Transamination in Tumors, Fetal Tissues, and Regenerating Liver*

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von Euler and co-workers (22), on the basis of results of essentially qualitative experiments, first reported that the transaminase activity of tumors was low. These investigators, using Jensen sarcoma and normal muscle, measured the rate of disappearance of oxaloacetic acid in the following reaction:

1) l(+)-glutamic acid + oxaloacetic acid $\underset{b}{\overset{a}{\rightleftharpoons}}$ α-ketoglutaric acid + aspartic acid

In addition to studies of reaction 1 in tumors, Braunstein and Azarkh (2) studied the reaction:

2) glutamic acid + α-keto acid $\underset{b}{\overset{a}{\rightleftharpoons}}$ α-ketoglutaric acid + amino acid

The amino acids investigated in the case of reaction 2b were the d- and l-forms of alanine, valine, leucine, and isoleucine. The rate of reaction 2a in which both d(−)- and l(+)-glutamic acid were used, plus pyruvic acid, was also studied. Braunstein and Azarkh (2) reported very low rates of transamination in a series of tumors, in some instances finding no evidence of transamination, and in the others, usually less than 15 per cent in 2 hours. These findings were interesting since Braunstein and Azarkh reported relatively fast rates for the same reactions in normal tissues. However, it has been recently shown (8) that the rates of reactions 2a and 2b are very slow in normal rat tissues, and that quantitatively the chief transamination substrates are those shown in reaction 1a.

Since some metabolic characteristics of tumors are also shared by embryonic tissue, it appeared of interest to study transamination in such tissues. In addition, regenerating liver was studied, since this represents a rapidly growing tissue and thus might be expected to show some metabolic similarity to tumor and embryonic tissue.

In this paper experiments are reported in which the rates of reactions 1 and 2 were studied quantitatively in 6 different mouse tumors. The rate of transamination with reaction 1a was also investigated with fetal, kitten, and adult cat tissues, in addition to regenerating rat liver.

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Tissue Sources

Tumors.—The mouse tumors employed in this investigation were the following:

1. United States Public Health Service No. 17—originally described as a neuroepithelioma (20).[1] 2. Sarcoma 37.[1] 3. Yale No. 1—an estrogen-induced mammary adenocarcinoma (5).[1] 4. No. 15091-A—spontaneous mammary medullary adenocarcinoma (6).[1] 5. No. 42—glioblastoma multiforme.[2] 6. No. 108—rhabdomyosarcoma.[2]

The tumors were transplanted at regular intervals to insure a uniform supply.

Fetal, kitten, and adult cat tissues.—Two pregnant cats provided the fetal tissue. The length of the pregnancy was uncertain, but was estimated to be in the last trimester in both instances. Hemihysterectomies were performed under nembutal anesthesia and 2 fetuses removed from each animal. The mothers were allowed to recover. One animal aborted the following day. The other animal delivered what appeared to be full term kittens 8 days later. These were allowed to nurse for 6 days.

Regenerating rat liver.—Seven albino rats, 150 to 180 gm. in weight were partially hepatectomized under ether anesthesia (11). In all instances, 60 to 70 per cent of the liver was removed. The excised tissue was used for control determinations of transaminase activity. The animals were then allowed to survive for varying lengths of time, and the transaminase activity measured in the regenerating liver.

Transaminase activity in different species.—The transaminase activity of liver, kidney, and skeletal muscle of mouse, rat, and cat are of the same order of magnitude; the per cent transamination in liver of

[1] Transplants of tumors No. 17, sarcoma 37, Yale No. 1 and 15091-A were obtained from Dr. M. Belkin, Department of Pathology, Yale University School of Medicine. The authors are indebted to Dr. Belkin for his assistance in providing these transplants and suitable strains of mice for carrying all these tumors.

[2] Transplants of tumors No. 42 and No. 108 were obtained through the kindness of Drs. H. M. Zimmerman and H. Arnold, of the Department of Pathology, Yale University School of Medicine. These tumors were originally produced by intracranial implantation of pellets of methylcholanthrene.

these 3 species under the same experimental conditions was found to be rat, 50; mouse, 46; and cat, 47.

