Autoradiographic Studies of Localization of Tritiated Methyl N-Carbamyl Maleamate in Ehrlich Ascites Tumor Cells.* (32480)

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It has been shown by us that maleuric acid and some related substances produce extensive morphological damage in mouse ascites tumor cells(1,2,3,4). Methyl N-carbamyl maleamate (MCM) and some other esters of maleuric acid had remarkable disruptive effects on spindle fibers of tumor cells(3). In the present work an attempt was made to delineate further the intracellular site of the action of MCM by radioautographic localization of H^3-labeled MCM.

Materials and methods. A mouse tumor, Ehrlich ascites tumor (hyperdiploid strain), was used in this experiment. The ascites tumor was transmitted serially as previously described(1) in Swiss/HaICR mice, weighing 18-20 g, fed on a stock diet of Purina chow and water. Test substances were injected intraperitoneally on the 7th day after transplantation, at which time the tumor was well established. The radioactive substances were injected in single doses of 0.06 mc of MCM-H^3 (H^3-methyl ester of monomaleyl urea, specific activity 1.0 c/mM) and 0.06 mc of H^3-methyl alcohol (specific activity 1.0 c/mM). Cold MCM in this experiment was administered at a level of 1 mg/kg.

Samples of tumor cells were removed by peritoneal puncture at 1, 3, 6, 12 and 24 hours after injection of the test substances. Smear preparations were made and fixed in Carnoy's solution (3:1) for 5 minutes after appropriate drying, and the autoradiographic film was applied. The autoradiographs were processed 14 days after exposure and were stained in toludine blue solution. Finally, slides were mounted in immersion oil(4).

Results. Incorporation of MCM-H^3 into tumor cells. Rapid incorporation of MCM-H^3 took place into interphase cells, approximately 55% of the cell being labeled in 1 hour after injection of this isotope, the earliest time at which a sample was taken. With the exception of the 3 hour period, there was a subsequent increase in percentage of labeled interphase cells up to 12 hours (Table I). Grains appeared at random in the cytoplasm and the nucleus of tumor cells that were fixed 1 hour after injection of this isotope. The numbers of grains were small. Grains seemed to localize around the nucleus in cells fixed at 3 hours after the injection and the numbers were increased over those seen at the 1 hour interval. With time, grains were distributed in the cytoplasm with little or no activity evident within the nucleus (Figs. 1-3).

Incorporation of methyl alcohol-H^3 into tumor cells. There was a possibility that the methyl ester of MCM might be hydrolyzed in the ascites to form methyl alcohol and free maleuric acid. That is, hydrolyzed MCM-H^3 in ascites might enter the tumor cells in the form of methanol-H^3.

To obtain data with regard to the latter possibility, methanol-H^3 was injected into tumor-bearing animals, and the experiments were carried out in the same way as in the case of MCM-H^3. Methanol-H^3 was incorporated into the interphase cells, approximately 35% of the cells being labeled in 1 hour after injection of this isotope. Thenceforth, there was an increase in percentage of labeled interphase cells up to 12 hours (Table I). Up to 6 hours after injection of this isotope, grains were scattered in the cytoplasm of tumor cells and the numbers of grains were small (Fig. 4). At 12 hours after the injection, grains in some of the tumor cells appeared around the nucleus.

In cases of both MCM-H^3 and methyl alcohol-H^3, grains in mitotic cells did not take any definite position, sometimes appearing around chromosomes and sometimes throughout the cytoplasm. However, when daughter nuclei began to be reconstructed in the telophase, after MCM-H^3 injection grains appeared around the nuclei (Fig. 3).

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TABLE I. Percentage of Interphase Cells with Label and the Number of Grains/Cell at Various Times After Injection of Methyl N-carbamyl Maleamate-H^3 and Methyl Alcohol-H^3.

