J. Pharm. Pharmac., 1971, **23**, 519–527 *Received November* 24, 1970

The site of the inhibitory action of salicylate on protein biosynthesis *in vitro*

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Salicylate (10 mM) affects neither the transfer of the amino-acid from leucyl-tRNA into protein by rat liver polysomes nor the formation of the leucyl-tRNA. However, lower concentrations of salicylate inhibit the activities of other aminoacyl-tRNA synthetases. The most sensitive enzymes are those which incorporate glutamate, aspartate and histidine. It is suggested that salicylate interferes with the *in vitro* biosynthesis of protein by preferentially inhibiting the production of certain aminoacyl-tRNA species. The drug, in concentrations of 0·6 mM and above, inhibits the ATP-pyrophosphate exchange reaction promoted by individual amino-acids, including leucine.

High concentrations of salicylate (10–15 mM) inhibit the *in vitro* incorporation of radioactive glutamate and proline into the protein of rat costal cartilage (Bellamy, Huggins & Smith, 1963) and of labelled threonine into the epithelial proteins of sheep mucosal scrapings (Kent & Allen, 1968). Protein biosynthesis in the rat isolated diaphragm is more sensitive to salicylate and concentrations from 0·5 to 5 mM interfere with the incorporation of glutamate, glycine, lysine and leucine (Manchester, Randle & Smith, 1958; Dawkins, Gould & Smith, 1966). The inhibition also occurs in cell-free systems prepared from rat liver (Reunanen, Hanninen & Hartiala, 1967). It is independent of the well-known uncoupling action of salicylate on oxidative phosphorylation (Brody, 1956) since it was observed with microsomal preparations supplied with an external energy source.

This paper describes experiments designed to elucidate the site of the inhibitory action of salicylate on protein biosynthesis *in vitro*. The overall process was studied in two main stages, the formation of aminoacyl-transfer RNAs and the transfer of amino-acids by polysomes from these complexes to protein. The ATP-pyrophosphate exchange reaction was also measured in an attempt to study activation of the amino-acids. A preliminary account of the work has been published (Burleigh & Smith, 1970).

MATERIALS AND METHODS

Materials

The following radiochemicals were used: [U-^{14}C]protein hydrolysate from *Chlorella* (54 mCi/mAtom of carbon); L-[U-^{14}C]aspartic acid (208 mCi/mmol); [U-^{14}C]glycine (109 mCi/mmol); L-[U-^{14}C]glutamic acid (249 mCi/mmol); L-[U-^{14}C]glutamine (42 mCi/mmol); L-ring-[2-^{14}C]histidine (57·8 mCi/mmol); L-[U-^{14}C]leucine (311 mCi/mmol); L-[U-^{14}C]lysine monohydrochloride (310 mCi/mmol); L-methyl[^{14}C]-methionine (53·6 mCi/mmol); L-[U-^{14}C]phenylalanine (459 mCi/mmol); L-[U-^{14}C]-proline (255 mCi/mmol); L-[U-^{14}C]threonine (208 mCi/mmol); L-methylene[^{14}C]-tryptophan (54·5 mCi/mmol); L-[^{35}S]cysteine hydrochloride (18 mCi/mmol) and

[^{32}P]tetrasodium pyrophosphate. These were purchased from the Radiochemical Centre, Amersham, U.K.

ATP and GTP (sodium salts), transfer RNA (tRNA) from Bakers yeast (Type III) and ribonuclease-A from bovine pancreas (salt-free) came from the Sigma Chemical Company. Triton X-100, PPO and POPOP came from Packard Instrument Co. Inc. and Sephadex G-25 from Pharmacia Fine Chemicals Inc. The Whatman GF/A glass fibre discs were obtained from Gallenkamp and Co.

Animals

Male rats, 400–500 g, of the Wistar strain, maintained on M.R.C. cube diet no. 41, were killed by stunning and cervical fracture.