Methods and Procedures

Experimental procedure.—The tissues were removed from the animals after exsanguination and kept in ice-cold saline. The tumors were dissected free of necrotic tissue. It was usually necessary to pool several tumors for a given experiment. Suitable samples were then weighed, after drying on filter paper, and homogenized (18) with M/10 phosphate buffer, at pH 7.4. This procedure was carried out at ice bath temperature. Aliquots of the homogenized preparations were then pipetted into the reaction flasks. All experiments with tumor tissue were carried out under anaerobic conditions, maintained by means of nitrogen gas and yellow phosphorus, in order to rule out aerobic reactions. In the case of the other tissues, anaerobic conditions were not necessary since tissue dilutions of 1:80 were used. All incubations were carried out at 38° C. with shaking.

Analytical methods.—In the experiments with the tumors, α-ketoglutaric acid was determined by the method of Krebs (15), pyruvic acid according to Westerkamp (23), and glutamic acid by a procedure previously described (4). The successful application of these analytical procedures to the measurement of transamination has been previously reported (5-7). In the case of the other tissues, reaction 1a was followed by measuring aspartic acid formation (6).

Substrates.—The substrates were all brought to pH 7.4 before addition to the homogenized tissue, with the exception of the amino acids used in the experiments shown in Table IV. In these experiments the amino acids were weighed directly into the cups, the necessary amount of alkali, or acid, was added to neutralize, and then M/3 phosphate buffer, pH 7.4, added to make up a volume of 0.3 ml. The final concentration of the substrates added was 0.016 M for each, except in the case of dl-methionine, which was 0.032 M. In the remaining experiments the substrate concentration was 0.014 M.

The source and preparation of the different substrates has been previously reported (6).

Results with Tumors

Transamination with reaction 1a.—In Table I typical results are presented for α-ketoglutaric acid formation by tumors according to reaction 1a. The per cent transamination values for the 6 tumors are considerably below those found for normal tissues, with the exception of testis, lung, and spleen (8), but indicate that at high tissue concentrations (1:10) tumors show a measurable transaminase activity when glutamic acid plus oxaloacetic acid serve as substrates.

Previous studies with purified transaminase (7) showed that the per cent transamination varied with the square root of the enzyme concentration. On this basis it would be expected that if a large amount of transaminase were present in a tissue, serial dilution would not show this relationship until a concentration of transaminase was reached which was just sufficient to catalyze reaction 1a at optimum speed. Below that concentration the per cent transamination would be expected to fall according to the square root relationship. However, if the transaminase content of the tumors was low to begin with, it would be expected that in addition to an initial low rate, the per cent transamination would decrease with dilution by an amount determined by the square root of the dilution factor. In Fig. 1 dilution curves are shown for the different tumors. The low initial rate and the rate of decrease of per cent transamination with dilution is seen to follow the square root relationship, indicating an initially low transaminase content. In contrast to this, most normal tissues show a much higher transaminase content (8).

Table I: α-Ketoglutaric Acid Formation from L(+)-Glutamic Acid and Oxaloacetic Acid

Tumor	Microliters α-ketoglutaric acid found: l(+)-Glutamic	Oxaloacetic	l(+)-Glutamic plus oxaloacetic		Per cent transamination
No. 42	105	54	670	471	35
No. 15091-A	72	41	478	365	27
No. 17	50	22	443	371	28
Yale No. 1	77	39	576	460	34
S-37	98	41	624	485	36
No. 108	65	52	514	397	30

Each flask contained 3 ml. of homogenized tumor, dilution 1:10, 0.3 ml. of 0.2 M l(+)-glutamic acid, and 0.3 ml. of 0.2 M oxaloacetic acid added as indicated. Incubation time, 15 minutes; N_2; yellow P; 38° C.

