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## Effect of Heavy Water (deuterium oxide) on Viability of Mouse Sarcoma and Melanoma.

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Since the discovery of the isotope of hydrogen<sup>1</sup> much interest has developed concerning biological properties of heavy water. Lewis<sup>2</sup> found that heavy water (99% H<sub>2</sub>O) completely inhibited the germination of tobacco seeds. Taylor, Swingle, Eyring, and Frost<sup>3</sup> showed that tadpoles of the green frog, *Rana clamitans*, died within one hour after being placed in the heavy water (92% H<sub>2</sub>O). Macht and Davis<sup>4</sup> obtained a slight inhibiting effect on germination of *Lupinus* seeds with less concentrated heavy water (0.05% H<sub>2</sub>O). On the other hand, Barnes<sup>5, 6</sup> has noted a stimulating effect of heavy water (0.05% H<sub>2</sub>O) on *Spirogyra*.

It is known that neoplasms are generally more sensitive to deleterious agents than are normal tissues. Since normal tissues are killed by water containing deuterium in high concentrations, and biological effects are alleged for concentrations of heavy water as low as 0.05%, the question at once arises of what will be the effect of heavy water upon tumor tissues. This can be determined readily by exposing tumor fragments to the heavy water, and subsequently transplanting into host animals.

Two concentrations of heavy water were used—14.8 and 40%. These were samples used by La Mer, Eichelberger, and Urey<sup>7</sup> in their freezing point determinations. Before each experiment, the water was purified by repeated distillation from potassium permanganate. Since it was distilled very slowly, it was probably saturated with air from the condenser.

The tumors used were the mouse sarcoma 180, and the Passey

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<sup>1</sup> Urey, H. C., Brickwedde, F. G., and Murphy, G. M., *Phys. Rev.*, 1932, **39**, 164, 864.

<sup>2</sup> Lewis, G. N., *J. Am. Chem. Soc.*, 1933, **55**, 3503.

<sup>3</sup> Taylor, H. S., Swingle, W. W., Eyring, H., and Frost, A. A., *J. Chem. Phys.*, 1933, **1**, 751; *J. Cellular and Comp. Physiol.*, 1933, **4**, 1.

<sup>4</sup> Macht, D. I., and Davis, M. E., *J. Am. Chem. Soc.*, 1934, **56**, 246.

<sup>5</sup> Barnes, T. C., *J. Am. Chem. Soc.*, 1933, **55**, 4332.

<sup>6</sup> Barnes, T. C., and Larson, E. J., *J. Am. Chem. Soc.*, 1933, **55**, 5059.

<sup>7</sup> La Mer, V. K., Eichelberger, W. C., and Urey, H. C., *J. Am. Chem. Soc.*, 1934, **56**, 248.

mouse melanoma. Aseptic precautions were taken in all experiments.

Into each of 2 weighing bottles were placed 2.5 cc. of Locke-Ringer solution\* which was evaporated to dryness over a covered water bath. One residue was then dissolved in 2.5 cc. of ordinary distilled water and the other in 2.5 cc. of heavy water, thus making isotonic solutions. The solutions were buffered to pH 7.0,† approximately (checked colorimetrically), by adding 0.088 cc. of 0.2 M  $\text{KH}_2\text{PO}_4$  and 0.052 cc. of 0.2 M KOH. Small pieces of tumor tissues (each weighing about 6 mg.) were placed in these solutions and left for 12 to 24 hours at 4-5°C. At the end of these periods of time, the tumor fragments were inoculated into mice. Parallel experiments were run with tumor fragments in ordinary distilled water and 40% heavy water, neither of which had been brought to isotonicity by the addition of the Locke-Ringer salts. This group of 4 sets involved 110 tumor implants.

The results showed that 40% heavy water has no effect upon the transplantability of the tumor fragments if isotonicity be maintained by a balanced salt solution. The tumor fragments which had been immersed in Locke-Ringer solution made up in ordinary and in 40% heavy water grew normally when transplanted into animals. Histological examinations of these treated tumor tissues showed no definite changes. On the other hand, the growth capacity of the mouse sarcoma 180 was markedly destroyed when subjected to hypotonic media for 24 hours, both in ordinary and heavy water (60 to 70% inhibition).

Experiments duplicating those with mouse sarcoma 180 were made with the Passey mouse melanoma. This group involved 108 tumor implants. The results obtained from these experiments were essentially the same as in the case of mouse sarcoma 180. The growth capacity of the melanoma was completely destroyed by 24 hours immersion in ordinary or 14.8% heavy water at pH 7.0. On the other hand, immersion in 40% heavy water or ordinary water solution, which were isotonic from the Locke-Ringer salts, did not affect the growth capacity of the mouse melanoma.

Barnes<sup>5</sup> has suggested " a stabilizing action of water containing

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\* A solution containing 0.15 moles of NaCl, 0.003 moles of KCl, and 0.003 moles of  $\text{CaCl}_2$  per liter.

† The growth capacity of mouse sarcoma 180 was completely destroyed by immersion in a Locke-Ringer solution at pH 4.0 or 10.0. At pH 5.0, 69% inhibition and at pH 9.0, 61% inhibition occurred. Tumor fragments immersed in solutions at pH 6.0, 7.0 and 8.0 for 24 hours at 4-5°C. grew normally when implanted into mice.

the heavy isotope . . . .", which he considers protective. Since exposure of the tumor fragments to hypotonic media is destructive, it is interesting to note if heavy water is less destructive than ordinary water.

Equal numbers of fragments of mouse sarcoma 180 were placed in 2.5 cc. portions of 14.8% heavy water and ordinary distilled water. These media had been buffered to pH 7.0 by means of 0.088 cc. of 0.2 M  $\text{KH}_2\text{PO}_4$  and 0.052 cc. of 0.2 M KOH. The weighing bottles containing the tumor fragments (from 10 to 20 pieces in each bottle) were allowed to remain for definite intervals of time of 6 to 48 hours in the refrigerator at 4-5°C. At the end of different periods of time, the fragments were inoculated into mice. The results show that immersion in heavy water or ordinary distilled water for 6 hours is without effect; the tumors grew normally. After immersion for 12 hours, the rate of growth was slightly retarded and partial inhibition of takes (20% in both ordinary distilled water and heavy water). Similar treatment for 24 hours produced from 50 to 71% inhibition in both ordinary and heavy water. Exposures for 30 hours produced 80% inhibition and marked retardation of growths, and 48 hours resulted in complete destruction of growth capacity.

Gross and histological examination of a number of tumor tissues after immersion in the heavy water and ordinary distilled water showed essentially the same things: *i. e.*, the swelling of the tumor tissues, their jelly-like appearance, and the hydropic degeneration of the cytoplasm, with nuclear degeneration.

In a separate experiment, it was found that 24 pieces of tumor tissue before immersion in 14.8% heavy water (pH 7.5) weighed 0.1698 gm., and after immersion for 24 hours at 4-5°C. they weighed 0.2646 gm. (156%). In a simultaneous experiment, 24 fragments before immersion in ordinary distilled water (pH 5.3) weighed 0.1698 gm., and after immersion they weighed 0.2200 gm. (130%). The swelling was greater in heavy water, but the disparity may be due to the difference in pH.

*Summary.* The proliferating capacity of the mouse sarcoma 180 and the mouse melanoma was unaffected by heavy water (14.8 and 40%  $\text{H}_2^{18}\text{O}$ ) when it contained salts of a Locke-Ringer solution in an isotonic amount.

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