Preparation of polysomes

Polysomes were prepared by an adaptation of the method of Munro, Jackson & Korner (1964). Livers from freshly-killed rats were rinsed in ice-cold 0·25M sucrose in Medium M (20 mM tris-HCl buffer, pH 7·8 at 4°; 10 mM $MgCl_2$; 40 mM NaCl; 100 mM KCl and 6 mM mercaptoethanol). The livers were blotted, weighed and minced with scissors in fresh medium (3 volumes per g liver) and homogenized using five strokes of an all-glass, loose-fitting Potter-Elvehjem homogenizer surrounded by an ice jacket. The homogenate was centrifuged at 4° in a Spinco Model L ultracentrifuge for 20 min at 18 000 *g*, and the separated supernatant for a further 10 min at the same speed, to remove cell debris, nuclei and mitochondria. The post-mitochondrial supernatant was added to one-ninth of its volume of 10% (v/v) Triton X-100 pH 8·2, to give a final concentration of 1% Triton (Korner, 1969) and aliquots (5 ml) layered over 2·5 ml of 0·5M sucrose in medium M, which has been layered over 2M sucrose in medium M, and the whole centrifuged for 4 h at 105 000 *g*. The polysome pellet was rinsed with medium M and stored overnight at −20°. Immediately before use, it was resuspended in medium M (one-quarter of the volume of original homogenate). Polysomes were characterized by the RNA:protein ratio which was between 1·0 and 1·2 in all preparations used. RNA was determined according to Munro & others (1964) and protein to Lowry, Rosebrough & others (1951).

Preparation of 105 000 g *supernatant fraction*

Livers from freshly-killed rats were homogenized as described above, in 2·5 volumes/g liver of medium H (35 mM tris-HCl buffer, pH 7·8 at 4°; 0·25M sucrose; 75 mM KCl and 10 mM $MgCl_2$). The homogenate was centrifuged for 20 min at 20 000 *g* and the supernatant centrifuged for 90 min at 105 000 *g* to remove microsomes. The middle, fat-free portion of the 105 000 *g* supernatant was collected by Pasteur pipette.

Preparation of pH 5 enzyme fraction and pH 5 supernatant

The pH of 105 000 *g* rat liver supernatant was lowered by dropwise addition of 0·01N HCl with constant stirring at 2° until a pH of 5·2 was reached. The pH 5 precipitate was collected by centrifugation for 10 min at 12 000 *g*, resuspended in a quantity of 0·25M sucrose equal to one-third of the original volume of supernatant, and re-isolated. The final precipitate was dissolved in 0·4M tris-HCl buffer, pH 7·5 at 4° (one-tenth of the original supernatant volume). The solution was diluted with four vol Medium H and dialysed against 3 × 1-litre changes of medium H for at least 24 h to remove endogenous amino-acids (Decken & Campbell, 1962). For the preparation of pH 5 supernatant for use as a source of transfer enzymes in the polysome incubations, the pH of the 105 000 *g* rat liver supernatant was lowered to 5·05

and the pH 5 precipitate removed by centrifugation for 20 min at 12 500 *g*. Both the 105 000 *g* supernatant and the pH 5 supernatant fractions were passed through a Sephadex G-25 column at 2°, equilibrated with 35 mM Tris-HCl, pH 7·5 at 20° to remove endogenous amino-acids and low molecular-weight materials.

Preparation of aminoacyl-tRNA

Transfer RNA from yeast and rat liver was esterified with [^{14}C]leucine plus the following unlabelled L-amino-acids: alanine, arginine, aspartic acid, asparagine, cysteine, glycine, glutamic acid, glutamine, histidine, isoleucine, lysine, methionine, proline, phenylalanine, serine, threonine, tryptophan, tyrosine and valine.