Hr after MCM-H^3‡ or CH_3OH-H^3§	MCM-H^3				CH_3OH-H^3			
	Alone*		Plus cold MCM (1 mg/kg)†		Alone*		Plus cold MCM (1 mg/kg)†	
	% of labeled interphase cells	No. of grains/cell	% of labeled interphase cells	No. of grains/cell	% of labeled interphase cells	No. of grains/cell	% of labeled interphase cells	No. of grains/cell
1	55.0 ± 8.2	20.9 ± 4.5	84.3 ± 1.3	81.2 ± 8.1	35.0 ± 4.0	11.78 ± 5.2	45.7 ± 4.05	12.7 ± 5.6
3	51.8 ± 6.3	34.5 ± 6.8	79.6 ± 2.9	83.2 ± 7.0	41.4 ± 5.4	14.62 ± 4.2	66.1 ± 6.2	15.7 ± 3.2
6	61.0 ± 6.7	36.7 ± 4.8	98.0 ± 0	93.3 ± 5.6	55.6 ± 4.1	17.6 ± 3.2	53.4 ± 0.5	16.7 ± 4.3
12	62.8 ± 6.5	36.8 ± 3.4	100.0 ± 0	94.4 ± 4.2	58.8 ± 7.3	18.3 ± 4.1	60.0 ± 0	18.6 ± 3.1
24	59.7 ± 4.4	35.6 ± 2.2	100.0 ± 0	93.2 ± 3.5	52.3 ± 8.2	17.3 ± 5.1	85.0 ± 1.0	18.7 ± 6.2

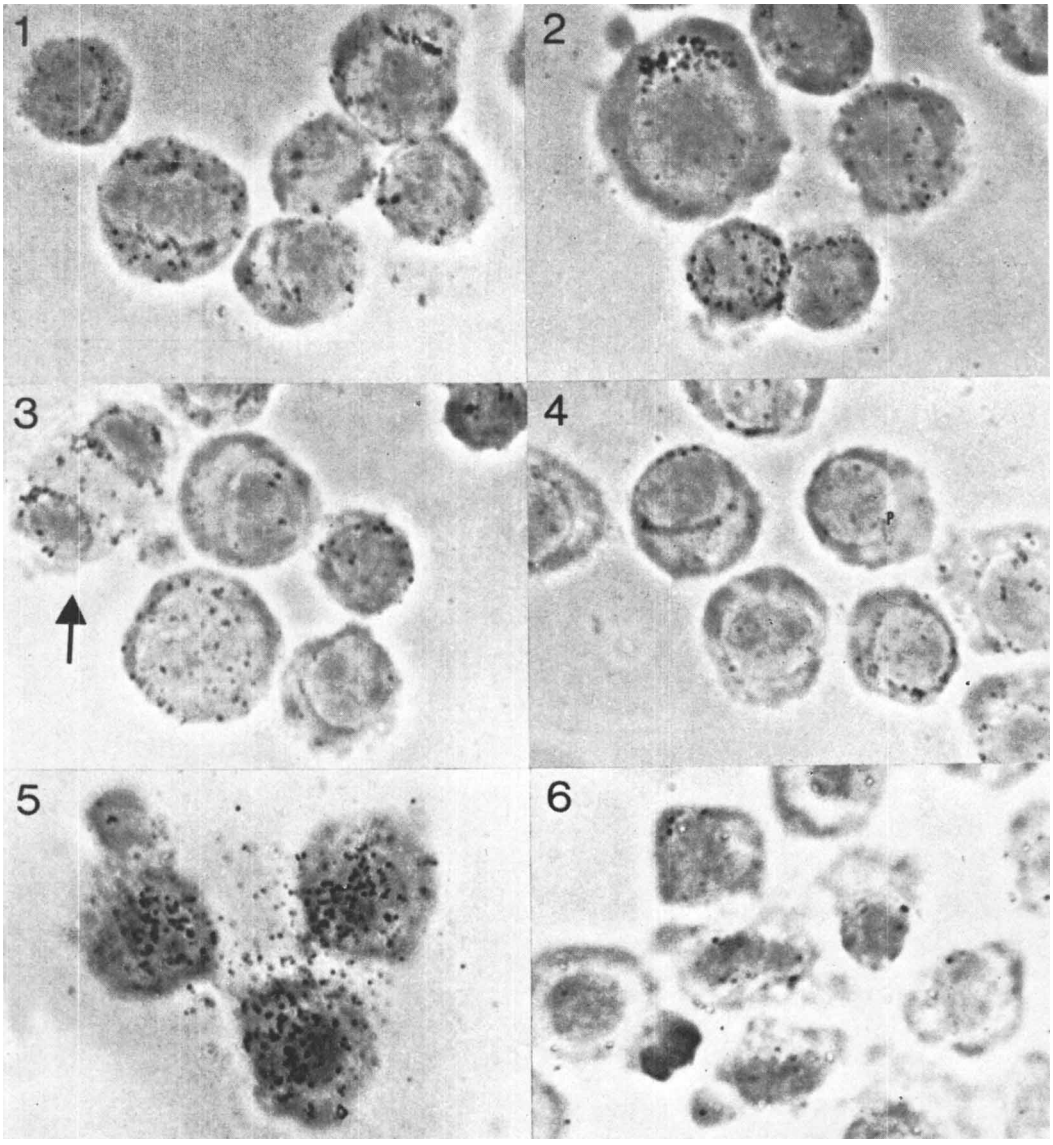
* Each individual figure is the mean value of determinations made in 6 animals.
† Each individual figure is the mean value from counts made from 100 cells in each of 2 animals.
‡ H^3-methyl N-carbamyl maleamate.
§ H^3-methyl alcohol.

