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INFLUENCE OF INTRAPERITONEALLY ADMINISTERED AMINO ACIDS ON AMINO ACID PATTERNS OF EHRLICH ASCITES TUMOR CELLS AND BRAIN IN MICE*

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Abstract—Experiments with individual amino acids intraperitoneally administered in isomolar amounts showed that Ehrlich ascites tumor cells have a high capacity for taking up and maintaining relatively large amounts of the nine essential amino acids *in vivo*. When these amino acids were taken up by the tumor cells, in some cases in extremely large amounts, there appeared to be, at most, minor disturbances in the relative concentrations of the various other easily extractable ninhydrin-reactive constituents found in the tumor cells. The one nonessential amino acid employed in this present study, arginine, did not appear to be taken up readily by the tumor cells. In contrast to the findings with the tumor cells, under the conditions of testing, small net increases in content of only four of the administered amino acids (methionine, threonine, isoleucine, and valine) were detected in the brain. Tyrosine, but not phenylalanine, was detected in the brain after phenylalanine administration. Quantatitive experiments were performed *in vivo* and *in vitro* on histidine uptake.

FOR many years a major concern of our laboratory has been the study of the contents and modes of maintenance of pools of nonprotein amino acids and related substances in normal and neoplastic tissues (see Refs. 1 and 2 for summaries and pertinent references). Much of the work, descriptive and kinetic, done in various laboratories on free amino acids in cells, tissues, and tissue fluids of a variety of organisms ranging from bacteria to man, has been discussed in a symposium.[3] Ascites tumor cells have proven especially valuable for studies *in vivo* of amino acid pools (see Refs. 1 and 2), since a relatively uniform cell population can be exposed directly to various chemical treatments by intraperitoneal injection of substances; and frequent samples, often from the same animal, can be obtained for chemical and/or cytological examination. Previously, we compared the effects *in vivo* on amino acid patterns of ascites tumor cells of high extracellular concentrations of glutamine and glutamic acid and α-γ-diaminobutyric acids (see Ref. 2 for summary). The present experiments were undertaken to extend our knowledge of the influence *in vivo* of exposure to high concentrations of extracellular amino acids on intracellular patterns of amino acids in Ehrlich ascites tumor cells and on the amino acid content of the ascitic fluid and to determine whether these patterns could be altered by a large uptake of amino acids which normally are found in the cells only in small amounts. Observations also were made on the amino acids of brains of the animals which had received the injections.

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Paper chromatographic procedures have been used extensively throughout this work because they are well suited for giving simultaneous information rapidly about the maximal number of ninhydrin-reactive constituents. By these methods valuable hints often can be obtained about which substances should be studied further with exact quantitative methods. In the present studies arginine and the nine amino acids essential for mice were studied individually. Changes from the control levels in these amino acids were readily detectable on the paper chromatograms because the levels of the essential amino acids and of arginine in the easily extractable form are low in ascitic tumor cells and fluid and in extracts of brain.

Quantitative measurements of histidine content were made in one set of experiments *in vivo*, and two correlative experiments *in vitro* were performed in which cell/fluid ratios of histidine were determined in cells which had been preloaded in solutions containing histidine.

MATERIALS AND METHODS

A mouse tumor, Ehrlich ascites tumor (diploid), was used for the examination of the effects of the administered amino acids. The ascites tumor was transmitted serially in Ha/ICR Swiss mice, weighing 30–40 g, fed on a stock diet of Purina chow and water. The tumor-bearing mice had received a transplantation of the Ehrlich ascites tumor (approximately 1×10^6 cells) 5 days previously and were given only sugar cubes and water for approximately 24 hr prior to the injection of amino acid. In the present experiments only those mice were employed in which the tumor had minimal contamination with nontumor, leukocytic cellular elements (approximately 5 per cent). It is obvious that erroneous results can be obtained in chemical studies in which there is a large proportion of leukocytes and erythrocytes, since many of the properties of the latter cell types differ greatly from those of the tumor cells. In each experiment a group of mice was given intraperitoneal injection of freshly prepared neutral aqueous solution or suspension of amino acid in 0·2-ml volume at a dosage level of 4 mmoles/kg.

Tumor and brain samples were obtained from control mice in each group before injection and at 15, 30, 45, 60, 90, and 120 min after the administration of the individual amino acids. The tumor samples were centrifuged for 20 min at 3500 rev/min in the cold (0–4°) in suitably calibrated prechilled centrifuge tubes; volumes of tumor cells and fluid were noted. The fluid was then removed for analysis, and the cells were resuspended in 10 volumes of ice-cold control ascitic fluid obtained from a pooled frozen reservoir of ascitic fluid and then were resedimented. The wash fluid was discarded. Numerous experiments in which extracts of cells and fluid were studied have shown that intracellular amino acids are not extracted from the tumor cells by this procedure. The tumor cells and fluid and the brain sample were extracted, and two-dimensional paper chromatography was performed in the phenol–lutidine solvent systems as previously described.[2] Chromatograms were prepared from extract equivalent to 75 mg of fresh weight of brain, 75 μliters of packed tumor cells, and 150 μliters of ascitic fluid. In making comparisons of amounts of an amino acid in the ascitic fluid with those in tumor cells or brain, it must be kept in mind that *twice* the volume of fluid as of the tissue samples had been used for the preparation of the chromatograms. Thus, an approximately equal amount of a substance on chromato-

grams of cells and fluid in a particular sample of tumor ascites would indicate that the cells had twice as much of the substances as had the fluid.

In the experiment *in vitro* the Ehrlich ascites cells were harvested at 6 days after intraperitoneal transplantation. The animals were sacrificed by cervical dislocation, and the ascitic fluid was drained through an abdominal incision into heparinized tubes (400 units/ml fluid). Ascitic fluid showing more than a trace of blood was not used. The ascites tumors were filtered through several layers of gauze and used without further treatment or washing, to minimize cell damage.[4] The packed cell volume was determined by centrifugation in capillary tube for 5 min at 10,000 *g*. The cells were preloaded with L-histidine by incubation for 30 min in a Dubnoff metabolic shaker (80 cycles/min at 37°; gas atmosphere 5% CO_2–95% O_2) of 3 ml of packed cells diluted with an equal volume of Krebs-Ringer bicarbonate* solution to which had been added 30 or 60 μmoles of L-histidine. Under these conditions uptake was shown to continue for at least another 15 to 30 min. The incubation was terminated by transferring the tubes to an ice bath. A 0·4-ml aliquot was pipetted into 3·6 ml of chilled Krebs–Ringer to determine histidine content of the cells, as described below, and used for the zero-time value. Subsequent efflux or uptake by the preloaded cells was studied by diluting 2·5 ml of the preloaded uptake samples (cell suspension) with 22·5 ml of chilled Krebs–Ringer with or without added histidine and incubating in the Dubnoff shaker as above. At different time intervals, 4·0-ml aliquots were removed into chilled centrifuge tubes. The cells were centrifuged at 2° by accelerating rapidly to 10,000 *g* and then decelerating. The supernatant liquid was decanted, and the tubes were swabbed to remove adhering fluid. Separated cells were extracted with 2 ml of 80% ethanol for 10 min at 70°. The precipitate was removed by centrifugation at 1200 *g* for 5 min and washed twice with 2-ml portions of 80% alcohol. The extracts and washings were combined and evaporated to dryness under infrared lamps with a current of air. The residue was taken up in 0·1 N HCl. Histidine determinations were performed on the cell extracts and extracellular fluid.[5] In the experiments *in vivo* a modification of the diazo reaction was employed[6] for determination of histidine contents.

RESULTS

Histidine, methionine, threonine, and lysine—amino acids taken up in large amounts by the tumor cells and retained for prolonged periods

Figure 1 through 18 (Plates I and II) show the results for histidine. The control patterns of ninhydrin-reactive constituents observed in the tumor cells, fluid, and brain (Figs. 1–3) are entirely consistent with those previously observed[2] and with subsequent control samples throughout this series of experiments. Fifteen minutes after the injection of histidine (H) a remarkably large amount of the amino acid had been taken up by the tumor cells, while the level in the fluid was already considerably lower than that observed in the cells. There was no evidence, either at this time or at any subsequent period, of any net uptake of histidine by the brain or of any alteration of the brain amino acid pattern during the experiment. The changes observed in the fluid consisted of increases in taurine (L) and glycine (K) contents at the 15 and 30-min periods and a small increase in glutamic acid (N) level. Increases in content of various

* Krebs-Ringer bicarbonate solution was modified to the extent that the NaCl and KCl levels were increased to 158 and 12 μEg/ml, respectively.

constitutents in the fluid may, of course, be attributable to outflow from tissues other than the tumor cells. There was a progressive decrease of histidine content of the fluid, but small amounts of this amino acid still were detectable even 2 hr after the injection of this amino acid. The levels of histidine in the cells decreased at a much slower rate than in the fluid. Thus, even though the concentration in the surrounding ascitic fluid fell to low levels, the tumor cells continued to retain extremely large amounts of histidine. After histidine administration, relatively small increases were observed in lysine (J) content of the tumor cells as well as in the amounts of valine (C), leucine and isoleucine (A), and tyrosine (B). Some minor unidentified ninhydrin-reactive spots appeared after the administration of histidine. These will be isolated and investigated in subsequent studies. However, marked losses of ninhydrin-reactive constituents from the cells did not take place even at the time that the maximal levels of histidine were found in the cells.

