EFFECT OF 8-MERCAPTOADENINE ON CELL METABOLISM

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Under the influence of 8-mercaptoadenine the synthesis of protein and RNA in HeLa cells is considerably stimulated, the duration of interphases is reduced (the duration of mitosis is unaffected) in HeLa and PEB cultures, the earlier appearance of certain enzymes is observed in HeLa cells and in primary trypsinized chick fibroblasts, and the survival rate of HeLa and PEB cells is increased.

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The object of this investigation was to study the effect of 8-mercaptoadenine (meradine) on cells in connection with results obtained previously in the writers' laboratory indicating that this substance can be used to stimulate interferon production.

EXPERIMENTAL METHOD

The action of meradine in doses of 0.1-300 μg/ml was studied on transplantable cell strains HeLa and PEB and on primary trypsinized chick fibroblasts. Strain HeLa was chosen because its cells possess an intensive metabolism and form clones readily. Cells of line PEB also form clones readily, but they are derived from normal cells and they proliferate more slowly than HeLa cells. The effect of meradine was studied in doses of 0.1-25 μg/ml on synthesis of RNA and protein by HeLa cells with the use of methionine-S^{35} and uracil-C^{14}. The intensity of incorporation was determined on a gas-flow counter 3-13 h after the addition of meradine. Activity of cytochrome oxidase, acid phosphatase, glucose-6-phosphatase, and NAD · H_2- and NADP · H_2-dehydrogenases was demonstrated in cells of the HeLa culture 8 h after addition of the compound. Meradine was added to the cells at the time of pouring. The activity of the same enzymes was determined in chick embryonic fibroblasts during the first 2 days of their cultivation in the presence of meradine and in the control. The mitotic activity of the HeLa and PEB cells was estimated every 3 h during the 2 days after addition of the compound.

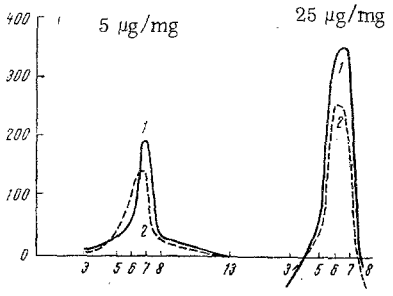

Fig. 1. Stimulation of synthesis of protein (1) and RNA (2) by the action of meradine. Abscissa, time (in h); ordinate, intensity of synthesis (in % of control).

The duration of interphase was compared in the control and experimental groups using the formula

$$T = \frac{0{,}693 \times tm}{M} \quad [8],$$

where M represents the duration of mitosis. It was determined by the colchicine method [4]. The survival rate of the HeLa and PEB cells was recorded by a modified Puck's method. The results were read 10 days after seeding of the cells. Colonies consisting of not less than 50 cells were regarded as surviving.

EXPERIMENTAL RESULTS

The experiments with the use of methionine-S^{35} and uracil-C^{14} showed that meradine, in doses of 0.1 and 1 μg/ml, does not affect the synthesis of protein and RNA. A dose of 5 μg/ml substantially increased

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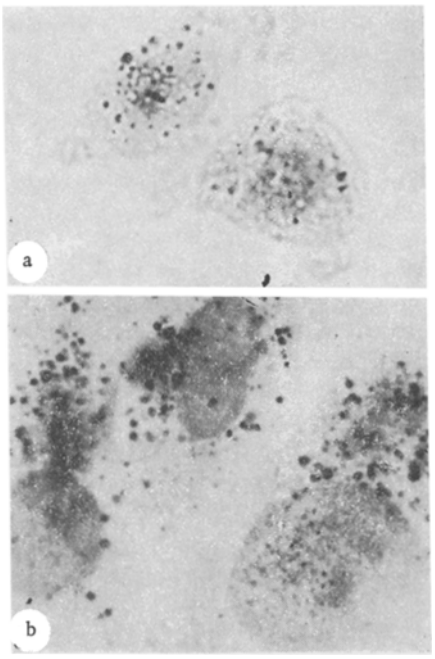

