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SOME METABOLIC AND ENZYMIC EXPERIMENTS WITH α-FLUORO-β-ALANINE

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SUMMARY

DL-α-Fluoro-β-alanine was found to be moderately toxic to rats, mice and chicks. The toxicity was reduced by the simultaneous administration of aminooxyacetic acid. Following injection of the fluoroamino acid into rats, a considerable proportion of the unaltered compound was recovered in the tissues and urine. The fluoro-analog was not utilized for transamination by a liver-enzyme preparation. It was a fairly good substrate for carnosine synthetase *in vitro*, but was poorly incorporated into skeletal muscle dipeptides in rats and chicks.

The α-fluoro-β-alanine was not active for pantothenic acid synthetase of *Escherichia coli*. It supported growth of a β-alanine-dependent strain of *Saccharomyces cerevisiae* only when used at high concentrations. By employing fluoro-[^{3}H]β-alanine under these conditions, it was possible to demonstrate labeling in the coenzyme A fraction of yeast.

INTRODUCTION

β-Alanine is formed in animal tissues from the catabolism of dihydrouracil[1], and it participates in transamination (or deamination) reactions in mammalian cells[2] and in microorganisms[3,4]. The biosynthesis of the β-alanyl dipeptides, carnosine and anserine, by enzymes of vertebrate muscle has been investigated[5–7], while the mode of formation of pantothenic acid from β-ala..ine and pantoic acid has been examined with an enzyme from *Escherichia coli*[8].

In connection with studies on organic fluorine compounds[9], it appeared of interest to synthesize an analog of β-alanine, with a fluorine atom substituted for hydrogen on the α-carbon position[10], and to explore the behavior and metabolism of this compound in animals. Also, the possible utilization of DL-α-fluoro-β-alanine for CoA synthesis was investigated in yeast. In addition, the analog was tested with three of the enzyme systems concerned with the metabolism of β-alanine: carnosine synthetase, pantothenate synthetase and a transaminase. In some of the experiments, the tritium-labeled fluoro compound was employed.

MATERIALS AND METHODS

Enzymes and their assay

Carnosine synthetase was an ethanol-precipitated protein fraction from chicken pectoral muscle[5]. The assay employed [1-^{14}C]histidine, non-isotopic β-amino acid, Mg^{2+} and ATP.

Pantothenate synthetase was prepared from *E. coli* cells by NOVELLI's method[8], but carried only to the stage of dialyzing the buffer-extracted acetone powder. His method of assay was modified as follows: The β-amino acid was used in radioactive form in the reaction mixture. At the end of the incubation, 4 volumes of ethanol were added, and the precipitated protein removed by centrifugation. Suitable aliquots of the supernatant solution were subjected to chromatography on Whatman No. 1 paper, with *n*-butanol–88% formic acid–H_2O (75:15:10) as solvent. R_F's were: β-alanine, 0.22; α-fluoro-β-alanine, 0.08; D-pantothenic acid, 0.65. The latter substance was eluted from the developed chromatogram with water, and its isotopic content determined.

The source of transaminase was an acetone powder prepared from fresh rat liver. 1 g powder was suspended in 20 ml of 0.05 *M* phosphate–0.05 *M* Tris (1:1) (pH 8.1). The suspension was dialyzed for 2 h at 0° against this buffer. Enzyme activity was measured by the method of ROBERTS AND BREGOFF[2], except that isotopic α-ketoglutaric acid was employed in the incubation mixture, together with a non-labeled β-amino acid and pyridoxal phosphate. Protein-free filtrates of the reaction mixtures were prepared as in the assay for pantothenic acid synthesis. For paper chromatographic separation of the glutamic acid formed, the solvent system water-saturated butanol–formic acid (95:5) was used. R_F's were: α-ketoglutaric acid, 0.55; glutamic acid, 0.17. The results were corrected for endogenous transamination (in the absence of added β-amino acid).

Biochemicals

CoA was purchased from Sigma Chemical Co., St. Louis (U.S.A.); aminooxyacetic acid hemihydrochloride from Distillation Products Industries, Rochester, (U.S.A.); and pantoyl lactone from Mann Research Laboratories, New York (U.S.A.); the latter compound was converted to pantoic acid by mild acid hydrolysis.

