TABLE II. Effect of Acid Mucopolysaccharides and Other Test Substances on HeLa Cell Growth as Measured by Total Dry Weight, Cell Count, Cell Volume. All test groups were treated with human lipemic serum.

Test substance	No. determinations	Total dry wt* Mean	Total dry wt* Standard error	Total dry wt* P value†	Cell No.* Mean	Cell No.* Standard error	Cell No.* P value†	Relative cell vol, cytocrit in %/ cell No. in %
Normal control	14	90.8	3.3	<.05	86.7	.2	<.001	1.08
Extract #1	7	112.1	4.5	<.05	115.3	4.5	<.01	.94
" #4	5	110.9	3.3	<.05	123.1	4.1	<.001	.90
Chondroitin sulfate A	6	112.3	2.4	<.001	109.2	2.8	<.05	1.11
Chondroitin sulfate C	5	103.2	1.9	>.05	112.6	2.7	<.05	1.05
Phospholipid	4	113.4	1.1	<.001	128.3	5.7	<.05	.86

* Numbers express a comparison of the test group with the corresponding HLS control group. 16 experiments of HLS in total; mean of each group of HLS controls taken as 100% or as a relative cell volume of 1.00.

† P value expresses comparison of the difference of mean values of test groups and corresponding HLS controls.

It appears that certain AMPS have appreciable growth stimulation in the HeLa cell systems. A comparable effect has previously been demonstrated in chicken aorta cells treated with extracts of calf aorta(1). Although decreases in relative amounts of lipid may not always accompany cell growth, the stimulating effect of AMPS may play a role in the lipid metabolic cycle. The degrees of stimulation of cellular activity which the various test substances demonstrate may influence different stages of intracellular metabolic activity and this is being investigated at present.

Summary. The growth stimulating effect of acid mucopolysaccharides on lipid saturated HeLa cell cultures has been examined by measurement of cytocrits, total dry weights, and cell counts; results demonstrate that certain AMPS have a significant growth stimulating effect.

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Dynamics of Amino Acid Transport in the Intact Intestine.*† (29966)

FRANCIS A. JACOBS AND ARLENE H. LANG

Guy and Bertha Ireland Research Laboratory, Department of Biochemistry, University of North Dakota, School of Medicine, Grand Forks

This laboratory has reported a 2-way exchange of L-tyrosine across the intestinal mucosa of the intact rat(1). We have also

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† Abbreviations and special terms used in this paper are as follows: Glycine (Gly), Alanine (Ala), Serine (Ser), Proline (Pro), Valine (Val), Threonine (Thr), Isoleucine (Ileu), Leucine (Leu), Methionine (Met), Phenylalanine (Phe), Tyrosine (Tyr), Aspartic Acid, (Asp), Glutamic Acid (Glu), Lysine (Lys), Histidine (His), Arginine (Arg). The term *efflux* refers to an outward movement of the amino acid into the lumen and perfusate; *perfusate* being the solution circulated through the cannulated lumen of an intestinal segment; *preperfusate*, the above solution before perfusion.

reported an influence on the secretion (efflux) of several other amino acids into saline perfusates with and without amino acids having been present in the initially perfused solution(2,3).

The problem was to demonstrate that amino acids could be absorbed actively against a concentration gradient in the intact animal. Refinements in methodology have made possible the study of absorption as well as the efflux of amino acids across the intestinal mucosa. This report is intended to demonstrate that the animal can absorb amino acids against a concentration gradient established with the plasma amino acids with a simultaneous efflux, thus presenting a dynamic system.

Methods. Animals were perfused with solutions of amino acids carried in either sodium chloride or Krebs-Ringer bicarbonate buffer. *In situ* perfusion was carried out employing an apparatus which was designed so that both efflux and absorption could be measured(4). The material studied was recirculated through the system, kept warm (37-38°C), mixed in a reservoir, pumped through a filter-flow meter system at a rate of 1.5 ml per minute, and perfused through the lumen of a 10 cm segment of the upper small intestine of an anesthetized (50 mg sodium pentobarbital per kg, intraperitoneally) adult male Sprague-Dawley strain rat. The system was designed so that simultaneous monitoring of the perfusate for radioactivity could be accomplished by passing through a monitor shunt and a detector system‡ described by Rapkin and Gibbs(5).

Chemical analyses were made by automatic column chromatography§(6) with simultaneous scintillation counting‡ of the effluent for levels of radioactivity of C^{14}-labeled amino acids in a manner similar to that employed by Elwyn(7).

