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Effects of acetylsalicylic acid on serum protein binding and metabolism of tryptophan in man

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In normal persons, ingestion of aspirin causes a release of tryptophan from its binding site on serum albumin. There is a fall in bound and total serum tryptophan concentrations and a rise in free tryptophan concentrations. The urinary excretion of 3-hydroxyanthranilic acid is decreased, that of xanthurenic acid is increased and that of 3-hydroxykynurenine was increased in 4 out of 6 subjects, indicating an effect on the enzyme systems involved in the metabolism of tryptophan. Conjugates of these metabolites were shown to interfere with the method of assay of the unconjugated hydroxy acids by column chromatography. To overcome this difficulty all urine samples were first boiled in molar hydrochloric acid to hydrolyse any conjugates present. Any results obtained using non-hydrolysed urines would be misleading. This work shows that it is important to take account of the drugs used in treatment before ascribing changes in tryptophan metabolism to pathological states.

Abnormal tryptophan metabolism has been reported in a variety of human pathological conditions including rheumatoid arthritis (Price, Brown & Yess, 1965). McMillan (1960) reported that patients with rheumatoid arthritis excreted increased quantities of the tryptophan metabolite 3-hydroxyanthranilic acid. Subsequently, increased urinary excretion of kynurenine (Bett, 1962), 3-hydroxykynurenine (Pinals, 1964; Flinn, Price & others, 1964) and xanthurenic acid (Speira, 1966) has been reported. Prolonged treatment with salicylates in rheumatoid arthritis was reported to have no effect on the excretion of kynurenine, 3-hydroxykynurenine, xanthurenic acid or kynurenic acid (Bett, 1963).

L-Tryptophan is the only amino-acid bound to human serum albumin to an appreciable extent (McMenamy & Oncley, 1958). Any variation in serum albumin concentration therefore would affect that of bound L-tryptophan. In rheumatoid arthritis low serum albumin concentrations may occur (Sydenes, 1963), so it would be expected that the amount of tryptophan bound to serum albumin would be less.

Competition for the L-tryptophan binding site on serum albumin has been demonstrated with a wide variety of compounds including salicylates (McArthur & Dawkins, 1969), fatty acids (Kotake, 1964), thyroxine (Tritsch & Tritsch, 1963) and clofibrate (O'Mahony, D. R., unpublished). It is possible that other acidic compounds may also compete with tryptophan for this binding site, and consequently affect tryptophan metabolism. In addition, it is known, that adrenal corticosteroids increase the activity of tryptophan pyrrolase (Altman & Greengard, 1966) and the urinary excretion of certain tryptophan metabolites (Rose & McGinty, 1968). These factors should be considered in any studies on the metabolism of tryptophan.

Although sodium salicylate has been shown *in vitro* to compete with tryptophan for the binding site on serum albumin (McArthur & Dawkins, 1969), aspirin

(acetylsalicylic acid) was used in the present study since this is the form in which salicylates are given to patients with rheumatic diseases. Aspirin was administered to normal persons and its effect on the binding of tryptophan to serum protein, and on the excretion of tryptophan metabolites, investigated.

The excretion of tryptophan metabolites in normal urines and urines collected after a tryptophan load has also been examined. The importance of hydrolysing any conjugates of 3-hydroxyanthranilic acid and 3-hydroxykynurenine in urine samples before assay of these metabolites has been investigated.

MATERIALS AND METHODS

Materials

Visking dialysis tubing (0·65 cm inflated diameter) was obtained from the Scientific Instrument Centre.

3-Hydroxy-DL-kynurenine and xanthurenic acid were obtained from Koch-Light Laboratories Ltd., Colnbrook, and L-tryptophan from BDH Chemicals Ltd., Poole, Dorset. 3-Hydroxyanthranilic acid and Dowex-50W x 12 (200–400 mesh) ion exchange resin were supplied by Sigma Chemical Company, St. Louis.

All other chemicals were of analytical grade and distilled water was used throughout.