Threonine (F) was rapidly concentrated from the fluid by the tumor cells shown in Figs. 19–36 (Plates III and IV). Even within 15 min, a much larger content of threonine was found in the cells than was detectable in the fluid. Small but detectable amounts entered the brain during the experiments. These amounts of threonine in brain were greater than were observed at any time during any of the other experiments. The threonine content of the tumor cells remained high for the entire period of observation, and no other significant changes from the control patterns were noted in the free amino acids of the tumor cells. The content of amino acids in the fluid was never greatly different from that of the controls, with the exception of the presence of threonine and of a higher taurine content at the 30-min period.

In the methionine experiment in Figs. 37–54 (Plates V and VI), the samples were oxidized with molybdate and peroxide after application on the paper[7] so that methionine was converted to its sulfone (T), which migrated to an easily identifiable position on the chromatograms. Methionine appeared to enter the brain and was detectable therein for the entire period of observation. The level of methionine in the ascitic fluid decreased progressively with time. The only change observed in the amino acid content of the fluid was an increase in alanine content. Methionine entered the tumor cells readily in large amounts, and relatively large amounts appeared to be retained within the cells for the period of observation. No other significant changes were noted in any of the detected intracellular ninhydrin-reactive constituents.

Lysine (J) entered the tumor cells in relatively large amounts, and even at the end of 2 hr large amounts still were retained within the tumor cells (Figs. 55–72; Plates VII and VIII). The only change noted during the experimental period in the free amino acid pattern of the cells was the appearance of tryptophan (U). The latter amino acid persisted throughout the experimental period. Tryptophan appeared in the fluid at the 15 and 30-min periods. No evidence, whatsoever, was obtained for a significant increment of lysine content in the brains of the experimental animals. The fall in lysine concentration in the fluid was very rapid, the biggest decrease occurring between 15 and 30 min after the administration. From 30 min to 2 hr after the beginning of the experiment lysine was present in the ascitic fluid in relatively constant small amounts.

The results for the four amino acids (histidine, methionine, threonine, and lysine) are quite similar in that they show a great avidity of the tumor cells for the amino acids, relatively slight changes in the content of other detectable, easily extractable

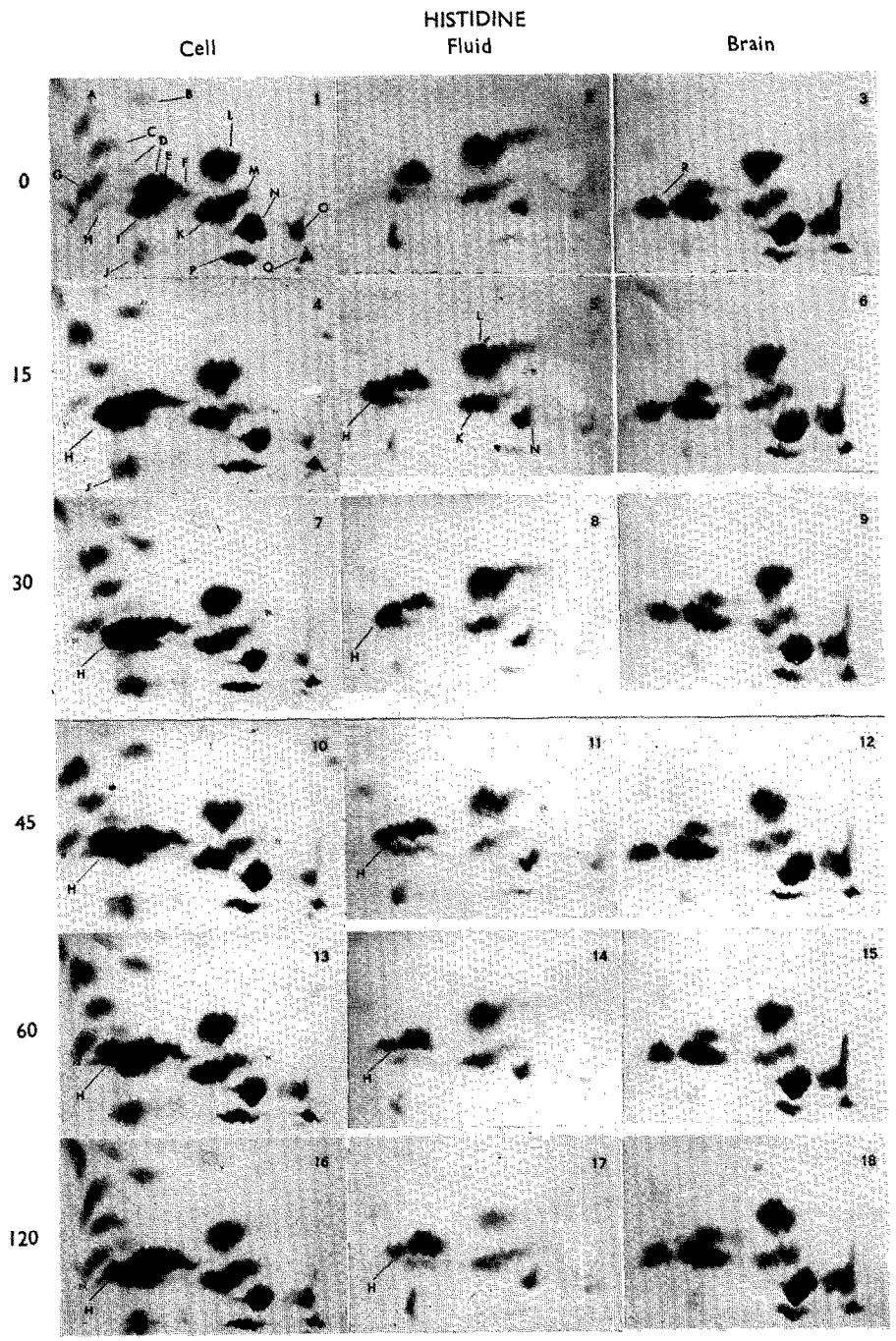

PLATE I. Figs. 1–9. Two-dimensional chromatograms of samples from histidine experiment performed as described under Methods. The sample was placed at the lower right corner. The phenol was run from right to left and the lutidine, bottom to top. A, leucine and isoleucine; B, tyrosine; C, valine; D, α-amino-n-butyric acid; E, alanine, F, threonine, G, proline; H, histidine; I, glycerylphosphoryl-ethanolamine and/or β-alanine and/or citrulline; J, lysine; K, glycine; L, taurine; M, serine; N, glutamic acid; O, aspartic acid; P, ethanolamine phosphate; Q, glutathione; R, γ-aminobutyric acid.

PLATE II. Figs. 10–18. See legend to Plate I.

