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The effects of salicylate on the concentrations of amino-acids in mouse tissues

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The concentrations of amino-acids in chopped preparations of mouse liver incubated with 0 to 20 mM salicylate were measured. The changes, observed with salicylate concentrations of 10 mM and above, were increased concentrations of aspartate, glutamine, tyrosine and ornithine and decreased concentrations of glutamate and γ-aminobutyrate. The effects of the intraperitoneal injection of salicylate, in doses ranging from 75 to 600 mg/kg body weight, on the amino-acid concentrations in mouse blood, kidney, liver and brain were studied. With a dose of 600 mg/kg, the amino-acid concentrations were decreased in the blood (except glutamate and aspartate which increased) and in the kidney, were increased in the liver (except glutamine, glutamate, glycine and alanine which decreased) and were unchanged in the brain (except alanine, valine and leucine which decreased and γ-aminobutyrate which increased). These changes may result from a combination of an inhibitory effect of salicylate on the renal tubular transport of the amino-acids and intracellular actions of the drug on aminotransferase and other enzyme activities.

The administration of salicylate has been shown to produce amino-aciduria in man (Andrews, Bruton & Knoblock, 1961) and in the rat (Berry & Guest, 1963). It has been suggested that this is due to the drug blocking the reabsorption of amino-acids in the kidney tubules (Segal & Blair, 1963). However, Andrews, Bruton & de Baare (1962) reported that the plasma concentrations of amino-acids were elevated in salicylate-poisoned patients suggesting that there was an intracellular accumulation of amino-acids leading to an increased entry of these metabolites into the circulation and hence an increased rate of urinary excretion. There is considerable evidence that salicylate inhibits several pathways of amino-acid metabolism *in vitro*. These include transamination reactions (Gould & Smith, 1965; Gould, Dawkins & others, 1966) and incorporation into proteins (Dawkins, Gould & Smith, 1966; Burleigh & Smith, 1970). In addition, there are some limited studies indicating that the administration of salicylate *in vivo* alters the concentrations of some amino-acids in rat tissues (Yoshida, Metcoff & Kaiser, 1961; Huggins & Smith, 1963). The present investigation is concerned with the effects of salicylate on the patterns and concentrations of amino-acids in mouse tissues, *in vitro* and *in vivo*, using an automated method for the separation and estimation of the amino-acids.

EXPERIMENTAL

Materials. Amino-acids were obtained from BDH Limited, Poole, Dorset; ninhydrin, A.R. grade, from Koch-Light Limited, Colnbrook, Bucks; hydrindantin was prepared by the method of Connell, Dixon & Haines (1955), reduced and oxidized gluatathione were obtained from the Sigma Chemical Co., St. Louis, and methylcellosolve from Union Carbide Chemicals Division, Rickmansworth, Herts. All

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

Animals and design of experiments. Male albino mice, 25 to 30 g, of the King's College Hospital strain, maintained on MRC cube diet no. 41B, were killed by cervical fracture. In the *in vitro* experiments the livers were removed as rapidly as possible, washed with Krebs-Ringer phosphate (Cohen, 1957), pH 7·4, blotted and either homogenized immediately in 10 vol of saturated picric acid or cut into blocks 0·3 mm × 0·3 mm × 0·7 mm with a tissue chopper (McIlwaine & Buddle, 1953). The wet weight of tissue initially used for each experiment was 2·0 g and the chopped tissue was suspended in either 5 ml of the phosphate medium or 5 ml of the medium containing sufficient sodium salicylate to give final concentrations of between 2·5 and 20 mM. Each mixture was incubated in a 50 ml glass-stoppered conical flask for 1 h at 37° with shaking and the tissue killed by the addition of 20 ml of saturated picric acid solution. Corresponding control mixtures were obtained by adding the picric acid solution either at zero time or after incubation for 1 h. After the addition of the picric acid the mixture was homogenized in an all-glass Potter homogenizer, centrifuged at 3000 *g* for 20 min and the supernatant removed for subsequent analysis.

In the *in vivo* experiments each animal was given an intraperitoneal injection (0·5 ml) of either saline or sodium salicylate in doses ranging from 75 to 600 mg/kg body weight. Each injection was adjusted by the addition of appropriate amounts of sodium chloride to give a final sodium content of 0·52 g-ions per 100 ml. The animals were killed at time intervals varying from 0·5 to 4 h after the injection and the livers removed, homogenized in 10 vol of saturated picric acid solution and centrifuged as described above. In some experiments the kidneys and brains and samples of blood were also removed and treated as described for liver except that the brains were homogenized in 20 vol of picric acid.

