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# Effects of salicylate on the incorporation of orotic acid into nucleic acids of mouse tissues *in vivo*

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In mice, given intraperitoneal injections of orotic acid-5-$^3$H, two peaks of incorporation of radioactivity at 30 min and 6 h occurred in the RNA of kidney and of liver and its subcellular components. The concurrent administration of salicylate, in doses of 200 mg/kg body weight and above, significantly inhibited the incorporation of the labelled orotic acid at 30 min but not at 6 h.

Salicylate, in concentrations of 3 mM and above, inhibits the activities of RNA and DNA polymerases prepared from rat liver (Janakidevi & Smith, 1969). These observations suggested that the drug may interfere with the biosynthesis of nucleic acids *in vivo*. The present paper describes the effects of the injection of salicylate on the incorporation of radioactivity from tritiurated orotic acid into nucleic acid fractions of mouse liver and kidney.

## EXPERIMENTAL

### *Materials and methods*

Male albino mice, 25–30 g, maintained on MRC modified cube diet no. 41B were used. Orotic acid-5-$^3$H (specific activity 1000 mCi/mmol) was obtained from the Radiochemical Centre, Amersham, Bucks., RNA from Boehringer Corporation (London) Ltd. and orcinol from the Sigma Chemical Co., St. Louis. The sodium salicylate was of British Pharmacopoeial grade, all other chemicals were of analytical grade and glass distilled water was used throughout. Each mouse received an intraperitoneal injection (0·5 ml) containing 25 $\mu$Ci of orotic acid-5-$^3$H plus either sodium salicylate at dose levels of either 50, 100, 200 or 400 mg/kg body weight or sufficient sodium chloride to contain the same final concentration of sodium. Groups, each of three mice, were killed by cervical fracture at appropriate time intervals (see Results section) and duplicate samples (approximately 0·25 to 0·5 g) of the excised liver and kidney were immediately homogenized, using an all glass pestle and mortar, at 0° with 10 volumes of 3% (v/v) $HClO_4$. The homogenates were centrifuged at 3000 *g* for 15 min at 0° and the sediment resuspended and centrifuged with two further quantities (5 vol) of perchloric acid and one of distilled water (5 vol). The original supernatant together with the supernatants from the subsequent acid and water extractions of the sediment were combined as the acid-soluble fraction. The final sediment was washed with two quantities of ice cold ethanol–ether mixture (3:1) to remove lipids, and the RNA was then extracted according to the directions of Tata & Widnell (1966). The specific activity of the extracted RNA was measured by estimating the RNA content by the orcinol method

(Hurlbert, Schmitz & others, 1954) and the radioactivity as described below. Sufficient M KOH was added to the acid-soluble fraction to adjust the pH to 8·0, the mixture was allowed to stand for 2 h at 0° and then centrifuged at 3000 *g* to remove the deposited $KClO_4$. Aliquots (0·1 ml) of the supernatant were removed for measurement of radioactivity and the pH of the remaining supernatant was adjusted to pH 1 with 6M HCl (Munro, Jackson & Korner, 1964). The extinction at 260 nm was measured in this mixture using a Unicam SP 800 spectrophotometer and taken to represent the acid-soluble nucleotide fraction. All radioactive counting was performed on samples dried on GF/A (2·1 cm) glass fibre discs in a Beckman LS 200B liquid scintillation system.

In some experiments the whole liver was homogenized in 10 vol of 0·32 M sucrose containing 3 mM $MgCl_2$ and fractionated by differential centrifugation. The crude nuclear pellet, obtained at 700 *g*, was further purified (Janakidevi & Smith, 1969), the mitochondrial fraction being separated at 15000 *g* for 20 min, the microsome fraction at 105 000 *g* for 1 h and the residual supernatant taken as the cell sap. The acid-soluble components and the RNA were extracted from each subcellular fraction as described above.