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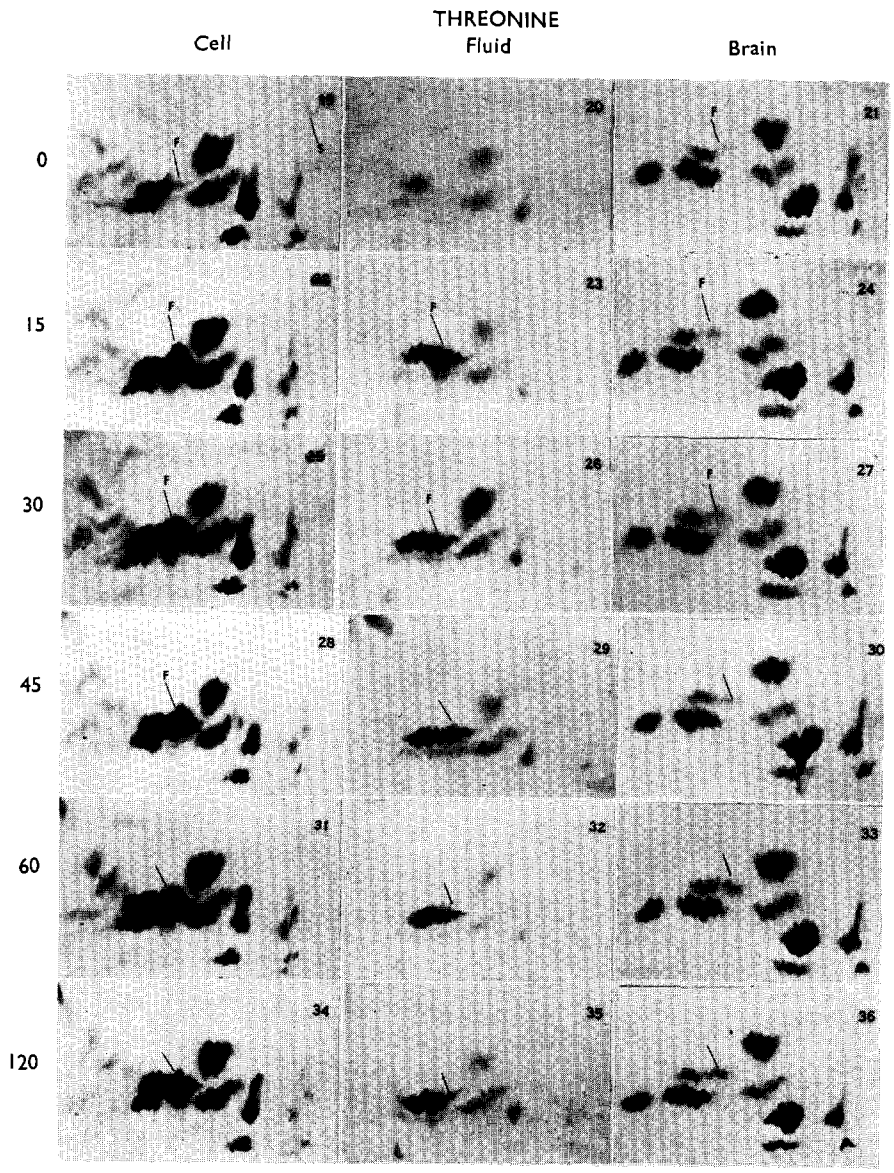

PLATE III. Figs. 19–27. Results for threonine experiment. F, threonine; S, cystine (cysteic acid). Unmarked arrows point to threonine spot.

PLATE IV. Figs. 28–36. See legend to Plate III.

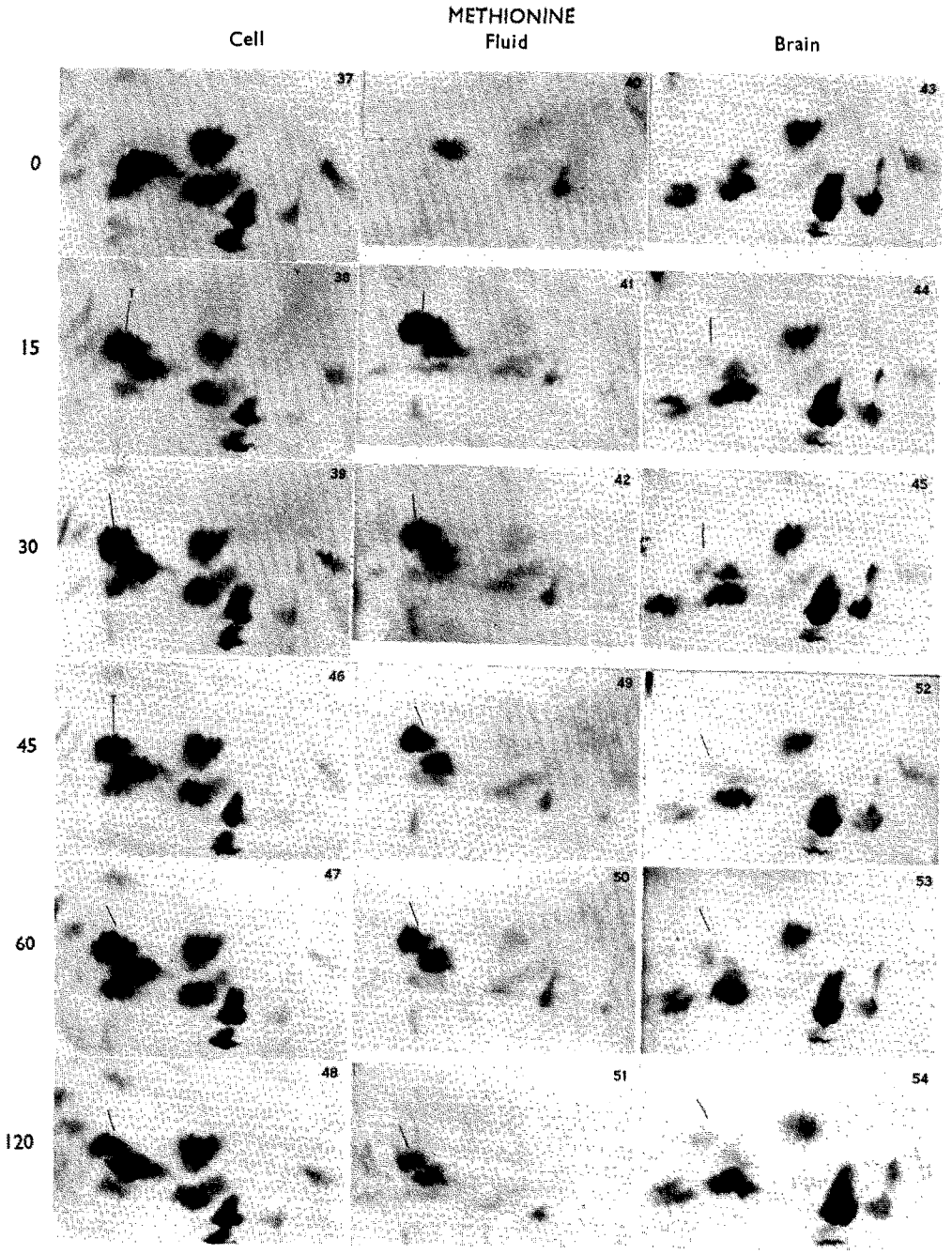

PLATE V. Figs. 37–45. Methionine experiment. T, methionine (methionine sulfone). Unmarked arrows point to methionine. Some of the amino acids appearing in small amounts appear to be lower in this set than in the others because of partial destruction by the treatment with H_2O_2 and molybdate.

PLATE VI. Figs. 46–54. See legend to Plate V.

