Summary. The *o*- and *p*-fluoro- analogues of phenylserine have been synthesized and tested for their inhibitory effects on bacterial growth. Only *p*-fluorophenylserine inhibits the growth of *Escherichia coli*, and its action is reversed by phenylalanine, tryptophan, and to a limited extent, tyrosine. These results seem to emphasize the existence of a phenylalanine-tryptophan metabolic interconversion in bacteria.

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Transportation of Human Cells Cultured *in vitro*.* (22391)

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The increased use of tissue cultures for studies in biology and medicine has made essential the transportation of living animal cells. Although cell cultures have occasionally been transported between laboratories, *e.g.*, mouse, strain L cells(1), detailed information and optimal conditions for shipment of cells are not on record. The extensive application of a strain of human epithelial cells, strain HeLa (Gey), to studies of poliomyelitis virus(2,3) made desirable the preparation of HeLa cell cultures in a central laboratory for distribution to virus laboratories. Preliminary studies showed that strain HeLa cells withstand shipment by ordinary train or plane(2). Since this information was inadequate for mass distribution of cultures from the central laboratory established at Tuskegee Institute, Ala., the following experiment was performed. The results of this experiment pertain to one type of cell; data describing shipment of another cell-type, *i.e.*, monkey kidney cells, has recently been reported(4). It is hoped that methods for transportation of other cells will soon be recorded.

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Materials and methods. Cultures of strain HeLa cells on glass were prepared 2-5 days before shipment, by transfer of cells in a fresh mixture of adult human serum, 40% and Hanks's balanced salt solution, 60%, (HuS-40, H-60), from stock bottle cultures to screw capped 16 x 125 mm test tubes. Each tube contained 70,000 to 100,000 cells at the beginning of shipment; 1341 tube cultures were sent.

Results. The experiment was designed on the premise that the following 6 factors relate fundamentally to successful transportation of animal cells and thus the experimental results are discussed in relation to each factor.

1) The *nutritional status* of cells at the beginning of shipment was evaluated by comparing survival of cells a) placed in fresh HuS-40, H-60 one day before shipment, and b) kept for 2-5 days in the HuS-40, H-60 medium used for preparation of the cultures. The experimental results (Table I) show that placement of cells in fresh medium for 1 day prior to shipment increased significantly the percentage of surviving cultures.

2) The *availability of nutrients* in relation to nutritional needs of cells during shipment and to a limited extent, 3) *mechanical trauma* to cells was studied by sending cells with and

TABLE I. Transportation of Strain HeLa Cells: Effects of Nutritional Status of Cells, Medium during Shipment, and Duration of Shipment.

Days between change of medium and shipment	Medium* during shipment	Cultures satisfactory† after shipment: Results by days in transit 1 Ratio‡	%	2 Ratio	%	3 Ratio	%	4 Ratio	%
1	Yes	124/142	87	65/69	94	88/122	72	62/122	51
1	No	69/ 69	100			67/112	60	50/120	42
2–5	Yes	8/ 69	12			0/175	0	0/ 46	0
2–5	No	50/ 71	70			96/178	54	1/ 46	2

* Medium—adult human serum, 40%, and Hanks' balanced salt solution, 60%.

† Satisfactory signifies a tube culture with at least 50,000 cells of normal morphology, 24-48 hr after arrival and incubation at 36°C. HuS-40, H-60, 0.5 ml, was added on arrival to cultures sent without medium.

‡ Numerator = No. of satisfactory cultures; denominator = No. of cultures shipped.

without liquid medium in the tubes. Supernatant liquid was neither essential nor detrimental to survival of well-nourished cells, *i.e.*, those fed 1 day before shipment (Table I). Contrariwise, survival rates for less well-nourished cells, *i.e.*, those fed 2-5 days before shipment, were lower in cultures with liquid medium because the cells were often detached from the glass evidently by movement of the fluid.

4) The *duration of shipment* was varied from 1-4 days by employing for shipment between Tuskegee Institute, Ala. and Minneapolis, Minn., air parcel post, special delivery for one-day delivery, air parcel post for 2-day delivery and regular parcel post for 3- and 4-day delivery. Strain HeLa cells survived periods of one or 2 days in transit better than 3 or 4 days (Table I).