Absorption studies were carried out on rats which had been fasted 18-20 hours. Since the animals were perfused with amino acid mixtures which were equal in concentration to those found in the plasma (or multiples thereof), it was necessary to establish these blood levels in our rats.

All amino acids used were of the L-configuration and chromatographically pure.

Results. Blood levels. The data for plasma levels of free amino acids of rats sacrificed in the fed state, and others fasted for periods extending to 172 hours are presented in Fig. 1. The data for the 18.5-hour fast were the reference base for the concentration levels of amino acid mixtures perfused.

Effect of salts. In the selection of a suitable solvent for these substances both normal saline and Krebs-Ringer bicarbonate buffer were employed. Rats perfused with either of these solutions presented characteristic patterns of amino acid efflux into the intestinal lumen. The findings are presented in Fig. 2. A solution of isotonic KCl was also examined and found to be slower in equilibrating than either of the other two systems. From these data it was apparent that there was an ionic influence upon the efflux pattern in the system examined, the buffer inducing a greater efflux.

Effect of alanine upon efflux. When one or several amino acids were perfused in either saline or buffer, the efflux was stimulated over the salt or buffer alone. Examples are shown (Fig. 3) for 1.0 mM L-alanine dissolved in saline and in buffer. This preparation was perfused for 60 minutes with an increase in efflux for all amino acids measured in the saline/alanine solution, and for all but the dicarboxylic acids for the buffer/alanine preparation. It can be interpreted that even a simple molecule such as alanine can disrupt the balance of the amino acid pool so as to result in such an efflux pattern. For this reason our studies on absorption were continued using mixtures of 17 amino acids‖ (5 of which were labeled with C^{14}) at the same concentrations or ratios as found in the free form in the plasma.

Perfusion of multiple mixtures. A 17-amino acid mixture at plasma concentration was perfused for 60 minutes. Fig. 4 presents

‡ Flow detector, Model 317, manufactured by Packard Instrument Co., LaGrange, Ill.

§ Qualitative and quantitative analyses were made using the Amino Acid Analyzer, Model 120, manufactured by Beckman/Spinco, Palo Alto, Calif.

‖ Tryptophan levels were in accord with blood levels reported by Ryan and Carver(9).

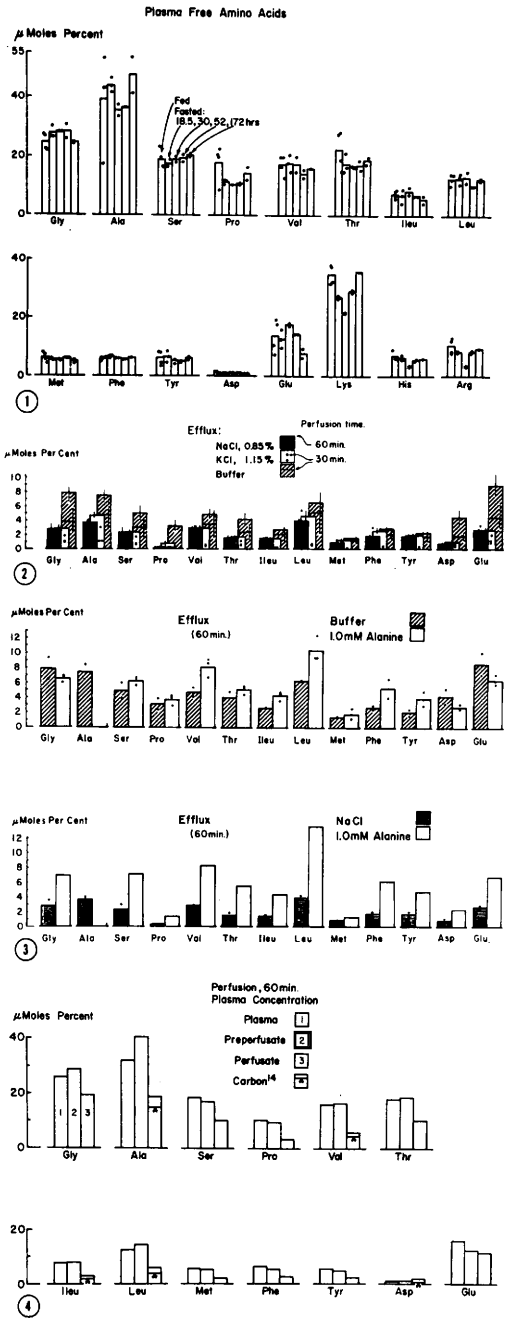


FIG. 1. Plasma free amino acids. Amino acid levels determined on deproteinized(8) plasma prepared from heparinized heart blood of the rat. Samples marked with an asterisk (*) were not measured.

