I would like to stress that the text, the formulas and the diagrams are very carefully executed so that the reviewer in examining them found practically no errors or inaccuracies. A great number of expertly selected problems proposed for solution augments the value of the book for students and other interested readers.

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sand selected cells.

## SPECIAL ARTICLES

## THE CENTRIOLE IN RADIATED TUMOR TISSUE

ALTHOUGH previous studies on the cytology of Walker rat carcinoma 256 had not emphasized the presence of centrioles<sup>1, 2, 3</sup> we have found paired bodies in the interkinetic stage of the cells of this tumor embedded in a surrounding mass of granular centrosomal substance. They are specific granules consistently revealed with hematoxylin stains following appropriate fixatives. We assume them to be centrioles. They apparently migrate to opposite poles in the early stages of mitotic activity. The metaphase spindle reveals a single compact body at each focal point although this body is not apparent if the spindle is wide at the poles. Our own Zenker-fixed unradiated material with the stains used in our previous studies do not show these bodies. We found that the Zenker-fixed material after radiation (single dose, 2400 r) did show them with a variety of stains. On further investigation other fixatives, such as Bouin, show these bodies whether the tissue has been radiated or not, particularly if stained with Heidenhain's iron hematoxylin. Thus we may follow the effect of radiation on them and this gives further evidence for their probable identification as centrioles.

The method of killing the animals, the nature of the tumor and the preparation of the tissue were the same as described in previous papers.<sup>4, 5, 6</sup> The type and dosage of radiation were as follows: 200,000 volt X-ray -50 cm distance-Filter 0.5 Cu, 1 mm Al-one dose 2400 r. The rate of radiation in r per minute was 40.6.

Control as well as radiated tumor tissue was fixed either in Bouin or Zenker. The stains used were Heidenhain's iron hematoxylin and eosin, eosin Y and methylene blue and phosphotungstic acid hematoxylin. 18, 48, 72, 96 and 120 hours after radiation animals were killed for study.

A quantitative study was made of cells showing centrioles. We assumed that all cells in the interkinetic stage properly fixed and stained would show centrioles in the centrosomal region. Only those cells

<sup>1</sup> W. R. Earle, Amer. Jour. Cancer, 24: 566-612, 1935. <sup>2</sup> E. Waldschmidt-Leitz and E. McDonald, Ztschr. f. physiol. Chem., 219: 115-127, 1933. <sup>3</sup> M. R. Lewis, Abst. Anat. Rec., 45: 268-269, 1930.

4 L. C. Fogg and S. Warren, Amer. Jour. Cancer, 31: 567-577, 1937

5 Ibid., 31: 578-585, 1937.

6 Ibid., Proc. Soc. Exp. Biol. and Med., 39: 91-93, 1938.

were counted which were lying in such a plane that the centrioles could be clearly identified. Since necrotic cells were not counted this study does not include all the cells in a given region. If radiation produces centriolar change, it should be apparent in one thou-

In control tissue there are some cells which show higher numbers of centrioles as 3, 4 or very rarely 5. The term "multiple centrioles" as used here will refer to any number over the normal unit 2. After radiation centrioles, not previously seen in Zenker-fixed control tumor cells, can be demonstrated and tumor tissue fixed at varying intervals after radiation shows a variation in the frequency of multiple centrioles.

The interval immediately following radiation when mitosis has been largely inhibited shows only a slight increase in the number of centrioles. With a dose of 2400 r this tumor renews mitotic activity by 24 hours or later.<sup>7</sup> This suggests that the increase in number of centrioles (Table 1) is associated with the cellular

TABLE 1 EFFECT OF 2400 r on Centrioles in Walker Rat Carcinoma 256

| Hours<br>after<br>radiation            | Number of<br>cells with<br>visible cen-                | Nu  | ımb                              | Per cent.<br>of multi-              |                                |                     |  |  |                       |                       |   |
|--|--|---|----------------------------------|-------------------------------------|--------------------------------|---------------------|--|--|-----------------------|-----------------------|---|
|  | trioles<br>counted                                     | 2   | 3                                | 4                                   | 5                              | 6                   | 7  | 8  | 9                     | 10                    | trioles   |
| 18<br>48<br>72<br>96<br>120<br>Control | $1000 \\ 1000 \\ 1000 \\ 1000 \\ 1000 \\ 1000 \\ 1000$ | $\begin{array}{r} 900 \\ 605 \\ 401 \\ 538 \\ 966 \\ 958 \end{array}$ | 81<br>97<br>62<br>77<br>26<br>39 | $17 \\ 274 \\ 355 \\ 236 \\ 6 \\ 2$ | $1 \\ 5 \\ 14 \\ 11 \\ 2 \\ 1$ | $0\\15\\113\\94\\0$ | $     \begin{array}{c}       1 \\       2 \\       7 \\       7 \\       0     \end{array} $ | $\begin{smallmatrix}&0\\&2\\41\\30\\&0\end{smallmatrix}$ | 0<br>0<br>1<br>0<br>0 | 0<br>0<br>6<br>7<br>0 | $     \begin{array}{r}       10 \\       40 \\       60 \\       46 \\       3 \\       4     \end{array} $ |

division processes. By 48 hours there is a sharp increase in the number of multiple centrioles per cell which continues until 72 hours and then decreases. By 120 hours the tissue has returned to normal for this feature. Cells with 6 or more centrioles are at their peak at 72 to 96 hours. There is a predominance of even numbered centrioles. This suggests that there has been one or more incomplete cell divisions where certain cell components have divided but where the separation of the cytoplasm has been suppressed. This hypothesis and the possibility that radiation may have fragmented the centrioles bears further investigation.

After radiation a greater variation in the shape, size and number of nuclei per cell occurs. Typically the control nuclei are single and roughly somewhat kidney

7 S. Warren, Am. Jour. Roent., 38: 899-902, 1937.

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 CAR-BON 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

TABLE 1 THE INFLUENCE OF CARBON DIOXIDE ON THE COURSE OF THE OXYHYDROGEN REACTION IN GREEN ALGAE\*

|   |               |   | Neg. pressure changes<br>(mm)  |   |  |  |  |  |
|---|---------------|---|--|---|--|--|--|--|
| Material  | Time<br>hours |   | Gas: H <sub>2</sub><br>0.3 cc of<br>5 per cent.<br>NaOH in-<br>side bulb | Gas: H <sub>2</sub><br>with 1 per<br>cent. to 4<br>per cent.<br>CO <sub>2</sub> |  |  |  |  |
| 0.030 cc of Scenedesmus D 3<br>in 5 cc of phosphate solu-<br>tion               | 9             | { | 114<br>110   | $\begin{array}{c} 174\\ 236\end{array}$   |  |  |  |  |
|   | 5             | { | $\begin{array}{c} 145\\ 148\end{array}$                                  | $\begin{array}{c} 230\\ 230\end{array}$   |  |  |  |  |
| 0.015 cc of Scenedesmus ob-<br>liquus in 2 cc of phosphate<br>solution (pH 5.3) | 7             | { | $\begin{array}{c} 110\\ 106 \end{array}$                                 | $\begin{array}{c} 196 \\ 199 \end{array}$                                       |  |  |  |  |
|   | 14            | { | $\begin{array}{c} 135\\ 140 \end{array}$                                 | $\begin{array}{c} 243\\ 245\end{array}$   |  |  |  |  |
| 0.045 cc of Ankistrodesmus<br>spec. in 3 cc of phosphate<br>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<sub>2</sub>. Complete conversion of the oxygen into water would result in a pressure change of - 150 mm B.

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.

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