4, but sometimes 8 liters of samples were taken for the radiochemical analyses.

Figure 1 shows the variation of the Sr^{89}/Sr^{90} ratio in rain. A sharp increase in the ratio was observed 10 days after the first French nuclear explosion, and the ratio reached a maximum approximately 3 weeks after the explosion. The second peak which followed the April explosion was small and not too well defined.

Results from our previous studies predicted that the Sr^{89}/Sr^{90} ratio in the stratosphere after February 1960 would have been something like the dotted straight line *ab* shown in Fig. 1, if the February and April nuclear explosions had not taken place.

If the debris from the French nuclear detonations did not enter the stratosphere, the Sr^{89}/Sr^{90} ratio in rain should have asymptotically approached the straight line *ab* 1 to 2 months after the April nuclear explosion. Figure 1 indicates, however, that a new Sr^{89}/Sr^{90} ratio, given by the straight line *cd*, was established in the stratosphere after the French nuclear detonations. This can be explained by the fact that some of the Sr^{89} and Sr^{90} atoms produced by the French nuclear explosions were transported from the troposphere to the stratosphere by the upward flow of air in the tropical region, described by Brewer and by Dobson (*1*).

It is possible to make a rough estimate of the increase in the stratospheric Sr^{90} inventory due to the French nuclear explosions. If it is assumed that the February explosion was chiefly responsible for the increase in the stratospheric Sr^{90} inventory, the Sr^{89}/Sr^{90} ratio in the stratosphere has increased from 0.15 ± 0.05 (curie/curie) to 0.5 ± 0.1 (curie/curie) because of this nuclear explosion. It is known that the fissionable material used in the bomb was plutonium. The Sr^{89}/Sr^{90} ratio in a fresh fission products mixture from a plutonium bomb explosion can be calculated to be approximately 170 (curie/curie), and hence

$$\frac{(0.15 \pm 0.05)P + 170\,Q}{P + Q} = (0.5 \pm 0.1) \qquad (1)$$

where P is the total quantity of Sr^{90} in the stratosphere prior to the nuclear explosion and Q is the quantity of Sr^{90} which entered the stratosphere from the French nuclear detonation. Solving Eq. 1 for Q/P, a value

$$Q/P = 0.0021 \pm 0.0009$$

is obtained.

It is possible that the April explosion was chiefly responsible for the increase in the stratospheric Sr^{90} inventory, and similar calculation yields a value

$$Q/P = 0.0010 \pm 0.0003$$

for this case. Thus, it would appear that the percentage increase in the Sr^{90} inventory of the Northern Hemisphere due to the French nuclear explosions was approximately 0.1 to 0.2 percent (*4*).

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4. This investigation was made possible by support from the U.S. Atomic Energy Commission. We are grateful to T. K. Chan for assisting in sampling work.
* On leave from the department of chemistry, Arkansas State College, State College, during the summer of 1960.

7 November 1960

Blockade of Deoxyribonucleic Acid Synthesis by Deuterium Oxide

Abstract. Interference with deoxyribonucleic acid replication need not be a *primary* mechanism in the blockade of cell division by deuterium oxide, but current hypotheses on the molecular basis of the blockade do suggest that such interference might take place under appropriate conditions. Autoradiographic experiments support the suggestion, for whereas normal sea urchin eggs incorporate H^3-thymidine into deoxyribonucleic acid from almost the beginning of development, cells immersed in deuterium-enriched media do not. Blockade of mitosis and inhibition of thymidine incorporation are simultaneously relieved when the eggs are returned to normal water.