Transamination with reaction 2a.—The per cent transamination with reaction 2a, in which the α-keto-acid was pyruvic acid, is shown for the different tumors in Tables II and III. The data shown in Table II are from experiments in which the disappearance of pyruvic acid was measured. As can be seen, there is no appreciable transamination with this system even after 60 minutes incubation and with a tissue concentration of 1:10. Some of these experiments were repeated and α-ketoglutaric acid formation was measured. As seen from Table III, in confirmation of the

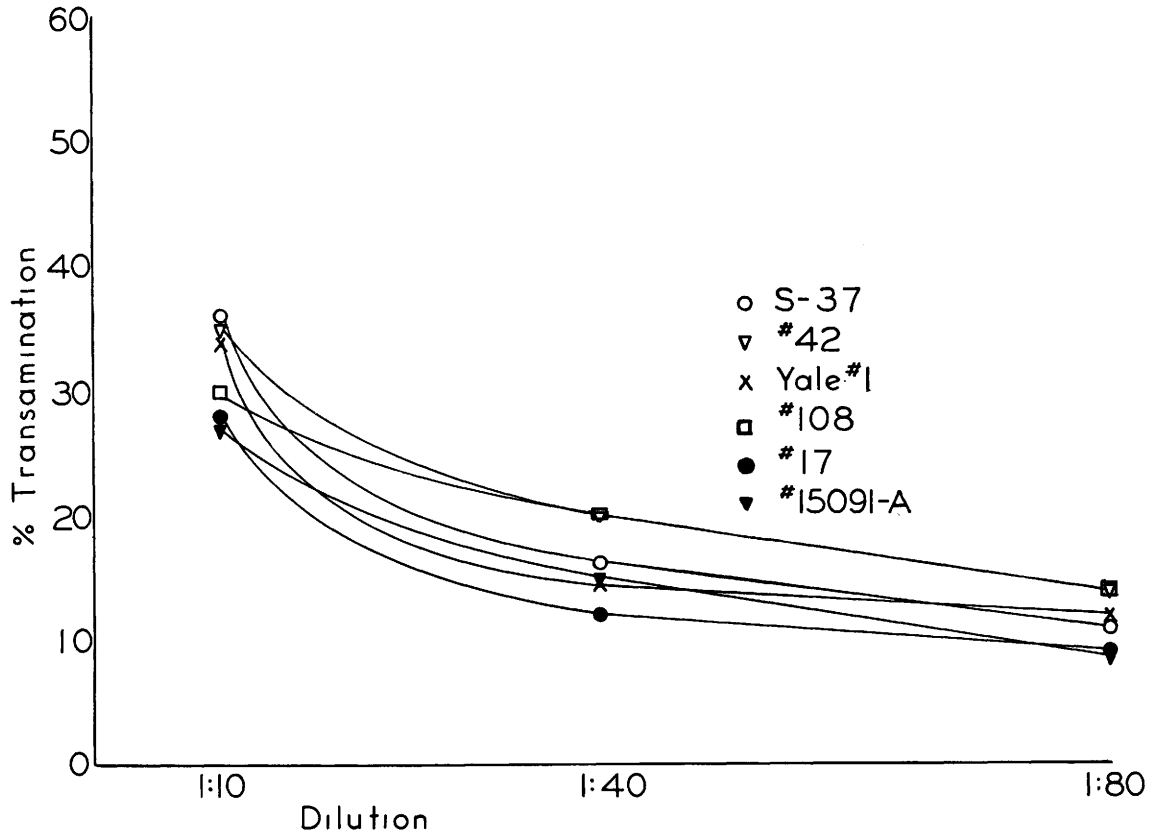

FIG. 1.—Transaminase activity of tumors at different dilutions. Substrates, l(+)-glutamic acid plus oxaloacetic acid 0.014 M. Incubation time, 15 minutes; N_2; yellow P; 38° C.

data in Table II, there is no significant formation of α-ketoglutaric acid.

