vitro since four tissues worthy of further investigation have been ascertained, and the active enzymes have been obtained in soluble form from two of them.

Summary. Liver mitochondria and spleen were found to contain dehydrogenases which attacked lecithin and sphingomyelin and required ATP. Muscle and intestinal mucosa had dehydrogenase activity with lecithin or sphingomyelin if DPN was present whereas under the same conditions homogenates of spleen were active only with sphingomyelin. The dehydrogenase systems were obtained in soluble form from acetone powders of liver mitochondria or intestinal mucosa.

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Phenylserine Studies. IV. Biochemical Activity of *o*- and *p*-Fluorophenylserine. (22390)

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Phenylserine was shown(1) to be an inhibitor of the growth of various species of bacteria. The inhibition was associated with certain enzymatic sequences in phenylalanine and tryptophan metabolism, since either of these two amino acids was able to reverse the inhibitory effect. Subsequently, the discovery of chloromycetin, which may be considered to be a phenylserine derivative, has stimulated increased interest in the bacteriostatic action of this type of compound(2). Fluoro-derivatives of various metabolites have frequently proven of interest in the study of metabolic sequences, presumably because the small atomic dimension of the fluorine atom does not prevent the reaction of the analogue with an enzyme, but does prevent completion of the enzymatic sequence. The fluoro-analogues of phenylalanine have previously been prepared and tested as to their bacteriostatic properties(3,4). It therefore seemed of interest to synthesize and test the properties of ring-substituted fluoro-derivatives of phenylserine. The present paper describes the microbiological effects of the *o*-fluoro- and *p*-fluoro- analogues of phenylserine.

Materials and methods. Fluorophenylserines. Into an evaporating dish are introduced 19.8 ml of a 2.5 M glycine solution, 9.9 ml of 95% ethyl alcohol, 9.0 ml of *o*- or *p*- fluorobenzaldehyde,* and 19.8 ml of 6.25 M sodium hydroxide. The mixture is stirred until it becomes clear, permitted to stand for one hour or until a waxy white precipitate forms, and the precipitate dried between sheets of filter paper. The precipitate is washed twice with 15 ml portions of ethanol and then twice with 10 ml portions of water. To the residue is added 500 ml of water with stirring, acetic acid is added to pH 6.0, and any residue is filtered off and discarded. The filtrate is evaporated to dryness, taken up in hot water, and recrystallized from the water

* Obtainable from Custom Chemical Laboratories, Chicago, Ill.

in the ice box. The crystals are dried between sheets of filter paper, and then in a vacuum desiccator over calcium chloride. Paper chromatography demonstrates in each case that a new amino acid has been formed almost quantitatively from the reactants. The *m*-fluoro- analogue is not readily prepared by this procedure.

o-fluorophenylserine, mpt. 178°C.

$C_9H_{10}O_3NF$ (199).

Calculated, N 7.04; found, N 7.67
Calculated, C 54.27; found, C 55.99
Calculated, H 5.02; found, H 5.66

p-fluorophenylserine, mpt. 193°C.

$C_9H_{10}O_3NF$ (199).

Calculated, N 7.04; found, N 7.18
Calculated, C 54.27; found, C 55.06
Calculated, H 5.02; found, H 5.28

Microbiological Assay. Neither the *o*- nor *p*-fluorophenylserine produces a visible precipitate with any of the recognized trace elements (5). They produce no observable effect on *Aspergillus niger* or *Serratia marcescens*. Against *Escherichia coli* and using previously described methods(1), only *p*-fluorophenylserine was inhibitory. The nature of this inhibition is described in detail in the following paragraphs.

Results. Complete inhibition of the growth of *Escherichia coli* with *p*-fluorophenylserine was achieved at levels of approximately 30 μg per ml, with 50% inhibition at 20 μg per ml. When an extensive series of metabolites was tested for reversal effects against *p*-fluorophenylserine, only tyrosine, phenylalanine, and tryptophan showed measurable effects. The effects of varying levels of these amino acids in reversing the *p*-fluorophenylserine inhibition are shown in Table I. A yeast extract solution reversed the inhibitor at levels equivalent to a phenylalanine content of five percent. Paper chromatography of the extract failed to show any fraction with activity other than the amino acids previously mentioned.

Discussion. The principles governing the elucidation of metabolic sequences on the basis of data obtained from inhibition studies have previously been described(6). Experience with phenylserine(1) and *p*-fluorophenylalanine(4) have indicated that the inhibitory action of both of these compounds is reversed by phenylalanine and tryptophan, and in some cases by tyrosine. Bergmann *et al.*(4) have analysed these data, and extended their significance by the study of similar relationships in phenylalanineless, tyrosineless and tryptophanless mutants of *Escherichia coli*. From these studies it was concluded that tryptophan acts as a phenylalanine precursor, and that phenylalanine acts as a tyrosine precursor, but that an alternate synthetic pathway exists between tryptophan and tyrosine.

TABLE I. Effect of Phenylalanine, Tyrosine, and Tryptophan in Reversing the *p*-Fluorophenylserine Inhibition of *Escherichia coli*.

Reversing agent (μg/ml)	*p*-Fluorophenylserine (μg/ml) 0	30	100	300	1000
	% maximum growth				
Control	100	0	0	0	0
L-Phenylalanine					
.003	100	60	0	0	0
.010	100	100	25	5	0
.030	100	100	100	55	0
.100	100	100	100	100	55
.300	100	100	100	90	50
L-Tryptophan					
10	100	20	0	0	0
30	100	70	15	0	0
100	100	85	70	15	0
300	100	100	100	65	60
1000	100	100	100	100	100
L-Tyrosine					
10	20	20	20	15	
30	20	20	15	15	
100	40	45	50	45	
300	40	35	30	10	

In the present study phenylalanine has been found to be approximately 10,000 times as effective as tryptophan in reversing the toxicity of *p*-fluorophenylserine. Tyrosine is roughly as effective as tryptophan, but since tyrosine is itself toxic to this strain of *Escherichia coli*(7), synergistic inhibitory effects appear and it is impossible to obtain consistent data. The data thus seem to support the straight line precursor relationship previously proposed between tryptophan, phenylalanine and tyrosine. The fact that large amounts of phenylalanine also tend to reverse the inhibition of microorganisms by 5-methyltryptophan suggests that the relationship between tryptophan and phenylalanine is a reversible one.

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