## RESULTS

The incorporation of radioactivity into the acid-soluble fraction and into the RNA of mouse liver at 5, 10, 15, 30 min, 1, 3, 6, 12 and 24 h after the intraperitoneal injection of orotic acid-5-$^3$H are given in Fig. 1. The results show that the incorporation of radioactivity into both fractions reached a peak at 30 min, and

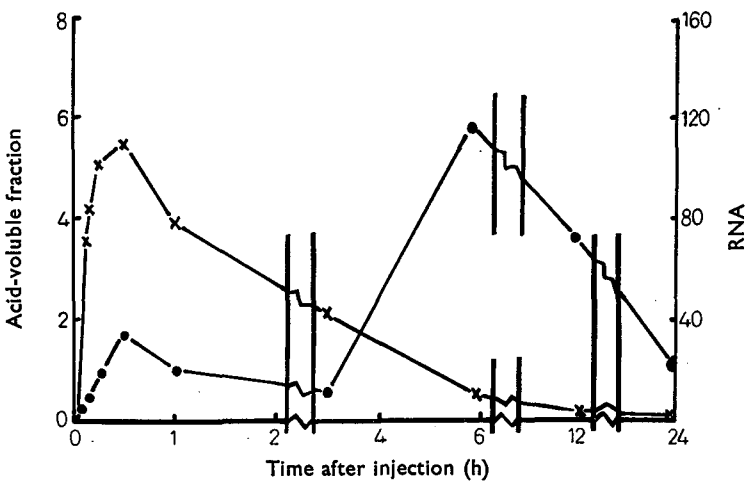


FIG. 1. Incorporation of orotic acid-5-$^3$H into the acid soluble fraction and RNA of mouse liver. The labelled orotic acid (25 $\mu$Ci) given by intraperitoneal injection at 0 min. Individual results represent the mean values from three mice. ×, incorporation of $^3$H into acid soluble fraction, expressed as counts/min × $10^{-2}$ per $\mu$g equivalent of RNA. ●, incorporation of $^3$H into RNA, expressed as counts/min × $10^{-2}$ per mg of RNA isolated.

subsequently declined for the acid-soluble fraction but that the initial peak at 30 min for the RNA was succeeded by a second peak at 6 h. Similar results were also observed with the mouse kidney.

The effects of an intraperitoneal injection of 400 mg/kg body weight of salicylate, given at the same time as the labelled orotic acid, on the incorporation of radioactivity into the acid-soluble fraction and into the RNA of mouse liver and kidney at 30 min and at 6 h are given in Table 1. The results show that the salicylate

Table 1. *Effect of the injection of salicylate on the incorporation of orotic acid-5-$^3H$ into the acid-soluble fraction and RNA of mouse liver and kidney.* The labelled orotic acid and the salicylate (400 mg/kg body weight), when present, were given by intraperitoneal injection at 0 min. The results are expressed as counts/min per $\mu$g RNA equivalent for the acid-soluble fraction and counts/min per mg RNA isolated for the RNA. Each value is given as the mean $\pm$ standard deviation for each group of three animals.

| Organ | Treatment | Radioactivity incorporated 30 min after injection | | Radioactivity incorporated 6 h after injection | |
|---|---|---|---|---|---|
| | | Acid-soluble fraction | RNA | Acid-soluble fraction | RNA |
| Liver | Control | 518 $\pm$ 103 | 2268 $\pm$ 377 | 715 $\pm$ 116 | 13528 $\pm$ 4744 |
| | Salicylate | 267 $\pm$ 96* | 732 $\pm$ 118* | 546 $\pm$ 124 | 14816 $\pm$ 5716 |
| Kidney | Control | 4336 $\pm$ 754 | 72290 $\pm$ 5193 | 1190 $\pm$ 278 | 194237 $\pm$ 22661 |
| | Salicylate | 2459 $\pm$ 1000* | 46018 $\pm$ 5772* | 1493 $\pm$ 301 | 236255 $\pm$ 54738 |

* Statistically significant difference between control and salicylate results. $P < 0{\cdot}05$.

injection caused significant inhibition of the incorporation of radioactivity into the acid soluble fraction and into the RNA of both the liver and kidney at 30 min but not at 6 h. The results of further experiments, in which the labelled orotic acid was administered at zero time and the salicylate injected after 5½ h, showed that the drug did not affect the incorporation of tritium into either the acid-soluble fraction or into the RNA in the liver and kidney of animals killed at 6 h.