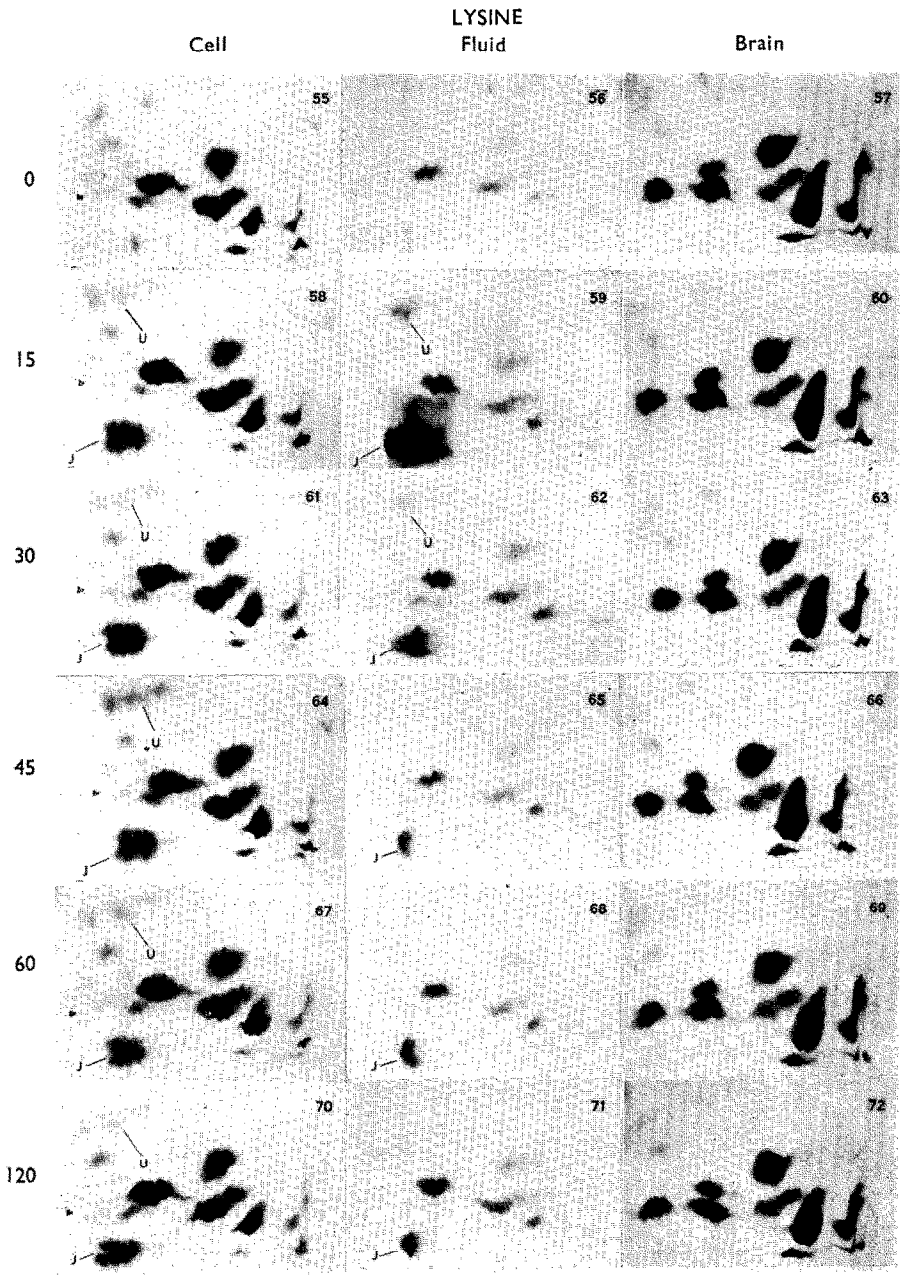

PLATE VII. Figs. 55–63. Lysine experiment. J, lysine; U, tryptophan.
PLATE VIII. Figs. 64–72. See legend to Plate VII.

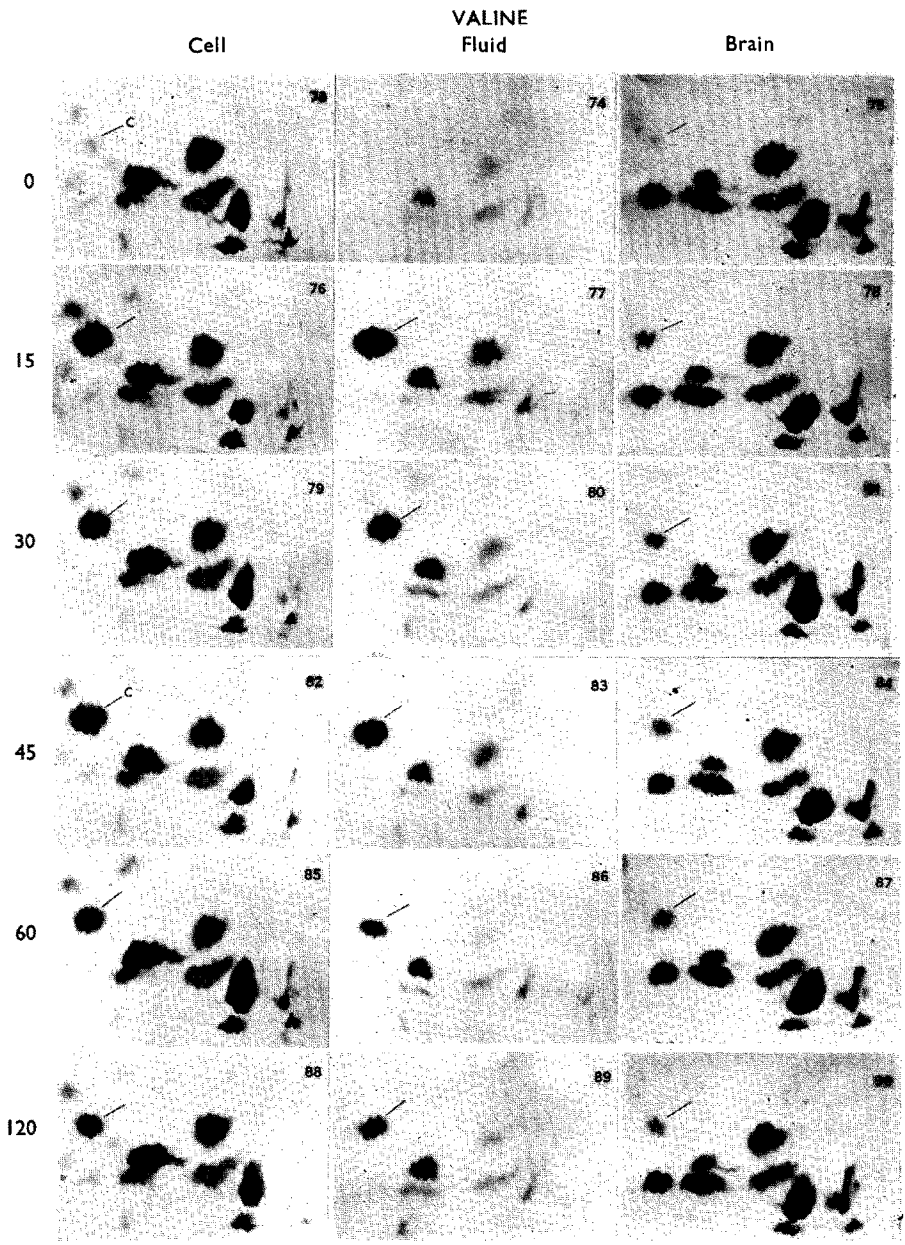

PLATE IX. Figs. 73–81. Valine experiment. C, valine. Unmarked arrows point to valine.
PLATE X. Figs. 82–90. See legend to Plate IX.

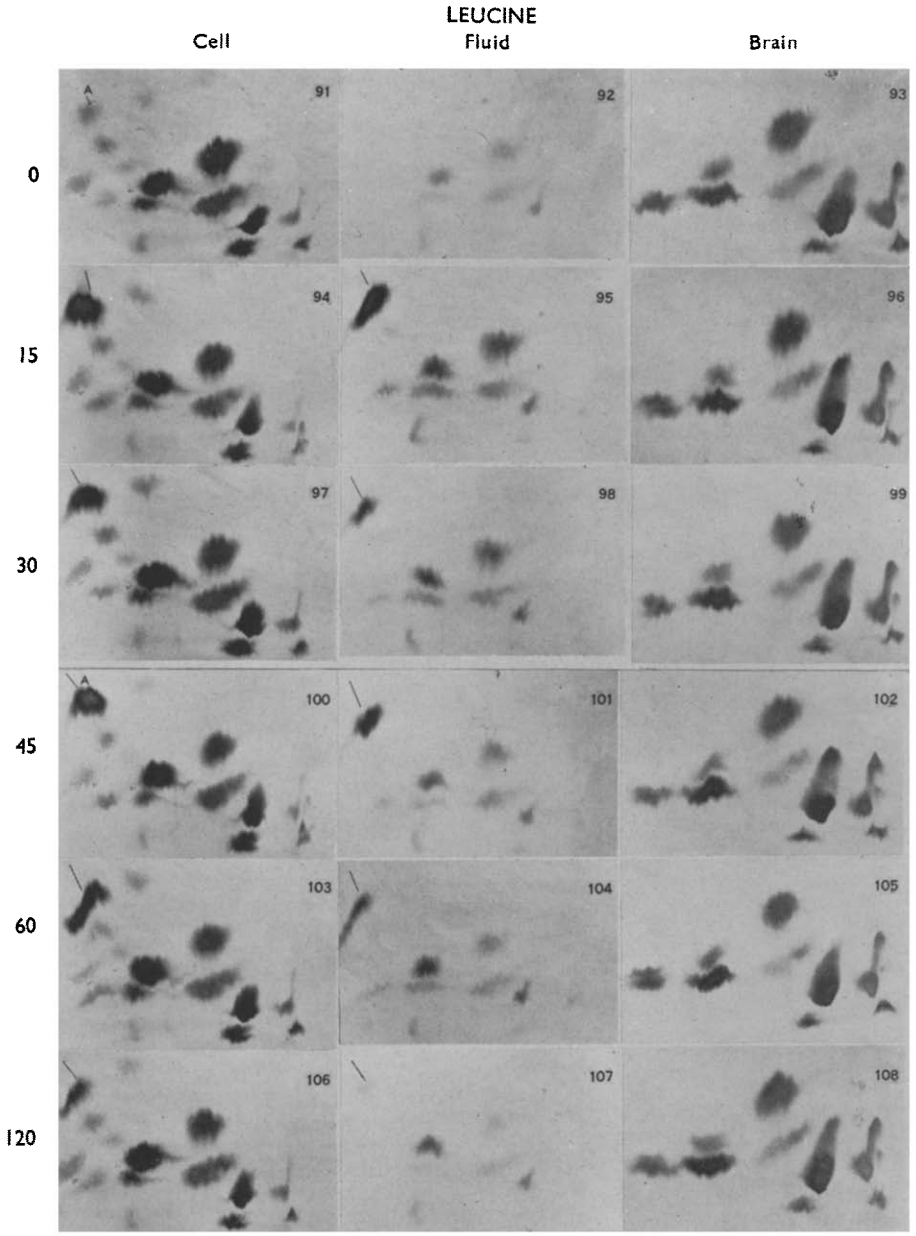

PLATE XI. Figs. 91–99. Leucine experiment. A, leucine. Leucine and isoleucine overlap in the chromatographic systems employed. The intensification of the spot in this instance is attributable to leucine, the amino acid administered.