Collection of samples

Eight healthy adult student volunteers (6 male, 2 female, aged 21–23 years) were studied. Blood samples (50 ml) were taken at 10 a.m., after a light breakfast of coffee or tea and toast. On the same day, 1800 mg of acetylsalicylic acid (6 tablets of aspirin) were ingested between 9 and 11 p.m. and a further 1800 mg between 8.30 and 9.30 a.m. on the following morning; 50 ml blood samples were taken at 10 a.m., allowed to clot at room temperature and then centrifuged at 3000 rev/min for 5 min. The serum was removed and samples taken for determination of tryptophan (free + total) and salicylate. Normal urine, and urine after administration of aspirin were collected for the 14 h from 11 p.m. on day 1 until 1 p.m. on day 2.

Tryptophan loading

L-Tryptophan (5 g) was administered orally as a suspension in yoghurt at 10 a.m. to six student volunteers. Blood samples (20 ml) were taken immediately after to obtain zero time levels of serum tryptophan and then at hourly intervals until 4 p.m., and the sera collected. On a subsequent occasion aspirin was administered to the same students as described above, and 5 g L-tryptophan ingested before collection of the first blood sample at 10 a.m. Blood samples (20 ml) were then taken at hourly intervals until 4 p.m. Urine was collected for 6 h after ingestion of the tryptophan load, acidified with hydrochloric acid and stored at −20°.

Dialysis of serum

Visking dialysis tubing was soaked in distilled water for 4 h before use. Sacs of the Visking tubing, containing 1 ml of distilled water, were immersed in 8 ml serum and dialysis allowed to proceed for 20 h at room temperature (McArthur & Dawkins, 1969). Samples of the dialysate were then taken for estimation of free tryptophan.

Determination of free and total tryptophan in serum

Tryptophan (free and total) was estimated by its conversion to the norharman derivative according to the method of Hess & Udenfriend (1959) and its fluorescence measured spectrofluorometrically.

Free tryptophan. Samples (0·5 ml) of dialysate were added to water (1·65 ml) and 20% w/v trichloroacetic acid (TCA) (0·85 ml); formaldehyde (0·2 ml 20% w/v) was then added and the solutions heated in a boiling water bath for 25 min, before addition of hydrogen peroxide (0·2 ml, 6% w/v). The solutions were then boiled for a further 25 min. After cooling, the fluorescence of the solutions was measured on an Aminco Bowman spectrofluorometer, activation 365 nm, emission 440 nm.

Total tryptophan. Samples (1 ml) of serum were deproteinized by addition of water (4 ml) and ice-cold TCA (2 ml 20% w/v). After centrifugation at 3000 rev/min for 5 min, 3 ml samples of the supernatant were taken for formation of the norharman derivative of tryptophan.

Bound tryptophan. The amount of tryptophan bound to serum protein was calculated by subtraction of the free from the total concentration.

Determination of serum salicylate

The salicylate levels (total and free) in serum were assayed using the method of Trinder (1954).

Determination of tryptophan metabolites in urine

Xanthurenic acid was determined according to Satoh & Price (1958). Separation of the tryptophan metabolites, 3-hydroxykynurenine and 3-hydroxyanthranilic acid was by ion-exchange chromatography using modifications of the methods of Brown & Price (1956) and Heeley (1965). The specificity of this method has been confirmed by comparison with gas-liquid chromatography (Rose & Toseland, 1967). Columns (3·5 × 1·2 cm) of Dowex-50W resin, H^+ form, were equilibrated with 0·5M HCl in glass chromatography columns (10 × 1·2 cm). They were operated under gravity. Since tryptophan metabolites are known to be conjugated in the body, the presence of conjugates of 3-hydroxyanthranilic acid and 3-hydroxykynurenine in the urine samples was studied as follows. Urine was collected for 6 h after oral administration of L-tryptophan (5 g) to one normal subject and to two patients with rheumatoid arthritis. Samples of these urines before and after acid hydrolysis were chromatographed. To hydrolyse any conjugates present, the urines were boiled for 1 h in 1M HCl, cooled and diluted to 0·5M HCl before application to the columns.

