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Meso-Inositol Requirement of HeLa and Human Conjunctival Cells.* (23205)

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A number of different human cells have been shown to require meso-inositol when grown *in vitro*(1). This is of considerable interest since it represents the first conclusive demonstration that meso-inositol is an essential nutrient for human cells. The report of Eagle *et al.*(1) was also of interest because no conclusive evidence was obtained that inositol was required by the cell strain derived from a human carcinoma, the HeLa cell.

In similar studies with the human conjunctival cells(2), regular success was obtained only after a greater concentration of inositol was used than the 1 μM suggested by Eagle *et al.*(1). In view of this, the requirement for inositol by the HeLa cell was also studied. These studies form the basis of the present report.

Methods. Human conjunctival cells(2) and HeLa cells(3) were used throughout these experiments. Stock cultures were propagated in 250 ml serum dilution, neutral glass bottles containing 10 ml of Eagle's basal medium(4) plus 10 to 20% human serum. Only stock bottles which showed the presence of a continuous sheet of polyhedral cells with clear cytoplasm and occasional mitoses were used. The actual experiments were conducted using sets of 4 roller tubes, 18 x 150 mm, containing a total volume of 1 ml of Eagle's medium plus 20% dialyzed human serum. Following inoculation, the tubes were slanted at a 2-5° inclination at 36°C for 16 to 20 hours and then were placed in a roller drum also at 36°C. The medium was replaced in all tubes on the third and fifth days. The cells were examined daily under 100x magnification. On the seventh day, the cells were trypsinized and counted with a Levy counting chamber (5). Dialysis of serum was accomplished as follows: 15 ml of serum was dialyzed in cellulose dialysis tubing† against 15 ml of Eagle's basal medium(4) for 24 hours; to maintain the pH at 7.2 to 7.4, the serum was dialyzed in a stoppered Erlenmeyer flask of 125 ml capacity. The dialysate was frozen at −80°C until used. The serum was dialyzed further against 4 changes of 0.85% NaCl, of about 1 liter each, for a total of 48 hours, and then against approximately 100 ml of Eagle's basal medium for one to 2 hours longer. Each dialysis procedure was carried out at 0 to 2°C with constant agitation. The volume of the dialyzed serum was routinely measured and usually showed an increase of 0.5 to 1.0 ml. The dialysate was routinely checked for serum protein contamination by the addition of

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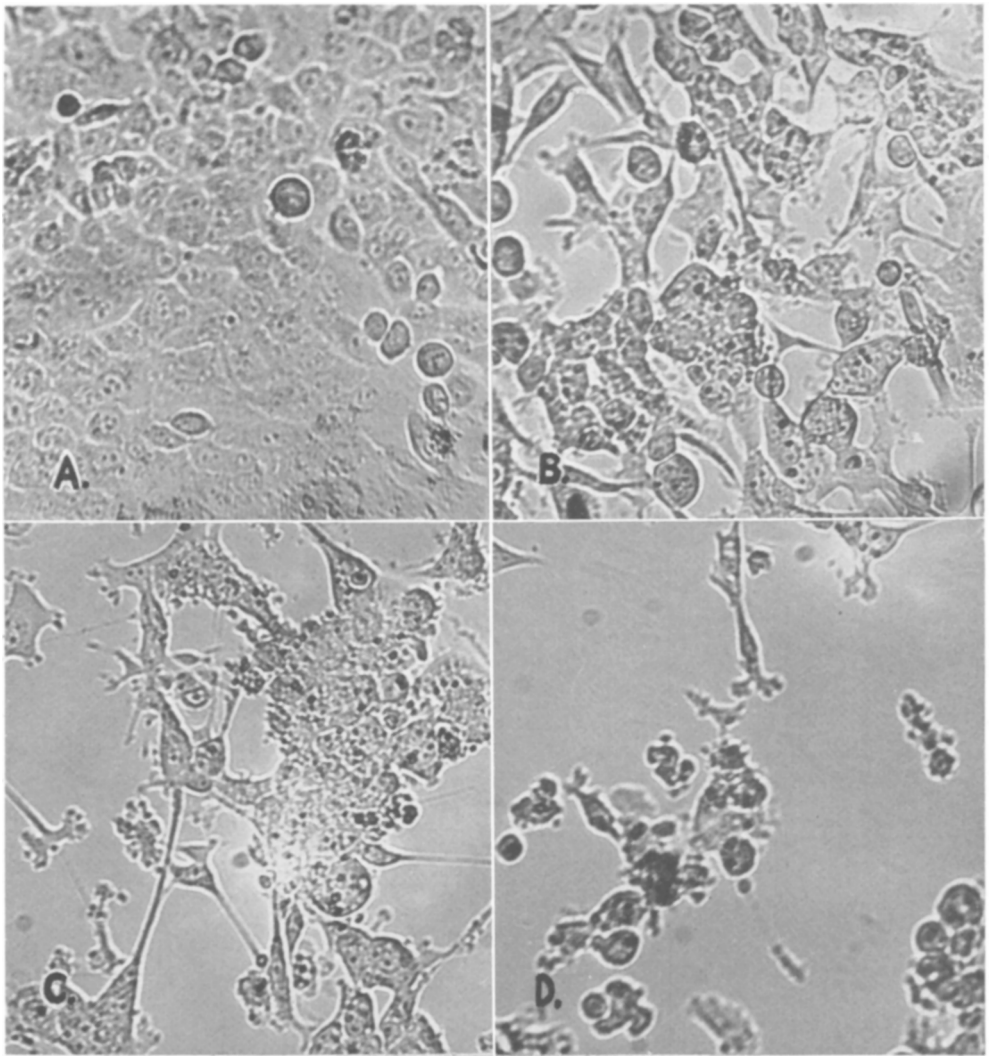