The 105 000 *g* supernatant fraction (approximately 4 mg protein per ml final incubation volume), after passage through Sephadex G-25, was incubated for ten min at 37° with 100 mM tris-HCl buffer, pH 7·5 at 37°, 4 mM mercaptoethanol, 10 mM $MgCl_2$, 10 mM KCl, 0·25 mM CTP, 2·25 mM ATP, 0·25 mM each amino-acid, 1·25 μCi/ml [^{14}C]leucine (311 μCi/mmol) and 1 mg/ml yeast tRNA. The reaction mixture was cooled to 0° and an equal volume of 90% (v/v) phenol added (Hoagland, Stephenson & others, 1958). After two further extractions of the separated phenol layer, the three aqueous fractions were pooled and the RNA precipitated by the addition of one tenth volume of 20% (w/v) potassium acetate, pH 5, and 2·5 volumes of ethanol; the mixture was maintained for at least 3 h at −20° before isolating the RNA by centrifugation. The ethanol precipitation was repeated to remove all traces of phenol and the final tRNA precipitate resuspended in water to give a concentration of 1·5 mg/ml (approximately 100 000 counts/min per mg RNA) and dialysed against 3 × 1 litre distilled water for a total of 5 h at 2°.

Extraction of protein for radioactive assay

The reaction for the incorporation of [^{14}C]leucine from aminoacyl-tRNA into protein (see Fig. 1) was stopped by the addition of an equal volume of 10% (w/v) trichloroacetic acid (TCA). The precipitate was washed in 5 ml of 5% (w/v) TCA,

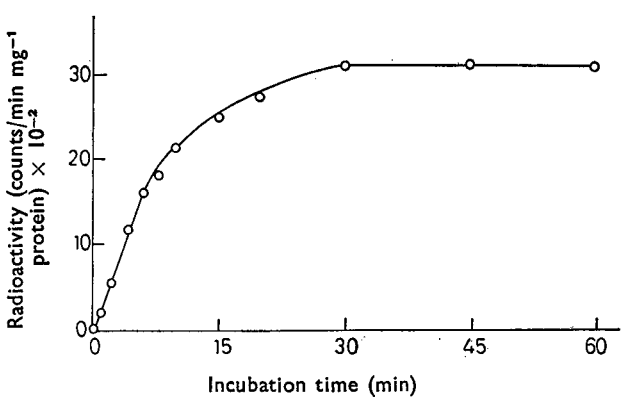

FIG. 1. Time curve for transfer of [^{14}C]leucine from aminoacyl-tRNA into protein by rat liver polysomes. Each reaction mixture contained, in 1·0 ml total volume, 3·0 μmol ATP; 0·5 μmol GTP; 300 μg aminoacyl-tRNA containing [^{14}C]leucyl-tRNA (0·015 μCi); 0·2 ml polysome suspension (140 μg RNA); 0·25 ml pH 5 supernatant (1·4 mg protein). The incubation medium was Medium M, the pH of which had been adjusted to 7·4 at 37°. The reaction was started by the addition of the polysome: pH 5 supernatant mixture and each tube was incubated at 37° with gentle shaking for an interval varying from 1 to 60 min. Counts for the zero time controls were in the range 0–5 counts/min per mg protein isolated. Each point represents the mean of at least two separate experiments.

then heated in 5 ml of 5% TCA for 20 min at 90° to remove tRNA. The precipitate was then washed successively with 5 ml quantities of 5% (w/v) TCA, ethanol–TCA (95:5 by vol), ethanol–ether–chloroform (2:2:1 by vol), acetone and ether and gently dried. The final precipitate was dissolved in 1 ml of N NaOH. All radioactive counting was performed on 0·1 ml samples dried on Whatman GF/A (2·1 cm) glass fibre discs in vials containing 5 ml of scintillation fluid [4 g 2,5-diphenyloxazole and 0·1 g 1,4-bis-(5-phenyloxazolyl-2)-benzene in 1 litre of toluene] in a Beckman LS 200B scintillation system with an efficiency of 94% for ^{14}C. Protein concentration was determined according to Lowry & others (1951) and the results expressed as counts/min per mg protein isolated.

