Methods: Total body irradiation with X-rays was given to white male mice. The doses were 500 and 1,000 r/air in one exposure or in fractions of 100 r daily. The radiation factors were: 200 kv., 10 Ma., 0.25 mm. Cu + 1.0 mm. Al filters, 50-cm. distance, 20 × 20 cm. field, 23.4 r/minute, HVL = 0.75 mm. Cu. The technic of exposure was the same as previously described (*2*).

Daily doses of 0.25 or 0.5 mg. desoxycorticosterone acetate[2] (in oil) were administered subcutaneously six times weekly. Total doses varying between 2.5 and 8.0 mg. were given within 10 to 18 days. The effects on mortality rate, fat content of the liver, and radiation changes in other organs were studied over a period of 40 days.

TABLE 1

Irradiated animals M = 1.43 σ = 1.42						
	Grading of fat					
X-ray dose	0	+	++	+++	++++	No. of animals
500 r	14	–	8	9	–	31
500 r frac.	8	1	2	5	3	19
1,000 r	11	1	3	3	1	19
1,000 r frac.	7	2	4	6	1	20
Total	40	4	17	23	5	89
Irradiated and desoxycorticosterone-treated animals M = 0.50 σ = 1.01						
	Grading of fat					
X-ray dose	0	+	++	+++	++++	No. of animals
500 r	21	1	3	3	–	28
500 r frac.	14	2	1	2	2	21
1,000 r	13	2	1	–	–	16
1,000 r frac.	13	–	1	–	–	14
Total	61	5	6	5	2	79

M = arithmetical mean = fat index; σ = standard deviation.

Microscopic sections of livers stained with Sudan III were used for the assay of fat, and the following grading was applied: 0 = no sudanophile fat; + = traces of sudanophile fat; ++ = increased amount of fat with definite arrangement around central vessels; +++ = considerable increase in fat; ++++ = fat making up an entire lobule. For the quantitative evaluation of these histological changes the "fat index," *i.e.* the arithmetical mean of the various grades of sudanophile fat, was used.

Results: The outstanding observation made in these studies was a striking reduction in the amount of sudanophile fat in the irradiated and desoxycorticosterone-treated group of animals as compared with those receiving irradiation only. The data are summarized in Table 1.

No striking difference in the radiation effects on bone marrow and spleen was noticed between the irradiated and desoxycorticosterone-treated group and the solely irradiated controls.

However, there was a slight decrease in the mortality rate produced by the various doses of X-rays in favor of the desoxycorticosterone-treated group.

Further details will be given in a later publication.

The protective action of desoxycorticosterone against X-ray-induced liver changes seems to be of interest from various points of view: (1) These results give experimental evaluation to, and support the clinical impression of, the value of desoxycorticosterone in the treatment of radiation illness. They also seem to support the histamine hypothesis of radiation effect. (2) The protective power of desoxycorticosterone against radiation effects in the liver appears of particular interest in the clinical application of radioactive substances known to be selectively deposited in the liver. In utilizing this effect the clinical efficiency of radioactive substances in the treatment of leukemias and cancers may be improved.

[2] We wish to express our gratitude to Dr. E. Henderson, of the Schering Corporation, for generously supplying us with desoxycorticosterone acetate.

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Redox Potentials and Photoreduction by Chloroplast Granules

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The over-all reaction for photosynthesis,

$$CO_2 + HOH \xrightarrow[\text{pigment-system}]{\text{Light}} (CH_2O) + O_2, \qquad (1)$$

is remarkable not so much for the apparent decomposition of water but for the ability of the system to utilize efficiently as poor an oxidant as CO_2. Light may be used to effect the decomposition of water by inorganic sensitizers, e.g. Hg, if the wave length is sufficiently short. Redox couples (as $Co^{+3} \rightarrow {}^{+2}$, $Ce^{+4} \rightarrow {}^{+3}$) with a favorable potential (E° greater than −1.23 volt) are able to oxidize water in the dark. The potential of CO_2—HCHO (formaldehyde) is +0.08 (*8*) and probably any "first product" of photosynthesis is of the order of +0.1 volt; that of $Fe^{+3} \rightarrow {}^{+2}$ is −0.771.

