lene, possess oral antifertility activity in rats, guinea pigs, and rabbits. Efficacy, following daily doses as low as 5 μg, is restricted to first 4 days of pregnancy. Antifertility activity has not been observed in hamsters. These compounds cause minimal uterotropic stimulation and also inhibit estradiol-induced uterine weight increases. Gonadotropin inhibiting activity is not observed at therapeutic doses. In contrast to many compounds reported to effectively inhibit pregnancy, both U-10520A and U-11100A possess very favorable therapeutic ratios.

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Distribution of Tritium Labeled β-3-thienyl-L-alanine in Tissues of Adult Male Rats Bearing Murphy-Sturm Lymphosarcoma.* (28071)

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β-3-thienylalanine (β3TA) is a phenylalanine analogue that has been studied in several laboratories for its ability to inhibit growth and protein synthesis in both microorganisms and mammalian tissues(1,2,3,4,5). The β3TA and a closely related compound, the β-2-thienylalanine (β2TA) were found to inhibit tumor growth in mice and rats(6,7,8, 9). In all the studies of which we are aware, the DL form of β3TA was used. Ferger and Du Vigneaud showed that L-β2TA is twice as potent an inhibitor as is the DL-β2TA (10). Experience with other amino acids further suggests that only the L-isomer of β3TA has a significant biological activity.

In view of the known oncostatic effect of β3TA, a localization study was undertaken using a new method of tritium analysis developed by Jacobson *et al.*(11).

Materials and methods. The tritium-labeled beta-3-thienyl-L-alanine was prepared from acetyl beta-3-thienyl-DL-alanine with the tritium label attached to the beta carbon of the alanine side chain. The synthesis of the radioactive acetyl-beta-3-thienyl-DL-alanine was carried out by W. G. Brown, Arthur R. Lepley and Frederick H. Greenberg who also prepared a non-radioactive sample of the same compound (Dept. of Chemistry, Univ. of Chicago). Infra red spectra of the 2 compounds were identical. The method of synthesis is to be published later. The specific activity of the radioactive compound was 170.4 mc/g. This compound was resolved into its D and L isomer, and only the L-isomer was used in this study. The method of resolution was patterned after the procedure for beta-2-thienylalanine used by Ferger and Du Vigneaud(10). The specific rotation of the brucine salt of the D-isomer in methanol was $[\alpha]_D^{25} = -37.9°$ and that of the L-isomer in pyridine was $[\alpha]_D^{25} = -61.5°$. These rotations resembled the values of −38.7 and −57.8° for the D and L brucine formyl beta-2-thienylalanine diastereoisomers reported by Ferger and Du Vigneaud(10).

After removal of brucine the resulting acetyl-L-beta-3-thienylalanine, dissolved in ethanol, gave a specific rotation of $[\alpha]_D^{25} = +39.8°$ and the acetyl-D-beta-3-thienylalanine $[\alpha]_D^{25} = -39.96°$. The free beta-3-

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thienyl-L-alanine was obtained by hydrolytic removal of the acetyl group. Optical rotation of the free amino acid was not measured because of the small amount available.

Tritium labeled L-isomer of β3TA was force fed to the Murphy-Sturm lymphosarcoma-bearing adult male rats. The animals were killed at intervals and samples of tissues, urinary water and urinary solids were analyzed for tritium.

Holtzman adult male rats of average weight of 277 g were injected subcutaneously with a suspension of Murphy-Sturm lymphosarcoma into the right subscapular region. The rats were then given Purina rat chow *ad libitum* for a period of 12 days. By that time the rats had reached an average weight of 317 g and tumors were growing rapidly. For 4 subsequent days the animals were force fed 24 cc of maintenance amino acid diet (MAA) daily in 3 equal doses as described previously(6); the dietary phenylalanine was, however, replaced by equimolecular amounts of DL-β3TA. By the end of the fourth day the tumors had stopped growing. The animals were divided into 3 groups, each comprising one control and 2 experimental animals. On the 5th day all rats received a last tube feeding of 8 cc of β3TA containing MAA. The 6 experimental animals received simultaneously an oral dose of 60 microcuries of tritium labeled L-β3TA. From then on, all rats were fasted but allowed to drink water *ad libitum*. Group I was bled to death under ether anesthesia 5 hours, Group II 29 hours and Group III 53 hours after the last feeding. The animals were then rapidly autopsied and the following organs removed, weighed, samples placed into preweighed tubes and immediately frozen in a deep freeze: kidneys, liver, tumor, testes, jejunal mucosa, pancreas, femoral bone marrow, spleen, gastrocnemius muscle, adrenals, thymus, cerebellum, xiphoid cartilage and tibial bone. Representative wet samples of all these tissues, weighing between 20-100 mg, were used for tritium analysis.

