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Uptake of L-Histidine Alone and in the Presence of Other Amino Acids by Carcinogen-induced Sarcomas of the Rat *in Vitro*

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SUMMARY

The uptake of free L-histidine by carcinogen-induced sarcomas and the effect of other amino acids on uptake have been investigated and compared with earlier work on a transplantable (RD3) sarcoma and on normal tissues.

The degree of uptake was considerably less than that found either with brain or with the transplantable sarcoma, and resembled more that found with intestinal mucosa, kidney, testis, and spleen. There was no relationship between degree of uptake and type of cell, weight of tumor, or age of tumor (as calculated from date of administration of carcinogen).

Neutral α-amino acids produced considerable inhibition of uptake, with greater effect by the L isomer than by the D isomer; this was similar in general to the effect found with normal tissues, but different in detail. Long-chain basic α-amino acids produced significant inhibition, but short-chain basic α-amino acids produced none, a pattern found with none of the normal tissues investigated previously, and the opposite to that found with brain. Acidic α-amino acids had no inhibitory effect, an effect opposite to that found with brain, but similar to that found in most cases with other normal tissues. ω-Amino acids had little or no inhibitory effect.

INTRODUCTION

Tumor tissue has been found to be able to take up free amino acids to a greater extent than have certain normal tissues (5, 16). This has been shown, for instance, for a transplanted carcinoma when compared with liver and muscle (1) and for a transplanted (RD3) sarcoma (15) when compared with intestine, spleen, testis, and kidney (9). The extents of uptake by the sarcoma and by brain, however, were found to be similar (9), suggesting that the actual nature of the transport processes for amino acids in the sarcoma might resemble those in brain but not those in other normal tissues.

An attempt has been made here to clarify the relation between uptake by tumor and uptake by brain and other normal tissues. Carcinogen-induced sarcoma was investigated by the same technic (uptake of L-histidine and its inhibition by the presence of other amino acids) that has previously been used for comparing brain, intestinal mucosa, spleen, testis, and kidney one with another (12, 13), thus allowing a direct comparison between sarcoma and those tissues.

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MATERIALS AND METHODS

Tumors. Young albino rats (Sheffield University stock) approximately 8 weeks old were injected s.c. in the right flank with 0.2 ml of a 2% solution (w/v) of 9:10-dimethyl-1:2-benzanthracene (DMBA) in paraffin. Past experience has shown that with this treatment subcutaneous sarcomas begin to appear about 5 months later and over 90% of the animals ultimately (over a period of 2 years) develop tumors.

Preparation of Tissue. The procedure followed as closely as possible the technic used for normal tissues (12). Animals bearing a tumor were killed by a blow on the head. The tumor was removed, weighed, and placed in Krebs-bicarbonate-saline (6) at about 2°C. Pieces of healthy tumor tissue were selected and thin slices cut by hand with a razor blade. These were placed in bicarbonate-saline at about 2°C, mixed by gentle stirring, and random samples of a total weight of about 200 mg transferred to filter paper. After removal of most of the adherent fluid, slices were suspended in 20 ml of bicarbonate-saline, in equilibrium with 5% CO_2-95% O_2, containing 2 mM L-histidine alone or together with an equimolar concentration of a second amino acid. They were incubated thus for 1 hr at 37°C (with agitation at a rate of 80 oscillations/min, amplitude 5 cm) after which tissue and suspending medium were then treated as described previously for mouse brain slices (8) in preparation for the estimation of free histidine.

With every tumor some of the slices removed from the cold bicarbonate-saline were fixed directly in 4% formaldehyde in saline for light microscopy. Such random selection ensured that the slices of tumor taken for histologic study were typical of those used for investigation of uptake of L-histidine, and permitted as far as possible an accurate estimate of the histologic status of the tissue used for incubation with amino acids. Formalin-fixed tissue was embedded in paraffin, sectioned at 5 μ, and stained with hematoxylin and eosin.

Amino Acids. The grade and source of amino acids used were those indicated elsewhere (11). The acidic amino acids were neutralized by an equivalent amount of $NaHCO_3$.

Estimation of Histidine. Histidine was estimated by a colorimetric method (7) using a Unicam (Cambridge) S.P. 500 spectrophotometer with a wavelength setting of 500 mμ.