TABLE II: TRANSAMINATION WITH L(+)-GLUTAMIC ACID PLUS PYRUVIC ACID (PYRUVIC ACID DISAPPEARANCE)

Tumor	Pyruvic acid found after incubation: Pyruvic acid, microliters	Pyruvic acid plus l(+)-glutamic acid, microliters	Δ microliters	Per cent transamination
No. 42	1,285	1,240	45	3
No. 15091-A	1,250	1,235	15	1
No. 17	1,330	1,300	30	2
Yale No. 1	1,270	1,225	45	3
S-37	1,260	1,215	45	3
No. 108	1,300	1,275	25	2

Each flask contained 3 ml. of homogenized tumor, dilution 1:10, plus 1 ml. of 0.06 M pyruvic acid, 0.3 ml. of 0.2 M l(+)-glutamic acid added as indicated. N_2; yellow P; 38° C.; incubation time, 60 minutes.

In normal tissues, reaction 2a proceeds at a slow but measurable rate (8) when compared to reaction 1a. In the case of these tumors it is apparent that reaction 2a does not take place at all, while reaction 1a proceeds at a much slower rate than in normal tissue.

Transamination with reaction 2b.—The formation of glutamic acid from α-ketoglutaric acid and different amino acids was determined in 3 mouse tumors. As can be seen from Table IV, the glutamic acid formation is considerable only in the case of aspartic acid. When compared with most normal tissues (8), the activity of reaction 1b in these tumors is also low.

TABLE III: TRANSAMINATION WITH L(+)-GLUTAMIC ACID PLUS PYRUVIC ACID (α-KETOGLUTARIC ACID FORMATION)

Tumor	Microliters α-ketoglutaric acid found: l(+)-Glutamic acid	l(+)-Glutamic acid plus pyruvic acid	Δ	Per cent transamination
No. 17	66	97	31	2
No. 42	74	90	16	1
No. 108	44	77	33	2

Each flask contained 3 ml. of homogenized tumor, dilution 1:10, 0.3 ml. of 0.2 M l(+)-glutamic acid and 0.3 ml. of 0.2 M pyruvic acid added as indicated. N_2; yellow P; 38° C.; incubation time 60 minutes.

Since reactions 2a and 2b have been shown to take place at very slow rates in normal tissues (8), it is not surprising to find that these reactions do not proceed

to any appreciable extent in tumor tissue, inasmuch as the fastest reaction in normal tissues, reaction 1a, has been shown to take place at a slow rate in tumor tissues.

Transamination with nonnatural amino acids.—Braunstein and Azarkh (2) reported that d-amino acids show some activity with tumor tissue in the presence of α-ketoglutaric acid. However, the analytical method employed by these workers was not satisfactory for measuring small amounts of glutamic acid as pointed out by Zorn (24). Inasmuch as reactions 2a and 2b have been shown in this study not to proceed at a significant rate with the l-amino acids it would hardly be expected that the d-forms would be active. However, since reaction 1a has been shown to proceed at a measurable rate in tumors, it seemed that d(−)-glutamic acid would be more suitable for studying transamination of d-amino acids. von Euler and coworkers (22) using qualitative tests previously reported that d(−)-glutamic acid was transaminated more slowly with oxaloacetic acid in Jensen sarcoma than in normal muscle.

Table IV: Glutamic Acid Formation from α-Ketoglutaric Acid and Different Amino Acids

Amino acid	Glutamic acid found, microliters			Increase due to added amino acid, microliters			Per cent transamination		
	No. 108	Yale No. 1	No. 15091-A	No. 108	Yale No. 1	No. 15091-A	No. 108	Yale No. 1	No. 15091-A
None	165	131	121	...	...	...	..	..	..
l(—)-Aspartic acid	357	273	270	192	142	149	14	11	11
l(+)-Alanine	205	167	191	40	36	70	3	3	5
l(—)-Phenylalanine	201	130	81	46	...	...	3	..	..
dl-Methionine	140	124	145	...	...	24	..	..	2
l(+)-Arginine	270	81	136	42	...	15	3	..	1
l(+)-Tryptophane	147	94	122	...	...	1	..	..	..

Each flask contained 3 ml. of homogenized tumor, dilution 1:10, plus 0.3 ml. of 0.2 M α-ketoglutaric acid. Amino acids weighed into flasks in amounts equivalent to 0.3 ml. of 0.2 M (0.4 M for dl-amino acids) solutions. Incubation time 60 minutes; N_2; yellow P; 38° C.