Extraction of aminoacyl-tRNA for radioactive assay

The reaction (see Table 1) was terminated by the addition of 2 vol of ice-cold M perchloric acid (PCA) containing 20 mM tetrasodium pyrophosphate (Chambon, Ramuz & others, 1968) and the tRNA allowed to precipitate for at least 45 min at 0°. The precipitate of protein plus tRNA was washed successively with 3 ml quantities of ice-cold 0·33M PCA containing 6·7 mM pyrophosphate (two washes), ethanol–0·33M PCA (5:1 by vol) and ethanol–ether (3:1 by vol). To increase the specificity of the extraction, the aminoacyl-tRNA was extracted twice in 0·5 ml of 10% NaCl for 10 min in a water bath at 100°. The tRNA was precipitated from the pooled supernatants with two vol of ice-cold ethanol followed by standing for at least 3 h at −20°. The final aminoacyl-tRNA precipitate was dissolved in 0·5 ml of 0·1M ammonium acetate buffer, pH 9, and incubated for 10 min at 20° with pancreatic ribonuclease-A (0·05 ml, containing at least 50 μg/ml). The digestion was terminated with 0·15 ml of 2·5M acetic acid and 0·15 ml ethanol. The protein precipitate was removed by centrifugation at room temperature and the supernatant retained for counting and RNA estimation (Hurlbert, Schmitz & others, 1954). The results were expressed as counts/min mg^{-1} tRNA isolated, when necessary divided by the protein concentration in the incubation mixture.

Extraction and estimation of [^{32}P]ATP

The [^{32}P]pyrophosphate-ATP exchange reaction (see Table 3) was terminated with 2 vol of M PCA. The protein precipitate was removed by centrifugation and the supernatant was added to 0·4 ml of acid-washed Norit-A suspension (15% w/v) in water to adsorb the labelled ATP (Tsuboi & Price, 1959). After vigorous mixing and standing for 5 to 10 min, the Norit was collected and washed with three 3-ml volumes of distilled water. The [^{32}P]ATP was eluted with 3 ml of 0·3M ammonium hydroxide in 50% (v/v) ethanol and the Norit removed by filtration. The ATP concentration was estimated by measurement of the absorbance at 260 nm of sample diluted 1 in 100. The radioactivity in the samples was counted on glass fibre discs and the counts/min of the [^{32}P]ATP and of the original [^{32}P]pyrophosphate solution were corrected to zero counting time to allow for the decay of ^{32}P radioactivity. The percentage exchange (counts/min μmol^{-1} ATP $\times$ 100 $\div$ total counts/min μmol^{-1}, ATP + PP_i) was calculated by the method of Hoagland (1955).

RESULTS

Transfer of [^{14}C]leucine from aminoacyl-tRNA to polysomal protein

The time curve for the transfer of radioactivity from [^{14}C]leucyl-tRNA into protein in the presence of nineteen other non-radioactive aminoacyl-tRNAs is given in Fig. 1.

The reaction mixtures contained a polysome fraction and a pH 5 supernatant prepared from rat liver. The rate of incorporation of the radiocarbon is linear up to 6 min and reaches a steady maximum at 30 min.

Salicylate, 0·5 to 10 mM, an incubation period of 30 min, to ensure maximum incorporation of radioactivity, and incubation periods of 15 or 5 min had no effect. Nor did lower concentrations of substrate and GTP in the reaction mixtures or the exclusion of mercaptoethanol from both the polysome and pH 5 supernatant fractions.

