INCORPORATION OF DNA BY CELLS OF THE
EHRLICH-LETTRE ASCITES CARCINOMA

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It has been reported previously that purified, highly polymerized DNA can be incorporated in vitro into cells of the Ehrlich-Lettre carcinoma (1-3). The incubation system employed in these studies consisted of a sucrose-phosphate buffer with supplements of glucose. These experiments have indicated that the uptake of DNA by the ascites cells is an energy-requiring process, dependent upon the involvement of both glycolysis and respiration. Similar experiments have been conducted to study the incorporation of protein by these cells under the same conditions (4, 5). This process, like the incorporation of DNA, is energy dependent.

Recently, Ryser has questioned the suitability of the incubation medium used in the above-mentioned studies (6). In so doing, Ryser stated that the observed uptake of protein reported for these cells by Choi and Kay (4, 5) was most likely an artifact due to the unphysiological pH of the incubation system and causing alterations in the cells. By inference, the same interpretation is used to explain the incorporation of DNA by these cells. We feel that such an interpretation is not justified in view of the results obtained from metabolic studies and also the results obtained from the viability tests which showed that cells maintained in the medium for periods up to 3 hr retained viability when transplanted into host mice.

In further attempts to assess the suitability of the incubation medium employed previously, experiments have been carried out in which the published method has been used and compared with an incubation method with Eagle’s medium (7), and a method with the Connaught medium CMRL1415 (8).

MATERIAL AND METHODS
DNA-$^{14}$C labeled from formate was isolated as described previously (1-3). Incubation of cells in a medium containing the labeled DNA was carried out as follows. (a) 2 ml of ascites fluid contained 0.5 ml of packed cells, 1 ml of 0.25 M sucrose-0.01 M phosphate buffer, pH 7.4, 0.8 ml of 0.25 M sucrose-0.1 M glucose, and 0.2 ml of water containing 15,000 cpm/mg DNA-$^{14}$C. (b) 2 ml of ascites fluid contained 0.5 ml of packed cells, 1.8 ml of Eagle’s medium, and 0.2 ml of water containing 15,000 cpm/mg DNA-$^{14}$C. (c) 2 ml of ascites fluid contained 0.5 ml of packed cells, 1.8 ml of Connaught medium CMRL1415, and 0.2 ml of water containing 15,000 cpm/mg DNA-$^{14}$C.

Incubations were carried out at $37^\circ$ for 15 min, after which the cells were centrifuged and fractionated as described previously (1, 2). The DNA was extracted from the nuclei, and the specific activities of adenine and thymine were determined (1, 2).

RESULTS AND DISCUSSION
Unpublished data for the uptake of DNA (9) have shown that the incorporation of DNA into the nuclear fraction of incubated cells is dependent upon a suitable pH of incubation. During a 15 min period the maximum incorporation took place at pH 7.4. Considerably less DNA was incorporated at pH values above or below this value. This finding would suggest that the uptake phenomenon is a normal physiological process and also that the stated conditions are suitable for such
studies at the short times of incubation used. During this time the pH did change, dropping at the most from pH 7.4 to pH 6.8 which we consider still a physiological pH. Longer times of incubation were not used since we had shown already (1, 2) that the uptake of DNA occurs during the first 15 min of incubation. Similar findings have been reported by Sirotnak and Hutchison (10) who showed that uptake of extraneous DNA takes place during the first 5 min of incubation. Ryser's criticism of the suitability of the sucrose-phosphate buffer medium seems to be based on dye uptake and protein adsorption studies, and he did not indicate the use of this medium in any studies on incorporation followed by a cell fractionation procedure to trace intracellular localization. We consider the latter step to be necessary in the identification of the place of localization of the incorporated DNA or protein.

Further evidence that normal physiological conditions prevailed in the observed uptake of DNA was found in the fact that labeled DNA was shown to be present in nuclei isolated from the incubated cells with a procedure employing dilute citric acid (11).

In further argument against the physiological state of cells incubated in the sucrose-phosphate buffer which was employed in our experiments, Ryser claims that the presence of $2 \times 10^{-2}$ M glucose is a starvation condition. In some unpublished experiments (9) the effect of various concentrations of glucose on the uptake of DNA was studied. In these experiments the cells were incubated with the extraneous DNA for 15 min, and the pH of the incubation medium was measured after the removal of the cells by centrifugation. It was found that the pH did decrease, as expected, with increasing glucose concentration, and that $2 \times 10^{-2}$ M glucose provided actually the optimum pH conditions.

There are two ways by which the pH of the medium could be lowered during incubation. One way is by an increased cellular glycolysis. Bloch-Frankenthal and Weinhouse (12) have shown that incubation with increased glucose results in an increased lactate production in the Ehrlich-Lettré cells, and that the rate of glycolysis is maximal at $10^{-4}$ M glucose. A second way by which the pH may be lowered is suggested by Cereijo-Santalo and Wenner (13) from their studies of the glycolysis of ascites cells. If the buffering capacity of the system is inadequate, rapid phosphorylation of glucose might release sufficient protons to result in a lowering of the pH.

The results of the uptake experiments are shown in Table I. It can be seen that in the three cases the specific activity ratios of adenine/thymine approximate closely those of the original experimental DNA. The close adherence to the A/T specific activity ratio between the DNA found in the isolated nuclei and the experimental DNA would suggest that in each case there has been an uptake of intact, high molecular weight DNA by the cells when incubated in vitro (1). The uptake of DNA by cells incubated in the sucrose-phosphate buffer medium is in agreement with previous studies (1, 2). The Connaught medium CMRL-1415, which is a superior, fully complemented...
medium for tissue cultures, also supported the uptake of DNA by the ascites cells, as did Eagle's medium. These results can be taken as further support for the physiological nature of this process as measured in earlier experiments.

This work was supported by Medical Research Council Grant No. MA 2705, Canada.

Received for publication 15 January 1968, and in revised form 8 April 1968.

REFERENCES