Preparation of tissue extracts for analysis. Aliquots (5 ml) of each picric acid supernatant were allowed to pass through 12 cm × 0·5 cm diameter columns of Dowex 1-acetate and the amino-acids eluted with 160 ml of 0·03 M acetic acid. Under these conditions, only the picric acid and glutathione remained on the column. It was necessary to remove glutathione because the tripeptide, either in the reduced or oxidized form, interfered with the subsequent analysis. Reduced glutathione obscured aspartate and oxidized glutathione could not be separated from glycine and alanine. The total effluent was evaporated to dryness in a rotary evaporator under reduced pressure at 40° and the residue stored at − 20° until required for analysis. The stability of the amino-acids under these conditions of storage was investigated by analysing aliquots of a bulk, one in ten homogenate of mouse liver, freed from picric acid and glutathione, at intervals of one and three months.

Amino-acid analysis. The apparatus was constructed from standard Technicon Autoanalyser units arranged according to the conventional plan. The column was filled to a height of 132 cm with Chromobeads A. The Haake heating bath was modified to allow the insertion of two contact thermometers controlled by a relay and time switch so that the apparatus could be operated for various time intervals at different temperatures. The Milton Roy pump was set to deliver 0·5 ml/min. The column was regenerated by filling the line from the Autograd to the tylock connection at the top of the column (volume 15 ml) with 0·2N NaOH, connecting up to a reservoir of pH 3·0 buffer and pumping for 2 h at a flow rate of 0·5 ml/min. The manifold consisted of a sample line (Orange Clear Tygon, 0·42 ml/min), a nitrogen line (Red Clear Tygon 0·8 ml/min) ninhydrin reagent line (Yellow Solvaflex, 1·06 ml/min) and a

return line (Yellow Solvaflex, 1·06 ml/min) and was cleaned as recommended by Technicon. The pump tubes were stretched one hole after each run. Nitrogen gas (white spot, British Oxygen Company) was continuously passed through the standard Technicon ninhydrin reagent. The heating bath was operated at 95°, only a single coil being used. Buffers were prepared according to the directions given by Technicon except that varying quantities of HCl or NaCl were added to give a range of pH values and salt concentrations.

The residue from the tissue extracts was dissolved in 5 ml of 0·1 M HCl containing 0·1 mM norleucine as an internal standard and 1 ml aliquots loaded on the top of the Chromobead column by forcing in under pressure. The amino-acids were eluted with the buffer gradient produced from the Autograd*.

The temperature of the column was maintained at 35° for the first 2 h and then allowed to cool to room temperature (20°) by the time control device switching off the Haake heating bath. After a further 2 h, the temperature of the column jacket was raised to 60° and kept at this temperature for the remaining 16 h of the run. Under these conditions the breakdown of glutamine was reduced to a minimum, and a good resolution of aspartate, threonine, serine and asparagine plus glutamine was obtained with the recorder pen returning to the base line between each peak. In addition, proline was eluted before and completely separated from glutamate, citrulline appeared as a separate peak between glutamate and glycine, β-alanine was separated after phenylalanine, γ-aminobutyrate, ethanolamine and ammonia occurred as separate peaks in that order and the elution of tryptophan was accelerated so that it formed a separate peak between ammonia and ornithine.

Ninhydrin-positive peaks were initially identified by comparing their elution times with those of authentic compounds. Samples containing five times the normal amount of amino-acids were loaded on the Analyser and eluted in the normal way for peak identification. The column effluent was split so that one tenth passed into the analytical system and the remainder was collected in a fraction collector holding 200 tube positions and set to collect a fresh sample every six min. The manifold for the analytical system consisted of a sample line (Orange Blue Clear Tygon, 0·05 ml/min); a nitrogen line (Black Clear Tygon 0·32 ml/min); a ninhydrin reagent line (White Solvaflex, 0·56 ml/min) and a return line (Orange Solvaflex, 0·42 ml/min). The recorder was fitted with an event marker to denote the changing of tubes in the fraction collector. The contents of tubes corresponding to each peak on the recorder chart were bulked and the combined fraction was desalted by passing it through a 15 cm × 0·5 cm diameter column of Zeokarb 225 H^+ and washing the resin with 30 ml distilled water. The amino-acids were then eluted with 30 ml 2N ammonium hydroxide. The residue, obtained after evaporating the ammoniacal solution to dryness in a rotary evaporator, was dissolved in 1 ml of 10% (v/v) isopropanol in 0·1 N HCl and transferred to a bijou bottle. The resulting solution was evaporated to dryness in a vacuum desiccator, containing concentrated H_2SO_4 and a beaker of NaOH pellets, and the residue dissolved in 50 μl of the acidified isopropanol. The amino-acids in 5 μl aliquots were identified by paper chromatography and high voltage electrophoresis. Identification of some of the minor peaks necessitated bulking the samples from several separate analyses.