Disturbances of cell division are the most obvious of the growth-inhibiting effects of heavy water on microorganisms. These effects have been the objects of several investigations (*1*). Katz and his co-workers have been able to adapt algae to growth in 99+ percent D_2O, and Flaumenhaft *et al.* observed changes in the patterns of nucleic acid synthesis and distribution in deuterated *Chlorella* and *Scenedesmus* (*2*). Serious disturbances of cell division follow also upon exposure of higher organisms to D_2O (*3*). Gross and Spindel (*4*) have found that D_2O inhibits reversibly the mitotic division of marine invertebrate eggs, and that the inhibition can be imposed at any stage, including even cytokinesis. Their interpretation of the data emphasizes a cooperative deuterium isotope effect upon the operation of multiple hydrogen-bonded structures in the cytoplasm, among them the mitotic apparatus and the cleavage furrows.

Calvin and his associates have supposed that cooperative effects of this type might appear at the molecular level, importantly so in the synthesis and function of proteins and nucleic acids (*5*). Since mitotic inhibition by D_2O can be effected in the sea urchin egg *after* deoxyribonucleic acid (DNA) replication is complete, and indeed long after metaphase (*4*), such a mechanism cannot alone account for the mitotic block. Yet cytochemical evidence does suggest alterations of nucleic acid synthesis in deuterated algae (*2*), and it is therefore of interest to determine whether DNA synthesis is affected by deuteration of cells other than microorganisms.

For theoretical and practical reasons (*4*), the sea urchin egg has been chosen for this work. A test of the hypothesis that DNA synthesis is affected by deuteration is afforded by autoradiography of cells exposed to tritiated thymidine during the period of DNA replication. Harding and Hughes (*6*) and Bucher and Mazia (*7*) have already shown that H^3-thymidine is incorporated into polymers during the early cleavages of sea urchin eggs, and the temporal relations between isotope incorporation and the appearance of mitotic figures have most recently been studied in injured lens epithelium by Harding and Srinivasan (*8*).

Eggs and sperm of *Arbacia punctulata* were obtained in the usual way. A large population of eggs (with jelly coats removed) was inseminated in normal sea water at 21.5°C. Exactly 5 minutes later, when 95+ percent of the cells had elevated normal fertilization membranes, the population was divided, half of the cells being placed in filtered sea water containing H^3-thymidine and the other half in a reconstituted deuterium sea water (90+ percent of D) containing H^3-thymidine. The thymidine had a specific activity of 1.9 c/m mole and was present in both incubation media at a level of 8.33 μc/ml.

Samples were removed from the cultures to acetic-alcohol and dilute formalin fixatives at brief intervals, beginning with the time when the control eggs (in normal sea water and thymidine) reached 50 percent first cleavage. One hundred and eight minutes after fertilization, when the controls were dividing from four to eight cells (the deuterated cells still undivided), the D_2O-treated population was freed of excess deuterium by repeated centrifuga-

tion through the normal sea water–thymidine medium. The cells remained therein until the experiment was terminated. Samples were observed and photographed throughout, these procedures serving to determine the cleavage intervals, the type and severity of mitotic inhibition, reversal thereof upon removal of D_2O, and the general morphology of the embryos. To the latter end, the cultures (both now in normal sea water–thymidine) were permitted to remain on the water-table overnight at 21.5°C.

Fixed cells were prepared for autoradiography by sectioning of paraffin-embedded material and by mounting of the sections on gelatin-coated slides. These were cleared and then covered with Kodak AR-10 stripping film. Exposures were for periods of 7 to 18 days, and the emulsions were developed without staining of the sections. Observations were made by means of bright-field and phase-contrast microscopy.

Eggs which had been placed in normal sea water–thymidine 5 minutes after fertilization cleaved normally at 57 minutes. The second division appeared on schedule. Cells of this population produced, ultimately, normal-appearing, free-swimming larvae. No significant deviations from the ordinary course of development were observed. This is surprising in view of the large dose of radiation supplied directly to the genetic material almost from the beginning of development (compare Harding and Hughes, *6*).