The urine samples collected from the students were acid hydrolysed before column chromatography. The hydrolysates were diluted with distilled water to 0·5M HCl and samples containing less than 2 mg of each metabolite applied to the columns. The columns were washed with 40 ml of 0·5M HCl and the eluates discarded. The columns were then eluted with 2M HCl followed by 5M HCl; 80 and 20 ml fractions were collected in each case and analysed separately to ascertain separation of the two metabolites. 3-Hydroxyanthranilic acid was contained in the 2M HCl fraction and 3-hydroxykynurenine in the 5M HCl fraction. Standard solutions of both metabolites were boiled in M HCl and chromatographed under the same conditions. Mean recoveries of these metabolites were 95–98%. These metabolites were only

determined in urines collected after ingestion of a tryptophan load since the method was insufficiently sensitive to detect the low levels present in normal urines.

Standard solutions of 3-hydroxyanthranilic acid and 3-hydroxykynurenine were prepared in 2 and 5M HCl respectively. Samples (3 ml) of these solutions containing 0–100 μg/ml were diazotized by treatment with 0·2 ml 0·25% w/v sodium nitrite. After 3 min, ammonium sulphamate (0·2 ml 10% w/v) was added and the absorbance of the solutions read immediately at 370 nm (Brown, 1957) against a water blank. Aliquots (3 ml) of eluates were analysed similarly. In addition, the absorbance of the eluates (3 ml + 0·4 ml 10% ammonium sulphamate) were measured and subtracted from the diazotized values.

Thin-layer chromatography of the 2 and 5M HCl eluates was carried out to ascertain complete separation of 3-hydroxyanthranilic acid and 3-hydroxykynurenine. The 2 and 5M HCl eluates obtained from the non-hydrolysed urines of a normal subject and patients with rheumatoid arthritis were chromatographed before and after boiling for 1 h in M HCl, and the results compared with those of the hydrolysed urines. Thin-layer chromatography was carried out using 10 cm plates, the adsorbent being cellulose powder MN 300. The solvent system was sodium acetate buffer M, pH 5·4. The metabolites and reference compounds were detected by their fluorescence in ultraviolet light (254 nm).

RESULTS

Effect of aspirin on serum tryptophan concentration in normal subjects

After ingestion of aspirin, the mean free tryptophan concentration in the serum of eight subjects rose from 4·0 to 6·0 μM and that of tryptophan bound to serum protein decreased from 32·0 to 17·0 μM (Table 1). The total (bound + free) tryptophan in serum decreased from 36·0 to 23·0 μM in the presence of salicylates. These results show that salicylates significantly reduce the binding capacity of serum protein for tryptophan with a consequent increase in the free tryptophan concentration. The serum concentrations of salicylate were 100–175 μg/ml. The concentration of tryptophan determined in the dialysates (free tryptophan) has been multiplied by

Table 1. *Tryptophan concentrations in serum of eight normal subjects before and after ingestion of aspirin.*

	Tryptophan concentration in serum (μM)					
	Total		Free		Bound	
Subjects	Normal level	After aspirin	Normal level	After aspirin	Normal level	After aspirin
I	32·7	17·2	3·3	4·1	29·4	13·1
II	25·8	21·8	3·7	4·7	22·1	17·1
III	32·1	21·8	3·3	7·8	28·8	14·0
IV	34·3	25·2	2·9	4·7	31·4	20·5
V	48·3	26·8	5·4	7·2	42·9	19·6
VI	32·3	23·3	4·3	6·3	28·0	16·9
VII	32·1	13·5	3·2	4·9	28·9	8·5
VIII	52·9	35·4	6·2	7·8	46·7	27·6
Mean μM ± s.d.	36 ± 9	23 ± 6	4 ± 1	6 ± 1	32 ± 8	17 ± 5
% Change in mean after aspirin		−36%		+50%		−47%
P Value		0·01		0·01		0·01

1·125 to correct for the dilution during dialysis. This correction factor does not, however, account for any small change in the equilibrium of bound to free tryptophan which may occur on dilution of the serum. The results have been analysed by the Wilcoxon Paired Difference test, the minimal acceptable level of significance being taken as $P = 0{\cdot}10$.