* Chambers Nos. 1–4 contained 0·2M sodium citrate† buffer pH 3·0; No. 5, 0·2M citrate† buffer pH 4·0; Nos. 6 and 7, 0·18M citrate buffer pH 5·0; Nos. 8 and 9, 0·2 M citrate buffer pH 5·0 containing M sodium chloride (Volume 75 ml). All buffers contained 0·1% (w/v) phenol and 1% (v/v) Brij 35 detergent. Those marked † also contained 0·5% (v/v) thiodiglycol.

Recovery of the amino-acids was studied by analysing portions of a mouse liver extract before and after adding known amounts of standard amino-acids and comparing their differences with separate determinations of the standard amino-acid solution. The results ranged from 93% for aspartate and glutamate to 105% for β-alanine, the overall mean recovery being 99%.

RESULTS

Amino-acid concentrations in mouse liver. The concentrations of the amino-acids in mouse liver preparations either homogenized in picric acid immediately after death, chopped into blocks before the addition of the picric acid or after incubating the chopped samples for 1h at 37° in Krebs-Ringer phosphate before adding the picric acid, are given in Table 1. The concentrations of the amino-acids in the picric acid homogenate were greater than those in the chopped preparation but subsequent incubation of the latter preparation for 1 h at 37° in the phosphate medium caused an appearance of γ-aminobutyrate, decreased concentrations of aspartate and glutamine,

Table 1. *Amino-acid concentrations in mouse liver preparations.* The whole liver was either homogenized in 10 vol of saturated picric acid or 2 g portions cut into blocks with a tissue chopper followed by homogenization in 20 ml picric acid either before or after incubation at 37° for 1 h in 5 ml of Krebs-Ringer phosphate buffer, pH 7·4. After centrifugation at 3000 *g* for 20 min each supernatant was passed through a Dowex 1-acetate column to remove the picric acid, the effluent evaporated to dryness and dissolved in 5 ml of 0·1 M HCl containing 0·1 mM norleucine. Aliquots (1 ml) were analysed for amino-acids using a Technicon AutoAnalyser system modified as described in the text. The results are given as the means ± s.d.

	Amino-acid concentration (μmol/g wet weight liver)		
Amino-acid	Whole liver (15 animals)	Chopped liver (7 samples)	Chopped liver after 1 h incubation (11 samples)
Tau*	11·07 ± 2·40	—	—
Asp	0·59 ± 0·18	0·46 ± 0·17	0·32 ± 0·03
Thr	0·18 ± 0·05	0·15 ± 0·08	0·44 ± 0·03
Ser	0·42 ± 0·07	0·42 ± 0·15	0·68 ± 0·12
Gln	3·43 ± 0·52	1·09 ± 0·54	0·87 ± 0·19
Pro	0·13 ± 0·06	0·16 ± 0·06	0·34 ± 0·11
Glu	1·93 ± 0·38	1·78 ± 0·48	2·98 ± 0·65
Cit†	0·03 ± 0·01	0·04 ± 0·02	0·06 ± 0·04
Gly	2·07 ± 0·29	1·38 ± 0·65	2·67 ± 0·33
Ala	2·08 ± 0·57	1·78 ± 0·53	6·04 ± 0·74
Val	0·18 ± 0·04	0·11 ± 0·04	0·56 ± 0·10
Met	0·03 ± 0·01	0·02 ± 0·01	0·10 ± 0·02
Ile	0·09 ± 0·03	0·04 ± 0·02	0·25 ± 0·03
Leu	0·18 ± 0·05	0·10 ± 0·04	0·45 ± 0·06
Tyr	0·07 ± 0·02	0·03 ± 0·01	0·02 ± 0·01
Phe	0·07 ± 0·02	0·04 ± 0·02	0·15 ± 0·02
β-Ala‡	0·14 ± 0·03	0·15 ± 0·03	0·57 ± 0·32
Amb§	0·00	0·00	0·82 ± 0·31
Eth‖	0·10 ± 0·04	0·14 ± 0·04	0·60 ± 0·38
Orn	0·34 ± 0·06	0·24 ± 0·11	0·39 ± 0·10
Lys	0·44 ± 0·12	0·25 ± 0·11	0·76 ± 0·11
His	0·43 ± 0·07	0·25 ± 0·12	0·45 ± 0·10
Arg	0·03 ± 0·01	0·01 ± 0·01	0·01 ± 0·01

