bean shaped. From 48 to 96 hours after radiation there are a number of cells with very large nuclei usually with more than two centrioles; cells with two nuclei usually have four centrioles between them and cells with several small and irregular shaped nuclei almost invariably have many centrioles up to ten. Stages in mitosis can be seen where there are typical multipolar spindles. The increase in multipolar mitoses seen after radiation may be related to multiple centrioles. Also there are many cells which show fully formed nuclear membranes with partial constriction of the cytoplasm. Observations also indicate that the greater the number of centrioles the greater the amount of nuclear material found in the cell. This would suggest further that there has been a delayed or inhibited cytoplasmic cleavage.

A study of Table 1 shows that there are fewer cells showing 6, 8 or 10 centrioles than might be anticipated if it is assumed that they arise by multipolar mitoses from the two-centriole cells. One may deduce from this that the viability of the cell decreases with the increase in the number of centrioles. Experimental data on the above are now being collected.

Therefore cytoplasmic division of certain cells may be inhibited even though nuclear division and the divisions of the centriole may continue within limits. This results in a transient increase in the number of centrioles.

It is the plan to extend this study to different types and dosages of radiation in relation to this kinetic component and to investigate some of the underlying causative factors.

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THE OXYHYDROGEN REACTION IN GREEN ALGAE AND THE REDUCTION OF CARBON DIOXIDE IN THE DARK

UNICELLULAR green algae when incubated for several hours in the dark in an atmosphere of nitrogen or hydrogen acquire metabolic capacities different from those observed under normal aerobic conditions. The most conspicuous change has been found with the algae Scenedesmus, Rhaphidium and Ankistrodesmus, which, after such treatment, are able to include molecular hydrogen in their metabolism. They behave like purple bacteria, in that they reduce in the light carbon dioxide with two equivalents of hydrogen.

Oxygen inhibits all the reactions with hydrogen in the algae, even the oxyhydrogen reaction. But the latter proceeds uninhibited if the oxygen is present in very small concentrations, the "hydrogenase" system remaining active. This process—a dark reaction of course—could be expected to result simply in the formation of water. Continued investigation, however, has now revealed that the oxyhydrogen reaction in green algae is somehow linked with the disappearance of carbon dioxide in the dark.

A closed vessel attached to a manometer and filled with hydrogen may contain a suspension of adapted algae in equilibrium with the hydrogen. Some oxygen is now brought into the vessel thereby producing a positive pressure change of, for example, 50 mm of the manometric fluid. If all the oxygen would disappear together with two equivalents of hydrogen one should observe a negative pressure change of 150 mm. Table 1 shows that in reality this is not the case. The course of the reaction is determined not only by oxygen and hydrogen but also by the absence or the presence of free carbon dioxide. In the absence of carbon dioxide the amount of hydrogen consumed is generally less than expected. This is not very surprising because many other substances besides hydrogen might be oxidized in the cell. In the presence of carbon dioxide, however, gas disappears in a large excess over that which appears possible assuming the complete conversion of the oxygen into water.

TABLE 1

THE INFLUENCE OF CARBON DIOXIDE ON THE COURSE OF THE OXYHYDROGEN REACTION IN GREEN ALGAE*

Material	Time hours	Neg. pressure changes (mm) Gas : H_2 0.3 cc of 5 per cent. NaOH inside bulb	Neg. pressure changes (mm) Gas : H_2 with 1 per cent. to 4 per cent. CO_2
0.030 cc of Scenedesmus D 3 in 5 cc of phosphate solution	9	114	174
		110	236
	5	145	230
		148	230
0.015 cc of Scenedesmus obliquus in 2 cc of phosphate solution (pH 5.3)	7	110	196
		106	199
	14	135	243
		140	245
0.045 cc of Ankistrodesmus spec. in 3 cc of phosphate solution	8	...	222

* Total pressure changes measured in mm of Brodie solution (10,000 mm B. = 760 mm Hg.) occurring in a manometer vessel containing a suspension of algae adapted to hydrogen after the introduction of 0.5 vol. per cent. of oxygen (= 50 mm B.). Temperature 25°. Gasphase: H_2 or H_2 with 1 per cent. to 4 per cent. of CO_2. Complete conversion of the oxygen into water would result in a pressure change of − 150 mm B.