Radioactive amino acids

α-[5-^{14}C]Ketoglutaric acid (2.7 mC/mmole) was purchased from California Corporation for Biochemical Research, Los Angeles (U.S.A.). [1-^{14}C]β-Alanine was a preparation previously synthesized[11]. DL-α-Fluoro-β-alanine[10] was tritiated by the WILZBACH technique[12], and after exchanging labile positions, the amino acid was purified by double paper chromatography, first with pyridine–*n*-butanol–H_2O (1:1:1), and subsequently with *n*-butanol–acetic acid–H_2O (75:15:10). Its specific radioactivity was approx. 0.12 mC/mmole.

Measurement of radioactivity

Analysis for ^{14}C was performed by counting samples on metal planchets in a flow-gas Geiger counter with an ultrathin window. When necessary, values were corrected for self-absorption. In the case of tritium, samples of negligible mass were

counted on planchets in a windowless flow-gas counter, while larger quantities of material were measured in a liquid scintillation counter. For these determinations, BRAY's mixture was used[13].

Analysis of animal tissues

α-Fluoro-[^{3}H]β-alanine was injected intraperitoneally into animals in specified dosages. After sacrifice, the corresponding tissues of each group of animals were pooled and treated as follows:

For total ^{3}H content, samples were homogenized with 80% dioxane. 1-ml aliquots of the homogenates were added to 12 ml of the phosphor mixture.

For chromatographic analysis of extracts, portions of the pooled tissues were homogenized with 9 parts of 5% trichloroacetic acid. After centrifuging, the supernatant solutions were extracted 3 times with an equal volume of ether (to remove the trichloroacetic acid), and then concentrated to 0.1 the original volume. Suitable quantities of these concentrates were chromatogrammed on paper, with the solvent system *n*-propanol–NH_4OH–H_2O (6:3:1) (see ref. 14). This method does not distinguish between fluoroacetic acid (R_F 0.62) and fluoro-β-alanine (R_F 0.64), but permits the separation of fluorocitric acid (R_F 0.24) as a sharp region. The desired sections of the chromatograms were eluted with water and dried on planchets for counting.

Analysis of urine

Small aliquots of the urine, collected from rats, were dried on planchets for determination of total ^{3}H. Other samples were chromatogrammed, as described above.

Isolation of muscle dipeptides

A 70% ethanol extract of muscle was concentrated to 0.1 its volume, and then added to a column of Whatman standard-grade cellulose powder, equilibrated with *n*-butanol–acetic acid–H_2O (75:15:10). After washing the column with this same solvent (thereby removing all constituents of the muscle extract except the dipeptides), the carnosine plus anserine fraction was eluted with pyridine–H_2O (2:1) as a single peak, and aliquots of the eluate were dried on planchets for counting.

Cultivation of yeast

Saccharomyces cerevisiae, ATCC 7752, was maintained on nutrient agar slants. Cell suspensions made from the slants were used to inoculate the synthetic culture medium[15]. The latter was cultivated in test tubes under aerobic conditions, but without aeration, usually at 30°. Maximum growth occurred in 2–3 days. With β-alanine supplementation under optimum circumstances, this growth corresponded to an absorbancy reading of about 0.8 (Klett colorimeter) and a yield of 1.6 g protein/l.

In some cases the effect of α-fluoro-β-alanine was tested, either alone, or together with β-alanine in the medium. In other experiments, in which [^{14}C]β-alanine was employed, the cells were subsequently centrifuged, and washed 3 times with water, before counting samples on planchets.

Analysis of yeast grown in the presence of labeled fluoro-β-alanine

The cells were carried through 3 successive series of cultivations in media con-

taining 1 mg fluoro-β-alanine/ml. without recourse to agar slants. This insured virtually complete elimination of endogenous β-alanine (derived from the original inoculum). Cells from the third stage were used to inoculate 10 ml of medium containing 5 mg of fluoro-[^{3}H]β-alanine. After a 3-day cultivation period, the yeast was centrifuged and washed 3 times with water. Small aliquots of the cells were used to measure the total ^{3}H content. The remainder of the yeast was extracted twice with 4 parts of 10% phenol[16]. The combined phenolic extracts were concentrated, and several aliquots were analyzed as follows: (a) directly for total ^{3}H, (b) hydrolyzed for 4 h at 110° in 4 N HCl, and subsequently chromatogrammed on paper with butanol–acetic acid–H_2O, in order to determine ^{3}H in the total fluoro-β-alanine, (c) chromatogrammed without prior treatment, in order to isolate free fluoro-β-alanine, CoA, and other ^{3}H-compounds. The solvent employed was ethanol–NH_4OH–H_2O (18:1:1).