* Taurine. † Citrulline. ‡ β-Alanine. § γ-Aminobutyrate. ‖ Ethanolamine.

and increased concentrations of the remainder except tyrosine and arginine. No significant changes in the concentrations of the amino-acids in the picric acid extract of mouse liver homogenate ocurred after storage at −20° for up to three months.

Effects of salicylate on the concentrations of amino-acids in mouse liver in vitro

The concentrations of the amino-acids in chopped preparations of mouse liver incubated for 1 h at 37° were measured in the presence and in the absence of salicylate concentrations ranging from 2·5 to 20 mM. No significant changes were observed with salicylate concentrations of 2·5 and 5 mM; at salicylate concentrations of 10 mM and above the most prominent effects were increased concentrations of aspartate, glutamine, tyrosine and ornithine and diminished contents of glutamate and γ-amino-butyrate. Alanine was reduced in the presence of only 20 mM salicylate (Table 2.)

Table 2. *Effect of salicylate on the amino-acid concentrations of chopped mouse liver preparations* in vitro. Experimental conditions as in Table 1. The results are expressed as percentages of the corresponding control values. Only those with values for $P < 0{\cdot}05$ (Student's *t*-test) are included.

Amino-acid	Salicylate concn (mM)	
	10	20
Asp	162	159
Gln	151	155
Glu	76	83
Ala	—	69
Tyr	314	496
Amb	60	26
Orn	145	123

Effects of salicylate injections on the concentrations of amino-acids in mouse tissues

A high dose of salicylate, 600 mg/kg weight, was chosen for the first set of experiments and the amino-acid concentrations in the livers of animals killed between ½ and 4 h after the injection are given in Table 3. Taurine, aspartate, proline, ethanolamine and histidine were not affected and have been excluded from the table but all the remainder showed changes. Glutamine and glutamate were significantly decreased over the 4 h period, glycine and alanine were reduced initially and the others were increased at one or more time intervals. The most widespread changes were observed at 1 h after injection. This time interval was used in the subsequent experiments in which the amino-acids were measured in blood, kidney, liver and brain of mice injected with either saline or salicylate. The results, after the administration of 600 mg/kg body weight salicylate are given in Table 4 and any changes observed with lower doses of the drug are described below. In blood the 600 mg/kg dose caused either significant decreases or no change in the concentrations of the amino-acids with the exception of aspartate and glutamate which increased significantly. This effect was less marked with lower doses of salicylate, with a 300 mg/kg dose, threonine, serine, glutamine, citrulline, alanine, valine, isoleucine, leucine and lysine were significantly decreased and with 150 mg/kg salicylate, serine, glutamine, alanine, valine, isoleucine and leucine were reduced. Similar changes were observed in the kidney except that glutamate and aspartate decreased but the brain showed a different pattern

Table 3. *Effects of* 600 *mg per kg body weight of salicylate on the amino-acids of the liver in mice killed at varying time intervals after the injection.* Each animal was given an intraperitoneal injection (0·5 ml) of either saline or sodium salicylate and killed at either ½, 1, 2 or 4 h. The liver was removed and homogenized in 10 vol of saturated picric acid and subsequently treated as described in Table 1. At each time interval six mice injected with saline and six with salicylate were used. The results are expressed as mean percentage differences from the corresponding control group. They have been analysed by Students' *t*-test and those marked * show a significant difference ($P < 0{\cdot}05$) between the control and salicylate-treated animals.