Presentation of Results

1. Experiments with L-Histidine Alone. The accumulation of free histidine in the tissue is presented as the concentra-

tion difference (concentration of histidine in tissue fluid less concentration in suspending medium) or concentration ratio (concentration of histidine in tissue fluid divided by concentration in suspending medium) at the end of the incubation period. The mode of presentation has been chosen in each case to facilitate comparison with earlier work (9, 12, 13). No correction has been made for adherent medium, and histidine was assumed to be evenly distributed throughout the tissue water. Mean tissue fluid, including adherent medium, was 89%.

Experiments were carried out in triplicate; three separate samples of slices from each tumor were carried through the entire procedure.

2. Experiments Involving Uptake of L-Histidine in the Presence of Another Amino Acid. These were carried out concurrently with Section 1., above, involving a single investigation for a given amino acid with each tumor. Uptake of histidine in each case has been calculated as a percentage of the mean of the triplicate results found in Section 1 for that particular tumor.

3. RD3 Sarcoma. This transplantable sarcoma of the rat was originally derived from a sarcoma induced by 1:2-5:6-dibenzanthracene but was not available for the experiments described in this paper. Since frequent reference is made to it, however, a brief note regarding its morphology and behavior is relevant. It had been transplanted serially in rats for some 20 years when the previous biochemical studies were carried out (15). In this time the tumor had altered from a slowly-growing fibrosarcoma to a mass of rapidly-growing pleomorphic cells with little stroma, which killed the host in about 2 or 3 weeks. The lack of stroma permitted the preparation of cell suspensions by the simple procedure of shaking pieces of tumor with a quantity of saline. With the carcinogen-induced sarcomas used for this paper a cell suspension could not be obtained and tissue slices had to be used. RD3 sarcoma differs from the carcinogen-induced tumor in two important respects: rapidity of growth and scant stroma.

RESULTS

Uptake of L-Histidine by Tumor.

Uptake of free histidine by different tumors was variable, the concentration difference varying from about 2 mM to about 6 mM (Chart 1). Results were consistent, however, for any given tumor, as the scatter of results in most cases was comparatively small for the triplicate samples (mean S.D. = 0.25 mM). Overall, the uptake figures for tumor tissue slices are of an order similar to those found *in vitro* for intestinal mucosa, kidney (Chart 2), testis, and spleen (13), but considerably less than those for brain or, by extrapolation, transplanted (RD3) sarcoma (Chart 2).

Uptake was unrelated to type of tumor cell involved (Chart 3), age of tumor (as calculated from the time between administration of carcinogen and the killing of the animal), or weight of tumor (Chart 1), although with the latter there appears to be the possibility of slightly greater uptake by the larger tumors.

Effect of Other Amino Acids on Uptake of L-Histidine (Charts 4–7).

The effect of other amino acids on L-histidine uptake may be compared with the effect in normal tissues under similar conditions. The normal tissues that have been investigated in this way are brain, intestinal mucosa, testis, spleen, and kidney (11–13). The ordinate and abscissa of Charts 4–7 have been drawn in the same relative proportions as those in the earlier papers, and so may be compared directly, in spite of the difference in the type of unit used on the ordinate. As mentioned elsewhere (13), it is the relationships between the effects of different amino acids in the different tissues that should be compared rather than the absolute values obtained.

1. Effect of Neutral D- and L-α-Amino Acids (Chart 4). As with normal tissues (13), there was an overall (but irregular) increase in inhibitory effect with increase in chain length of certain of the inhibitory amino acids, and in all cases the L isomer had a greater inhibitory effect than the D isomer. Otherwise, there was no consistent similarity in inhibitory pattern with any one normal tissue.

2. Basic L-α-Amino Acids (Chart 5). The two shorter diamino acids (α,β-diaminopropionic acid and α,γ-diaminobutyric acid) showed no inhibitory effect on the uptake of L-histidine. The longer two (ornithine and lysine) produced moderate inhibition. None of the normal tissues show a similar overall pattern (13). With intestinal mucosa and testis a fairly consistent amount of inhibition is produced by all four basic amino acids. With brain (and perhaps kidney), the inhibitory pattern tends to be the reverse of that found with tumor, the longer 2 amino acids showing no inhibition, while the shorter 2 produce considerable inhibition.

3. Acidic D- and L-α-Amino Acids (Chart 6). None of the acidic amino acids produced any inhibition with tumor tissue, nor were they found to do so with intestinal mucosa or testis (12). With kidney (and possibly spleen), on the other hand, the D isomer appears to produce some inhibition, but with brain considerable inhibition is produced by all the acidic amino acids used (12).