PLATE XII. Figs. 100–108. See legend to Plate XI.

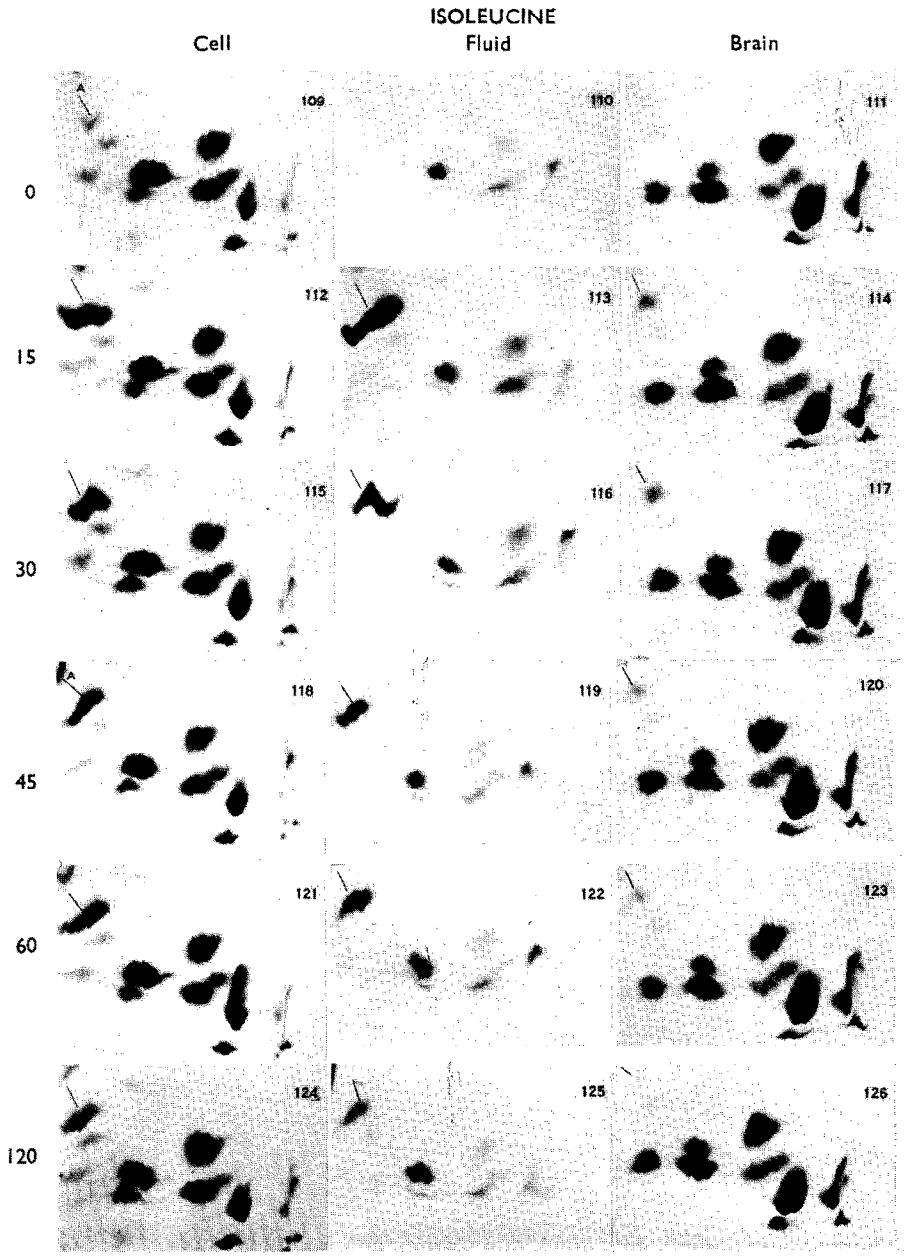

PLATE XIII. Figs. 109–117. Isoleucine experiment. A. isoleucine. See legend to Plate XI.
PLATE XIV. Figs. 118–126. See legend to Plates XI and XIII.

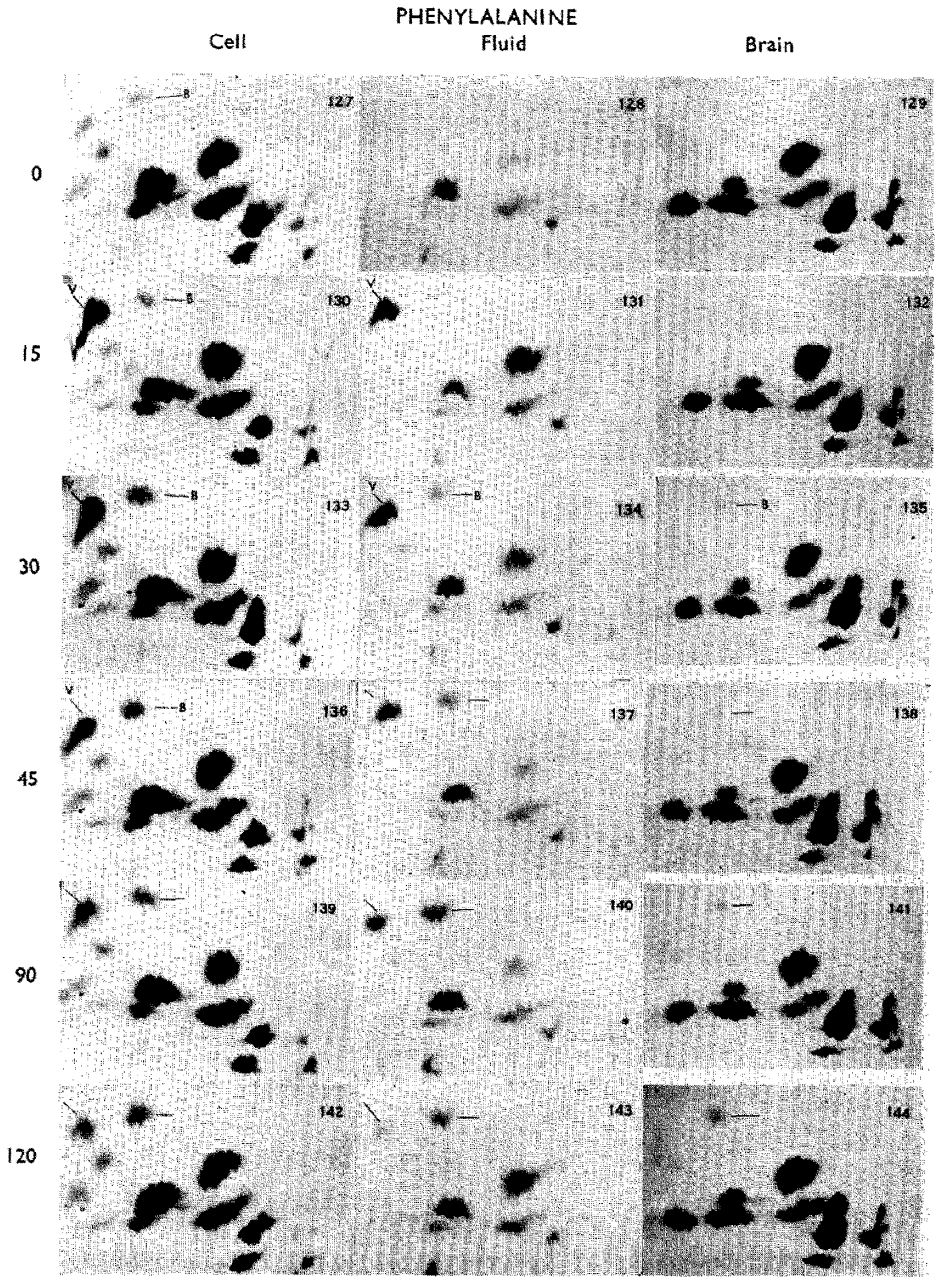

PLATE XV. Figs. 127–135. Phenylalanine experiment. V, phenylalanine; B, tyrosine.
PLATE XVI. Figs. 136–144. See legend to Plate XV.