Simultaneous injection of cold MCM with MCM-H^3 or methyl alcohol-H^3. From the above observations, it is clear that no significant differences between MCM-H^3 and methyl alcohol-H^3 were noted in incorporation rate and distribution pattern into cells. However, it was thought that differentiation between MCM-H^3 and methyl alcohol-H^3 might be obtained by injecting cold MCM simultaneously with both the radioactive materials and by observing whether or not cold MCM had any effects on the entry and distribution of either or both of the radioactive materials.

One mg of cold MCM was injected simultaneously with MCM-H^3 or with methanol-H^3 into tumor-bearing animals. As control, MCM-H^3 or methanol-H^3 alone was injected in the same amounts. When cold MCM was used, incorporation of MCM-H^3 took place more rapidly into interphase cells than when MCM-H^3 was given alone, approximately 85% of the cells being labeled in 1 hour after the injection (Table I) and the extent of labeling was much greater (Table I, Fig. 5). Thereafter, the percentage of labeled interphase cells increased. Numerous grains appeared all over the cells (Fig. 5). Administration of cold MCM plus methanol-H^3 gave results similar to those obtained with methanol-H^3 alone (Fig. 6, Table I). Only relatively few grains were found scattered in the cytoplasm.

Although the total amount of MCM given produced, at most, minimal morphological abnormalities in the tumor cells, the simultaneous injection of cold MCM together with MCM-H^3 markedly enhanced the extent of incorporation of isotope from the MCM-H^3 (Fig. 5, Table I). It seems likely, therefore, that the entry of intact MCM takes place into the tumor cells and that the rate of entry is concentration dependent. The finding that administration of MCM together with H^3-methanol did not enhance incorporation of the label into the cells (Fig. 6) suggests strongly that the entry of the MCM and methanol take place by different mechanisms.

Discussion. Our previous report(3) showed that within 1 hour after the injection of cold MCM (3 mg/mouse), spindle fibers in tumor cells began to break down into fragments and



FIGS. 1-6. Autoradiographs of Ehrlich ascites tumor stained by toluidine blue solution. × 1000 (phase contrast).