4. ω-Amino Acids (Chart 7). With the exception of glycine, none of the ω-amino acids had a consistent inhibitory effect. It is impossible to make a comparison with normal tissue owing to the wide scatter of results when using tumor. Inhibition with normal tissues is relatively slight or else is absent (13).

Histologic Investigations.

The object of the histologic study was two-fold: (*a*) to distinguish well-differentiated and presumably slowly-growing, less aggressive tumors from the anaplastic variety which are held to be more virulent in their behavior; (*b*) to determine the purity and quality of the samples used in the chemical studies.

For establishing the degree of differentiation or anaplasia, criteria similar to those routinely employed by histopathologists dealing with human biopsy material were used. It was found that the rat sarcomas could be divided into three groups. In the first were placed well-differentiated tumors, which showed well-formed spindle cells arranged in whorls and with a fair amount of collagen fibers. In such tumors few or no giant cells occurred and mitoses were rarely encountered. The second or intermediate group contained tumors where some areas showed elongated spindle cells, while other areas contained loosely-arranged pleomorphic cells and large and small round cells. Fiber production was scanty. Mitotic figures and giant cells were fairly frequently encountered. In the third group were placed frankly anaplastic tumors, which

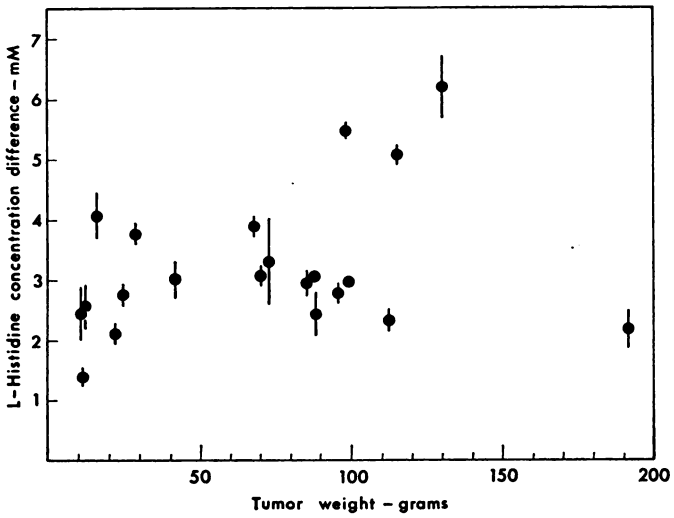


CHART 1. Uptake of L-histidine by different carcinogen-induced sarcomas. Each *circle* represents the mean uptake, with standard deviation, of three samples from 1 tumor. The initial concentration of L-histidine in suspending medium is 2 mM. Incubation for 1 hr at 37°C.

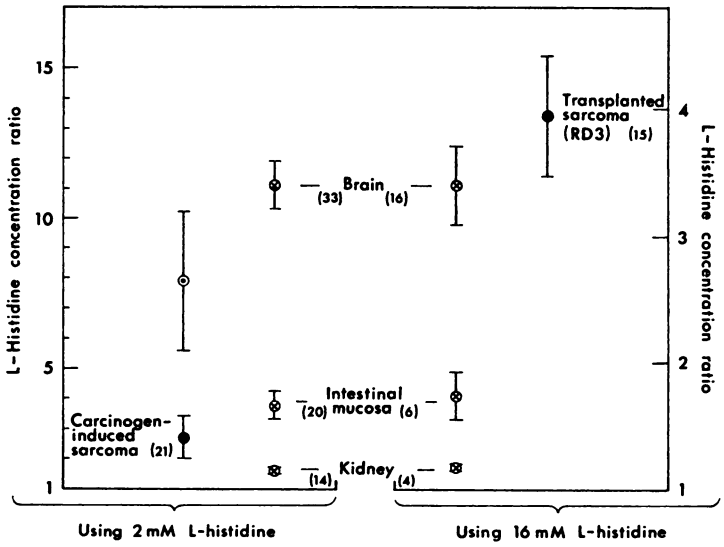


CHART 2. Comparison of uptake of L-histidine by carcinogen-induced sarcoma with that by transplanted (RD3) sarcoma and normal tissues. The scale on each ordinate has been adjusted so that the concentration ratio found with brain when using L-histidine at a concentration of 2 mM is the same distance from the base line (concentration ratio = 1.0) as that found when using it at a concentration of 16 mM. Concentration ratio is shown as the mean with standard deviation. The number of tissue samples are shown in parentheses. ●, Tumor tissue; ⊗, normal tissue; ⊙, maximum possible theoretical uptake for carcinogen-induced sarcoma (n = 21) (for interpretation see Results—Control Investigations). Data for experiments on normal tissue using 2 mM L-histidine are taken from Neame (13). Data for experiments using 16 mM L-histidine are taken from Wiseman and Ghadially (15) and Neame (9).