Effect of aspirin on serum tryptophan concentration after a loading dose of tryptophan

Total, free and bound serum tryptophan concentrations rose to a maximum between 1 and 2 h after ingestion of a load of L-tryptophan, the results showing a 10–12 fold increase in bound and total and a 30-fold increase of free tryptophan (Table 2). Similar results were obtained after ingestion of tryptophan and aspirin. The tryptophan concentrations at 6 h however, were markedly lower in the presence of salicylates and approached normal.

Table 2. *Tryptophan concentrations in serum of six normal subjects after ingestion of* (a) 5*g* L-*tryptophan and* (b) 1·8 *g aspirin and* 5 *g* L-*tryptophan.*

Time after ingestion of tryptophan (h)	Tryptophan concentrations in serum (μM ± s.d.)					
	Total		Free		Bound	
	Without aspirin	After aspirin	Without aspirin	After aspirin	Without aspirin	After aspirin
0	34 ± 7	22 ± 3	4 ± 1	6 ± 1	30 ± 6	16 ± 3
1	413 ± 166	365 ± 44	109 ± 38	123 ± 42	304 ± 138	242 ± 24
2	435 ± 102	396 ± 25	148 ± 49	120 ± 23	287 ± 71	276 ± 34
3	324 ± 94	309 ± 53	95 ± 53	75 ± 32	233 ± 79	234 ± 32
4	200 ± 32	196 ± 53	46 ± 18	52 ± 9	154 ± 15	144 ± 55
6	126 ± 62	67 ± 8	18 ± 7	22 ± 11	108 ± 61	45 ± 5

In the aspirin experiments, the total (free + bound) salicylate concentration in serum from six subjects was determined at hourly intervals for 6 h after ingestion of tryptophan. The total salicylate values were in the range 100–175 μg/ml serum. Free salicylate in serum was calculated by estimation of free salicylate in the dialysates. Free salicylate varied between 65 and 90% of the total concentration in normal sera and in sera after a tryptophan load.

Excretion of tryptophan metabolites

The excretion of the tryptophan metabolites 3-hydroxykynurenine, 3-hydroxyanthranilic acid and xanthurenic acid was determined in urines before and after the ingestion of aspirin (Table 3).

In each subject studied there was a significant decrease in the excretion of 3-hydroxyanthranilic acid and an increase in xanthurenic acid in the urines collected after ingestion of aspirin. An increase in the excretion of 3-hydroxykynurenine was observed in 4 out of the 6 subjects studied. Aspirin produced a large increase in the ratio 3-hydroxykynurenine : 3-hydroxyanthranilic acid and a decrease in the ratio 3-hydroxykynurenine : xanthurenic acid and in the ratio 3-hydroxyanthranilic acid : xanthurenic acid.

The effect of aspirin on the normal excretion of xanthurenic acid without prior administration of tryptophan was studied. In the presence of salicylates the excretion

Table 3. *Excretion of tryptophan metabolites,* 3OH-kynurenine, 3OH-anthranilic acid and xanthurenic acid were measured in urine collected for 6 h from 6 normal subjects after oral administration of (*a*) 5 g L-tryptophan and (*b*) 1·8 g aspirin + 5 g L-tryptophan. Each value represents the mean (μM/6 h ± standard deviation).

Metabolite	After 5 g L-tryptophan μM ± s.d.	After 5 g L-tryptophan + 1·8 g aspirin μM ± s.d.	% Increase or decrease after aspirin	*P* Value
3OH-Kynurenine ..	176 ± 60	211 ± 99	+20%	N.S.*
3OH-Anthranilic acid	97 ± 32	62 ± 33	−36%	0·10
Xanthurenic acid ..	50 ± 22	96 ± 48	+92%	0·05
$\frac{\text{3OH-KYN}}{\text{3OH-ANT}}$	1·81	3·40		0·05
$\frac{\text{3OH-KYN}}{\text{XA}}$	3·52	2·19		0·05
$\frac{\text{3OH-ANT}}{\text{XA}}$	1·94	0·65		0·05

* Not significant.

of xanthurenic acid increased by 45% from 12·9 ± 6·9 to 18·7 ± 8·9 μM (*P* 0·05) without a tryptophan load compared with 92% after a tryptophan load (Table 3).