Table V: Transamination of d(—)-Glutamic Acid

Tumor	Microliters α-ketoglutaric acid found		
	d(−)-Glutamic	Oxaloacetic	d(−)-Glutamic plus oxaloacetic
S-37	73	42	85
No. 42	66	63	102
No. 15091-A	63	76	96

Each flask contained 3 ml. of homogenized tissue, dilution 1:10, 0.3 ml. of 0.2 M d(−)-glutamic acid and 0.3 ml. of 0.2 M oxaloacetic acid added as indicated. Incubation time, 60 minutes; 38° C.; N_2; yellow P.

Quantitative measurements of this reaction are shown in Table V. As will be noted, in none of the 3 tumors studied was there any evidence of transamination with d(−)-glutamic acid. This finding is in keeping with previous experiments with purified transaminase preparations (6) and pigeon breast muscle (5).

Results with Cat Tissues

Transamination in fetal, kitten, and adult cat tissues.—In Fig. 2 data on transaminase activity, as measured by reaction 1a, are presented for fetal, kitten, and adult cat tissues. The fetuses were removed by hemihysterectomy 8 days *prepartum,* as determined by the date of delivery of the remaining fetuses. The newborn kittens were allowed to nurse for 6 days before they were killed. The mother cats served as the sources of adult cat tissues. Transaminase activity was measured in kidney, liver, and brain.

As can be seen from Fig. 2, the per cent transamination rapidly rises from fetal to adult tissues. Liver shows the greatest increase. The adult cat values for liver are of the same order as those for rat, while those for kidney and brain are somewhat lower (8). The per cent transamination values for fetal cat tissues are of the same order of magnitude as those found for mouse tumors.

Transamination in regenerating liver.—Data for the transaminase activity of regenerating liver are shown in Table VI. Transamination in these experiments was measured with the substrates l(+)-glutamic acid plus oxaloacetic acid (reaction 1a). As can be seen from the data, there is a significant fall in per cent transamination between 5 and 9 days after partial hepatectomy, after which the per cent transamination returns to a normal value.

Table VI: Transamination in Regenerating Rat Liver

Days after partial hepatectomy	Mgm. dry weight of liver	Per cent transamination	Qtransamination
0	11.5	52	243
2	10.2	40	212
5	10.0	39	210
9	11.2	35	168
12	11.5	51	238
24	11.6	53	245

Substrates, l(+)-glutamic acid plus oxaloacetic acid (equimolar concentration, 0.014 M). Tissue dilution, 1:80; incubation time, 15 minutes; air; 38° C.

The $Q_{transamination}$ values are also decreased significantly, indicating that the fall is not due to an increase in water content of the livers. These experiments, while not striking, fall in line with those for tumor and fetal cat tissues, and suggest that where growth is taking place rapidly, the transaminase activity is decreased.

TABLE VII: $Q_{TRANSAMINATION}$

Tumors		Fetal tissues (cat)		Adult tissues (cat)	
S-37	57	Kidney	78	Kidney	220
No. 42	72	Brain	77	Brain	210
Yale No. 1	54	Liver	64	Liver	230
No. 15091-A.	54	Placenta	94		
No. 108	72				
No. 17	53				