FIG. 2. Effect of salts on amino acid efflux. Vertical bars represent amino acid levels after 60 min of perfusion; points within the bars indicate values after 30 min of perfusion. Vertical lines within bars represent ± standard deviations from the means of each. Efflux of proline in presence of normal saline was negligible.

FIG. 3. Influence of a single amino acid upon efflux. Effect of 0.1 mM L-alanine upon amino acid efflux into Krebs bicarbonate buffer or normal saline is presented, the bars representing mean values obtained from these groups of rats. Upper bars show the efflux into buffer alone (diagonal shading) and into buffer-alanine perfusate (open bars). Lower graph shows the efflux into normal saline alone (horizontal shading) and into saline-alanine perfusate (open bars).

FIG. 4. Absorption of neutral and acidic amino acids against concentration gradient with the plasma. Alanine, valine, isoleucine, leucine and aspartic acid were measured for both net and absolute absorption; C^{14} measurements indicated by the symbols present in the third bar for these amino acids show a greater absolute than net absorption. These differences correspond to amounts of these amino acids in efflux into the intestinal lumen.

the data obtained. These findings demonstrated several things: 1. The amino acid concentrations after 60 minutes in this perfusate (bar 3) were lower than originally perfused (bar 2) or the level in the plasma (bar 1). This was found for 13 of the amino acids perfused except for aspartic acid. Basic amino acids were not measured.

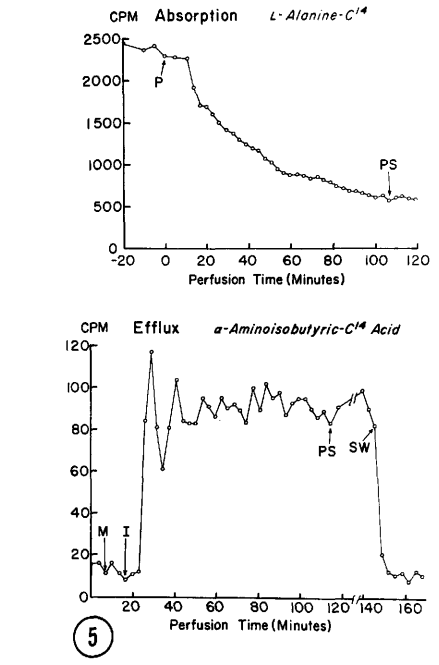
2. Those amino acids labeled with C^{14}, namely, alanine, valine, isoleucine, leucine, and aspartic acid all showed definite absolute absorption.

3. The difference between the chemical and tracer analyses accounted for the efflux of these compounds.

4. The amino acids in all instances, except for the dicarboxylic acids, were found to be absorbed against the concentration gradient of the plasma amino acids, even though efflux was present.

These experiments were extended to concentrations at twice the plasma level and at one-half the plasma level(4). The fact that there was both net and absolute absorption at plasma levels (and below) left little doubt that an "active process" was progressing. The system was dynamic, since the 2-way transport was apparent.

In vivo *monitoring of the perfusion.* A rat perfused with L-alanine-C^{14} (1.0 mM) in a mixture of neutral and acidic amino acids was monitored by scintillation counting as the experiment progressed. The findings are shown graphically in Fig. 5. Aside from the delay inherent in the perfusion system it can