CHART 3. Relationship between uptake of L-histidine and type of cell. Each *circle* represents the mean uptake of three samples from one carcinogen-induced sarcoma. Initial concentration of L-histidine in suspending medium was 2 mM. Incubation for 1 hr at 37°C.

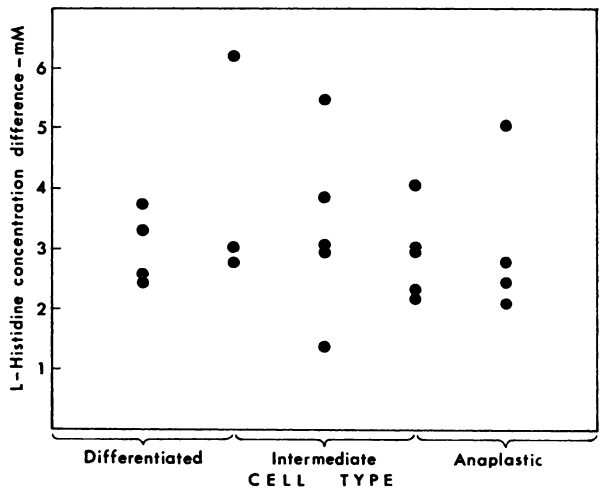
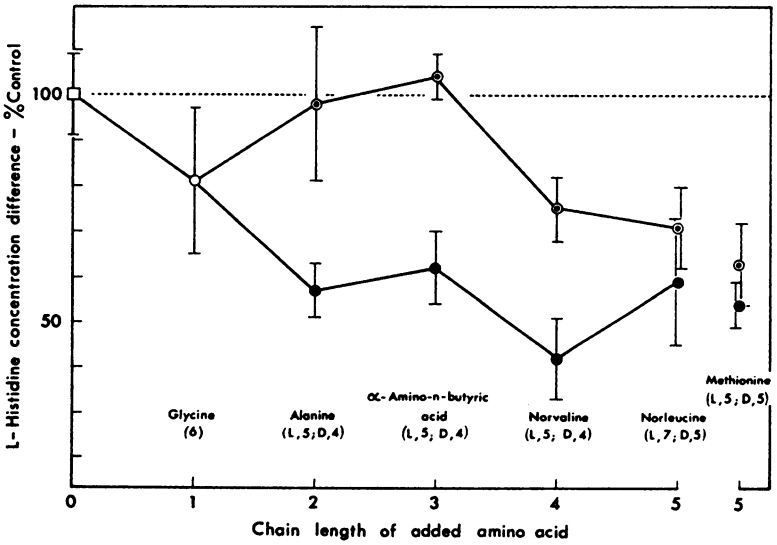


CHART 4. Uptake of L-histidine by carcinogen-induced sarcoma in the presence of neutral α-amino acids. The uptake of L-histidine in the presence of added amino acid (single estimation for each tumor) is calculated as the percentage (±S.D.) of mean uptake by triplicate samples in the absence of added amino acid. Chain length of added amino acid is calculated as the number of carbon (or sulfur) atoms in chain less one carboxyl group. The number of tumors is shown in parentheses. Initial concentrations of L-histidine and added amino acid are each 2 mM. □, No added amino acid (L-histidine alone); n = 21. ○, ω-Amino acids; ⊙, ●, D isomer and L isomer, respectively, of α-amino acids.



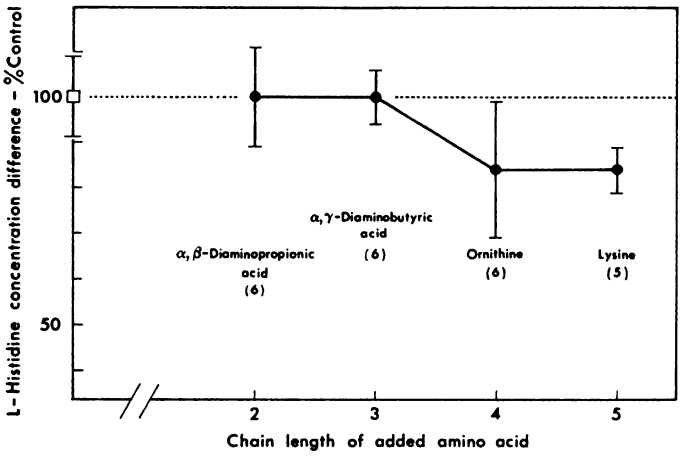


CHART 5. Uptake of L-histidine by carcinogen-induced sarcoma in the presence of basic α-amino acids. Details as in legend to Chart 4.