FIGS. 1-2. Labeled interphase cells 6 hrs after intraperitoneal injection of MCM-H^3, showing grains around the nucleus.

FIG. 3. Labeled mitotic cell (indicated by arrow) after intraperitoneal injection of MCM-H^3.

FIG. 4. Labeled interphase cells 6 hrs after intraperitoneal injection of H^3-CH_3OH.

FIG. 5. Heavily labeled interphase cells 6 hours after simultaneous injection of MCM-H^3 and cold MCM.

FIG. 6. Slightly labeled interphase cells 6 hrs after simultaneous injection of CH_3OH-H^3 and cold MCM.

dissolve. Fragmented spindle fibers were scattered at random in the cytoplasm. Thereafter, the number of abnormal mitotic cells with pycnotic chromatin masses and without spindle fibers continued to increase. Furthermore, observations of the percentage of labeled mitotic cells (normal and abnormal) employing H^3-labeled thymidine(3), showed that very few labeled mitotic cells were noted at any time when the injection of MCM

preceded the administration of the thymidine, even though relatively large proportions of the interphase cells had become labeled. Even when the agent was given 30 minutes after the addition of nucleoside and the usual normal proportions of interphase cells were labeled, there was a remarkable inhibition of the appearance of the grains in the mitotic cells.

Since it was not known whether MCM might exert its effects directly or indirectly on the nuclear apparatus, it was of interest to see if there was any preferential accumulation of MCM in a particular region of the cell. In the present experiment, 3 hours after the injection of MCM-H^3, grains began to appear around the nucleus and increased in number. No grains were observed within the nucleus. Therefore, it can be concluded that the MCM-H^3 became associated with cytoplasmic and not nuclear components. These components into which MCM-H^3 was incorporated may play a significant role in the formation of the spindle fibers. Experiments are under way in which an attempt is being made to isolate the H^3-labeled cytoplasmic constituents.

Summary. The intraperitoneal injection into mice with Ehrlich ascites tumor of trace amounts of H^3-methyl N-carbamyl maleamate, a substance which in larger amounts produces destruction of spindle fibers, resulted in the appearance on autoradiography of grains chiefly in the cytoplasm and only rarely in the nucleus. In a number of instances the grains appeared to be distributed in a perinuclear fashion. Somewhat similar results were obtained when H^3-methanol was administered. The administration of cold methyl N-carbamyl maleamate (1 mg) together with the labeled substance resulted in a marked enhancement of the uptake of the labeled material into the cells, while a similar experiment with nonradioactive methyl N-carbamyl maleamate and H^3-methanol showed no increase in uptake of isotope by the tumor cells. It was concluded that H^3-methyl carbamyl N-maleamate enters the tumor cells as an intact molecule and associates with cytoplasmic components. The relationship of some of these components to spindle fiber structures or precursors is suggested.

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Insulin Secretion *in vitro* by Islets from Insulin-Deficient Rats.* (32481)

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Following intravenous or intraperitoneal injection of guinea pig anti-insulin serum, transient hyperglycaemia induces rapid secretion of insulin(1) and more rapid depletion of pancreatic insulin than can be induced by hyperglycaemia due to intravenous administration of glucose(2). On the other hand, glucose induces the same rate of insulin secretion from isolated islets whether guinea pig anti-insulin serum is present in the incubation medium or not(3). An explanation for this discrepancy was sought in the present experiments by incubating islets obtained from normal rats and from rats treated with guinea pig anti-insulin serum for different times.

Materials and methods. Well fed male albino rats (250-350 g, Holtzman, Wisconsin) were injected either intravenously (1.5 ml) or intraperitoneally (2-5 × 5 ml) with guinea pig anti-insulin serum (GPAIS, Lots 401 or 404; binding 2.5 to 3.0 units bovine insulin/ml). Rats were anesthetized 60 or 150 minutes after a single intravenous injection and

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