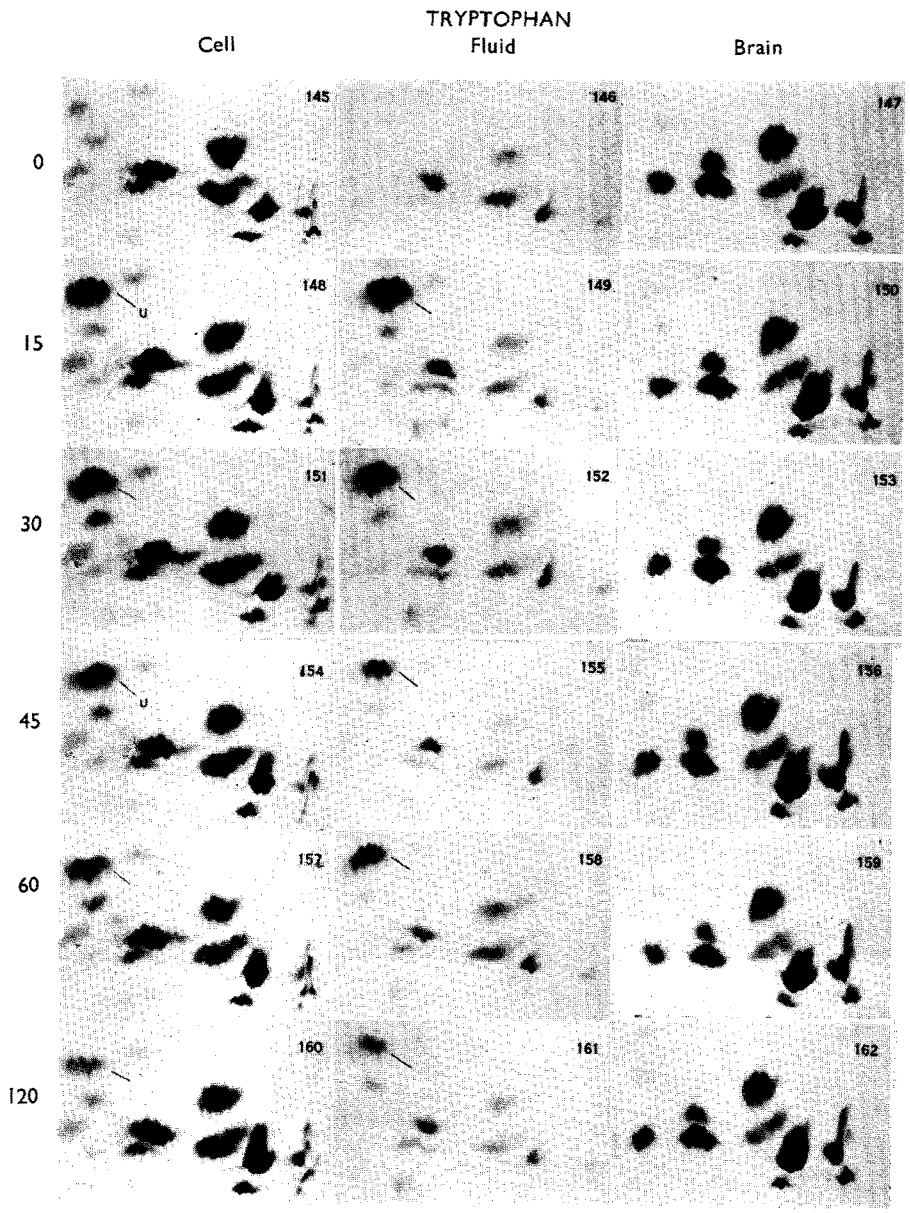

PLATE XVII. Figs. 145–153. Tryptophan experiment. U, tryptophan.
PLATE XVIII. Figs. 153–162. See legend to Plate XVII.

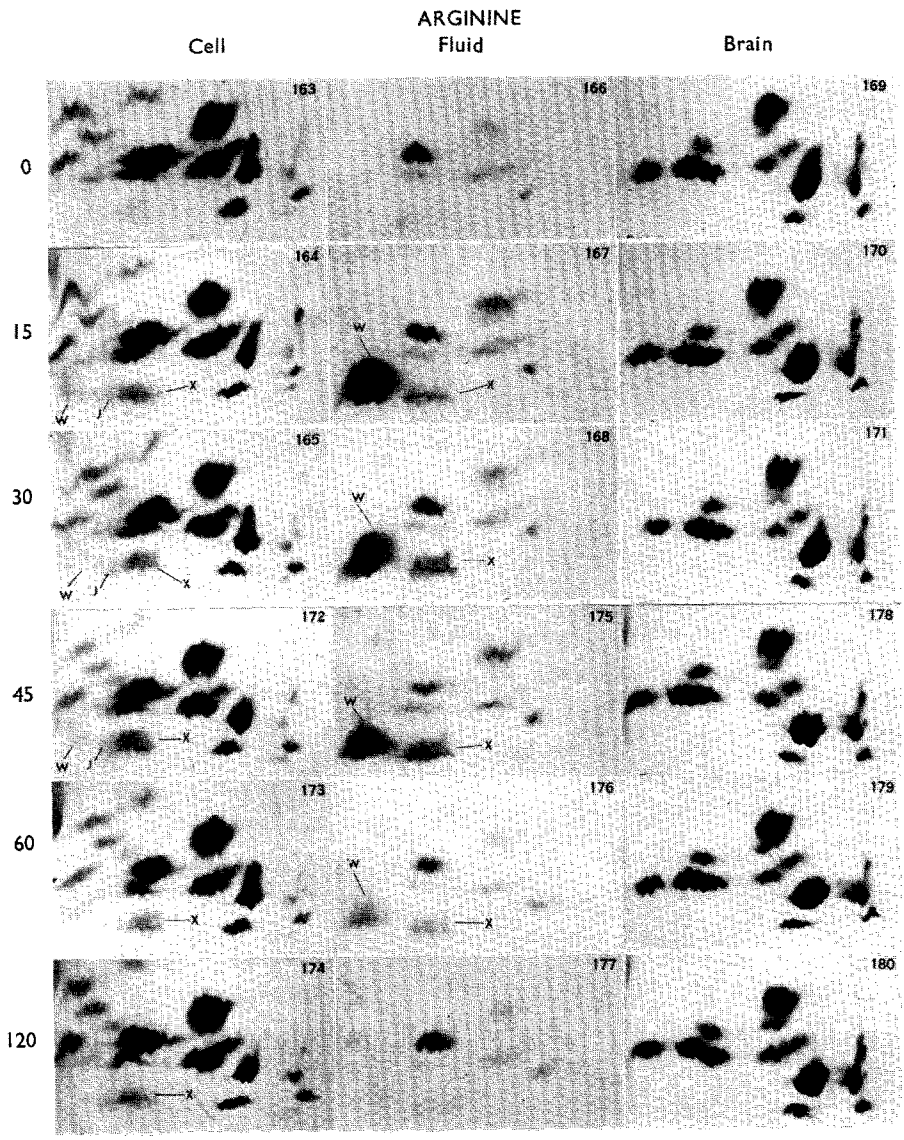

PLATE XIX. Figs. 163–171. Arginine experiment. W, arginine; X. ornithine; J, lysine.
PLATE XX. Figs. 172–180. See legend to Plate XIX.

ninhydrin-reactive constituents at the height of uptake, and only a slow decline in the intracellular content of the amino acid taken up in spite of a rapidly declining concentration in the surrounding fluid. The results indicate that these amino acids are not metabolized rapidly in the tumor cells and that they do not appear to compete strongly for retention with other amino acids which are present normally in the tumor cells in large amounts. Under the conditions of this experiment only two of the four administered amino acids, threonine and methionine, were taken up by brain in amounts sufficient to be detectable by the methods employed.

Valine, leucine, and isoleucine

Valine, leucine and isoleucine showed quite similar results in general (Figs. 73–126; Plates IX–XIV). In all instances the levels of administered amino acid in the fluid fell relatively rapidly after the injection of the amino acid and somewhat less rapidly in the cells. Detectable amounts of the injected amino acids were still present in the fluid at the end of 2 hr. Increased concentrations of isoleucine and valine were readily detectable in the brain even at the 15-min period. However, increases in leucine content of brain were not detected at any time after the administration of leucine. In the ascitic fluids, in each instance, there appeared to be an elevation in the alanine and taurine levels in the 15 and 30-min periods. The fall in levels of the amino acids in the tumor cells from the 15-min to the 2-hr period was much more notable for the three amino acids under consideration in this section than for the four discussed in the preceding section, possibly because of more rapid rate of exit and/or metabolic degradation. Valine, leucine, and isoleucine attained smaller intracellular/cellular ratios than the four previously discussed amino acids.

In the experiments with valine, leucine, and isoleucine, virtually no changes in distribution of any of the other detectable cellular constituents were noted either in the tumor cells or brain at any time after the injection.