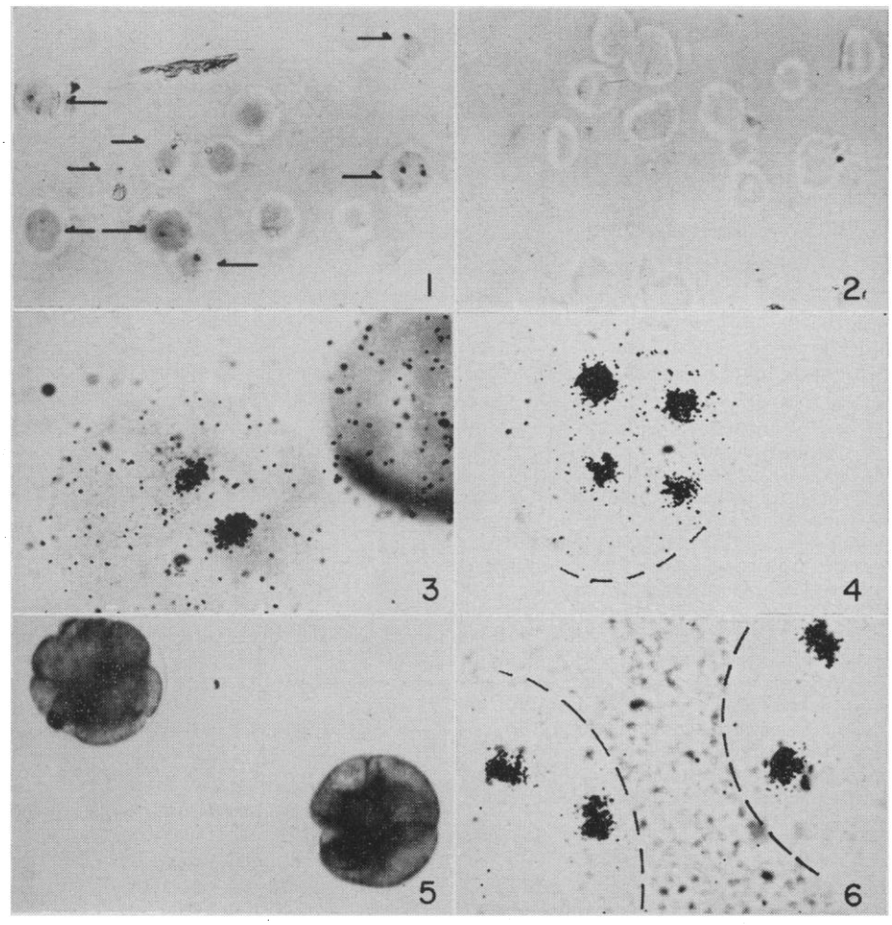

Fig. 1. Autoradiogram of control eggs, placed in normal sea water with H^3-thymidine 5 minutes after fertilization and fixed 103 minutes after fertilization. Arrows mark sections with labeled nuclei. Fig. 2. Autoradiogram of sectioned eggs, treated like the controls, except that the medium contained 90+ percent D_2O. No radioactivity above background. Fig. 3. High-power photomicrograph of sections from the control cell population. Unstained, bright-field illumination, focus on the emulsion grain. Two nuclei labeled. Fig. 4. High-power photomicrograph of sections from control cell population, fixed 149 minutes after fertilization. Dashed line indicates cell border. Four labeled nuclei. Fig. 5. Cells placed in sea water with 90+ percent D_2O and H^3-thymidine 5 minutes after fertilization, then washed free of excess deuterium after 108 minutes. Shown are two living cells from this population, 149 minutes after fertilization, with multiple cleavages. Fig. 6. High-power photomicrograph of sections from eggs shown in Fig. 5. Cells were not labeled during their 108 minutes in D_2O-thymidine, but 41 minutes after removal of D_2O, all nuclei in blastomeres resulting from the multiple cleavages were radioactive. Dense material between cells is an inert protein binder.