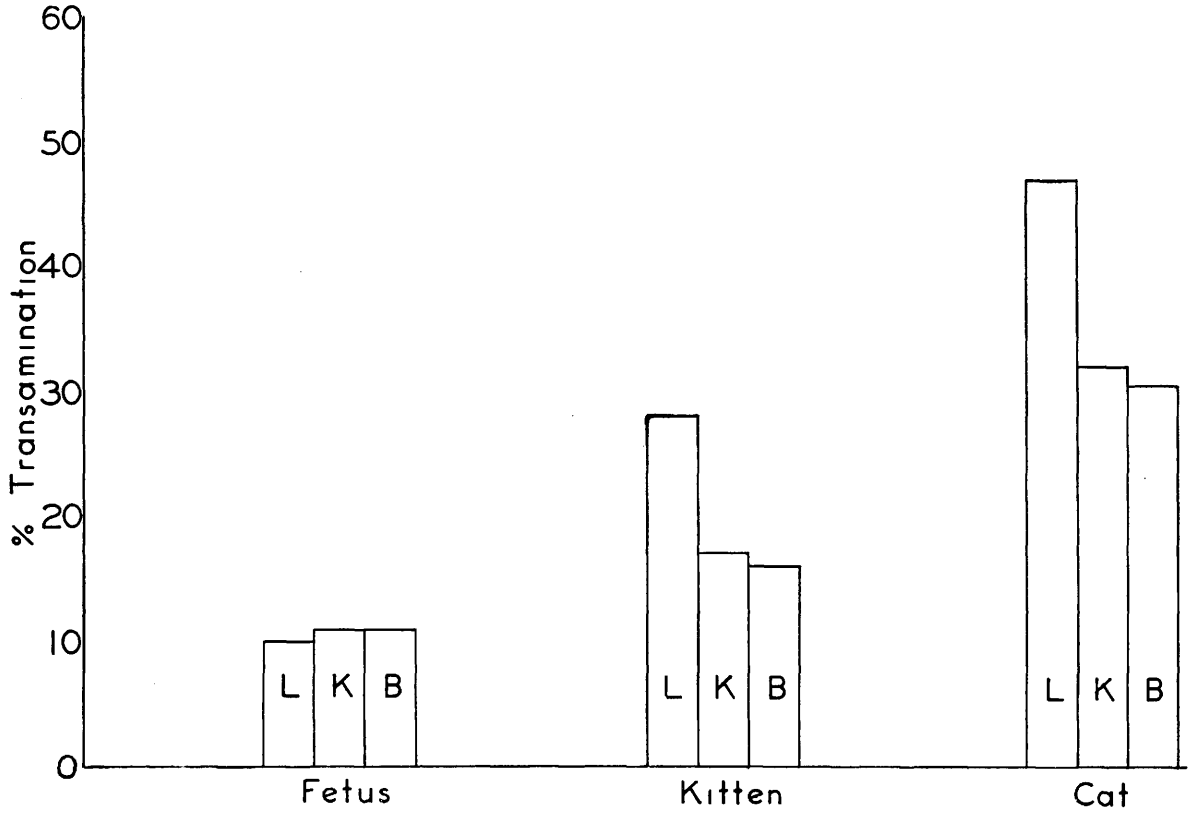

FIG. 2.—Transaminase activity in fetal, kitten, and adult cat tissues. Substrates, l(+)-glutamic acid plus oxaloacetic acid, 0.014 M. Tissue concentration 1:80; incubation time, 15 minutes, air; 38° C.

$Q_{transamination}$.—The rates of transamination in the different tissues can best be compared in terms of $Q_{transamination}$.

$$Q_{transamination} = \frac{\text{microliters of substrate transaminated}}{\text{mgm. dry weight} \times \text{hours}}$$

In Table VII are listed $Q_{transamination}$ values for mouse tumors, fetal cat, and adult cat tissues. It is seen that the $Q_{transamination}$ values for the fetal and tumor tissues are of the same order of magnitude. However, the values for the adult tissues are several times greater than those for the corresponding fetal tissues. The $Q_{transamination}$ value for regenerating rat liver 9 days after partial hepatectomy (Table VI) is of the same order of magnitude as that of kitten liver, but apparently never reaches the low value of fetal cat liver.