Determination of LD_{50} of fluoro-β-alanine

For each species studied, groups of animals (usually 5–6 individuals per group) were injected (I.P.) with a neutralized solution of the fluoro compound, at several closely spaced levels. The results are considered accurate within ± 10%.

RESULTS

Toxicity of DL-*α-fluoro-β-alanine in animals*

Table I summarizes the findings with three species. Fluoropyruvic acid has approximately the same degree of toxicity as α-fluoro-β-alanine, *i.e.*, they both have an LD_{50} of 80 mg/kg in rats and mice[17]. In contrast, fluoroacetic acid with an LD_{50} of about 5–7 mg/kg in chickens and 0.1–5 mg/kg in rats[18], and fluoro-acetoacetate with LD_{50} = 2.5 mg/kg in rats[19], are far more potent compounds.

In case of fluoro-β-alanine, there were no immediate symptoms of distress in the animals. Disturbances (convulsions) were apparent only after about 2 h. At, or slightly above the 50% threshold level, death occurred within 2–4 h after administration of the compound, or else the animals recovered without apparent permanent injury.

Another interesting finding was that aminooxyacetic acid, H_2N–O–CH_2–COOH, afforded a marked degree of protection against the toxicity and lethal action of fluoro-β-alanine in mice. Since the LD_{50} of the former compound was found to be about 40–50 mg/kg, it was not used above half this level. It may be seen (Table I) that

TABLE I

TOXICITY OF FLUORO-β-ALANINE IN BIRDS AND MAMMALS

Compound(s) tested	*Species*	*Number of animals*	*Weight range (g)*	*LD_{50} (mg/kg)*
Fluoro-β-alanine	Chicks	20	55–60	85
Fluoro-β-alanine	Rats (white)	12	92–101	90
Fluoro-β-alanine	Mice (white)	22	13–18	100
Fluoro-β-alanine + aminooxyacetic acid*	Mice	16	13–15	> 250

* Used invariably at 20 mg/kg, calculated as the free acid. The two neutralized amino acids were injected together.

aminooxyacetic acid raised the LD_{50} of the fluorooanalog to at least 250 mg/kg. There was no noticeable toxicity at the 200 mg/kg level, and no fatalities at 250 mg, in the presence of the oxy compound.

It may be mentioned that the injection of large quantities of pyridoxal phosphate (100–200 mg/kg) in chicks failed to overcome or prevent the toxic action of fluoro-β-alanine (data not included).

Elimination of tritium in urine of rats

The fluoro amino acid was eliminated at a moderate rate from the animal body, as judged by the ^3H appearing in the urine. Some 56 % of the isotope was recovered in less than three days (Table II). In experiments with [2-^{14}C]fluoroacetate, 32 % of the ^{14}C was recovered in the urine of rats in four days[14].

Chromatographic analysis of the 5-h urine of Table II revealed that 88 % of the ^3H was in a narrow region (R_F 0.62–0.65) corresponding to fluoro-β-alanine. It must be pointed out that fluoroacetate would have the same R_F (see ref. 14). However, no appreciable labeling was found in the fluorocitrate zone (R_F 0.21–0.25), nor elsewhere along the paper. In view of the relative volatility of fluoroacetic acid under the conditions employed, its detection would have been difficult in any case. Results quite comparable to those in Table III were obtained with a different solvent: water-saturated *n*-butanol–formic acid (95:5). GAL and coworkers[14] found that, following administration of [2-^{14}C]fluoroacetic acid, more than half of the urinary ^{14}C was due to fluoroacetate (steam-volatile), while less than 10 % represented fluorocitrate.

TABLE II

RECOVERY OF RADIOACTIVITY FROM THE URINE, FOLLOWING ADMINISTRATION OF FLUORO-[^{3}H]β-ALANINE TO RATS

The young animals (average weight, 118 g) were each injected (I.P.) with 1.5 mg of fluoro-[^{3}H]β-alanine. The pooled urine was collected.

Time elapsed (h)	*5*	*18*	*30*	*42*	*68*
Isotope recovered (%)	40	10.5	2.9	1.8	1.2

TABLE III

TRITIUM CONTENT OF CERTAIN ORGANS OF THE RAT, FOLLOWING INJECTION OF FLUORO-[^{3}H]β-ALANINE

Rats weighing 100–105 g were each given 1.5 mg of labeled amino acid. Two animals were sacrificed at each time interval.