The deuterated cells did not divide at all during their sojourn in heavy water and thymidine. At 108 minutes after fertilization, they were washed with, and placed in, normal sea water–thymidine. Thirty-seven minutes later most of the cells had divided, and the divisions were mainly multipolar. It is difficult to be certain that the multiple cleavages observed were truly simultaneous, but, in any case, cytokinetic activity in these cells accomplished in 37 minutes what had required more than 100 minutes in the controls.

Examination of the "reversed" deuterium culture after an overnight incubation revealed that, whereas most of the cells had continued to divide, little or no differentiation had taken place, so that except for a few scattered swimming forms the 'embryos" were multicellular masses lying inert on the bottom of the culture dish. Thus the cell division (or cytokinetic) mechanism survived the period of deuteration, but irreparable damage had been done to the sources of information for differentiation.

The numerous peripheral clear zones which appeared in the deuterated cells were no longer visible after "reversal," but in fixed (unwashed) material stained with hematoxylin, the "clear zones" (referred to as "mottling" in an earlier paper, *9*) resembled cytasters. Perhaps the multiple furrowing of the deuterium-treated cells is related to the appearance of these structures, a possibility which would weaken a previous supposition (*4*, *9*) concerning the continuance of synthetic activity in cells immersed in D_2O.

Cells maintained in normal sea water and thymidine were already labeled when they were first sampled, that is, at 50 percent first cleavage. The label was in the nuclei when present and in mitotic figures when these were included in the section. *No isotopic incorporation* took place in the deuterated cells. Figure 1 shows a typical low-power field of control cells sampled at 103 minutes after fertilization. The cells were at this time entering the second division. The microscope was focused on the emulsion grain, and groups of grains appear as black deposits. Of the 14 sections in the field, eight contain nuclei or mitotic figures, and all such are labeled. Figure 2 is a low-power photomicrograph of sections of deuterated cells sampled at the same time as those in Fig. 1. Among the ten full-diameter sections shown, at least five include nuclei, but none show radioactivity above background.

Figures 3 and 4 show sections, at high power, of the control cells. Those in Fig. 3 were sampled 103 minutes after fertilization. The two nuclei are strongly labeled. Figure 4 is a section

of a control cell fixed 149 minutes after fertilization, a time when the population was entering the fourth division. The cell shown in the figure had evidently been sectioned in a plane including four nuclei, each of which was labeled, as evidenced by the accumulation of silver grains.

The multiple cleavages in the cells from which the D_2O had been removed are shown in vivo in Fig. 5, and an autoradiogram of the same material in section is shown in Fig. 6. *The nuclei are now labeled.* The sections shown in the figure include two nuclei each, and all four of these exposed the emulsion strongly. Thus, the release of the block to cell division releases as well the block to the synthesis of DNA. It is not yet possible to conclude that this rather remarkable "switching-on" of DNA synthesis is matched by an equally abrupt "switching-off" upon deuteration, since we do not know whether or not synthesis had begun when the eggs were immersed in D_2O. Because the pronuclei had not yet fused, it is possible that DNA replication had in fact not begun, which would make the mechanism of action of D_2O in the present case an inhibition of the *onset* of synthesis, rather than the blockade of a process already in full career. These qualifications are mitigated, however, by the interesting finding of Bucher and Mazia (*7*) that H^3-thymidine is incorporated into the DNA of *Strongylocentrotus purpuratus* eggs whether the pronuclei have fused or not.

Failure of the H^3-thymidine to penetrate the deuterated cells, rather than any chemical isotope effect, might explain these results. Such an absolute permeability differential for normal and deuterated sea urchin eggs is rather unlikely, but the possibility does require experimental study (*10*).

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29 December 1960

Bulbar Control of Cortical Arousal

Abstract. In the cat, the cortical arousal induced by reticular or nociceptive stimulation is more intense and more long-lasting after elimination of the caudal and medial part of the medulla than in preparations with intact brain. This difference is explained by the intervention of a phasic-ascending inhibitory bulbar control secondarily triggered by the mesencephalic activating system.

