bacterial enzymes must, however, also be considered.

Study of a few specimens of lecithin purified from the gallbladder bile of subjects with gallstone disease encourages a wider study of fatty acid composition of the lecithins of bile collected from subjects with various liver affections. It will be of interest to compare fatty acid composition of the various lipids of the liver with corresponding fatty acids in the bile. This is now under investigation.

Summary. 1) Total fatty acids of human gallbladder bile and hepatic bile were analyzed qualitatively and quantitatively by gas-liquid chromatography. The 3 major fatty acids in human bile are palmitic, oleic and linoleic acid, representing around 80% of total fatty acids. 2) Analysis of composition of total fatty acids in uncomplicated gallstone disease failed to reveal any abnormality in this disease. 3) In one subject with occluding gallbladder stone and beginning absorption of bile pigments, and one subject with white bile, a reduction of the proportion of polyunsaturated fatty acids and an excess in saturated fatty acids were observed. 4) The significance of differences in relative concentrations of fatty acids of lecithins in subjects with gallstone disease is discussed.

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Metabolism of Arginine by Mouse Fibroblast, Strain L.* (25781)

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It has recently been reported(1) that periodic additions of L-arginine to cultures of mouse fibroblast cells (Earle's strain L) growing in suspension obviated the need for a medium change for periods up to 10 days or more. During this time growth rate and viability were maintained at a high level. This report presents results which may explain the need for periodic addition of L-arginine for sustained growth of the L cells.

Methods. Spinner cultures of L strain cells were set up as previously described(1), using Eagle's basal medium. The bidaily additions of L-arginine-HCl, L-citrulline and L-aspartic acid were such that final concentration of the supplement was 0.2 micromole. Initial cell concentrations were approximately 2×10^5 cells per ml. Samples were removed daily for estimations of cellular proliferation and viability and of ammonia content of the growth medium. Free ammonia in the medium was determined as follows: The cells were removed by a short centrifugation; 1.0 ml of the supernatant fluid was placed in a 16 x 150 mm test tube fitted with an air inlet; 2.0 ml of a saturated solution of potassium carbonate and 1 drop of octyl alcohol were added; the ammonia was driven off by vigorous aeration for 30 minutes into 4 ml of 1 N sulfuric acid(2). Amount of ammonia trapped in the acid was determined by nesslerization(3). Chromatographic analysis of the reaction products was carried out with a newly available sulfonic acid type cation exchange resin paper,† which, according to Tuckerman

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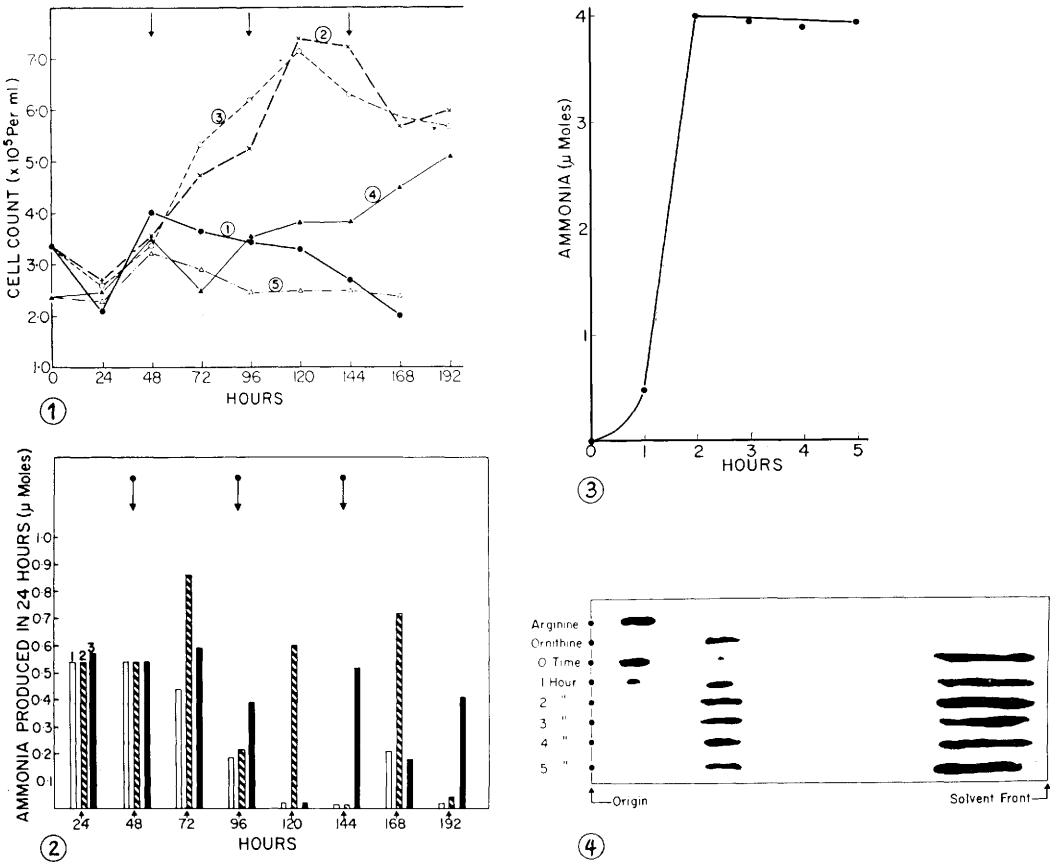

FIG. 1. Growth of L cells in spinner culture. Curve 1, control; curve 2, arginine supplemented; curve 3, citrulline + aspartate supplemented; curve 4, citrulline supplemented; curve 5, aspartate supplemented. Vertical arrows indicate time of a supplementation.