Amino-acid	Time after injection (h) ½	1	2	4
Thr	+27	+64*	+79*	+141*
Ser	+5	+61*	+36*	+59*
Gln	−60*	−64*	−54*	−41*
Glu	−64*	−47*	−44*	−18*
Cit	+84*	+104*	+125	+131
Gly	−20*	−27*	−21	+8
Ala	−26*	−56*	−5	−11
Val	+19	+72*	+65*	+113*
Ile	+8	+61*	+72*	+51*
Leu	+9	+33*	+24	+21
Tyr	−15	+35*	+21	+8
Phe	+2	+52*	+21*	+24*
β-Ala	+51	+73*	+89*	+97*
Orn	+52	+50*	+56*	+63*
Lys	+92*	+62*	+52*	+56*
Arg	−19	+79*	+27	+47

in that the only significant changes were decreased concentrations of alanine, valine and leucine and an increased concentration of γ-aminobutyrate at the 600 mg dose. An additional experiment was performed in which mice received a lethal dose of salicylate, 800 mg/kg, and were killed when they started to convulse, between 75 min and 2 h after the injection. The only changes observed in the brain amino-acids were decreased concentrations of alanine and valine. The results in the liver were similar to those given in Table 3 except that with lower doses, 300 and 150 mg/kg, only glutamine was significantly reduced and methionine and β-alanine increased.

DISCUSSION

Suspensions of animal tissues prepared with a mechanical chopper are superior to tissue homogenates for studying some aspects of metabolism because cellular structure is largely retained (McIlwaine & Buddle, 1953) and are convenient systems for the investigation of possible effects of substances added *in vitro*. The present results (Table 1) show that the concentrations of many of the amino-acids are lower in the chopped liver than in the liver homogenized in picric acid directly after removal from the animal. This decrease is probably due to transfer of amino-acids from the cut tissues into the wet filter paper on which the liver is placed during the chopping procedure. The values found in the chopped liver before incubation have been used as zero time values for comparison with those found after incubation and are not strictly comparable with values in the picric acid homogenate, which have been taken to be

Table 4. *Effects of salicylate on the concentrations of amino acids in mouse blood, liver, kidney and brain.*

Each animal was given an intraperitoneal injection (0·5 ml) of either saline or sodium salicylate and killed after 1 h. The livers, kidneys, brains and weighed samples of whole blood from groups of five animals were homogenized in either 10 or 20 vol of saturated picric acid and subsequently treated as described in Table 1. Only the results for the 600 mg per kg body weight are given and they are expressed as the means ± standard deviations, the numbers in parentheses being the numbers of groups, each of five animals. They have been analysed by Student's *t*-test and * indicates a significant difference ($P < 0.05$) between the control and salicylate-treated animals.