FIG. 1. Appearance of HeLa cells in medium without inositol. A. 3rd day. B. 5th day. C. 6th day. D. 7th day. (Magnification 100X.)

0.1 ml of 10% trichloracetic acid to 1 ml of the dialysate; the formation of turbidity was taken as indicative of the presence of proteins in the dialysate, and the dialysate was discarded. The dialyzed serum and dialysate were used at a final concentration equivalent to 20% whole serum. Meso-inositol‡ was used in the experimental tubes at concentrations from 0.27 μM to 16 μM. Pertinent data and results are given in the appropriate table.

‡ Corn Products Refining Co.

Results. In dialyzed serum diluted with Eagle basal medium, both the conjunctival and the HeLa cells showed characteristic progressive degenerative changes beginning on the fourth to sixth day. Such changes are illustrated in Fig. 1. Meso-inositol at a concentration of 1 μM did not prevent degenerative changes with 5 of the 6 sera tested in 5 separate experiments. Representative results are shown in Table I. By increasing the inositol concentration to 4 μM, degenerative changes were completely prevented with all sera tested. Further increase to 16 μM did

TABLE I. Effect of Meso-Inositol on Multiplication of Human Conjunctival Cells *In Vitro*.*

Exp.†	Donor	Supplement‡	Avg No. of cells × 10^3/tube	
1	Human A	None	0	(3+)‖
		Dialysate	106 ± 8	(0)
		Inositol§	19 ± 6	(2+)
2	Human B	None	0	(3+)
		Dialysate	214 ± 22	(0)
		Inositol	105 ± 25	(+)
	Horse L	None	5 ± 3	(3+)
		Dialysate	150 ± 24	(0)
		Inositol	65 ± 17	(+)
3	Human C	None	0	(3+)
		Dialysate	128 ± 19	(0)
		Inositol	182 ± 14	(0)

* These experiments show extremes when using different sera. A total of 5 experiments have been done using 6 different sera.

† Inoculi: 6,800, 10,000 and 12,000 cells/tube for Exp. 1, 2 and 3 respectively.

‡ Basal medium contained dialyzed serum at final conc. of 20% in Eagle's medium.

§ Meso-inositol used at final conc. of 1 μM.

‖ Morphological appearance of cells on 7th day: (0) = No degeneration, (+) = moderate, (2+) = advanced, (3+) = complete degeneration.

not significantly alter the multiplication rate. These results are presented in Table II. Thus the report of Eagle *et al.*(1) that meso-inositol is an essential nutrient for the conjunctival cells has been confirmed.

The HeLa cells, like the conjunctival cells, required inositol for rapid multiplication (Table III, Fig. 1). It is interesting that 1 μM inositol did not prevent degeneration of the HeLa cell in dialyzed human serum A and horse serum L diluted with Eagle basal medium as shown by results of Experiment 1 in Table III. However, when the experiment was repeated, satisfactory multiplication without grossly visible degeneration did occur (Exp. 3 in Table III). Such quantitative variations occurring in similar experiments have been frequently observed(6). This is probably caused by a difference in the physiological state of the initial cell inocula. In this instance, the amount of inositol carried along with the inoculum may be responsible. At any rate, in the present studies the HeLa cells invariably showed marked degeneration after seven days in media without inositol.