All animals had been kept in individual metabolism cages after the last feeding and the urine was collected and preserved for analysis. Feces and expired air were not analyzed.

Tritium analysis. Wet tissue samples weighing 20-100 mg were dried under vacuum until no further decrease in weight was obtainable. Dry sample weights ranged from 7 to 20 mg after completion of the procedure. The tissue water was counted; the dried tissues were further processed to determine their radioactivity by the method of Jacobson, *et al.*(11). Scintillation counting was performed in a tri-carb counter. It was ascertained that the efficiency of the instrument remained stable during single counting sessions while it varied between 0.7%-3.1% from day to day. In order to make determinations performed on different days comparable, it thus became necessary to report all results in absolute number of disintegrations per minute per mg of dry tissue (DPM/mg dry tissue), rather than in efficiency dependent CPM/mg of dry tissue.

A similar procedure was used for determination of urinary tritium content; 300 mg samples of filtered urine were frozen and the urinary water evaporated under vacuum, collected and its radioactivity determined. The remaining urinary solids, weighing between 7-18 mg, were combusted and processed in the same manner as the dry tissue samples.

Results. The tritium content of various tissues is listed in Table I. The turnover of the label was studied in kidney, liver, tumor and testis. No conclusions should be drawn from results obtained for rat F because of its death about 4 hours before the scheduled killing; late antemortem pathophysiological changes such as congestion of the liver and postmortem enzymatic action may have altered the relative tritium content of the tissues.

As expected with a major excretory organ, the tritium turnover of the kidney was rapid while that of the other tissues was slower. For all tissues studied, the decrease in radioactivity was much sharper during the first 24 hour period than during the second one.

The absolute uptake of label does not vary markedly for organs of rats of the same group. On the other hand, it can be readily seen from Table I that there was a stable

TABLE I. Distribution of Tritium in the Organ Solids after Feeding of Tritium Labeled β-3-thienyl-L-alanine.

	Group I Killed 5 hr after feeding radioactive β3TA			Group II Killed 29 hr after feeding			Group III Killed 53 hr after feeding		
	Control	Rat A	Rat B	Control	Rat C	Rat D	Control	Rat E	Rat F*
Kidney	0	7.00†	3.40	0	3.00	1.20	0	.59	.96
Liver	0	2.20	1.80	0	1.00	1.10	0	.58	1.10
Tumor	0	.82	.61	0	.31	.26	0	.12	.18
Testis	0	.65	.33	0	.19	.16	0	.11	.13
Jejunum				0	2.30	1.30			
Pancreas				0	1.30	1.00			
Bone marrow				0	—	.46			
Spleen				0	.35	.23			
Gastrocnemius muscle				0	.40	.06			
Adrenal				0	.16	.16			
Thymus				0	.15	.17			
Cerebellum				0	.16	.09			
Xiphoid cartilage				0	.00	.00			
Bone				0	.00	.00			

* Rat F died shortly before scheduled killing.
† All results are expressed in 10^3 DPM/mg of dried tissue.

hierarchy of relative uptake by various tissues. The study of urinary tritium shows that only about 2% of the total radioactive dose was excreted during the first 5 hours (Table II). No tritium was detected in the urinary solids. About one-fifth of the total administered dose was excreted in urine during the first 29 hours; 35-40% of this amount was present in urinary solids.

Although this experiment was not designed to account for the total amount of labeled material, available data permit a very gross estimate of various fractions of the total dose. While there was considerable variation from tissue to tissue and from rat to rat, the water evaporated from individual tissues contained on the average approximately 240 DPM/mg of H_2O. With this and other presented data it may be estimated that the total administered dose of 60 μc of tritium was distributed 29 hours after ingestion as follows: 1. 20-30 μc as tritiated tissue water; 2. 10-15 μc tissue bound (as protein, polypeptide, free amino acid or amino acid metabolite); 3. 10-15 μc in urine (about 60% as tritiated water and remainder as urinary solids); 4. 5-15 μc elsewhere (*e.g.*, in feces, expired air, evaporated as tritiated water from collecting vessel in metabolism cage). This, admittedly very rough, estimate would indicate that, after 29 hours, more than 50% of the total administered label appears in the form of tritiated water.

