrogen or androgen, respectively.*

A single dose of 20 mg of 7,12-dimethylbenz[*a*]anthracene (DMBA) in 1 ml of sesame oil fed by stomach tube to female rats (Sprague-Dawley) invariably induces mammary cancer; the tumors appear as early as 30 days after treatment (*1*). Twenty-four hours after oral administration of either 3-methylcholanthrene (3-MC) or DMBA to rats, the hydrocarbon is located mainly in the fatty or breast tissues; amounts in other tissues are either very small or insignificant (*2*). When female rats are fed a single dose of DMBA and their mammary glands are subsequently transplanted into female recipients receiving no carcinogen, cancer develops in the mammary grafts in the hosts (*3*). In many instances, tumors later develop in mammary glands which have been transplanted as early as 4 hours after the donor animal has been fed a single dose of DMBA.

Although this evidence suggests early action of the carcinogen on the mammary gland, microscopic examination reveals no changes in the gland for some time. Some biochemical events occurring within days of carcinogen administration can, however, be measured. Our results suggest that, after treatment with DMBA, there is an early alteration in RNA synthesis which is dependent on sex hormones.

Sprague-Dawley rats (60 to 70 days old) from the Holtzman Company, fed on a commercial ration (Rockland diet) and given water as desired, were fed 20 mg of DMBA in 1 ml of sesame oil. After they were killed by cervical dislocation, the abdomino-inguinal mammary glands were excised. Five hundred milligrams of tissue were incubated in 5 ml of Robinson's salt solution containing 1 mg of glucose per milliliter and 1.0 μmole of sodium C^{14}-formate (specific activity, 4 to 5 mc/mmole). The flasks were incubated for 2 hours

Table 1. Effect of hormones and DMBA on the incorporation of C^{14}-formate into RNA of mammary gland of castrated female and male rats. Results are expressed as counts/min per microgram of RNA. All rats within one experiment were of the same age (60 to 65 days) and were castrated on the same day. One week after castration daily injections of 10 μg of estradiol or 1 mg of testosterone propionate were started. Injections were given for 7 days. Twenty milligrams of DMBA were fed to the appropriate animals on the day of the last injection. All rats were killed 24 hours later. Numbers in parentheses are number of rats in each group.

Treatment	Castrated female		Castrated male	
	Experiment 1	Experiment 2	Experiment 1	Experiment 2
Control	13.59 ± 2.58 (8)	11.28 ± 1.45 (8)	7.29 ± 1.76 (8)	7.93 ± 1.60 (9)
DMBA		10.95 ± 2.44 (7)	7.62 ± 1.48 (8)	7.87 ± 1.91 (9)
Estradiol-17β	17.42 ± 3.29 (8)*			9.95 ± 1.63 (9)†
Estradiol-17β + DMBA	12.08 ± 3.07 (8)‡			7.63 ± 1.35 (9)§
Testosterone propionate		13.16 ± 2.82 (8)‖	7.99 ± 1.39 (8)	
Testosterone propionate + DMBA		15.04 ± 3.00 (8)¶	8.49 ± 1.64 (8)	

* $P < .02$ (from control); † $P < .02$ (from control); ‡ $P < .01$ (from estradiol); § $P < .01$ (from estradiol); ‖ $P < .01$ (from control); ¶ $P < .01$ (from control). All P values were calculated by Student's t-test.

at 37°C in air and the incubation was stopped by chilling. Enough trichloroacetic acid (TCA) was then added to provide a final concentration of 5 percent, and the tissue was homogenized. The insoluble portion was centrifuged and washed with 5 percent TCA and organic solvents, then incubated for 16 hours at 37°C in 1 ml of 0.3N NaOH. Then 1N HCl was added to the suspension. The supernatant which remained after chilling and centrifugation contained RNA nucleotides. The optical density of the solution was measured at 260 mμ. Portions of the solution were plated out and counted in a windowless gas-flow counter. The DeDeken-Grenson and DeDeken values for yeast RNA (*4*) were used to calculate the specific activity of the RNA.

In female rats, inhibition of the incorporation of formate into mammary gland RNA was observed 24 hours after the feeding of DMBA. Incorporation decreased to 63 percent of the control value. On the 2nd day after DMBA feeding, the incorporation was 49 percent that of the control value. From the 3rd day on, the incorporation of C^{14}-formate into RNA gradually rose to the control value. In male rats, contrary to the response found in the females, incorporation of C^{14}-formate into mammary gland RNA was immediately stimulated. The incorporation had risen to 150 percent that of the control value by the 5th day. An appreciable stimulation was still present on the 10th day (Fig. 1).