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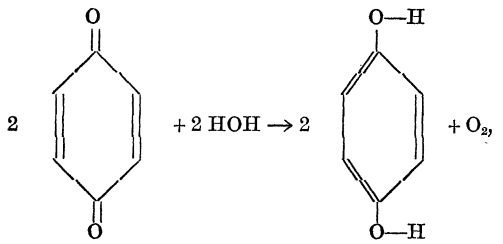
It has long been known (*2*) that disintegrated leaves or isolated chloroplasts have lost the largest part (*4*) of their ability to assimilate CO_2 and evolve O_2. However, Hill (*5*) was able to show that, in the presence of ferric oxalate, isolated chloroplasts evolve oxygen in considerable amounts (measured at first spectrocolorimetrically via $Hb + O_2 \rightarrow HbO_2$, and later manometrically) according to the reaction,

$$4\ Fe^{+3} + 2\ HOH \rightarrow 4\ Fe^{+2} + 4\ H^+ + O_2, \qquad (2)$$

which, of course, bears some analogy to that of photosynthesis. Further validation of the course of the reaction as following Equation (2) is afforded by recent work of Holt and French (*6*), where the reaction is followed titrimetrically (*i.e.* for H^+). The analogy to photosynthesis was even more evident when Warburg and Lüttgens (*9*) showed that chloroplasts were able to use o-benzoquinone as the oxidant, viz.,



(3)

since the oxidant here is actually the hydrogen acceptor.[2] These authors point out that chloroplast granules were also able to give the reaction.

The similarity of Equations (2) and (3) suggests that a certain generality exists in the ability of the chlorophyll system to effect the decomposition of water with the aid of oxidants and light. We have added to this generality by showing that various types of quinones are able to serve as oxidants. The rates of evolution of oxygen with the various quinones are, however, a function of their redox potentials (Fig. 1). One might have expected that if the photochemical reaction could proceed at all, then it should proceed independently of the potential. That the reaction is potential dependent appears to indicate that under these circumstances this system is not as capable of preventing those back reactions which, in normal photosynthesis, permit as poor an oxidant as CO_2 to be used to dissociate water.

One should not construe from linear extrapolation of the data that oxidants with a couple lower than −0.05 are incapable of giving the reaction. The values given in the figure are maximal, and deviation from linearity of the benzoquinone (as compared with the naphthaquinones) may be due to the greater number of runs available for the former rather than actuality. The necessity for this manner of presentation arises from the relatively rapid inactivation of the material, both Hill and Kumm and French (*7*) having noted a half-life of about two hours. In agreement with these authors we also found that different preparations (in our case, of market spinach) gave variable results. Material left overnight invariably lost almost 90 per cent of its activity (with no accompanying spectroscopic change), and such granules were often incapable of yielding oxygen with anthraquinone sulfonate. It is thus possible that the material, at the moment of isolation, is capable of utilizing oxidants even in the plus-voltage range, but because of the length of time in preparation (about two hours) sufficient deterioration has occurred to prevent its experimental verification.

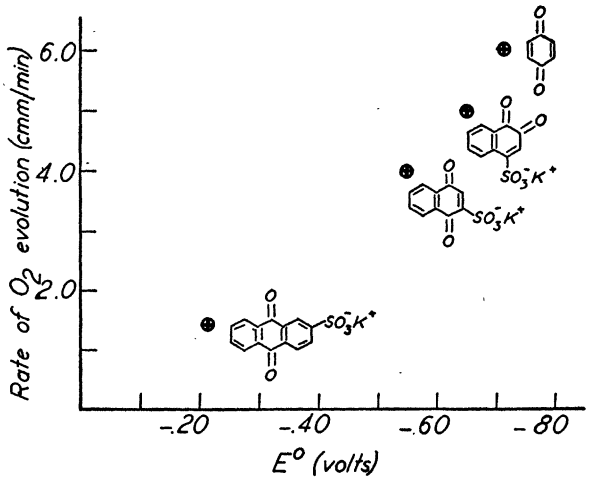


FIG. 1

Experimental details and other pertinent data will be discussed in a later publication.

Addendum. Additional work has shown that the rates of oxygen liberation by the various quinones are not equally proportional to the light intensity. With decreasing flux the poorer oxidants become relatively more efficient, until at one-third the intensity used above (30,000 lux) the rates are actually reversed. Furthermore, at the maximal flux, practical saturation has been reached, the rate leveling off more rapidly, the poorer the oxidant.