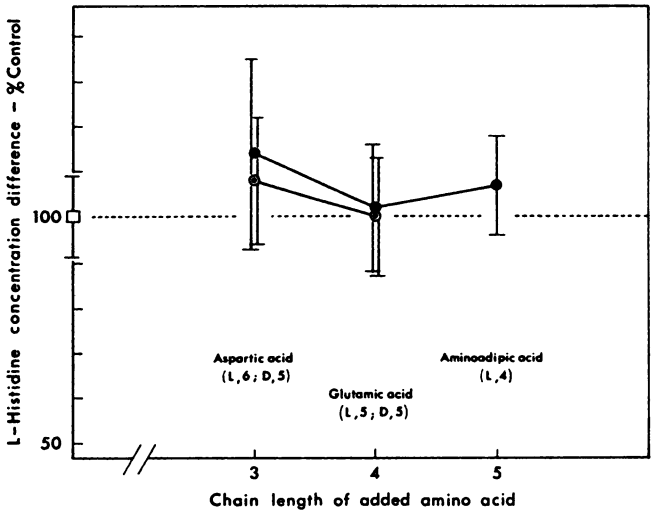


CHART 6. Uptake of L-histidine by carcinogen-induced sarcoma in the presence of acidic α-amino acids. Details as in legend to Chart 4.

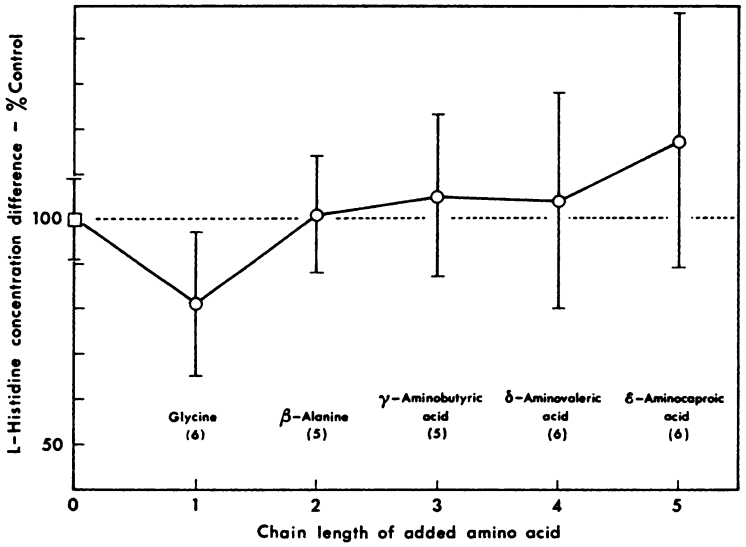


CHART 7. Uptake of L-histidine by carcinogen-induced sarcoma in the presence of ω-amino acids. Details as in legend to Chart 4.

were composed almost entirely of large pleomorphic cells with virtually no attempt at collagen fiber production. Such tumors showed many mitotic figures and giant cells. Classification of the tumors was made by one of us (F.N.G.) prior to any knowledge of the experimental results for uptake.

The uptake of histidine as related to the 3 groups of tumor is shown in Chart 3. Where it was difficult to decide to which of two groups a tumor belonged, the symbol has been placed between those groups. It is clear that the degree of differentiation or anaplasia in the carcinogen-induced sarcoma did not influence the degree of uptake of L-histidine.

Histologic study resulted in the detection of three unsuitable samples, the results of which have been rejected. Two of these contained a fair amount of surrounding normal muscle infiltrated by tumor and the third contained much edematous stroma but very few tumor cells.

Control Investigations

Blank Value of Amino Acid in Tissue. The apparent concentration of histidine in the tissue after incubation with no amino acid in the suspending medium was 0.55 ± 0.13 mM (n = 7). For comparison, blank values for normal tissues varied from 0.14 mM to 0.50 mM (10).

pH of Suspending Medium. In all experiments the pH of the suspending medium at the end of the incubation period was found to lie within the range pH 7.3–7.5.