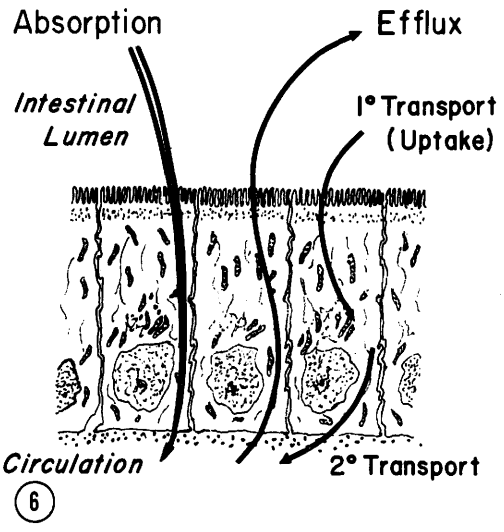


FIG. 5. Monitored absorption and efflux of amino acids across intestinal mucosa. *Upper curve* shows a progressive absorption of L-alanine-C^{14} from perfused intestinal lumen as measured by a steady decrease in radioactivity of the contents. *Lower curve* is a plot of radioactivity of intestinal contents after buffer had been perfused and the rat injected intravenously with α-aminoisobutyric-C^{14} acid. Point *P* indicates point at which perfusion was begun; at *PS*, perfusion of intestinal lumen was terminated. *M* indicates level of background count; at point *I* the animal was injected intravenously with the radioactive amino acid. At point *SW* the perfusion system was washed free of its contents and replaced with water, to present again the background count.

FIG. 6. Dynamics of amino acid transport in the intestine. Diagrammatic representation of absorptive and secretory transport of amino acids across intestinal mucosal cells.

be seen that absorption proceeded to levels below the concentration of the plasma alanine, (which would amount to an equivalent of 1000 cpm under these experimental conditions).

Efflux was likewise demonstrated to occur by perfusing an animal with buffer and then injecting *via* the vena cava 0.5 ml of α-aminoisobutyric acid solution (0.5 ml = 51.5 mg with an activity of 10 μcuries). There was a perfusion circuit delay and then an immediate appearance of the radioactive molecule in the luminal contents (Fig. 5), demonstrating a very rapid efflux of this model amino acid.

Discussion. Evidence has been presented showing that there is an efflux of amino acids into the intestinal lumen of the intact animal. This is influenced by ions present in saline, KCl or Krebs-Ringer bicarbonate buffer solutions which might well have been expected since amino acid transport has been related to the ionic environment of the cell (10,11,12). Efflux is stimulated by only a single amino acid such as alanine or phenylalanine(2,3) in the perfused solution, suggesting a disruption of balance in the amino acid pool brought about by an overload of even a single amino acid.

Amino acid mixtures in ratios and levels found in plasma are actively absorbed from the intestinal lumen against a concentration gradient established by the free amino acids present in the plasma. Net absorption was found for all amino acids tested except aspartic acid (and possibly glutamic acid). In the case of aspartic acid there was net efflux of the acid into the lumen during the experimental period; however, by tracer measurements absolute absorption against a concentration gradient was demonstrated. Concurrent intestinal efflux with absorption observations were made possible by measuring the changes in amino acid concentration in the perfused solution (perfusate) by both chemical and tracer means.

In the transport of amino acids across the normal intestinal mucosa the magnitude of the absorptive process exceeds the efflux with an ultimate gain by the host of the nutrient amino acids from the luminal contents of the intestine in a dynamic fashion (Fig. 6).

Summary. Net active absorption of amino acids, as mixtures at blood level, has been demonstrated. Both absorption and efflux occur simultaneously across the intact intestine. The transport of amino acids is an active process and is indeed dynamic.

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Effects of Dietary Iodinated Casein on Components of the Electron Transport System of Chicken Liver.* (29967)

D. N. MOURY,† F. L. CRANE AND J. C. ROGLER (Introduced by M. X. Zarrow)

Departments of Biological Sciences and Animal Sciences, Purdue University, West Lafayette, Ind.

Several recent studies of the effects of thyroxine on rat liver(1,2,3,4,5) and skeletal muscle(4) have presented evidence that the effects of thyroxine on electron transport are secondary to an effect on protein synthesis. Studies in our laboratory on rat liver(1) have shown a general thyroxine-induced increase of all components of the succinoxidase system. Since this should be a basic metabolic phenomenon, it was decided to investigate a second vertebrate class.

Methods and materials. Hubbard White Mountain chickens were placed on experiment at 4 weeks of age, treated for 4 weeks and sacrificed by severing the neck arteries. For treatment, the birds were divided into 8 groups of 5 males and 5 females, and while Groups 1-4 were kept at 70°F, Groups 5-8 were kept at 85°F. Groups 1 and 5 received the basal diet and in all other groups, iodinated casein was substituted for glucose monohydrate in the basal diet at levels of 0.05% (Groups 2 and 6), 0.10% (Groups 3 and 7) and 0.20% (Groups 4 and 8). Livers were rapidly removed following sacrifice and chilled in 20 ml of the homogenizing medium (0.25 M sucrose).

Previously described assays were used for determination of cytochrome oxidase activity

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† Present address: Dept. of Biochemistry, Bowman Gray School of Med., Winston-Salem, N. C.