TABLE III. Relationship Between Treatment Given, *i.e.* Demerol Alone in Varying Amounts or Demerol and N-allylnormorphine, and % of Mice Having Convulsions. Time of onset of first convulsion.

Demerol, mg/kg	% having convulsions	% mice showing convulsions who died during exp. period	Avg time between inj. of Demerol and 1st convulsion, min	Time between inj. and 1st convulsion, min
175	66	65	18	2-65
200	75	50	13	2-66
190, followed within 1 min by 20 N-allylnormorphine	14	60	29	5-93

treated with 200 mg/kg of Demerol and 18 minutes for those receiving 175 mg/kg of Demerol. There was a wide range of time intervals before the onset of the first convulsion, but in some mice convulsions developed within 2 minutes after the injection of Demerol.

Discussion. Contrary to the findings of Huggins, Glass, and Bryan(3) using dogs, N-allylnormorphine does have a protective action against Demerol when tested on mice. There would seem to be at least two possible explanations for their failure to find this effect. First, barbitalization of their dogs may have obscured the action of N-allylnormorphine, although this did not occur with the other narcotics they tested. The second possibility is that the very rapid action of Demerol in mice, and perhaps in dogs, necessitates the immediate use of N-allylnormorphine if any reduction in mortality is to be effected. For example, if N-allylnormorphine is given 5 minutes after the injection of Demerol, the number of mice dying approaches 50%, while Unna(2) found protection against an LD 50 of morphine for as long as 15 minutes after its injection. The rapidity with which Demerol may act is also indicated by the onset of convulsion within 2 minutes after its subcutaneous injection in some of the mice.

Summary. (1) N-allylnormorphine in amounts of 10 mg/kg or more, if given within one minute after an LD 50 of Demerol, provides significant protection in mice. (2) If N-allylnormorphine is given 5 minutes after the LD 50 of Demerol, it provides little protection even if 20 mg/kg is given.

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Nutrition of Animal Cells in Tissue Culture. VI. Low Toxicity of Barium.*† (19251)

JOSEPH F. MORGAN, HELEN J. MORTON, GEORGE M. HEALY, AND RAYMOND C. PARKER.

From the Connaught Medical Research Laboratories, University of Toronto, Toronto, Canada.

During studies on the development of a synthetic medium for animal cells in tissue culture(1-4), it was observed that small

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amounts of certain heavy metals exerted marked effects on cell survival and multiplication. Iron, *e.g.*, was found to be beneficial(1) whereas cobalt was somewhat inhibiting(4). Because several ingredients of the synthetic mixture were obtained commercially as barium salts, and were converted to sodium salts before use, it was thought desirable to determine the degree of toxicity of barium and the barium content of natural and synthetic media.

Materials and methods. Strain cultures of fibroblast-like spindle cells (hereafter referred to as *fibroblasts*) were derived from the leg muscle of 11-day-old chick embryos and maintained in Carrel D-3.5 flasks in a coagulum consisting of fowl plasma, chick embryo extract(5) and balanced salt solution(6) for at least 5 passages (weeks) before use. Roller-tube cultures of chick mesenchyme tissues were prepared from the leg muscle of 11-day-old embryos and maintained in synthetic Mixture 199(1-3). Full details of the various assay procedures have already been described (1,3,4). A stock solution of barium acetate (reagent grade), containing 10 mg barium per ml, was prepared in glass-distilled water, sterilized by autoclaving, and diluted to the desired levels shortly before use. Barium was added to Mixture 199 in place of an equal volume of water, and to the plasma cultures in place of equal volumes of balanced salt solution. The barium content of natural and synthetic media was determined by the mixed-crystal method of Yagoda(7). Before making the assays, the samples (10 ml) were repeatedly ashed with concentrated nitric acid, and lead and iron were removed by successive extraction with chloroform solutions of diphenylthiocarbazone and 8-hydroxyquinoline.

Experiments and results. A. Effect of barium on fibroblasts cultivated in a medium containing plasma and embryo extract. Barium was added to fibroblast cultures at final concentrations ranging from 0.01 to 1000 μg per ml. Below 1 μg per ml there was no appreciable effect, but a progressive inhibition was observed as the barium concentration was increased from 1 to 1000 μg per ml (Table I). Because the barium precipitated at 100 and 1000 μg per ml, higher levels were not tested.