To learn whether the difference in the incorporation of C^{14}-formate into rat mammary gland RNA in male and female rats after DMBA administration is hormonally controlled, castrated female and male rats were treated with hormones and DMBA, and the incorporation of formate into RNA from their mammary glands was studied. In castrated females, DMBA induced only a slight and insignificant inhibition of the incorporation of formate into mammary gland RNA (Table 1). In male castrated rats, DMBA no longer stimulated the incorporation of formate into mammary gland RNA (Table 1). Administration of estradiol-17β for 7 days to castrated female rats caused a marked increase in formate incorporation. When DMBA was given to estradiol-treated castrated female rats, the incorporation of formate into mammary gland RNA was significantly depressed. Also, in castrated male rats estradiol-17β induced a similar stimulation of formate incorporation. If DMBA was given in addition to estradiol-17β, however, this stimulatory effect was nullified, and a significant inhibition of formate incorporation was observed.

Treatment of castrated rats of either sex with testosterone proprionate induced slightly greater incorporation than occurred in the control castrated rats, but the increase was not statistically significant. If the androgen-treated, castrated male or female rats were given DMBA 24 hours prior to sacrifice, the incorporation of formate further increased.

To determine whether the radioactivity in the acid-soluble solution was indeed due to purines, the specific activities of portions of the solution were measured. The remainder of the solution was subjected to hydrolysis at 100°C for 1 hour to release purines from purine nucleotides. Adenine and guanine (5 μmole each) were added as carrier to known volumes of the solutions, containing known amounts of radioactivity. Adenine and guanine were adsorbed onto charcoal; they were eluted with 10 percent pyridine in 50 percent ethanol (*5*). The eluate was dried, taken up in a small volume of 1N HCl, and spotted for chromatography. Chromatography was carried out in methanol, formic acid, and water (80 : 15 : 5). Areas containing adenine and guanine were eluted from the chromatograms. After determination of the radioactivity (count/min) the concentrations of adenine or guanine were calculated from the absorption at 262 mμ for adenine and at 248 mμ for guanine (*6*). Calculations showed that the radioactivity in the solutions were attributable solely to adenine and guanine. In one experiment, female rats were killed 1 day after being fed DMBA. The specific activity of the RNA nucleotides of the experimental animals was 73 percent of the control value. In the control animals, 94.7 percent of the radioactivity could be attributed to adenine and guanine combined; in the experimental animals

Table 2. Incorporation of C^{14}-formate into RNA of various tissues after treatment with DMBA. Results are expressed as counts/min per microgram of RNA. Female rats (65 days old) were fed 20 mg DMBA and killed one day later, with untreated control animals. Mammary gland, liver, and kidney were excised, sliced, and incubated. Numbers in parentheses are number of rats in each group.

Mammary gland	Liver	Kidney
	Control	
19.63 ± 1.04 (4)	2.31 ± 0.26 (4)	2.90 ± 0.51 (4)
	DMBA	
15.31 ± 2.56 (4)*	4.85 ± 1.19 (4)†	3.69 ± 0.57 (4)

* $P < .02$ (from control); † $P < .01$ (from control). P calculated by Student's t-test.

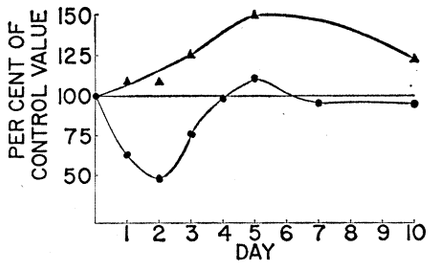

Fig. 1. Effect of DMBA on the incorporation of sodium C^{14}-formate into RNA in the rat mammary gland. DMBA was fed to rats on day 0. Incorporation is expressed as percentage of control values. Control values for female rats (●——●) were 7.9 count/min per microgram of RNA. Control values for male rats (▲——▲) were 6.3 count/min per microgram of RNA.

adenine and guanine accounted for 100.9 percent of the radioactivity. In a second experiment, male rats were killed 3 days after they were fed DMBA. The specific activity of RNA in the experimental animals increased 23 percent over that of control values. Adenine and guanine accounted for 98 percent of the radioactivity in the control animals and for 94 percent in the experimental animals. The data suggest that the specific activity is due to incorporation of C^{14}-formate into the RNA purines.