	Amino-acid concentration (μmol/g wet weight)							
Amino-	Blood		Kidney		Liver		Brain	
acid	Control	Salicylate	Control	Salicylate	Control	Salicylate	Control	Salicylate
Tau	0·52 ± 0·09 (7)	0·56 ± 0·13 (5)	6·26 ± 1·71 (9)	3·07 ± 0·48 (10)*	10·69 ± 3·18 (9)	11·24 ± 2·53 (10)	7·02 ± 1·19 (7)	6·53 ± 0·39 (5)
Asp	0·11 ± 0·03 (7)	0·16 ± 0·03 (4)*	1·41 ± 0·20 (9)	1·07 ± 0·25 (8)*	0·46 ± 0·11 (7)	0·52 ± 0·08 (5)	3·88 ± 0·28 (7)	4·09 ± 0·13 (5)
Thr	0·17 ± 0·01 (5)	0·09 ± 0·02 (5)*	0·27 ± 0·04 (9)	0·16 ± 0·02 (8)*	0·24 ± 0·07 (7)	0·40 ± 0·03 (5)*	0·49 ± 0·05 (7)	0·48 ± 0·04 (5)
Ser	0·19 ± 0·02 (5)	0·13 ± 0·03 (5)*	0·44 ± 0·07 (9)	0·20 ± 0·05 (10)*	0·43 ± 0·09 (7)	0·63 ± 0·06 (5)*	1·10 ± 0·14 (7)	1·09 ± 0·06 (5)
Gln	0·54 ± 0·05 (7)	0·33 ± 0·05 (5)*	1·49 ± 0·26 (9)	0·28 ± 0·07 (10)*	4·63 ± 0·71 (5)	1·81 ± 0·16 (5)*	5·79 ± 0·58 (7)	6·20 ± 0·79 (5)
Pro	0·14 ± 0·08 (6)	0·06 ± 0·02 (4)	0·08 ± 0·05 (5)	0·03 ± 0·02 (4)	0·21 ± 0·07 (7)	0·26 ± 0·04 (4)	0·13 ± 0·02 (6)	0·12 ± 0·03 (6)
Glu	0·21 ± 0·04 (7)	0·29 ± 0·04 (4)*	5·63 ± 0·75 (9)	3·00 ± 0·66 (12)*	1·87 ± 0·43 (7)	1·28 ± 0·15 (5)*	13·42 ± 2·41 (7)	13·60 ± 1·74 (5)
Cit	0·07 ± 0·01 (7)	0·05 ± 0·01 (5)*	0·04 ± 0·01 (4)	0·04 ± 0·03 (3)	0·02 ± 0·01 (7)	0·04 ± 0·01 (5)*	0·05 ± 0·01 (4)	0·05 ± 0·02 (4)
Gly	0·46 ± 0·04 (5)	0·35 ± 0·09 (5)*	3·86 ± 0·45 (9)	0·98 ± 0·18 (12)*	1·93 ± 0·27 (7)	1·51 ± 0·19 (5)*	2·11 ± 0·54 (7)	2·08 ± 0·15 (5)
Ala	0·35 ± 0·04 (5)	0·14 ± 0·04 (5)*	0·70 ± 0·11 (9)	0·31 ± 0·06 (12)*	2·55 ± 0·61 (7)	1·75 ± 0·27 (5)*	0·89 ± 0·11 (7)	0·66 ± 0·08 (5)*
Val	0·17 ± 0·02 (6)	0·09 ± 0·01 (5)*	0·16 ± 0·02 (9)	0·12 ± 0·03 (12)*	0·24 ± 0·08 (7)	0·43 ± 0·05 (5)*	0·11 ± 0·01 (7)	0·07 ± 0·02 (5)*
Met	0·03 ± 0·01 (5)	0·02 ± 0·01 (3)	0·07 ± 0·02 (9)	0·04 ± 0·01 (9)*	0·04 ± 0·01 (5)	0·09 ± 0·02 (4)*	0·04 ± 0·03 (7)	0·02 ± 0·02 (5)
Ile	0·06 ± 0·01 (7)	0·03 ± 0·01 (5)*	0·08 ± 0·01 (9)	0·05 ± 0·01 (12)*	0·13 ± 0·05 (7)	0·24 ± 0·03 (5)*	0·04 ± 0·02 (7)	0·03 ± 0·01 (4)
Leu	0·13 ± 0·01 (6)	0·07 ± 0·01 (5)	0·25 ± 0·05 (9)	0·12 ± 0·02 (12)*	0·29 ± 0·04 (7)	0·42 ± 0·03 (5)*	0·12 ± 0·03 (7)	0·08 ± 0·01 (5)*
Tyr	0·08 ± 0·02 (7)	0·07 ± 0·01 (5)	0·08 ± 0·02 (9)	0·08 ± 0·02 (12)	0·10 ± 0·04 (7)	0·17 ± 0·03 (5)*	0·10 ± 0·01 (7)	0·11 ± 0·02 (5)
Phe	0·06 ± 0·01 (7)	0·05 ± 0·02 (5)	0·07 ± 0·03 (9)	0·07 ± 0·02 (11)	0·10 ± 0·04 (7)	0·18 ± 0·03 (5)*	0·08 ± 0·02 (7)	0·08 ± 0·02 (5)
β-Ala	0·02 ± 0·01 (3)	0·03 ± 0·01 (2)	0·44 ± 0·12 (9)	0·15 ± 0·09 (9)*	0·14 ± 0·03 (7)	0·20 ± 0·03 (5)*	0·09 ± 0·03 (2)	0·11 ± 0·02 (2)
Amb	0·00	0·00	0·00	0·00	0·00	0·00	3·04 ± 0·45 (7)	3·59 ± 0·25 (5)*
Eth	0·03 ± 0·01 (3)	0·03 ± 0·01 (3)	0·50 ± 0·24 (9)	0·37 ± 0·16 (12)	0·11 ± 0·05 (7)	0·13 ± 0·05 (5)	0·73 ± 0·01 (3)	0·74 ± 0·03 (3)
Orn	0·08 ± 0·01 (6)	0·05 ± 0·01 (5)*	0·05 ± 0·01 (9)	0·04 ± 0·01 (12)*	0·31 ± 0·08 (7)	0·43 ± 0·08 (5)*	0·05 ± 0·03 (5)	0·04 ± 0·01 (4)
Lys	0·34 ± 0·04 (6)	0·26 ± 0·05 (5)*	0·22 ± 0·02 (9)	0·17 ± 0·03 (12)*	0·48 ± 0·18 (7)	0·85 ± 0·12 (5)*	0·34 ± 0·02 (7)	0·30 ± 0·04 (5)
His	0·05 ± 0·01 (7)	0·06 ± 0·03 (5)	0·14 ± 0·03 (9)	0·07 ± 0·01 (12)*	0·43 ± 0·13 (7)	0·41 ± 0·04 (5)	0·10 ± 0·03 (7)	0·11 ± 0·02 (5)
Arg	0·20 ± 0·02 (5)	0·14 ± 0·01 (5)*	0·16 ± 0·03 (9)	0·09 ± 0·02 (11)*	0·03 ± 0·01 (7)	0·05 ± 0·01 (5)*	0·21 ± 0·03 (7)	0·19 ± 0·03 (4)