DISCUSSION

Tumor, fetal cat, and regenerating liver tissue have in common the property of rapid growth, or rapid protein synthesis. These tissues, particularly the first two, also show a low transaminase activity. This would suggest that an inverse relationship exists between protein synthesis and transaminase activity. The exact mechanism by which the transamination reaction could influence protein synthesis is not clear. However, if one considers the substrates involved in reaction 1, it is seen that we are dealing with substances which are known to play special roles in intermediary metabolism. Thus, oxaloacetic acid and α-ketoglutaric acid act as respiratory mediators in the Szent-Gyorgyi-Krebs cycle (16). On the other hand, glutamic acid and aspartic acid play important roles in protein metabolism. Schoenheimer and Rittenberg (19) have

stated, "The results obtained with N^{15} strongly support the theory that the dicarboxylic acids play a central role in protein metabolism." These workers observed that whenever isotopic amino acids, or ammonia, were fed, the dicarboxylic acids showed a much higher isotope concentration than the other amino acids, with the exception of the amino acid fed. Glutamic acid always showed a higher concentration of isotope than aspartic acid.

While the facts are too few to permit a satisfactory formulation of the mechanism by which transamination influences protein synthesis, for purposes of a working hypothesis the interrelationship might be pictured as follows. Glutamic acid plays a central role in protein synthesis. From the work of Schoenheimer and Rittenberg (19) it is clear that the protein molecule cannot be considered as a stable substance, but rather as a highly reactive compound which continuously participates in exchange reactions with free amino acids. Thus the protein molecule can be pictured as existing in a dynamic equilibrium with free amino acids and other metabolites. In the presence of high concentrations of transaminase, the amount and rate of protein synthesis would be determined by the rate at which glutamic acid is "pulled out" of the protein-amino acid equilibrium system. Since reaction 1a proceeds at a rate 2 to 3 times as fast as reaction 1b (7, 8), aspartic acid synthesis would take place at the expense of glutamic acid, with the result that protein synthesis would be limited. The high transaminase activity of adult tissues with a slow rate of protein synthesis, and the low activity of tumor and embryonic tissues with a high rate of protein synthesis, fit into such a hypothesis.

In addition to low transaminase activity, low values for cytochrome C (12, 17, 21), coenzymes I and II (1, 14) and riboflavin (13, 14) have been reported for tumor and embryonic tissue. These deficiencies would be expected to limit the oxidative capacity of tumor and embryonic tissue and consequently would result in a more limited oxidation of glutamic acid. In the case of adult tissues, the high transaminase content would act in such a way as to "pull out" glutamic acid, while the higher content of cytochrome C, coenzymes I and II, and riboflavin would insure rapid oxidation of glutamic acid via α-ketoglutaric acid, succinic acid, and finally, oxaloacetic acid. The latter could then be used in transamination with another molecule of glutamic acid. The fact that of the amino acids, glutamic acid alone is rapidly oxidized by most tissues lends support to the above speculations. In the case of tumor and embryonic tissue, the low values for transaminase, cytochrome C, coenzymes I and II, and riboflavin would serve to insure a high glutamic acid content for purposes of protein synthesis.

The recent finding that tumors and embryonic tissue are low in arginase (10) may represent another mechanism by which an amino acid is "preserved," as it were, for the purpose of protein synthesis in rapidly growing tissues.

Summary

1. The transaminase activity of a series of 6 mouse tumors, regenerating rat liver, and fetal kitten and adult cat tissues was determined with the reaction:

1) l(+)-glutamic acid + oxaloacetic acid $\underset{b}{\overset{a}{\rightleftharpoons}}$ α-ketoglutaric acid + aspartic acid

The tumors and fetal tissues showed low activities and the regenerating liver a somewhat lowered activity when compared to normal adult tissues.

2. No transaminase activity was observed with three different tumors when d(−)-glutamic acid was used in reaction 1a.

3. The reaction:

2) l(+)-glutamic acid + α-keto acid $\underset{b}{\overset{a}{\rightleftharpoons}}$ α-ketoglutaric acid + amino acid

was also studied with different tumors. In the case of reaction 2a, with pyruvic acid as the α-keto acid, no measurable transamination occurred. In the case of reaction 2b, the amino acids l(−)-aspartic acid, l(+)-alanine, l(−)-phenylalanine, dl-methionine, l(+)-arginine and l(+)-tryptophane were used. The transaminase activity was appreciable only in the case of l(−)-aspartic acid (reaction 1b).

4. The suggestion from these experiments that an inverse relationship exists between transaminase activity and protein synthesis is discussed.

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