5) *Temperature* as a factor in transportation was difficult to study in detail. Despite efforts to keep the temperature between 15° and 30°C by employing insulated cardboard boxes, temperatures recorded by maximum-minimum temperature thermometers for several shipments during the period February and March 1954 ranged from 5-20°C. During the summer, cells died in several boxes where the maximum temperature was 43°C; during cold weather cells were destroyed by freezing.

6) The *method for revival of cells* following transportation is obviously important and must furnish cells with adequate nutrients at a proper temperature. A standard procedure was employed in this experiment to revive cells after shipment, *i.e.*, each culture was incubated at 36°C for 24-48 hours and HuS-40, H-60, 0.5 ml, was added to cultures sent without medium. This procedure brought the cells within 24-48 hours into a healthy state (*i.e.*, thin delicate membranes, clear agranular cytoplasm, polygonal shape and evidence of acid production by change in color of phenol red).

Discussion. The results of this experiment revealed that survival of strain HeLa cells shipped by train or plane was enhanced: a) by placement of cells in fresh medium 1 day before shipment, b) by completion of travel within 2 days, and c) by protection of cells from excessive heat and from freezing. Moreover, the lack of a detrimental effect of supernatant liquid upon cells well-nourished at the beginning of shipment made feasible the shipment of medium in the cultures to avoid the laborious addition of medium to each tube after shipment.

With this information, the laboratory at Tuskegee Institute proceeded with the shipment of large quantities of HeLa cell cultures. Tube cultures each containing 70 to 100 thousand cells at the beginning of shipment and bottle cultures with 5 to 10 million cells, received fresh medium (HuS-40, H-60) 1 day before shipment. The medium was left in the tubes during transit but removed from the bottle cultures since fluid tended to detach from glass the sheets of closely packed HeLa cells in bottle cultures and because cells shipped as well without as with medium (Table I). Shipments were completed within 3 days by air parcel post, air express or regular parcel post. During warm weather, *i.e.*,

April to September, one or 2 cans of sodium sulfate decahydrate ("Equitherm")(5) were enclosed in each package to help keep the temperature near the cultures below 38°C.

During the period April 1 through Sept. 30, 1954, approximately 133,000 tube cultures and 1,800 bottle cultures were sent from Tuskegee Institute to laboratories in 23 cities located in all sections of the United States. Although it was not possible to determine precisely the outcome of each tube and bottle, reports from recipient laboratories indicated that more than 90% of the cultures were received in satisfactory condition. By application of the principles established in this experiment, large numbers of strain HeLa cells in suspension were shipped and successfully used for detection of neutralizing antibodies for poliomyelitis virus(6). Since the percentage of successful shipments may be influenced by factors such as seasonal temperature, only further experience in shipment will reveal the extent to which this percentage fluctuates.

The conclusions derived from these studies that may be applicable to transportation of other animal cells follow: a) Cell survival is related inversely to duration of travel. b) Cell nutritional status at the beginning of shipment is a critical factor; placement of strain HeLa cells in fresh medium for a day before shipment significantly increased survival. c) The temperature of cells during shipment should be controlled to prevent death of cells from excessive heat or cold and to keep metabolic requirements of cells at a level met by the supply of nutrients. Evidence that cells can be preserved at subzero temperatures(7) may make it feasible to keep cells during transit at the temperature of storage. d) The nutritional requirements for maintenance of cell viability must be met during transit. For example, the nutrients needed by strain HeLa cells, well-nourished at the beginning of 1-2 day shipments, at temperatures that assure low metabolic rates were supplied by the few residual drops of medium left after removal of the supernatant fluid. For longer periods in transit or for other types of cells, a larger supply of nutrients may be essential for survival. However, as cell damage increases with duration of shipment, mechanical trauma to cells from movements of supernatant liquid may result in detachment and death of cells. Indeed, for strain HeLa cells in transit for 3 or more days, limitation of the supernatant liquid to several drops seems advisable. Alternatively, cells could be embedded in plasma or in gel foam(8) to prevent detachment from glass and to keep nutrients near cells.

Summary. Living human epithelial cells, strain HeLa (Gey), readily survive transportation over periods of 1-3 days. Six factors in survival, *i.e.,* 1) nutritional status of cells at the beginning of shipment, 2) availability of nutrients in relation to nutritional needs of cells during shipment, 3) mechanical trauma to cells, 4) duration of shipment, 5) temperature near cells and 6) the method for revival of cells following shipment, were studied to define satisfactory conditions for routine shipment of strain HeLa cells.

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