more representative of concentrations of amino-acids existing *in vivo*. After incubation of the chopped mouse liver preparations in Krebs-Ringer phosphate medium, the concentrations of almost all the amino-acids increased markedly. The appearance of γ-aminobutyrate in the chopped liver preparations after incubation is consistent with the results of other workers who have shown that the presence of the amino-acid is not restricted to the brain and may occur in the liver of man (Zachmann, Tocci & Nyhan, 1966) and of the cat (Tallan, Moore & Stein, 1954) and that it appears in homogenates and sterile autolysates of mouse liver and tumours after incubation for varying periods of time (Roberts & Frankel, 1950). The mechanisms controlling the amino-acid concentrations in chopped preparations of mouse liver incubated in phosphate medium are not very sensitive to salicylate. Drug concentrations below 10 mM produced no effects and the changes observed with 10 and 20 mM salicylate were restricted to a few amino-acids (Table 2).

In contrast to the relative insensitivity of chopped preparations of mouse liver to salicylate *in vitro* the injection of salicylate into whole animals produced more marked and widespread effects on the amino-acid concentrations in the liver (Table 3). Previous work (Sturman, Dawkins & others, 1968) indicated that the expected concentrations of the drug in the livers of mice given an intraperitoneal injection of 600 mg/kg body weight of salicylate would range from 3 mM at 30 min to 0·5 mM at 4 h after the injection. Significant differences in the concentrations of a variety of the amino-acids occurred at each time interval, the effects being most marked at 1 h, when only aspartate, proline, ethanolamine and histidine were not affected. One h between injection and killing was therefore used for the later experiments which were extended to include other tissues (Table 4) and a range of salicylate doses.

The concentration of most of the free amino-acids in kidney and blood were significantly reduced after injection of high doses of salicylate, aspartate and glutamate were increased in blood. The essential amino-acids, threonine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine, histidine and arginine may be considered as a group with respect to their response to salicylate in that most of these compounds were decreased in the blood and kidney, unaffected in the brain and increased in the liver. Non-essential amino-acids which behaved in a similar manner included taurine, serine, citrulline, β-alanine and ornithine. In contrast to the group of amino-acids whose concentrations increased in the liver, there was a small group comprising glutamine, glutamate, glycine and alanine whose concentrations decreased in the liver. The decreased plasma concentrations of the amino-acids in the salicylate-treated animals may be caused by a primary action of the drug in blocking the renal tubular reabsorption of the amino-acids. Salicylate causes amino-aciduria in man (Elliot & Murdaugh, 1962; Ben-Ishay, 1964) but it has been reported (Andrews & others, 1962) that the drug also increases the plasma α-amino nitrogen concentrations and that the pattern of amino-aciduria resembled that found in hepatic injury rather than in renal tubular damage (Andrews & others, 1961). However, in the rat a dose of 450 mg/kg weight of salicylate induces a gross generalized amino-aciduria (Berry & Guest, 1963) which suggests a renal origin, although the authors considered that the pattern of amino-acid excretion was similar to those observed in several types of human liver disease. The intravenous administration of salicylate also causes amino-aciduria in the dog (Simoes & de Barros, 1955) and in this species there is some evidence that there may be competition between salicylate and some amino-acids for active sites in the renal tubular transport system (Weiner, Washington & Mudge, 1959).