FIG. 2. Ammonia production during growth of L cells. For significance of 1, 2, and 3, see Fig. 1. Vertical arrows indicate time of supplementation.

FIG. 3. Ammonia production by resting L cells. Amount produced by control incubation without arginine has been subtracted.

FIG. 4. Chromatogram of products produced by L cells metabolizing arginine. For methods used, see text. All monoamino acids travel as a single wide band at solvent front.

(4), allows separation of the basic amino acids by descending chromatography at room temperature with 0.2 M acetate buffer in 90 minutes. These conditions were found adequate for separating arginine and ornithine.

Results. In Fig. 1 are shown the effects of periodic addition of L-arginine and of simultaneous addition of L-citrulline and L-aspartic acid. Addition of either arginine or a mixture of citrulline and aspartic acid permitted sustained growth of the cultures. Addition of either citrulline or aspartic acid alone at this concentration was found to be not nearly as effective as the combination of the two. At twice the concentration used in these experiments, citrulline alone will sustain growth of L cells almost as well as arginine.

Amounts of ammonia formed each day are shown in Fig. 2. A large ammonia evolution occurs during the first 24 hours after an arginine addition. During the next 24-hour period, quantity of ammonia formed is the same as that in the control cultures. Amount of arginine added at each supplementation was 0.2 μM per ml and amount of additional ammonia evolved during the 24-hour period following a supplementation was 0.4-0.6 μM per ml. After supplementation with citrulline and aspartic acid, again 0.4-0.6 μM per ml

of additional ammonia was liberated, but over a period of 48 hours instead of 24 hours. Urea determinations were made on the suspension culture fluids. No significant increase or decrease in urea was observed at any time.

It appeared from these observations that arginine was being catabolized during growth of L cells in the spinner culture. To determine the products of the reaction, actively multiplying L-cells were harvested from a suspended culture and placed in fresh growth medium at a concentration of 5 x 10^7 cells per ml. The culture was divided into 2 portions of 10 ml each. To one (the control) was added 0.1 ml saline, to the other 0.1 ml of a 0.2 M solution of arginine. The 2 flasks were incubated at 37° in a rotary shaker. Samples were removed every hour, placed in a boiling water bath for 10 minutes, cooled, centrifuged for 15 minutes at 25,000 x g, decanted and frozen until analyzed. Amount of ammonia formed in the flask containing arginine, in excess of that produced in the control, is shown in Fig. 3. The maximal ammonia production of 3.98 μM per ml was observed in 2 hours. Since the initial arginine concentration was 2 μM per ml, it appears that each μM of arginine was catabolized to 2 μM of ammonia. Fig. 4 is a diagrammatic representation of the paper chromatogram obtained by spotting 50 μl of the samples on the ion exchange resin paper. At zero time, only the arginine spot can be seen. At one hour, the arginine spot is still present, although reduced in size, and the ornithine spot has appeared. At 2 hours, arginine has completely disappeared and only the ornithine spot can be seen. Thus complete disappearance of arginine coincides with appearance of ornithine and 2 equivalents of ammonia.

Discussion. The growth of cells in suspended culture provides a convenient means of obtaining large numbers of cells for metabolic studies. One drawback to the procedure has been the need for periodic media changes. Thomas *et al.*(1) have reported that periodic addition of L-arginine eliminates the need for a medium change for 6-10 days. This study provides a possible explanation for the need for periodic arginine supplementation of the media. The evidence seems to indicate that L cells contain enzymes which carry out the following reactions:

(1) arginine → ornithine + 2 NH_3

(2) citrulline + aspartate → arginine

Reaction (1) seems to take place at a more rapid rate than reaction (2). Since exogenous arginine must be supplied for continued growth to occur, conversion of ornithine to citrulline must be blocked in these cells. Further studies will be required to determine which step in this conversion is not taking place.

Summary. 1. For sustained growth of mouse fibroblast cells (strain L) in suspended culture in Eagle's medium, periodic addition of arginine is essential. This requirement can be provided by arginine alone or by equimolar amounts of citrulline and aspartic acid. 2. These cells contain enzymes which catalyze the breakdown of arginine to ornithine and 2 equivalents of ammonia. 3. The requirement for periodic addition of arginine or its precursors is explained by continuous rapid breakdown of arginine observed during growth.

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