Discussion. The phenomenon of *in vivo* incorporation of essential amino acid analogues into tissue proteins is yet to be firmly established(12). In this study, the relatively slow turnover of labeled β3TA in liver, tumor and testis would indicate that a sizable proportion of the tritiated compound remains

TABLE II. Urinary Excretion of Tritium After Feeding of Tritium Labeled β-3-thienyl-L-alanine.

	Group I (5 hr)			Group II (29 hr)		
	Control	Rat A	Rat B	Control	Rat C	Rat D
Total vol of urine excreted	9 cc	6 cc	8.5 cc	42 cc	36 cc	25 cc
No. of μc excreted in urinary water	0	1.4	1.0	0	7.7	6.0
% of total dose fed	0	2.3%	1.7%	0	13%	10%
No. of μc excreted in urinary solids	0	0	0	0	6.5	3.3
% of total dose fed	0	0	0	0	11%	5.6%
Total μc excreted in urine	0	1.4	1.0	0	14.2	9.3
% of total dose fed	0	2.3%	1.7%	0	24%	16%

tissue-bound. It is more likely that β3TA is incorporated into a larger molecule rather than being adsorbed or broken down into free metabolites. In both of these latter situations the labeled substance would be expected to diffuse rapidly out of the cell by equilibration with the extracellular compound of the same chemical nature.

The fact that a high tritium content was found in metabolically or mitotically most active tissues is also best explained by β3TA incorporation into cellular proteins.

The high tritium uptake by the pancreas is particularly noteworthy. In a parallel study, this finding was confirmed autoradiographically and, in addition, an increased concentration of the label was observed in apical portions of pancreatic acinar cells. In this connection it is well to recall the work of Hansson, who has shown fast incorporation of labeled methionine, cystine, glycine and phenylalanine into presumably newly formed digestive enzymes rather than into structural pancreatic protein(13). As an analogue of phenylalanine, β3TA may behave similarly.

While liver, pancreas, kidney and jejunal mucosa showed a large tritium uptake, that of Murphy-Sturm lymphosarcoma was unexpectedly low. It was clearly shown that under the conditions of this experiment, there was no selective uptake by the tumor. This may be partially due to the fact that the growth of the tumor was already inhibited after 4 days of force fed phenylalanine-deficient, β3TA-containing diet(6,8). Endogenous phenylalanine, resulting from tissue breakdown may have competitively prevented a more substantial uptake of labeled β3TA (7). If we accept the postulate that the oncostatic effect of an amino acid analogue is related to the amount of the compound taken up by the tumor, we can readily see that a complete inhibition of the growth of Murphy-Sturm lymphosarcoma cannot be expected from β3TA administration alone.

Since more than 50% of the β3TA tritium label appears in urinary solids or water within 29 hours after feeding, it is important to consider the possible pathways by which the tritium atom, placed in the beta position on the β3TA molecule, could rapidly appear in water. The stability of the label in aqueous solution of tritiated β3TA in various dilutions was demonstrated by evaporation of the water *in vacuo*. All radioactivity was found in the solute, no radioactivity was detected in the evaporated water. *In vivo,* however, β3TA may be rapidly broken down into simple metabolites, one of which eventually is water. Loss of tritium could also occur during enolization of the keto acid derived from beta-3-thienyl-L-alanine by transamination or oxidative deamination.

Summary. The distribution of tritium-labeled L-isomer of beta-3-thienylalanine in the tissues of Murphy-Sturm lymphosarcoma-bearing rats was studied 5, 29 and 53 hours after force feeding of the radioactive compound. For 4 days prior to that, the rats had been force fed a phenylalanine deficient, β3TA-containing amino acid maintenance diet. After 29 hours the highest tritium content was found in kidneys, liver, jejunal mucosa and pancreas. More than 50% of the total dose appeared as tritiated water within 29 hours. The tumor uptake was low.

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