Segal & Blair (1963) have also shown that salicylate *in vitro* inhibits the transport of phenylalanine, lysine and histidine into slices of rat and human kidney cortex and also accelerates the efflux of the amino-acids from these tissues. This effect may explain the decreased concentrations of amino-acids in the mouse kidney observed in the present work (Table 4). If the amino-aciduria observed in animals given high dose levels of salicylate is due to an overflow mechanism, the plasma concentrations of the amino-acids would be expected to either exceed or equal those in the controls. However, the present results do not support such a mechanism, except for aspartate and glutamate, where an overflow mechanism cannot be excluded. Webber (1963) has shown that in the dog there is a separate mechanism of renal tubular reabsorption for the acidic compared to the basic and neutral amino-acids, and the failure to see a decrease in these may be due to this transport system not being affected by salicylate. The changes in the blood amino-acid concentrations caused by the acute dose of salicylate described here, are qualitatively similar to changes in the plasma amino-acid levels produced by chronic doses of salicylates which did not produce amino-aciduria. Rats fed on a diet containing 0·5% aspirin showed elevated levels of plasma glutamate while the peak concentrations of the other amino-acids were depressed (Vaughan, Korty & Steele, 1969).

If salicylate depletes the intracellular amino-acid pools in the mouse kidney cells, other than the tubules, by decreasing influx from and increasing efflux to the circulation, this effect of the drug is far less evident in the brain, and in the liver must be antagonized and overcome by other actions of salicylate. Such actions must lead to increased formation of amino-acids in the hepatic cells and may comprise an increased rate of protein breakdown, inhibition of protein synthesis and interference with transamination. There is experimental evidence that salicylate may promote all these. The drug is known to cause a marked depletion of liver glycogen in whole animals (Lutwak-Mann, 1942) and it may also increase other catabolic processes in the liver, including protein breakdown, as part of a general stimulating action on substrate breakdown due to its uncoupling action on oxidative phosphorylation reactions (Smith, 1966). Salicylate has been shown to inhibit the incorporation of radioactive amino-acids into the protein of cell-free preparations from rat liver (Dawkins & others, 1966; Reunanen, Hanninen & Hartiala, 1967) by a mechanism involving an interference with the formation of aminoacyl-t-RNAs (Burleigh & Smith, 1970). The drug also inhibits the activities of rat tissue aminotransferases involving the interaction of 2-oxoglutarate with leucine, isoleucine, valine, serine, threonine, methionine, phenylalanine, ornithine and arginine (Gould & Smith, 1965). If salicylate differentially interfered with aminotransferase activities in the mouse liver, then this could explain the increased concentrations of the above amino-acids plus tyrosine, β-alanine and lysine, and the decreased concentrations of glutamate and alanine, observed in the present work. Any effects of salicylate on amino-acid patterns due to interference with aminotransferase activities would be expected to be most pronounced in the liver since this organ is concerned with the metabolism of dietary amino-acids entering it from the portal circulation.

One prominent difference between the effects of salicylate on the amino-acid levels in brain and the other tissues is the lack of sensitivity of the brain towards the drug. It is known (Sturman & others, 1968) that salicylate penetrates the mouse brain after injection and attains concentrations approximately equivalent to those in the other tissues. The levels of aspartate and glutamate are very much higher in the

brain than in other tissues and there are different mechanisms controlling the levels of the amino-acids in brain (Haslam & Krebs, 1963; Balazs, 1965) compared with other tissues (Williamson, Lopes-Vieira & Walker, 1967). In the brain the non-essential amino-acids are formed mainly from glucose (Gaitonde, Dahl & Elliott, 1965). The levels of essential amino-acids in this tissue are maintained to a large extent by protein breakdown within the brain (Vrba & Cannon, 1970) and the rate of entry of these amino-acids from the blood across the blood brain barrier is a much slower process than in other tissues (Neuberger & Richards, 1964). In addition, there is evidence for compartmentation of some of the amino-acid pools within the brain (Berl & Purpura, 1966) and not all the compartments may be accessible to salicylate.

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