Fig. 2. Change in activity of acid phosphatase in culture of HeLa cells by the action of meradine in a dose of 5 μg/ml. a) Control; b) meradine in a dose of 5 μg/ml. 480×.

the intensity of incorporation of methionine and uracil (Fig. 1). Protein synthesis was increased 3 h after addition of the compound by 13.8%, and RNA synthesis by 10% compared with the control, while 5 h after addition of meradine the increases were by 23 and 28%, and 6 h after its addition by 50 and 103%, respectively. The greatest increase in the intensity of synthesis of protein (by 199%) and of RNA (by 133%) was observed 7 h after addition of meradine. After 8 h the intensity of incorporation of methionine and uracil remained higher than in the control by 30 and 20%, respectively, while 13 h after addition of meradine it was the same in the experimental as in the control series. Under the influence of meradine in a dose of 25 μg/ml, synthesis of protein and RNA was stimulated still more strongly; protein synthesis was increased 5, 6, and 7 h after addition of the compound by 73, 331, and 350%, respectively, and RNA synthesis at these same times by 51, 253, and 235%. This marked increase in the intensity of incorporation of label was followed by a decrease in the intensity of incorporation of methionine by 10% and of uracil by 55% relative to the control. On the basis of these experiments, for use later in the investigation a dose of 5 μg/ml of meradine was chosen, for this gave marked stimulation of synthesis of protein and RNA without causing the subsequent sharp inhibition.

In the cytochemical study no activity of any of the studied enzymes could be detected in most cells of the control cultures during 8 h after seeding. On the contrary, in cells cultivated in the presence of meradine, activity of all enzymes appeared very clearly 8 h after the beginning of the experiment (Fig. 2). Products of the cytochemical reactions in places of activity of the enzymes were detected uniformly in all cells, and the intensity of the reactions corresponded to that observed in the control after 24 h. In the control 8 h after seeding, the population consisted of small cells with a little cytoplasm, while in the presence of meradine the cells were much larger and had a wide layer of cytoplasm.

The addition of meradine to the HeLa culture in the first 16 h did not affect its mitotic activity; later, after 18 h, the number of mitoses was reduced by 26%, the difference not being statistically significant, and after this an increase in mitotic activity began to take place, reaching its maximum after 24 h. At this time the number of mitoses in the experimental group was 1.5 times higher than in the control ($P < 0.05$). After 27 h the number of mitoses began to return to normal. A similar picture of changes in mitotic activity was observed in the PEB culture; it differed only by a shift in time. A decrease in the number of mitoses in the experimental group by 35%, likewise not statistically significant, was observed after 24 h, and an increase in mitotic activity to twice the control level was observed after 27 h, after which the mitotic activity in the experimental group became the same as in the control.

On the basis of these experiments the duration of interphase was compared in the experimental and control series. For cells of the control HeLa culture its duration was 29 h, and for cells of the experimental group 24 h. For the slowly growing PEB culture, with a few mitoses in the control and a large increase in mitotic activity, the duration of interphases was reduced by meradine from 53.3 h in the control to 43.3 h, but the duration of mitosis itself was unaffected by meradine, amounting to 2.2 h for the control HeLa culture and 2.1 h for the experimental HeLa culture.

Determination of the effect of meradine on the survival of HeLa and PEB cells, based on their ability to form colonies, revealed the influence of the compound on the separate cells and on their succeeding generations. Meradine in these experiments was added in doses of 0.1-100 μg/ml. Under the influence of meradine in doses of 0.01-10 μg/ml the survival rate in the HeLa line was increased by 223-290%, and in the PEB line by 231-313%. A dose of 25 μg/ml stimulated the formation of colonies by cells of line PEB by 473%, and by HeLa cells by 173%. A dose of meradine of 50 μg/ml produced weaker stimulation of colony formation by cells of both lines, while a dose of 100 μg/ml proved to be toxic.