Phenylalanine administration—rapid appearance of tyrosine in tumor cells

A higher level of phenylalanine (V) was found in the tumor cells at 15 min after the administration of this amino acid than was present in the ascitic fluid (Figs. 127–144; Plates VX and XVI). A significant increment in tyrosine (B) content of the tumor cells also was noted at this time, while this amino acid was below the level of detection on the chromatograms prepared from ascitic fluid and brain. The maximal tumor cell level of tyrosine was attained at 30 min, and relatively constant amounts of this amino acid were found in the tumor cell extracts for the remainder of the experimental period. The phenylalanine content of the cells fell progressively so that at 120 min the phenylalanine and tyrosine levels were approximately equal. The intracellular levels of tyrosine were considerably higher at all times than those found in the ascitic fluid. The possibility is under further investigation that hydroxylation of phenylalanine may take place in the tumor cells, themselves, as well as in the liver. The phenylalanine levels fell rapidly in the ascitic fluid, whereas tyrosine content increased progressively up to 90 min after phenylalanine injection. In the sample of fluid studied at 120 min the tyrosine level was greater than that of phenylalanine. Small amounts of tyrosine, but not phenylalanine, were detected in brain in 30 min and in all subsequent samples. There was an increase in taurine content of the ascitic

fluid at 15 and 30 min. No other significant changes were noted in the patterns of tumor cells, fluid, or brain.

Tryptophan

Tryptophan (U) produced results similar in many respects to those found for phenylalanine (Figs. 145–162; Plates XVII and XVIII). The tryptophan was high in the ascitic fluid at 15 min, but only relatively low levels were seen at 2 hr. At all time intervals the intracellular/extracellular ratios were lower than those found for the previously discussed amino acids. The decrement in content proceeded in essentially parallel fashion in both cells and fluid. No other changes in the amino acid pattern in the cells were observed, and minimal changes were produced in the fluid. No increase in tryptophan content was observed in the brain.

Arginine

In contrast to the previously discussed nine amino acids, all of which are essential amino acids for growth and all of which were taken up in considerable quantities by the tumor cells under the experimental conditions employed, arginine (W) did not enter the tumor cells rapidly (Figs. 163–180; Plates XIX and XX). At most, traces of arginine were noted in the tumor cells at a time when the fluid levels were extremely high. Arginine also was not detected in the brain. Ornithine was detected in the ascitic fluid even in the 15-min sample and was still found 2 hr after the injection of arginine, at which time arginine itself was no longer detectable. Ornithine may have had its origin in the liver, which has by far the highest arginase activity of any tissue in the body. As in the ascitic fluid, ornithine made its appearance in the tumor cells at 15 min and persisted therein. The ornithine concentration in the tumor cells appeared to reflect the ornithine content of the ascitic fluid. Additionally, the arginase of tumor cells could convert entering arginine into ornithine.[8] No changes from the control levels were noted in the case of any of the other amino acids in the tumor cell or brain extracts.

Cytological examination of tumor cells at various times after administration of amino acids

Samples were studied cytologically at all time intervals employed. In most instances, when cytological changes were noted to have taken place after amino acid administration, tumor samples also were taken at the 4-, 5-, and 7-hr time intervals in order to note the time of persistence of the effect.

In general, when changes were noted they consisted of swelling of the cells, cytoplasmic blebbing, and the increased occurrence of droplets and vacuoles in the cytoplasm. In some instances cytoplasmic blebs appeared to have become pinched off, producing debris floating in the tumor fluid. In the cases of methionine, tryptophan, and isoleucine, crystal-like formations were seen in the cytoplasm during the first hour after the administration of the amino acid. Further work is in progress to determine whether these formations are amino acid crystals formed because the amino acids had been taken up in amounts exceeding the solubility in cytoplasm. In most instances recovery of normal morphological features had occurred by 2 hr after the administration of a particular amino acid, but in the cases of valine, leucine, isoleucine, methionine, and tryptophan some abnormalities were noted even at 5 hr.

Surprisingly, histidine and threonine, which were intensely concentrated by the cells, produced virtually no observable morphological alterations.

Quantitative studies of uptake of histidine in vivo *and* in vitro *by Ehrlich tumor cells*

Quantitative analytical results obtained in a separately performed experiment (Table 1) confirmed the previously discussed chromatographic observations with

TABLE 1. HISTIDINE CONTENT OF TUMOR CELLS AND ASCITIC FLUID AT VARIOUS TIMES AFTER INTRAPERITONEAL ADMINISTRATION TO MICE

Time (min)	Number of samples*	Histidine content	
		Cells (μmoles/ml $\pm$ S.D.)†	Fluid (μmoles/ml $\pm$ S.D.)†
15	8	24·8 $\pm$ 13·2	4·2 $\pm$ 2·2
30	8	25·8 $\pm$ 11·3	2·5 $\pm$ 0·6
45	7	21·9 $\pm$ 12·5	1·9 $\pm$ 1·2
60	7	23·7 $\pm$ 6·5	2·0 $\pm$ 0·9
75	8	21·2 $\pm$ 8·7	2·1 $\pm$ 0·5
90	6	17·8 $\pm$ 7·0	2·5 $\pm$ 1·4
105	7	13·6 $\pm$ 5·1	2·0 $\pm$ 0·8
120	8	11·9 $\pm$ 5·9	2·2 $\pm$ 1·5
240	7	8·3 $\pm$ 3·7	1·2 $\pm$ 1·1

* Each sample was obtained from an individual tumor-bearing mouse.

† Corrected for average "blank" values found in 8 sepearate samples obtained from uninjected controls.

regard to histidine. At 15 min after injection of the histidine, the mean concentration in the cells already was six times as high as that found in the ascitic fluid. The high intracellular levels of histidine were maintained at approximately constant levels for 75 min, the maximal intracellular/extracellular ratio of 11·8 being attained at 60 min. At these times the histidine was calculated to comprise approximately 1·5–2% of the dry weight of the cells. At subsequent times the intracellular levels of the histidine fell so that at 2 and 4 hr the mean values were approximately one half and one third, respectively, of the maximal earlier levels. Relatively low levels of histidine were observed in the fluid even at 15 min after the injection, and even lower values were found subsequently. Chromatography of a number of the extracts showed that the analytically determined values were attributable to histidine itself, and not to metabolites thereof. The large standard deviations indicate the inevitable variability encountered in this type of experiment *in vivo*. The total volumes of tumor ascites varied from animal to animal as did the percentages of the total volume attributable to cells and fluid.

The ascitic fluid is in communication with the blood and tissues as well as with the tumor cells, and the concentrations of substances found at any time in the fluid are resultants of exchanges taking place at differing rates between these compartments. It, is therefore, not possible to deduce accurately the quantitative relationships in the situation *in vivo* for a constituent distributed between the cells and fluid. It was desirable to perform a quantitative study *in vitro* with one of the essential amino acids that is greatly concentrated by tumor cells. Histidine was chosen because it is readily determined quantitatively and because recoveries showed that there was little or no

loss of total histidine from the system even on prolonged incubation (see Methods). The results of two experiments are shown in Chart 1. Between 60 and 120 min an equilibrium was attained between the concentration of histidine in the cells and that in the fluid. An intracellular/extracellular steady-state ratio of histidine of approximately 25/1 was approached from either direction and was approximately twice as

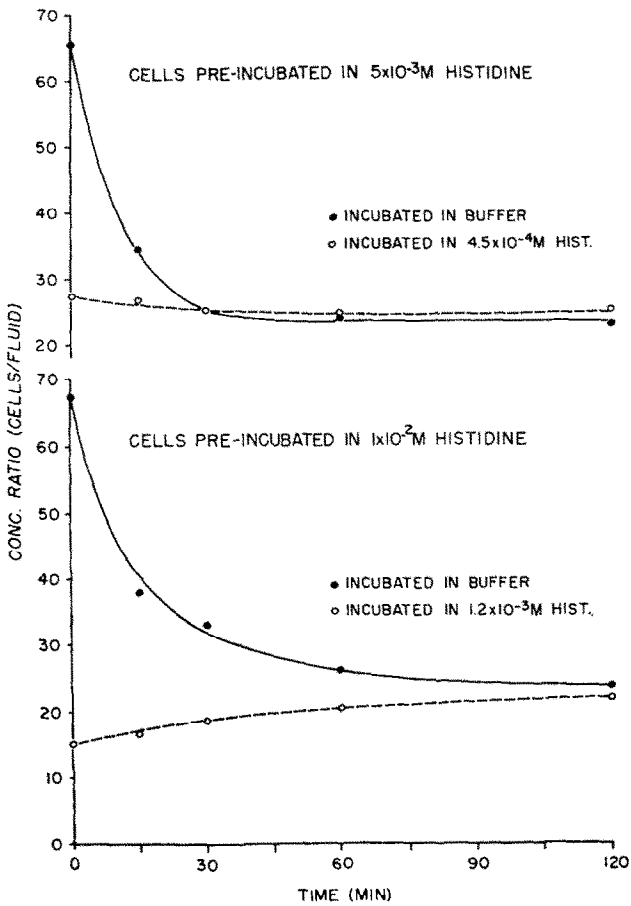

CHART 1. Attainment of equilibrium cell/fluid ratios of histidine by preloaded cells incubated subsequently in solutions containing different concentrations of histidine.

high as that reached *in vivo*. It did not matter whether the cells initially contained quantities which required them to lose or gain histidine or to show no change in intracellular concentrations to attain the equilibrium ratio. Relevant to the subsequent discussion was the finding that the extracellular/intracellular Na^+ ratios were approximately 3/1.