Change in Amount of Amino Acid during Incubation.

1. Histidine. There was moderate apparent loss of free histidine in the system as a whole during incubation, amounting to about 4% of the total histidine initially present in the suspending medium (40 μmoles). The amount apparently lost in terms of tumor mass was 10.4 ± 4.1 (S.D.) μmoles/ml tissue water in 1 hr. Some or even perhaps most of the apparent loss of histidine was due to unavoidable removal of tissue during procedures after incubation but before weighing, in which an unknown and variable amount of tissue adhered to filter paper used for removing much of the adherent suspending medium.

Involvement of an amino acid in metabolic processes within the cell might possibly account to some extent for low uptake by removing some of the amino acid which would otherwise accumulate. In the present case, however, much of the apparent loss of histidine may be considered as artifact due to actual loss of tissue. It has been felt advisable, however, to indicate a "maximum possible theoretical uptake" (Chart 2), calculated by assuming that all histidine lost, whether by removal of tissue or by actual metabolism, was present as free histidine in the tissue measured. It has no significance other than the setting of an absolute upper limit for hypothetical uptake ability.

Values for actual uptake found are related to the amount of tissue weighed and are therefore not affected by the tissue loss.

2. Other Amino Acids. Paper chromatography was used as described elsewhere (11) to detect any gross change (>25%) in added amino acid in suspending medium during incubation. In no case was any such change detected.

DISCUSSION

Uptake of L-Histidine by Sarcoma. The ability of tumors to grow at the expense of other tissues (16) implies an enhanced ability either to incorporate amino acids into tissue protein, as has been shown with spontaneous and transplanted tumor (4, 16), or to transport amino acids into the intracellular fluid, as has been shown with transplanted tumor (1, 9, 14, 16). Carcinogen-induced tumor, however, does not appear to show enhanced transport, as judged by results with L-histidine (Chart 2), although it is possible that a small part of the difference between carcinogen-induced and transplanted (RD3) sarcoma could be attributed to differences in the form of tissue used (3).

It seems likely that such a difference in uptake between the two types of tumor is related to differences in age of tumor or rate of tumor growth. The cells of the transplanted (RD3) sarcoma, after twenty years of growth, multiply rapidly and show a high degree of uptake, while those of the carcinogen-induced sarcoma, with less than a year of growth, multiply relatively slowly and show poor uptake.

Effect of Other Amino Acids on L-Histidine Uptake. Competition between amino acids for entry into a tissue by a common transport system can be seen experimentally as a reduction in the uptake of one amino acid in the presence of another. Thus those amino acids can be found which share a common transport system, and the different inhibitory effects found in different tissues may be used to compare certain transport systems in those tissues (12, 13). There are certain resemblances between normal tissues in this respect, but no two tissues present a generally identical pattern of inhibition. Similarly, the inhibitory patterns for carcinogen-induced sarcoma bear some resemblance to those found with certain normal tissues, but do not resemble entirely any one tissue. The effect which shows the greatest difference is that produced by the acidic amino acids. Only in brain do the latter produce considerable inhibition of L-histidine uptake (12). In sarcoma (Chart 6) and in most cases in the other normal tissues (12) they produce none. In this respect sarcoma is quite unlike brain, although in view of the negative nature of the findings it cannot necessarily be said to resemble other normal tissues.

In the case of the neutral amino acids no definite conclusions can be drawn from any differences, involving as they do single amino acids. But the general trend towards greater inhibition by the L as opposed to the D isomer suggests a basically similar transport system for neutral amino acids in both normal and tumor tissue, yet with considerable differences in detail.

For the basic amino acids the inhibitory pattern seen with sarcoma is quite unlike that found with any of the normal tissues (Chart 6) (13); none of the latter show an inhibition that is greater with the longer basic amino acids than with the shorter ones, as is seen with sarcoma. The effect is different from that found with the Ehrlich carcinoma cell, in which the long-chain basic amino acids produce no inhibition of histidine uptake (2).

It has been suggested (12, 13) that the degree of uptake of a particular amino acid in a tissue may be related to the number of transport systems involved, and this seems also to apply in tumor tissue. The lack of any common transport system involving both histidine and the acidic amino acids eliminates one means of entry for histidine, and there appears to be a similar lack in relation to the short-chain basic amino acids. Thus, with the carcinogen-induced sarcoma, histidine enters only by routes involving neutral amino acids and the long-chain basic amino acids, and this could account, at least in part, for the relatively poor uptake.

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