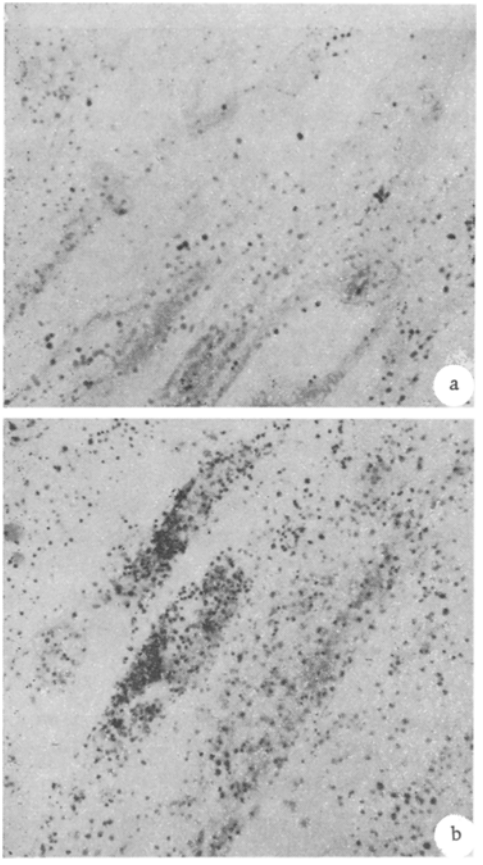

Fig. 3. Changes in succinate dehydrogenase activity in a culture of fibroblasts. a) Control; b) meradine in dose of 5 μg/ml. 480×.

Experiments to study the effect of meradine in a dose of 5 μg/ml on adaptation of chick fibroblasts of a primary trypsinized culture to in vitro conditions gave the following results. In the control, most fibroblasts were still round after 24 h, and under the influence of meradine they became straighter and acquired their usual elongated shape. In the presence of meradine 24 h after seeding the fibroblasts they exhibited definite activity of all the studied enzymes, while in the control activity of the enzymes was detected only in some fibroblasts and to a slight degree (Fig. 3). Higher enzymic activity in the cells of the experimental group than in the control was still observed after 48 h, although the difference between the two groups was less marked on account of the increased enzymic activity in the control.

Under the influence of meradine, synthesis of protein and RNA in cells of the HeLa culture was thus considerably increased, and this may explain the decrease in the duration of interphase described above. The increase in enzymic activity of HeLa cells under the influence of meradine also indicates that the compound stimulates cell metabolism. During adaptation of primary trypsinized cells in the vitro conditions, the phenomenon of masking of the enzymes was observed during the first 2-3 days of cultivation of the cells [5]. The mechanism of this phenomenon has received inadequate study. Addition of meradine caused demasking of enzymes of the different types in chick embryonic fibroblasts during the first 24 h of their cultivation, whereas in the control in the present experiments enzymic activity appeared only after after 48 h.

In view of the results described above, it can be postulated that this phenomenon is also connected with activation of metabolism of the fibroblasts under the influence of meradine.

Experiments to study the survival of HeLa and PEB cultures showed that the addition of meradine increased by 2 or 3 times the number of cells forming viable colonies. Usually in the control only a proportion (40-60%) of cells could give rise to several generations and form colonies containing 50 cells, regarded as viable. The increase in the number of colonies under the influence of meradine was evidently connected with a decrease in the number of cells dying after seeding, and this in turn could be related to the stimulation of cell metabolism. These experiments suggest that meradine, while stimulating metabolism of the parent cell, has no unfavorable action on the viability of its subsequent generations.

The facts described above demonstrate the stimulant effect of meradine on cell metabolism.

At the same time, previous investigations [1, 2, 3, 7] show that cells whose metabolism has been increased in this manner possess increased resistance to certain harmful factors. A comparison of these data indicates that further benefits may be expected from the use of meradine.

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