Time (h)	*Percent of ^3H recovered/g fresh tissue*			
	Intestine	*Kidney*	*Liver*	*Skeletal muscle*
4	3.95	2.60	2.05	0.76
20		0.49	0.35	0.14
40		0.29	0.22	0.12
66		0.24	0.16	0.11

Distribution of tritium in body tissues

At 4 h after injection of fluoro-[^{3}H]β-alanine into rats, the intestinal mucosa had the highest ^3H concentration of the tissues tested, followed by kidney and liver,

while skeletal muscle was considerably lower (Table III). In the study[14] with [2-^{14}C]-fluoroacetate at 4 h the highest level of isotope was found in brain, followed by heart, liver and kidney, while intestine had somewhat lower activity than these organs. It may be seen in Table III that significant concentrations of radioactivity were still present in tissues after 2–3 days.

Chromatography of tissue extracts

Using the solvent system *n*-propanol–NH_4OH–H_2O with samples of rat tissues from 4-h experiments, a well-defined radioactive region was found on paper chromatograms, corresponding to the R_F of fluoro-β-alanine. This radioactivity accounted for 71% of the ^{3}H in liver, and 84% of that in intestine. Insignificant labeling was present in the fluorocitrate region.

Labeling in muscle dipeptides

Table III showed that after about 20 h, skeletal muscle lost its remaining radioactivity rather slowly. Carnosine and anserine are known to have a long half-life in muscle[11], and it was of interest to determine whether the above isotopic content reflected the incorporation of fluoro-[^{3}H]β-alanine into these peptides. However, analysis revealed (Table IV) that the fluoro analog was rather poorly utilized for this purpose in rats and chicks. It can be estimated that less than 1/5 of the ^{3}H of rat skeletal muscle represented dipeptides after one day. Table IV shows that isotopic β-alanine was incorporated approx. 9 times more extensively than fluoro-β-alanine in

TABLE IV

COMPARISON OF FLUORO-β-ALANINE WITH β-ALANINE AS PRECURSOR OF MUSCLE DIPEPTIDES

Groups of 2 chicks (50 g body weight) and 2 young rats (60–70 g) were injected with either [1-^{14}C]β-alanine or fluoro-[^{3}H]β-alanine at a level of 30 mg/kg. The animals were sacrificed after 24 h.

Tissue	*Incorporation of isotope into peptides (per cent administered dose/g muscle)*	
	With [^{14}C]β-alanine	*With fluoro-[^{3}H]β-alanine*
Chick pectoral muscle	0.30	0.035
Rat gastrocnemious muscle	0.21	0.025

both species. However, this comparison does not take into account possible differences in metabolic behavior of the D- and L- forms of the fluoro compound. Attempts to favorably influence the incorporation of radioactive fluoro-β-alanine into carnosine peptides by the simultaneous administration of aminooxyacetic acid to mice were unsuccessful (data not shown).

Utilization of fluoro-β-alanine by yeast

Fig. 1 shows that the fluoro analog was less than 1% as effective as β-alanine in supporting growth of *S. cerevisiae*. Even at a level of 100 mg/l, optimum growth was not achieved with the fluoro-amino acid.

It was found (Fig. 2) that fairly high concentrations of the analog inhibited neither the utilization of β-alanine for growth, nor the incorporation of [^{14}C]β-alanine into yeast cells. The maximum radioactivity attained corresponds to the uptake of 65% of the labeled amino acid added to the medium. Analysis of an acid hydrolysate of the cells showed that over 90% of the ^{14}C was in the form of β-alanine.

Attempts to adapt *S. cerevisiae* to fluoro-β-alanine by prolonged cultivation in media containing high concentrations of this amino acid were not successful. After three successive 3-day cultivations in media containing 1 mg fluoro-β-alanine/ml,

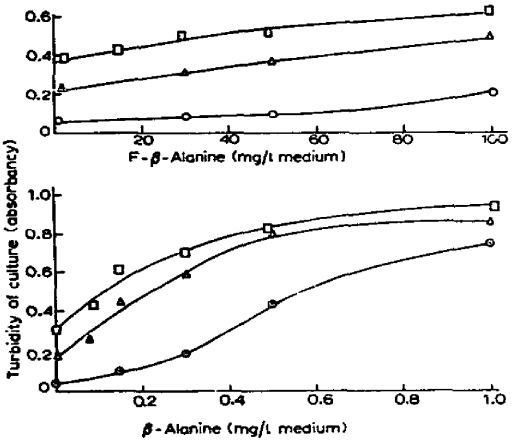

Fig. 1. Comparison of fluoro-β-alanine with β-alanine in promoting growth of *S. cerevisiae*. Cultivation time: O—O, 28 h; △—△, 40 h; □—□, 56 h.