Incorporation of radioactive amino-acids into aminoacyl-tRNAs

The effects of salicylate, in concentrations ranging from 0·5 to 10 mM, on the incorporation of radioactivity from the [^{14}C]amino-acids of a *Chlorella* protein hydrolysate into aminoacyl-tRNAs by the 105 000 *g* supernatant from rat liver are given in Table 1. In contrast to the results obtained with the transfer of amino-acid from

Table 1. *Effect of salicylate on incorporation of a radioactive protein hydrolysate into aminoacyl-tRNA.* Each reaction mixture contained, in 1·0 ml total volume, 2 μmol ATP; 10 μmol $MgCl_2$; 100 μmol tris-HCl (pH 7·5 at 37°); 500 μg yeast tRNA; 0·25 ml 105 000 *g* supernatant (0·4 mg protein); 2 μCi *Chlorella* protein hydrolysate (54 mCi/mAtom of carbon) and sufficient of a mixture of KCl and potassium salicylate to yield final salicylate concentrations ranging from 0–10 mM and a constant K^+ concentration of 20 mM. The reaction was started by the addition of the 105 000 *g* supernatant and each tube was incubated at 37° for 10 min. Counts for the zero time controls were in the range 80–130 counts/min and have been deducted from the experimental values.

Salicylate concn. (mM) ..	0	0·5	1·0	2·0	3·0	6·0	10
Counts/min* ..	10114± 493	9835± 958	9381± 457**	8786± 899**	8443± 248**	8048± 980**	7645± 404**

* Means ± standard deviations of five separate experiments.

** Statistically significant difference ($P < 0·05$) between the control and salicylate values by t-test.

the aminoacyl-tRNA to polysomal protein, the drug caused a significant inhibition at concentrations of mM and above. The specificity of the inhibition was further studied by using individual radioactive amino-acids, representing each chemical type, either in the absence or the presence of 2 or 10 mM salicylate. The results show that the amino-acids can be divided into three groups on the basis of the effects of different salicylate concentrations on their incorporation into the corresponding aminoacyl-tRNAs. The first group, comprising aspartate, glutamate, glutamine and histidine, is sensitive to 2 mM salicylate (Table 2), the second which includes cysteine, methionine, threonine and tryptophan is affected by 10 mM but not by 2 mM drug (Table 2) and the incorporation of the remainder is not inhibited by 10 mM salicylate.

ATP-pyrophosphate exchange reaction

The effect of salicylate on the ATP-pyrophosphate exchange, which has been considered to reflect the first stage in the formation of aminoacyl-tRNAs, was studied in an attempt to further define the site of action of the drug on protein biosynthesis

Table 2. *Incorporation of aspartate, glutamate, glutamine, histidine and cysteine, methionine, threonine and tryptophan into aminoacyl-tRNA.*

Salicylate conc. (mM)	Amino-acid	Counts/min mg^{-1} tRNA isolated per mg enzyme protein†	Amino-acid	Counts/min mg^{-1} tRNA isolated per mg enzyme protein‡
0		740 ± 14		6584 ± 420
2	Aspartate	708 ± 20*	Cysteine	6940 ± 241
10		654 ± 10*		5185 ± 170*
0		1339 ± 37		873 ± 32
2	Glutamate	1232 ± 25*	Methionine	831 ± 62
10		842 ± 17*		746 ± 18*
0		2978 ± 68		3242 ± 137
2	Glutamine	2778 ± 57*	Threonine	3132 ± 60
10		2330 ± 76*		3049 ± 104*
0		295 ± 10		570 ± 18
2	Histidine	274 ± 13*	Tryptophan	575 ± 18
10		246 ± 10*		534 ± 10*

* Statistically significant difference between the control and salicylate values by the *t*-test.

† Conditions as in Table 1 except that each reaction mixture contained 0·4 ml of 105 000 g supernatant (5 mg protein) and 2·0 μCi (0·05 μmol) of the individual radioactive amino-acid. Counts for the zero time controls were in the range 0–20.

‡ Conditions as in † except that 1·7 μCi (0·1 μmol) of [^{35}S]cysteine HCl plus 1 mM mercaptoethanol were used.