Table 4 gives the results obtained for the estimation of 3-hydroxyanthranilic acid, 3-hydroxykynurenine and any conjugates of these compounds in the 2 and 5M HCl eluates from the column chromatography of urines before and after acid hydrolysis. In each subject there appears to be more 3-hydroxyanthranilic acid excreted than 3-hydroxykynurenine in the eluates obtained from non-hydrolysed urines. This is reversed if these urines are hydrolysed before column chromatography.

Thin-layer chromatography of the 2 and 5M HCl eluates, from hydrolysed urines revealed complete separation of 3-hydroxyanthranilic acid from 3-hydroxykynurenine, i.e. 3-hydroxyanthranilic acid in the 2M HCl eluate and 3-hydroxykynurenine in the

Table 4. *Column chromatography of urine, before and after acid hydrolysis.* Excretion of 3OH-anthranilic acid and 3OH-kynurenine after administration of 5 g L-tryptophan to one normal subject (*a*) and to patients with rheumatoid arthritis (*b*) and (*c*).

		3OH-anthranilic acid (2M eluate) mg/6 h	3OH-kynurenine (5M eluate) mg/6 h	$\frac{\text{3OH-KYN}}{\text{3OH-ANT}}$
Non-hydrolysed urine	(a)	18*	14	0·78
	(b)	220*	104	0·47
	(c)	91*	65	0·71
Hydrolysed urine	(a)	14	30	2·14
	(b)	57	374	6·50
	(c)	20	148	7·40

* 2M eluate of non-hydrolysed urine contained 3OH-anthranilic acid and a conjugate of 3OH-kynurenine.

5M HCl eluate. The fluorescence and R_F values of two spots observed corresponded to the authentic reference compounds. Thin-layer chromatography of the 2M HCl eluates from the non-hydrolysed urines however, revealed several spots. One spot gave the fluorescence as 3-hydroxykynurenine and another similar to 3-hydroxyanthranilic acid but with different R_F values to these compounds. A third spot corresponded to 3-hydroxyanthranilic acid.

Thin-layer chromatography of these 2M HCl eluates after boiling resulted in the appearance of a spot identical with 3-hydroxykynurenine. The 5M HCl eluates from the non-hydrolysed urines revealed one spot identical with 3-hydroxykynurenine.

DISCUSSION

Salicylates and tryptophan binding to serum protein

The results presented indicate competition of salicylate with tryptophan for the binding site on serum protein *in vivo* since bound tryptophan was reduced by about 50% after ingestion of aspirin. A similar results has previously been demonstrated *in vitro* after adding sodium salicylate to pooled human serum (McArthur & Dawkins, 1969). Free tryptophan, however, *in vivo* did not rise to the concentrations obtained *in vitro*, and the total serum tryptophan (free + bound) was much reduced in the presence of salicylates. Presumably, *in vivo* the tryptophan freed from its binding site diffuses into tissues and becomes available for metabolism or excretion, hence the observed reduction in total serum tryptophan.

In the presence of salicylates, there was no significant difference in the concentration of bound tryptophan 2–4 h after the 5 g tryptophan load; the normal bound serum tryptophan concentrations before loading and that 6 h after the tryptophan load, were significantly reduced (Table 2). However, the values obtained between 2 and 4 h were difficult to interpret since the time curves and concentrations obtained for total serum tryptophan varied considerably during this time in three of the six subjects studied. These differences may have been due to variation in the rate of absorption of tryptophan from the small intestine. After a load of tryptophan, the very high serum concentrations occurring between 2 and 4 h appear to result in preferential binding of tryptophan to serum albumin. However, at the lower physiological concentration of tryptophan, salicylates are able to compete with the binding sites giving rise to the observed reduction of bound tryptophan. At zero time (i.e. before loading) the ratio total salicylate : tryptophan is 30 : 1; at 6 h after ingestion of the tryptophan load the ratio is 8 : 1. At these concentrations the displacement of tryptophan by salicylates is marked. During the 2–4 h after the tryptophan load however, the ratio is between 2 : 1 and 5 : 1. From these ratios it would seem that tryptophan is more firmly bound to serum albumin than salicylates, since relatively high concentrations of salicylates are required to show a marked displacement of tryptophan.