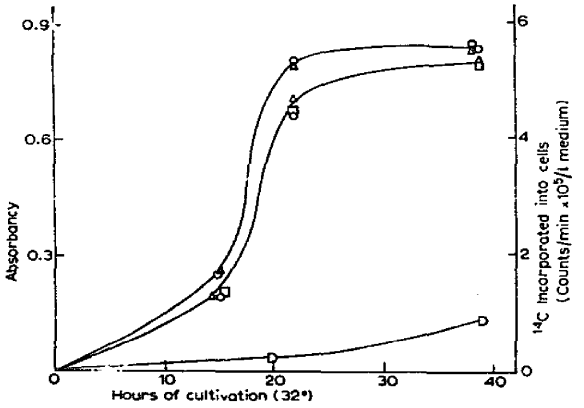

Fig. 2. Utilization of β-alanine by yeast cells in the presence of fluoro-β-alanine. O—O, 0.9 mg [^{14}C]β-alanine/l; □—□, 1 mg (non-labeled) β-alanine/l; △—△, 0.9 mg [^{14}C]β-alanine + 30 mg fluoro-β-alanine/l. D—D, no β-amino acids added (control). Open symbols, absorbancy. Solid symbols, radioactivity.

the yeast cells showed no change in sensitivity to low concentrations of this amino acid. However, by using a high level of ^{3}H-labeled fluoro derivative, it was possible to introduce considerable isotope into yeast cells (Table V). The latter took up 6.8 mg of fluoro compound/l of medium in 3 days. Of this radioactivity, 70% was extracted by aqueous phenol, 3% appeared to be in protein combination (washed trichloroacetic acid precipitate), and 27% was unaccounted for. Approximately 91% of the ^{3}H in the phenolic extract was recovered as fluoro-β-alanine, following hydrolysis with strong acid. This compound could be easily distinguished chromatographically from β-alanine. Direct chromatography of the (unhydrolyzed) extract (Fig. 3) showed that the isotope was largely distributed among three major fractions: CoA (36%), free fluoro-β-alanine (27%), and a component (14%) whose chromatographic properties closely resembled those of pantothenic acid. Results similar to those in Fig. 3 were obtained with a different solvent system, butanol–formic acid–H_2O (75:15:10). It must be emphasized that this present evidence for the incorporation of fluoro-β-alanine into CoA and pantothenic acid is still of a tentative character.

Enzyme systems involving fluoro-β-alanine

It was found (Fig. 4) that carnosine synthetase of muscle utilized the DL-fluoro-

β-amino acid about 40% as well as an equivalent amount of β-alanine. This result is comparable to those obtained for certain other β-alanine analogs, such as γ-amino butyric acid, and confirms the view that the substrate specificity requirements of the enzyme are not too rigid[20]. As in the experiments *in vivo*, it is not known whether both D- and L-isomeric forms of the fluoro derivative were substrates for dipeptide synthesis.

Table VI shows that higher concentrations of fluoro-β-alanine partially inhibited the incorporation of [^{14}C]β-alanine into pantothenic acid. The figures (marked with

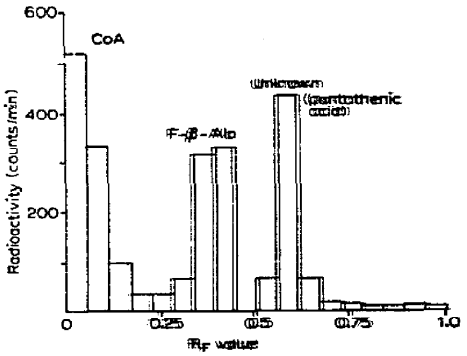

Fig. 3. Paper chromatography of a portion of phenolic extract of labeled yeast. Solvent: ethanol–NH_3–H_2O ((18:1:1)). 1 M NH_4OH was used to eluate the radioactive regions from the developed chromatogram.