Table 3. *Effect of salicylate on ATP-pyrophosphate exchange promoted by a mixture of amino-acids.* Each reaction mixture contained, in 1·0 ml total volume, 50 μmol tris-HCl (pH 7·5 at 37°); 10 μmol NaF; 10 μmol ATP; 0·5 μmol of each of the 20 amino-acids (see Methods: preparation of aminoacyl-tRNA); 4·4 × 10^6 counts/min (10 μmol) tetrasodium-[^{32}P]pyrophosphate; 0·25 ml pH 5 enzyme fraction (1 mg protein) and sufficient $MgCl_2$, KCl and potassium salicylate to yield constant Mg^{2+} and K^+ concentrations of 10 mM and 20 mM respectively and final salicylate concentrations ranging from 0–10 mM. The reaction was started by the addition of the enzyme and each tube was incubated at 37° for 40 min. Endogenous exchange, measured in the absence of the amino-acid mixture, was 0·1% .

Salicylate concn (mM)	0	0·3	0·6	1·0	2·0	3·0	6·0	10·0
% exchange*	16·00 ± 0·40	15·56 ± 0·35	15·41 ± 0·27**	15·28 ± 0·51**	15·08 ± 0·47**	14·40 ± 0·37**	13·79 ± 0·46**	12·82 ± 0·40**

* Means ± standard deviations of 6 separate experiments and calculated as percentages exchanged.

** Statistically significant difference between the control and salicylate values by the *t*-test.

in vitro. This was done using an equimolar mixture of 20 amino-acids and the results (Table 3) show that the exchange reaction was significantly inhibited by salicylate concentrations of 0·6 mM and above. In addition, the exchange promoted by certain individual amino-acids, was also studied. The results (Table 4) show that the ATP-pyrophosphate exchange supported by aspartate and histidine was significantly inhibited by 2 mM salicylate whereas that associated with leucine and phenylalanine was only sensitive to 10 mM salicylate. The addition of 10 mM glutamate did not increase the ATP-pyrophosphate exchange above the endogenous level.

Table 4. *Effect of salicylate on ATP-pyrophosphate exchange promoted by individual amino-acids.* Conditions as in Table 3 except that 10 μmol of each amino-acid was added. In the experiments with aspartate and leucine each reaction mixture contained 0·25 ml pH 5 enzyme fraction (0·72 mg protein) and $4{\cdot}7 \times 10^7$ counts/min [^{32}P]pyrophosphate but in the histidine and phenylalanine experiments each tube contained 0·25 ml enzyme (0·60 mg protein) and $7{\cdot}2 \times 10^7$ counts/min -[^{32}P]pyrophosphate. The endogenous exchange was measured in each experiment and subtracted from the corresponding experimental value. In the absence of salicylate the maximum value for the endogenous exchange was 0·094 and with 10 mM salicylate the minimum value was 0·040.

Amino-acid	Salicylate concn (mM)	% exchange
Aspartate	0	0·172 ± 0·009
	2	0·154 ± 0·009*
	10	0·089 ± 0·014*
Leucine	0	0·478 ± 0·007
	2	0·428 ± 0·094
	10	0·396 ± 0·021*
Histidine	0	0·081 ± 0·016
	2	0·166 ± 0·001*
	10	0·139 ± 0·006*
Phenylalanine ..	0	0·250 ± 0·013
	2	0·251 ± 0·006
	10	0·193 ± 0·007*

* Statistically significant difference between the control and salicylate values by the *t*-test.

DISCUSSION

The present results indicate that salicylate interferes with protein biosynthesis *in vitro* by inhibiting the activities of certain aminoacyl-tRNA synthetases and not by affecting the transfer of amino-acid from esterified tRNA into polysomal protein.

In the polysome experiments the transfer of only one radioactive amino-acid was followed because a single set of enzymes acts on all the aminoacyl-tRNAs (Fessenden & Moldave, 1961). The use of a pH 5 supernatant, from which the endogenous amino-acids had been removed, ensured that no esterification of tRNA could occur during the incubations. We found that 10 mM salicylate did not affect the transfer of radioactive leucine to the protein. That reduction of the aminoacyl-tRNA concentrations in the reaction mixtures made no difference, shows that the substrate concentrations were not sufficiently high to mask an inhibition due to salicylate. The GTP concentration in the incubation mixtures was also varied because it had been reported that the action of an inhibitor (Hoagland, Scornik & Pfefferkorn, 1964) present in the microsomal fraction of normal rat liver is antagonized by the nucleotide. Since salicylate remained inactive its interference with protein synthesis in the rat liver microsomal-cell sap preparation cannot be mediated via the inhibitor.