The carboxyl group of tryptophan is one of the stereospecific requirements for its binding to serum albumin (McMenamy & Oncley, 1958). These workers concluded that the *N*-terminal group of albumin is the ionizable group involved at the protein-binding site. Similar results were obtained in studies of the binding of L-thyroxine to human serum albumin; the α-amino-group of the amino-terminal aspartic acid residue of the protein being a vital part of the strong binding site (Tritsch & Tritsch, 1963). At pH 7·35 tryptophan is a competitive inhibitor for thyroxine binding but

not at pH 8·70. Tritsch & Tritsch concluded that "other groups of presently unknown identity are involved in both sites, and that the binding sites of tryptophan and thyroxine are not identical but overlap at least around the region of the amino-group of the aspartic acid residue". Acidic drugs containing carboxyl groups, such as salicylates, may bind to albumin by competition with tryptophan for the carboxyl group binding site. Clofibrate (chlorphenoxyisobutyric acid), another acidic drug, was reported to combine with plasma proteins and to displace albumin-bound tryptophan in rats (O'Mahony, D. R., unpublished). In addition, a fall in the serum tryptophan was observed. Competitive displacement of tryptophan and other naturally occurring metabolic substrates, like hormones and fatty acids, may be a factor in the mode of action of such drugs.

Urinary excretion of tryptophan metabolites

The procedure used for assay of 3-hydroxyanthranilic acid and 3-hydroxykynurenine will also detect conjugates of these compounds. Any such conjugates present in the urine will therefore also be determined. The results obtained (Table 4) indicate the presence of conjugates of these metabolites in non-hydrolysed urines. This was confirmed by thin-layer chromatography in which a conjugate of 3-hydroxykynurenine was detected in the 2M HCl eluate of non-hydrolysed urine. This conjugate of 3-hydroxykynurenine, possessing different physical and chemical properties to the free hydroxy acid, is eluted in column chromatography by 2M HCl rather than by 5M HCl which elutes the free hydroxy acid. This finding explains the decrease in 3-hydroxyanthranilic acid and increase in 3-hydroxykynurenine in the eluates from the chromatography of hydrolysed urine compared with non-hydrolysed urine in all the subjects. The figures obtained for the total excretion of 3-hydroxyanthranilic acid and 3-hydroxykynurenine in the 2 and 5M HCl eluates are less with non-hydrolysed urine than with hydrolysed urine. It is possible that other conjugates of both compounds were lost from the columns in the initial washings with 0·5M HCl. In the present work it was not necessary to identify the conjugates of these metabolites since all were hydrolysed by boiling in M HCl to give the free hydroxy acids. To obtain complete separation of these metabolites it is therefore necessary to hydrolyse any conjugates which may be present in the urine before column chromatography. Since the degree of conjugation clearly differs from subject to subject, any results obtained using non-hydrolysed urines will be misleading.

Overall effects of salicylates on tryptophan metabolism

In the presence of salicylates the rate of metabolism of tryptophan and the excretion of its metabolites may be affected by the associated changes in serum tryptophan concentrations and by the effect of salicylates on the enzymes involved in the metabolism of tryptophan. Since free serum tryptophan is increased in the presence of salicylates it is possible that more tryptophan would enter the kynurenine pathway and this could perhaps account for the observed increase in xanthurenic acid, but not the decrease in 3-hydroxyanthranilic acid. Tryptophan, itself at very high levels, is known to activate the first enzyme of this pathway namely tryptophan pyrrolase in various species (Knox & Mehler, 1950). However, in man, given a test load of 2 g L-tryptophan there was no increase in the activity of tryptophan pyrrolase (Altman & Greengard, 1966). It is therefore unlikely in the present work that this enzyme

is activated by the small increase in free tryptophan observed *in vivo* after aspirin, without a tryptophan load.