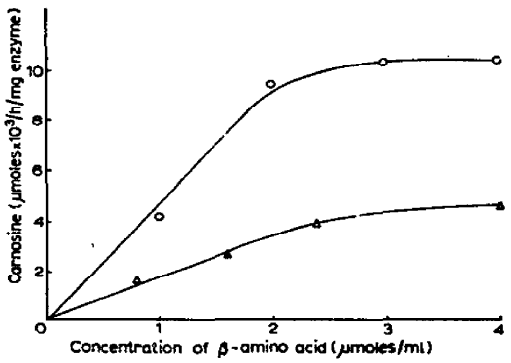

Fig. 4. Comparison of fluoro-β-alanine with β-alanine as substrates for carnosine synthetase. ○—○, β-alanine; △—△, fluoro-β-alanine.

TABLE V

ANALYSIS OF ^{3}H-LABELED YEAST

Cells were cultivated for 72 h in 10 ml of medium containing 6 mg fluoro-[^{3}H]β-alanine (total of 1860000 counts/min).

Fraction analyzed	*^{3}H-content counts/min*
Washed cells	21 800
Phenolic extract of cells	15 200
Total fluoro-β-alanine in acid hydrolysate of phenolic extract	13 800
Cellular protein	700

TABLE VI

EFFECT OF FLUORO-β-ALANINE ON PANTOTHENIC ACID SYNTHESIS

The assay system contained 10 μmoles D-pantoic acid, 4 μmoles [1-^{14}C]β-alanine, 20 μmoles ATP, 20 μmoles $MgSO_4$, 10 μmoles KCl, 20 μmoles Tris buffer (pH 8.5), 0.2 ml enzyme, and specified quantities of fluoro-β-alanine, in a total volume of 1 ml. The mixtures were incubated for 0.5 h at 37°.

Fluoro-β-alanine added (μmoles)	*0*	*20*	*50*	*120*	*150*	*4**
^{14}C found in pantothenate (%)	47	48	39	9.5	9.0	1*

* In this experiment fluoro-[^{3}H]β-alanine was used in place of [^{14}C]β-alanine.

TABLE VII

RELATIVE TRANSAMINATION OF β-ALANINE AND α-FLUORO-β-ALANINE

Each tube contained 0.3 ml liver-powder suspension, 4 μmoles [5-^{14}C]α-ketoglutaric acid, 16 μmoles β-amino acid, 0.15 μmole pyridoxal phosphate, and 0.05 *M* Tris buffer (pH 8.1) to give a total volume of 0.5 ml. The mixtures were incubated at 37°.

β-Amino acid employed	*Per cent of ^{14}C found in glutamic acid*		
	0.5 h	*1 h*	*1.5 h*
β-Alanine	7.2	12.2	14.3
α-Fluoro-β-alanine*	— 3.1	— 3.9	— 4.8

* The negative values indicate the percentages by which the radioactivity was depressed below the endogenous level of transamination.

asterisks) on the extreme right-hand side of the table indicate that fluoro-[^{3}H]β-alanine itself was not utilized appreciably by the *E. coli* enzyme.

An attempt to transaminate [^{14}C]ketoglutaric acid with fluoro-β-alanine in the presence of a liver preparation was unsuccessful (Table VII). β-Alanine was reasonably well utilized under the same conditions. Other experiments with higher concentrations of the fluoro analog gave added indication that the latter compound inhibited the transamination process.

DISCUSSION

The present work reflects certain of the difficulties inherent in the tritium labeling method as applied to systems *in vivo*. Only three stable positions for labeling with ^{3}H were available in the fluoro-β-alanine molecule. While decarboxylation could have proceeded without the loss of tritons, the deamination process would have removed at least one, and possibly two, radioactive atoms. Hence the ^{3}H-labeled compound was not well suited for degradative studies, and no exhaustive attempts were made to follow its pathways of catabolism. However, information has been obtained on other aspects of the metabolic behavior and stability of the fluoroamino acid.

It is well known that β-alanine is very rapidly deaminated and catabolized in animals[11,21,22]. It has been suggested that the first degradation product is malonic acid semialdehyde[22]. While the carboxyl carbon of β-alanine is extensively converted to CO_2, the α- and β-carbons appear to be utilized to a considerable extent for acetic acid formation.