TABLE I. Effect of Barium on Area of Outgrowth of Representative Cultures of Chick Fibroblasts Propagated in Carrel Flasks in a Medium Containing Fowl Plasma and Chick Embryo Extract.

Barium conc. (μg per ml)	No. of cultures	Avg inhibition, %*
.01	11	10†
.1	10	10†
1	10	20
10	11	22
100	7	35
1000	5	60

* Inhibition was calculated as percentage of the area of outgrowth of sister control cultures not exposed to barium.

† Inhibitions of 10%, or less, are not considered significant.

TABLE II. Effect of Barium on Length of Survival of Chick Embryo Mesenchyme Tissues Cultivated in Roller Tubes in Synthetic Mixture 199.

Barium conc. (μg per ml)	No. of cultures*	Avg survival, days
0†	12	37
.1	12	33
1	6‡	29
10	14	31
100	14	36
1000	13	27

* Data summarized from results of 3 typical experiments.

† Mixture 199.

‡ Several cultures in this group were lost through bacterial contamination.

B. Effect of barium on chick embryo mesenchyme tissues cultivated in a synthetic medium. In roller-tube cultures maintained in synthetic Mixture 199, barium was tested at levels ranging from 0.1 to 1000 μg per ml (Table II). In each of three separate experiments, no consistent effect upon the survival of the cultures was noted even at barium concentrations that inhibited, very appreciably, the outgrowth of fibroblasts cultivated in plasma and embryo extract. In general, however, cultures exposed to barium did not appear quite as healthy as the control cultures. As in the previous experiments, it was difficult to keep the barium in solution in Mixture 199, and the highest levels tested (100 and 1000 μg per ml) were very turbid.

C. Barium content of natural and synthetic media. Barium determinations were made on 8 samples of Mixture 199, on 2 samples each of hexose diphosphate and adenosinetriphos-

phate (metabolites that had been converted from barium to sodium salts) and on 5 samples each of fowl plasma and chick embryo extract. In all instances, the barium content was less than 5 μg per 10 ml, an amount too low to cause an appreciable effect upon the cultures. Although the assay method used is not extremely sensitive, the quantitative recoveries obtained when known amounts of barium were added to the samples showed that the results were reproducible.

Discussion. The results of the present experiments, based on more than 300 cultures, indicate that barium, in moderate amounts, is slightly toxic to tissues cultivated in flasks in naturally-occurring media but is relatively non-toxic to roller-tube cultures supplied with synthetic media. Verne and Sannié(8) and Hogue(9) have previously reported that barium is only slightly toxic to short-term hanging-drop cultures in natural media.

The observation that relatively high concentrations of barium do not shorten the period of survival of tissues cultivated in synthetic medium is somewhat surprising since these same concentrations reduce the area of outgrowth of fibroblasts in natural media. In a previous publication from this laboratory (4) it was shown that small amounts of cobalt are extremely toxic to tissues cultivated in natural media, but are non-toxic to tissues maintained in synthetic media. This lack of toxicity of cobalt was found to be due to the protective action of the L-histidine contained in the synthetic medium. No explanation for the lack of toxicity of barium under similar conditions has yet been obtained.

Many metabolites of nutritional interest are available commercially only as barium salts. The low solubility of these compounds makes it important to convert them to soluble forms before adding them to the culture media. In most instances, the corresponding sodium salts have suitable properties. The conversion is conveniently carried out by dissolving the barium compound in dilute mineral acid and adding saturated sodium sulfate until no further precipitate forms. The barium sulfate precipitate is centrifuged off and the pH of the supernatant adjusted to neutrality with dilute sodium hydroxide. Because barium sulfate has a low solubility product, the conversion is quantitative and there is no carry-over of barium. The effectiveness of this method is confirmed by chemical assays on the media.

Summary. Relatively high concentrations of barium (1 to 1000 μg per ml) reduce the area of outgrowth of chick fibroblasts propagated in a medium containing fowl plasma and chick embryo extract. But these same concentrations of barium do not shorten the period of survival of chick embryo mesenchyme tissues maintained in a synthetic medium. Chemical analyses showed the barium content of the natural and synthetic media that were used to be less than 5 μg per 10 ml.

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