Experiments with *Chlorella* protein hydrolysate (Table 1) showed that salicylate, 1 mM and above, inhibited the formation of aminoacyl-tRNAs. This was due to a differential action of the drug on the activities of the aminoacyl-tRNA synthetases, the most sensitive being those incorporating aspartate, glutamate, glutamine and histidine (Table 2).

It has been suggested that the biosynthesis of aminoacyl-tRNA occurs in two stages, activation of the amino-acids and their transfer to the tRNA (Hoagland, Zamecnik & Stephenson, 1957). The ATP-pyrophosphate exchange reaction has been considered to reflect the first stage. The results in Table 3 suggest that salicylate interferes with this proposed stage. However, the ATP-pyrophosphate exchange promoted by phenylalanine and leucine (Table 4) was significantly inhibited by 10 mM salicylate whereas the formation of the corresponding aminoacyl-tRNAs was not affected by this concentration of the drug. A possible explanation for the discrepancy is that a rat liver supernatant and a pH 5 fraction respectively, were used to study the aminoacyl-tRNA synthetase reaction and the ATP-pyrophosphate exchange. An experiment in which the pH 5 enzyme preparation was used to measure the formation of leucyl-tRNA showed that no inhibition occurred in the presence of 10 mM salicylate.

These results suggest that salicylates interfere with the biosynthesis of proteins *in vitro* by preferentially inhibiting the formation of certain aminoacyl-tRNA species. A deficiency of any one aminoacyl-tRNA would be expected to block translation on the polysome at the corresponding messenger codon (Anderson, 1969). Thus, an impaired synthesis of glutamyl-, aspartyl- and histidinyl-tRNA in the presence of salicylate could explain the reported effects of the drug in inhibiting the incorporation of leucine into the protein of cell-free systems and of other amino-acids into the protein of a variety of animal tissues *in vitro*. The observation that salicylate inhibits the incorporation of phenylalanine by a poly U-directed system from rat liver ribosomes (Reunanen & others, 1967) is less explicable. One possibility is that salicylate could inhibit the binding of the poly U to the ribosomes. This binding is qualitatively different from the binding of natural messenger RNA (Brawerman & Eisenstadt, 1966) which only occurs to a negligible extent in cell-free systems.

The concentration of salicylate (2 mM) which significantly inhibits the activities of the aminoacyl-tRNA synthetases utilizing aspartate, glutamate, glutamine and histidine (Table 2) is within the range of the concentrations of the drug attained and maintained in animal tissues for some hours after the injection of single doses of sodium salicylate of 400 mg/kg and above (Sturman, Dawkins & others, 1968; McArthur, Dawkins & Smith, 1970). An inhibitory action of salicylate on the formation of one or more of the aminoacyl-tRNAs could therefore explain the reported effects of the drug in protein synthesis *in vivo*. It has been shown that high doses of the drug interfere with the synthesis of collagen in carrageenan granuloma in the rat (Fukuhara & Tsurufuji, 1969) and with the incorporation of radioactive histidine into liver proteins in the intact mouse (Dawkins, McArthur & Smith, 1971). The daily administration of 200–300 mg/kg of salicylate caused lower rates of weight gain and impaired skeletal growth in immature rats (Limbeck, Conger & others, 1966) and in growing chicks (Thomas, Nakaue & Reid, 1967).

Acknowledgements

We wish to thank the King's College Hospital and Medical School Research Committee for financial aid and the Nuffield Foundation for a grant towards the purchase of some of the instruments used in the work.

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