DISCUSSION

From a large body of work[2] it has been possible to conclude that, under normal conditions for a given species at a particular stage of development, each normal tissue,

including every type of blood cell, has a distribution of easily extractable ninhydrin-reactive constituents which is relatively constant and characteristic for that tissue. However, it was found that quite similar patterns of free amino acids are found in many different types of transplanted and spontaneous tumors in rodents. The latter results indicate that steady-state concentrations of small molecules reflect the physiological and enzymatic differentiation of normal tissues, and the similarities found in tumors agree with Greenstein's generalization[9] based on enzyme assays: "No matter how or from which tissues tumors arise, they more nearly resemble each other chemically than they do normal tissues or than normal tissues resemble each other."

Their characteristic amino acid patterns were largely maintained in both normal and neoplastic tissues when drastic changes were induced in the external environment of the animal (starvation, dehydration, vitamin deficiency), or when major disturbances were produced experimentally in the homeostatic mechanisms of the *animal as a whole* (thyroxin injection, thyroidectomy, hypophysectomy, adrenalectomy, diabetes).[2] The patterns of amino acids did not even appear to depend on intactness of microscopically observable intracellular structures, as shown by the failure to induce any major changes in amino acid patterns of ascites tumor cells after treatment with drugs which produced severe damage in individual cells as indicated by abnormalities at cell surfaces as well as in mitochondrial and nuclear structures. Similarly, it was found that alkylating agents which impaired the ability of sarcoma-37 ascites cells to produce tumors when inoculated into mice did not effect the capacity of cells to concentrate tyrosine.[10] Especially striking were the findings made in a study of the influence of myocardial infarction on the free amino acids of dog heart at various times after the ligation of the anterior descending coronary artery.[11] Comparisons were made of the infarcted and noninfarcted areas of the left ventricle. Up to 8 hr after the infarct only relatively small changes in free amino acid distribution were noted in the infarcted area. At this time the muscle fibres had begun to show segmentation, necrosis, and loss of cross-striations. Taken together, the above results suggest that the maintenance of free amino acid (metabolite) pools are related to some minimal basic properties of living mammalian cells. Many experiments were consistent with the suggestion that the steady-state concentrations in tissues of the various detectable constituents are regulated largely by their own separate servomechanisms.[2] There were observed a number of examples of metabolically induced changes in the concentration of one intracellularly contained ninhydrin-reactive constituent without marked effects occurring in any of the others, even though close metabolic relations are known to exist between the constituents that changed and those that remained essentially constant.

Previously[12] it had been shown that glutamic acid did not enter the tumor cells readily when this amino acid was injected intraperitoneally into rats bearing an ascites tumor (Yoshida sarcoma). On the other hand, glutamine was taken up rapidly in large amounts by the cells and metabolized rapidly, in one experiment complete disappearance of the injected glutamine (50 mg) having taken place from both cells and fluid within 1·5 hr after administration. Even when glutamine was at its maximal level in the tumor cells, little change was noted in the contents of other ninhydrin-reactive substance in the cells. Similar experiments with α, γ-diaminobutyric acid in mice bearing the Ehrlich ascites tumor[2] showed there was a rapid uptake of large amounts of this amino acid. Small amounts of this slowly metabolized substance

were still detectable at 24 hr after the injection, but not in the ascitic fluid. At no time after the administration of α, γ-diaminobutyric acid were marked changes observed in the concentrations of amino acids normally found in the cells, even though cytological examination showed chromosome abnormalities and marked diminution in the number of mitotic cells. In *in vitro* studies[13] accumulation of this amino acid by tumor cells was accompanied by a marked loss of potassium from the cells and an entry of chloride. In the present experiments it was found that all nine of the essential amino acids could be taken up readily by Ehrlich tumor cells *in vivo*; in no instance did marked losses occur of other ninhydrin-reactive substances. Histidine was accumulated to such an extent that at its maximal intracellular level it accounted for approximately 1·5–2% of the dry weight of the cell. Yet in the latter instance no cytological abnormalities were produced, and separate experiments showed that the transplantability of the cells was unaffected. In the present study the relative capacities of the tumor cells for concentrating the above amino acids were generally in good agreement with estimates of the equilibrium ratios *in vitro* for these amino acids.[13, 14]

The high capacity of the tumor cells for accumulating all the essential amino acids and some of the nonessential ones may give them the selective advantage for growth which they seem to possess in a wasting host. At a time when the normal tissues are losing their substance, tumor cells often can grow and multiply at a rapid rate. In experiments in which mice with the Ehrlich ascites tumor were fed glycine or L-alanine, the tumor cells were more active in the accumulation of the free amino acids than were the cells of liver or muscle.[15] One wonders whether it would be possible to employ this high concentrative capacity for amino acids as an adjunct to various approaches to therapy. An extremely high intracellular level of a single amino acid which normally occurs in only small amounts intracellularly would be expected to play a disruptive role because of possible inhibitory effects on the metabolism, utilization, and uptake of other amino acids, etc. Gross dietary imbalances of amino acids are well known to have deleterious effects on the health of an animal as a whole. If it should be found that one or more the the amino acids that are intensely concentrated by tumor cells are taken up to a much smaller extent by various normal tissues than by tumors, it would be of interest to precede a therapeutic procedure (X-irradiation, chemotherapy, surgery) with the administration of large amounts of these substances. Experiments are now in progress to determine whether a clear-cut differential uptake exists for any of the essential amino acids.

The data from the quantitative study with histidine *in vitro* (Chart 1) are completely consistent with the existence of a carrier-mediated transport system for this amino acid in the tumor cells.[16–23] Recent evidence suggests that the energy for operation of such a transport system for amino acids (and possibly many other metabolites) may be furnished by the Na^+-ion gradient.[16–19] Therefore, there would not be a requirement for the linkage of metabolically generated ATP to each transport carrier, but a utilization of ATP for the operation of the Na^+ pump which maintains the gradient could presumably enable the many Na^+-requiring membrane carriers to carry out active transport. In order for a relatively unspecific experimental treatment to have a marked effect on amino acid pools of cells bathed in a fluid of relatively constant composition, the treatment would have to have a severe direct effect on the membrane carrier systems or on the operation of the Na^+ pump. It is unlikely that cell life could be maintained for long under these conditions. It is therefore understandable now why

so many experimental manipulations have failed to alter greatly the amino acid patterns of living cells.[2] Since in the presence of carrier-mediated systems there is a tendency to maintain constant intracellular/extracellular ratios of various cellular constituents, it is obvious that the constancy of the amino acid patterns in free cells (blood cells and ascites tumor cells) in an intact organism would be partially dependent on the maintenance of minimally fluctuating plasma levels of these constituents, largely through the metabolic activities of the liver and the excretory and reabsorptive activities of the kidney. Parenchymal tissues would be buffered further against environmental concentration changes by the activities of the cells in the capillaries found in these tissues. Thus, brain slices can take up many amino acids to a considerable extent *in vitro*, while the same substances penetrate the hemato-encephalic barrier to a much more limited extent (see Ref. 24 for pertinent discussion).

At present there is a question whether or not specific transport systems exist for glutamic acid and arginine in Ehrlich ascites tumor cells. The current experiments showed that arginine was not concentrated from the ascitic fluid, and previous studies[12] showed that there was no net uptake of glutamic acid by Yoshida tumor cells and little or no exchange of the intracellular pool of glutamic acid in these cells with ^{14}C-labeled glutamate in the medium. Specific studies are under way to settle these points.

All the present and previous results are consistent with the conclusion that when some amino acids are taken up in extremely large amounts by ascites tumor cells there are, at most, quantitatively only minor disturbances in the relative concentrations of the various other detectable, easily extractable ninhydrin-reactive constituents. In experiments on glycine uptake by ascites tumor cells it was found that while 42 μmoles of glycine were being accumulated by 813 mg of cells, the cells lost only 3·5 μmoles of all other compounds giving color with ninhydrin.[25] However, it cannot by inferred that even such minor changes are not physiologically important. Even when no quantitative changes are noted in the content of a particular constituent during the uptake of another substance, important shifts may take place between intracellular compartments. Important losses or gains in ninhydrin-reactive substances of small molecular weight which are present in small amounts, or in ninhydrin-negative substances, may have taken place in the present experiments, but such changes would not have been detected by the procedures employed.

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