The reasons for the differences between the results of Eagle(1) and those reported here are not clear. The sera used in these studies were routinely dialyzed for 2 to 4 days to insure adequate removal of dialyzable factors. There are, however, 2 extraneous sources of inositol which may be of importance in obtaining non-consistent results either between experiments or between various sera. One of these is inositol carried along in the cell inoculum, since the stock medium contains adequate inositol. The other is inositol which was not completely removed by the dialysis procedure. Such inositol may be either in the form of phosphoric esters or inositides. The latter would be of especial importance for several reasons. First, phosphatides being colloids in aqueous solutions dialyze with difficulty(7). Second, phosphatides occur bound to serum protein(8). Third, McKibbin and Brewer(9) have reported that human plasma contains 74 μmoles of lipid inositol per liter. This concentration is sufficiently high so that sufficient inositol could be retained during dialysis due to the first and second factors. In this regard it is of interest that dialysis does not cause degradation of serum lipopro-

TABLE II. Effect of Varying Concentration of Meso-Inositol on Multiplication of Conjunctival Cells.

		Avg No. of cells × 10^3/tube at following concentrations of inositol					
Exp.*	Medium†	0	.25 μM	1 μM	4 μM	16 μM	Dialysate 20%
1	Human A	0 (3+)‡	2 (3+)	116 ± 40 (+)	294 ± 62 (0)	262 ± 31 (0)	238 ± 45 (0)
1	" Hu	0 (")	5 (")	27 ± 9 (+)	117 ± 50 (0)	130 ± 53 (0)	352 ± 46 (0)
2	" Ha	0 (")		220 ± 43 (0)	296 ± 49 (0)		132 ± 41 (0)

* Inoculi: 10,000 and 20,000 cells/tube in Exp. 1 and 2 respectively.

† Eagle's basal medium containing 20% dialyzed serum from the specified donor and meso-inositol or serum dialysate at the specified concentrations.

‡ Morphological appearance of cells on seventh day: (0) = No degeneration, (+) = moderate degeneration, (2+) = advanced degeneration, (3+) = complete degeneration.

TABLE III. Effect of Meso-Inositol on Multiplication of HeLa Cells.

Exp.*	Medium†	Avg No. of cells × 10^3/tube at following concentrations of inositol: 0	.25 μM	1 μM	4 μM	16 μM	Dialysate 20%
1	Human A	0 (3+)‡		11 ± 6 (2+)			103 ± 69 (0)
	Horse L	2 (")		60 ± 13 (+)			203 ± 40 (0)
2	Human W	0 (")					101 ± 17 (0)
3	Human A	22 ± 2 (3+)	50 ± 9 (2+)	272 ± 28 (0)	280 ± 20 (0)	284 ± 32 (0)	217 ± 43 (0)
	Horse L	35 ± 17 (3+)	131 ± 27 (+)	275 ± 36 (0)	292 ± 20 (0)	191 ± 13 (0)	281 ± 31 (0)

* Inoculi: 15,000, 13,000 and 27,700 cells/tube in Exp. 1, 2 and 3 respectively.
† & ‡ See footnotes to Table II.

teins(10).§ The extent to which complex forms of inositol are utilized by human cells is a matter for future study.

Summary. A concentration of meso-inositol of at least 4 μM was necessary for maximum multiplication of human conjunctival cells grown *in vitro*. Inositol was found to be essential for multiplication of HeLa cells.

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§ In our experiments analysis of human and horse sera before and after 48 hours of dialysis showed little alteration in cholesterol content.

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Lipid Mobilizer (LM) from Posterior Pituitary of Hogs.* (23206)

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Injection of plasma or plasma dialyzate, obtained from animals administered cortisone or subjected to stress, induced hyperlipemia in intact, adrenalectomized or hypophysectomized rats previously fasted and sensitized by exposure to potential hepatotoxins(1-4). The dialyzate and highly potent material crystallized from it differed from other hyperlipemia inducing agents in not requiring the presence of either the adrenals or pituitary for its action(2-4). These studies suggested that LM was released from the pituitary gland. The data in this paper establish the presence of a

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