The effect of DMBA on the incorporation of formate into the RNA of the uterus in the castrated rats was also determined. Whereas C^{14}-formate incorporation into the uterine RNA of the castrated female rat was 13.67 ± 9.1 count/min per microgram of RNA, it was 15.02 ± 2.21 in rats treated with DMBA. DMBA appears to have little or no effect on uterine formate incorporation. When a single injection of estradiol-17β was given to other castrated rats, a two-fold increase in the formate incorporated into uterine RNA was observed (25.90 ± 9.89 count/min per microgram of RNA). The data suggest that a carcinogenic hydrocarbon like DMBA does not possess estrogenic activity.

The inhibitory effect of DMBA on the incorporation of formate is evidently a specific effect on the mammary gland. When incorporation of C^{14}-formate into mammary gland, liver, and kidney RNA from female rats treated with DMBA is measured, incorporation of formate into RNA is inhibited in the mammary gland; it is stimulated in liver and kidney (Table 2). These results are comparable to those that show increased incorporation of $C^{14}O_2$ into RNA uridine from the livers of rats fed 3-MC (*7*).

Under certain conditions one effect of chemical carcinogens on the target tissue is a decrease in macromolecular synthesis. There is a decrease in the amount of messenger RNA synthesized in the liver in animals 1 month after their treatment with 4′fluoro-4-dimethylaminoazobenzene (*8*). In addition to the decrease in total synthesis, there is a change in the pattern of countercurrent distribution. Hulten and Arrhenius (*9*) have observed a decrease in the ability of microsomes from the livers of rats treated with 2-aminofluorene to incorporate leucine into protein. This effect is apparent 4 hours after feeding, but only in rats that have been subjected to an experimental vitamin E deficiency. Although the adrenal cortex of the rat does not develop cancer after DMBA treatment, it is profoundly affected by this carcinogen. Thymidine incorporation is inhibited in the adrenal glands of rats treated with DMBA (*10*).

Similarly, DMBA affects the incorporation of C^{14}-formate into mammary gland RNA. In the female Sprague-Dawley rat, this incorporation is severely decreased within one day of the feeding to the animal of 20 mg of DMBA. When similar experiments are done with male control rats and male rats fed DMBA, incorporation of C^{14}-formate into mammary gland RNA of the experimental animals is stimulated. These effects are directly due to the sex hormones present in the animals (Table 1). Incorporation of C^{14}-formate is unchanged in castrated animals, whether or not the rats have been fed DMBA. In the presence of estradiol, DMBA feeding causes a marked decrease in incorporation; in the presence of testosterone, DMBA causes a slight increase in incorporation. It is unlikely that these effects are secondary to that of DMBA on the adrenal, since the effect on the adrenal is constant with animals of either sex (*11*).

Whether the differences between male and female rats in the incorporation of formate may be related to another physiological difference in the mammary gland, breast cancer in females and none in males after DMBA feeding, is not known. Likewise, it is not known whether a decrease in RNA synthesis may be related to tumorigenesis. It is of interest that there is a slight decrease in tumorigenesis in rats fed DMBA if the rats are concomitantly treated with either uracil or thymidine (*12*).

PAUL R. LIBBY
THOMAS L. DAO

Department of Breast Surgery, Roswell Park Memorial Institute, Buffalo, New York

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Evolutionary Significance of Polyploidy in the Pteridophyta

Abstract. *Polyploidy occurs in the heterosporous and homosporous Pteridophyta, but with a much higher frequency in the latter. Ninety-six percent of the homosporous Pteridophyta show a gametic chromosome number greater than 27, whereas 90 percent of the heterosporous ones possess a gametic chromosome number less than 28. Ultrafrequent establishment of polyploidy in the homosporous Pteridophyta appears to be necessary to create and maintain genetic variation in the face of the homozygotizing effects of habitual self-fertilization in the monoecious gametophytes of these plants.*

The frequency with which interspecific hybrids are found and the allopolyploid origins which have been demonstrated for many fern species (*1*) has led to the assumption that ferns are typically outbreeding organisms and, consequently, have a tendency to-