[2] One must note the experiments of Fan, *et al.* (*3*), who presented evidence that benzaldehyde, acetaldehyde, and nitrourea may be reduced by algae, in the absence of external CO_2, with the evolution of oxygen. The effects mentioned therein are but slightly greater than those of respiration and therefore not as conclusive as the above-mentioned experiments. Furthermore, Boichenko (*1*) has noted not only evolution of oxygen (detected by oxidation of leuco dyes) but assimilation of CO_2 (as noted by change in pH of medium) by chloroplasts. We have as yet been unable to duplicate these results manometrically, possibly due to insufficient experimental details.

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Direct Observation of the Transfer of Pronuclei in Living Conjugants of *Paramecium bursaria*[1]

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At the present time three sexual processes have been described for *Paramecium* in which three micronuclear (pregamic) divisions lead to synkaryon formation. In one, called conjugation (cross-fertilization), there is a temporary union of two individuals involving three micronuclear divisions, *nuclear exchange,* and the establishment of a new synkaryon in each conjugant. Thus, a sine qua non of conjugation is nuclear exchange. The second process, called autogamy (self-fertilization), was described by Diller (*2*) for *P. aurelia* as occurring in *single* animals only. Here, three micronuclear divisions lead to the formation of gametic nuclei which fuse and form a synkaryon in the single animal without the cooperation of another individual.

Earlier investigators used killed and stained paramecia for their cytological studies. Beginning in 1936 the author approached the problem of cytological study of nuclear behavior in joined paramecia with an entirely new method. Studies were made on *living* joined pairs of paramecia which were isolated and placed in a precision microcompression chamber so that direct observations could be made under the microscope. This method led to the discovery of a third and new sexual process called *cytogamy.* In this process, the three pregamic divisions occur as in conjugation, but, instead of a transfer of pronuclei as in true conjugation, the pronuclei remain within the confines of each joined individual and a synkaryon is formed in each (*8*). The classical accounts of conjugation wherein nuclear exchange is supposed to be a constant feature were questioned.

More recently, a rather lively interest has developed in the problems of exchange of pronuclei and cytoplasm during the conjugation process. On the general problem papers have appeared giving cytologic, genetic, and serologic evidence of the transfer of pronuclei or cytoplasm or both during conjugation. Chen (*1*), using fixed and stained preparations of *P. bursaria* presents cytological evidence of pronuclear transfer. In addition, he reports some cases in which the two pronuclei remain in the same conjugant and fuse to form a synkaryon (cytogamy). Sonneborn (*7*) presents genetic evidence to show that there is nuclear and cytoplasmic transfer in *P. aurelia* during conjugation, and he also shows genetic evidence for cytogamy. He reports that the transfer of cytoplasm is crudely measured by the extent of the time interval between separation of conjugants at their anterior ends and separation at their paroral cones. If the interval is less than 3½ minutes, exchange of cytoplasm is not detected, regardless of what races were crossed, but when the interval is 20 minutes or more, "cytoplasmic factor" is invariably transferred. Further, he reports that exchange of cytoplasm at conjugation never occurs in crosses in certain races (although nuclei are exchanged in these crosses) but does occur in others. Concerning nuclear exchange, Sonneborn (*6*) reported that there is a definite temperature factor involved in cross-fertilization and some indication that calcium increases the frequency of it and that sodium decreases it. Harrison and Fowler (*4*) present serologic evidence of cytoplasmic interchange during conjugation of *P. bursaria.* They believe that the antigen involved in the reaction is very largely, if not exclusively, cytoplasmic in character and that there is an extensive interchange of cytoplasm.

In view of what has been done thus far, it was thought desirable to study the conjugation process in the *living* condition in an effort to obtain information on nuclear and cytoplasmic behavior and determine the time relationships involved. From a large number of races of five different species of *Paramecium,* two races of *P. bursaria,* of opposite mating type, seemed pre-eminently suited for direct observation on living conjugants when placed in the microcompression chamber. One race, B9, is composed of large green specimens due to the presence of zoochlorellae, while the other race, 255 (kindly supplied by Dr. J. A. Harrison), consists of smaller paramecia which are colorless because of the complete absence of zoochlorellae. At any time or in any stage one is able to identify the members of each race in the conjugation process. The mating reaction is much like that reported for this species by Jennings (*5*). Here the organisms begin showing a feeble mating reaction at 4:45 A.M. at 25° C., concomitant with the appearance of daylight. The reaction gradually increases in intensity until at 8:00 A.M. it is strong

[1] Aided by a grant from the Committee on Research and Publication, Temple University.