We have recently published experimental data which led to the conclusion that a brief stimulation of the mesencephalic reticular facilitating system is able to set secondarily into action a phasic-descending bulbar inhibitory control. This control was evidenced by the decreased amplitude of a sympathetic response, the galvanic skin response recorded on the cat's foot pad (*1*). During these experiments, we noticed that the depression of the sympathetic response was accompanied by decreased intensity of the cortical response to the same mesencephalic stimulation. Because of the simultaneity of both these descending and ascending effects which follow reticular stimulation, we assumed that the decrease of the cortical response might also be the consequence of a bulbar control mechanism. The present investigation was designed to test this hypothesis of bulbar control of cortical arousal.

The experiments were performed on acute spinal cats (cord section at T_2 level), which had been operated under ether. A few hours after the surgical procedure, the preparations were paralyzed with Flaxedil, artificially ventilated and slightly nembutalized (3 to 7 mg/kg). This narcosis, by depressing the normally predominant reticular activating influence, facilitates the "unmasking" of coexisting inhibitory processes. The spontaneous electrical cortical activity of such preparations shows sleep patterns (see Fig. 1); a cortical arousal, however, may easily be evoked by the stimulation of the mesencephalic reticular activating system or by the stimulation of an afferent nerve of the anterior limb (median, cubital, or superficial radial nerve). Because of the spinal section, these stimuli do not affect the cardiovascular sympathetic tone.

The electroencephalographic responses to reticular or afferent stimulation have been compared in preparations (i) with the intact brain and (ii) after elimination of the effects of the bulbar inhibitory formation (*2*) by either a prebulbar (retropontine) tran-

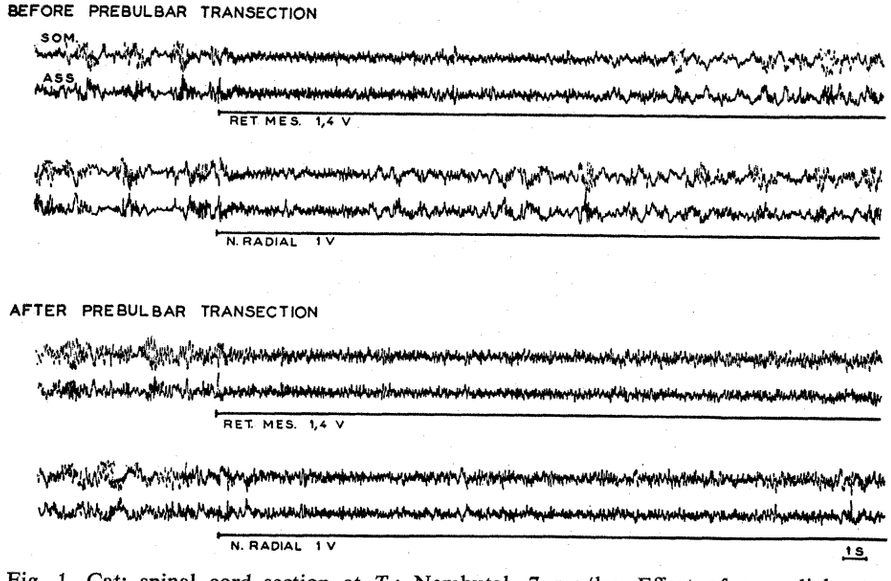

Fig. 1. Cat; spinal cord section at T_2; Nembutal, 7 mg/kg. Effect of a medial prebulbar section of the brain stem on the duration of the cortical arousal induced by long-lasting reticular (mesencephalic) and afferent (superficial radial nerve) stimulation. Before the section (upper traces) the arousal is short and soon followed by the return of the initial sleep pattern. After the section (lower traces), the arousal remains sustained as long as the stimuli are applied.