The analysis of the gas exchange in the presence of carbon dioxide yielded results such as the figures of Table 2. Simultaneously with oxygen and hydrogen a certain amount of carbon dioxide disappears. The facts presented in both tables permit the conclusion that in these algae the oxyhydrogen reaction promotes the reduction of carbon dioxide in the dark. This means that all three types of carbon dioxide assimilation observed in living organisms, chemical reduction

TABLE 2

DISAPPEARANCE OF CARBON DIOXIDE IN THE COURSE OF THE OXYHYDROGEN REACTION IN ALGAE*

Material	Time hrs.		cmm O_2 introduced	cmm H_2 consumed	cmm CO_2 present before exp.	cmm CO_2 present after exp.
Rhaphidium polymorphium ...	20	exp.	196	567	109	50
		contr.	0	43	115	112
Scenedesmus D 3	18	exp.	218	620	118	65
		contr.	0	6	116	125
Scenedesmus D 3	20	exp.	417†	871	173	50
		contr.	0	14	163	179

* About 0.03 cc of cells in 3 cc of slightly acid phosphate solution. Gasphase: 99 per cent. H_2, 1 per cent. CO_2. Temperature: 25°.

† Exp. was stopped before all the oxygen had been consumed.

in the dark, photochemical reduction with hydrogen donors in the light and photosynthesis as specified by the liberation of oxygen, may occur in the same plant cell.

The decomposition of carbon dioxide in plants is generally believed to be intimately linked with, and absolutely dependent upon, the effect of light on chlorophyll. The finding here reported suggests, however, that the photochemical process and the reduction of carbon dioxide are more widely separated reactions than has been hitherto assumed.

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INACTIVATION OF PITUITARY LACTOGENIC HORMONE BY IODINE[1]

OUR preparation of pituitary lactogenic hormone has shown itself to be a homogeneous substance with respect to electrical charge.[2] Solubility studies[3] have also indicated that both beef and sheep preparations are almost if not actually pure. Although we have not yet been able to secure this protein in *uniform* crystalline state, the above mentioned physico-chemical behavior seemed to justify further chemical studies.

There is no indication that the hormone has a prosthetic group; yet the study of effects on biological activity of specific modifications of the molecule by chemical reagents might enable us to detect "functional" groups in the molecular makeup, for example, specific spatial arrangements of amino acids.

We have already reported the results obtained by the action of ketene[4] and nitrous acid[5] on the lactogenic hormone. These studies indicated the essentiality of the free amino groups for biological action of the hormone. This conclusion has recently been confirmed by Bottomley and Folley[6] in their experiments using phenyl isocyanate. Question as to the essentiality of the tyrosine molecule is next in line.

It has already been demonstrated that tyrosine is an essential component of certain protein hormones, enzymes and antigens.[7] Harrington and Neuberger[8] found that iodinated insulin retains only 5–10 per cent. of the physiological activity of the parent substance. Herriott[9] obtained an iodinated pepsin which has less than 1 per cent. of the original proteolytic activity. The iodination of these two substances showed that only the tyrosine molecule changed into the diiodo-compound which gives no Millon reaction. The present study also demonstrates that iodine acts only on the tyrosine component of lactogenic hormone.

Lactogenic hormone (L 283) as prepared from beef[10] pituitary contains 5.84 per cent. tyrosine and 1.34 per cent. tryptophane as determined by Lugg's modification of the method[11] of Folin and Ciocalten. Iodinated lactogenic hormones were prepared by the treatment of 100 mgm L 283 with 0.024 N iodine solution in 10 cc 0.5 M phosphate buffer of pH 7.0. The reaction was completed within an hour. The excess iodine was removed by a few drops of 0.2 N thiosulphate reagent. The colorless suspension was then dialyzed and its iodine[12] and nitrogen contents were analyzed. As shown in Table I, decrease in biological potency accompanied the absorption of iodine.

TABLE I

Material	N per cent.	I_2 per cent.	Tyrosine per cent.	Tryptophane per cent.	Med* mgm
L 283	15.26	0	5.84	1.34	0.2
Iodinated for ½ hr. (L 41)	F: 14.50 T: 14.52	F: 4.96 T: 5.38	2.00	1.38	1.0
Iodinated for 1 hr. (L 36A)	F: 14.00 T: 14.12	F: 7.90 T: 8.18	0.00	1.30	<1.5

* The minimum effective dose is defined as the smallest amount of the preparation which on intramuscular injection in 30-day-old squabs (4 daily injections of 0.5 cc) causes the minimum crop sac reaction in at least two out of three squabs.

The "F" and "T" in Table I denote the found and theoretical values. The latter is calculated and based

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[3] Results to be published.

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[10] Lactogenic hormone from sheep pituitary invariably has a lower tyrosine content—about 4.5 per cent. (to be published).

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[12] We are indebted to Jane Conrat for iodine determinations.