It has recently been reported that salicylates at a concentration of 1 mM inhibit the enzyme tryptophan pyrrolase *in vitro* by 74% (Satoh & Moroi, 1969). Since this was the mean serum salicylate level determined *in vivo*, it is more likely that the tryptophan pyrrolase activity is inhibited by salicylates rather than activated by the small increase in free tryptophan. Any increase in the excretion of tryptophan metabolites may simply be due to the presence of more tryptophan available for entrance to the pathway. Salicylates, *in vitro* inhibit the enzyme 3-hydroxyanthranilic acid oxidase (Vescia & di Presco, 1962). One would therefore expect a block in the pathway at this point with an increase in the excretion of 3-hydroxyanthranilic acid. Since this is not observed it seems probable that salicylates may either be exerting an inhibitory effect on the kynureninase enzyme responsible for the conversion of 3-hydroxykynurenine to 3-hydroxyanthranilic acid or activation of the amino-transferase enzyme involved in the formation of xanthurenic acid (Fig. 1). Salicylates are known to be generally inhibitory with regard to transaminases (Gould & Smith, 1965). However, salicylates have been found to activate L-tryptophan-α-oxoglutarate aminotransferase (Gould & Smith, 1965).

The present work shows that it is important to take account of the effects of drugs used in treatment before ascribing changes in tryptophan metabolism to pathological states. Patients with rheumatoid arthritis frequently take aspirin. This may influence tryptophan metabolism by increasing the level of free tryptophan in the blood and by changing the pattern of the urinary excretion of its metabolites.

In conclusion, the presence *in vivo* of salicylates in blood results in the competitive

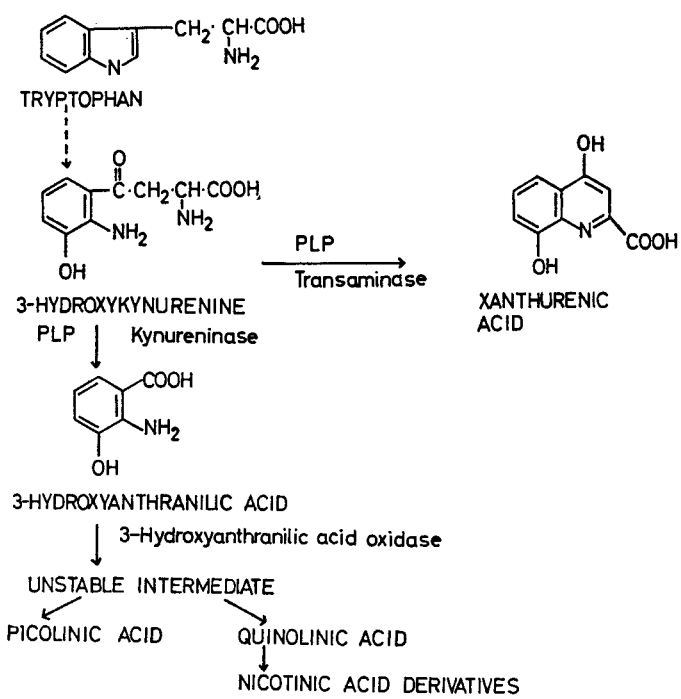

FIG. 1. Pathways of tryptophan metabolism. PLP = Pyridoxal phosphate.

displacement of tryptophan bound to serum albumin and a lowering of the total tryptophan in serum, and to an alteration in the pattern of excretion of at least two of the tryptophan metabolites in the kynurenine pathway, indicating an effect on the enzyme systems involved in the metabolism of tryptophan. The presence in the urines of conjugates of the metabolites studied clearly indicates the necessity to hydrolyse all urine samples before using column chromatography to estimate these compounds.

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