The observation that extracts of liver acetone powder do not promote the transamination of ketoglutarate by fluoro-β-alanine appears to be significant in relation to the considerable quantities of this amino acid recovered in body tissues and in urine of rats. Despite the absence of significant amounts of ^{3}H in the fluorocitrate regions of chromatograms, the possibility cannot be excluded that a minor proportion of the fluoroamino acid was deaminated and transformed into fluoroacetic acid *in vivo*.

BAXTER AND ROBERTS[23] found that the injection of aminooxyacetic acid into rats resulted in an increased β-alanine concentration in liver and in kidney. They suggested that this effect represented an *in vivo* inhibition of the transamination process. Likewise, MCCORMICK AND SNELL showed that the same compound was an inhibitor of pyridoxal phosphokinase *in vitro*. In the absence of similar evidence in the

case of fluoro-β-alanine, no explanation can presently be offered for the observed protective action of the oxyamino acid against the toxicity of the fluoro compound, nor is the latter effect understood.

It may be of interest to mention that β-fluoro-α-amino isobutyric acid was found by us to be non-toxic in rats and mice, with an LD_{50} greater than 250 mg/kg. This compound resembles in structure "non-metabolizable" α-aminoisobutyric acid[23].

Fluoropyruvate is thought not to be converted to fluoroacetate or fluorocitrate[17,26], nor is the cause of the convulsions produced by this substance known.

Unlike animal tissues, yeast cells were not injured nor inhibited by an excess of fluoro-β-alanine (Fig. 2). This may reflect an inability of the yeast to readily absorb or catabolize this compound. In contrast, fluoropyruvic acid completely inhibits growth of *E. coli* cultures at 20 μg/ml[17]. When a very high concentration of the fluoro analog was employed in the yeast nutrient medium, a moderate degree of growth occurred, although not as good as in the presence of β-alanine.

In Fig. 2, the maximum uptake of ^{14}C corresponded to 650 μg β-alanine, or 7.3 μmoles/l of medium. From Fig. 3 and Table V, it can be calculated that 1700 μg or 15.7 μmoles of fluoro-[3H]β-alanine were incorporated presumably into (fluoro) CoA/l. It is possible that the radioactive CoA fraction contained other labeled substances, such as higher fluoro-CoA intermediates, and acyl-, or other fluoro-CoA derivatives. In any case, the agreement between the ^{14}C and ^{3}H values seems reasonable.

Although the synthetase of *E. coli* was unable to condense fluoro-β-alanine with pantoic acid (Table VI), it is likely that yeast cells did succeed in synthesizing fluoropantothenic acid. The component of high R_F in Fig. 3 probably signifies an accumulation of the latter substance, and may reflect a partial block in the pathway of CoA synthesis.

Future studies with ^{14}C-labeled fluoro-β-alanine and with synthetic fluoro-pantothenic acid may help to elucidate in greater detail the metabolic transformations of these analogs in animals and in microorganisms.

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THYROIDAL IODIDE TRANSPORT

IV. THE ROLE OF ION SIZE

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SUMMARY

1. The ability of various anions to react with the iodide-transport system of sheep-thyroid slices has been investigated. K_m and K_{ii} values ranged from $3–5 \cdot 10^{-7}$ M to $2 \cdot 10^{-2}$ M to give a series of increasing K values: $TcO_4^- \leqslant ClO_4^- < ReO_4^- < BF_4^- < SeCN^- \simeq SO_3F^- < SCN^- < I^- < NO_3^- \ll NO_2^- < OCN^- \simeq Br^-$.

2. These were compared with the partial molal ionic volumes at infinite dilution, Φ_0, which shows that this series also follows a decreasing order of Φ_0 (with the exceptions of ReO_4^-, SO_3F^- and $SeCN^-$). A linear relation exists between the pK values and the partial molal ionic volumes over the range of 25–46 ml/mole. Although the pK's decline with larger volumes, a clear-cut maximum was not observed. No similar correlation exists between the pK and other size parameters.

3. All of the anions (except TcO_4^-, which was not tested) were shown to be competitive inhibitors of iodide transport by double reciprocal plot analysis.

4. The importance of size, univalency and shape for anion transport in thyroid tissue are briefly discussed in relation to certain physical properties of the ions.

INTRODUCTION

The role of ionic size in anion transport by thyroid tissue has been suspected ever since it was found that the Br^- ion is concentrated to a lesser extent than iodide[1]. It was subsequently found that the halide, $^{211}_{85}At^-$, was well concentrated by thyroid glands[2], whereas the smaller ions Cl^- and F^- were not. The complex anions of periodic