The results in Table 2 show the effects of the injection of 400 mg/kg body weight of salicylate on the incorporation of isotope into the acid-soluble fraction and into

Table 2. *Effect of the injection of salicylate on the incorporation of orotic acid-5-$^3H$ into the acid-soluble fraction and RNA of subcellular fractions of mouse liver.* Experimental details as for Table 1

| Subcellular fraction | Treatment | Radioactivity incorporated 30 min after injection | | Radioactivity incorporated 6 h after injection | |
|---|---|---|---|---|---|
| | | Acid-soluble fraction | RNA | Acid-soluble fraction | RNA |
| Nuclei | Control | 99 $\pm$ 23 | 36600 $\pm$ 3961 | 116 $\pm$ 25 | 50175 $\pm$ 5392 |
| | Salicylate | 31 $\pm$ 14* | 14216 $\pm$ 9599* | 87 $\pm$ 16 | 74066 $\pm$ 6890 |
| Mitochondria | Control | 193 $\pm$ 23 | 259 $\pm$ 22 | 82 $\pm$ 22 | 12421 $\pm$ 1690 |
| | Salicylate | 98 $\pm$ 21* | 138 $\pm$ 40* | 64 $\pm$ 12 | 9400 $\pm$ 1596 |
| Microsomes | Control | 154 $\pm$ 36 | 670 $\pm$ 128 | 21 $\pm$ 10 | 16415 $\pm$ 1560 |
| | Salicylate | 103 $\pm$ 15 | 194 $\pm$ 64* | 42 $\pm$ 15 | 15240 $\pm$ 1468 |
| Cell sap | Control | 193 $\pm$ 70 | 767 $\pm$ 155 | 60 $\pm$ 21 | 3306 $\pm$ 456 |
| | Salicylate | 146 $\pm$ 46 | 266 $\pm$ 67* | 54 $\pm$ 14 | 3589 $\pm$ 520 |

* Incorporation of $^3H$ significantly decreased in salicylate-treated animals. $P < 0{\cdot}05$.

the RNA of the subcellular components of liver from mice killed after 30 min and 6 h. In general, the drug caused significant inhibition of incorporation of radioactivity at 30 min in all the fractions except the acid-soluble fraction of the microsomes and cell sap, but did not inhibit the corresponding incorporations at 6 h. In Table 3, the effects of varying the size of the injected dose of the salicylate on the

Table 3. *Effect of varying the size of the injected dose of salicylate on the incorporation of orotic acid-5-$^3H$ into the acid-soluble fraction and RNA of mouse liver.* Experimental details as for Table 1

| Organ | Salicylate mg/kg body wt | Radioactivity incorporated 30 min after injection Acid-soluble fraction | RNA |
|---|---|---|---|
| | 0 | 641 ± 85 | 2203 ± 345 |
| | 50 | 583 ± 146 | 1870 ± 264 |
| | 0 | 723 ± 48 | 2709 ± 440 |
| | 100 | 654 ± 106 | 2642 ± 502 |
| Liver | 0 | 742 ± 69 | 2085 ± 355 |
| | 200 | 248 ± 38* | 812 ± 115* |
| | 0 | 518 ± 103 | 2268 ± 377 |
| | 400 | 267 ± 96* | 732 ± 118* |
| | 0 | 7547 ± 1399 | 68027 ± 6059 |
| | 50 | 5732 ± 1899 | 68891 ± 10470 |
| | 0 | 6956 ± 403 | 59211 ± 8622 |
| | 100 | 6223 ± 1142 | 51337 ± 8045 |
| Kidney | 0 | 7909 ± 1736 | 90265 ± 3423 |
| | 200 | 3765 ± 708* | 68646 ± 1422* |
| | 0 | 4336 ± 754 | 72290 ± 5193 |
| | 400 | 2459 ± 1000* | 46018 ± 5772* |

* Statistically significant difference between control and salicylate results. $P < 0{\cdot}05$.

30 min incorporation into the acid-soluble fraction and the RNA of whole liver and kidney are given. Significant inhibition was only observed with salicylate doses of 200 mg/kg body weight and above.

## DISCUSSION

Orotic acid-5-$^3H$ is incorporated into the acid-soluble fraction and into the RNA of mouse liver and kidney *in vivo*, the initial peak of incorporation occurring 30 min after administration of the labelled precursor. The concurrent administration of salicylate, in doses of 200 mg/kg body weight and above, significantly inhibits the 30 min incorporation into the two organs and into the main subcellular fractions of the liver. Packman, Esterly & Peterson (1969) have recently reported that salicylate inhibits the incorporation of tritiurated uridine into the nucleic acids of human peripheral lymphocytes.

Salicylate could produce this effect by one or more of several mechanisms. It has been shown (Janakidevi & Smith, 1969) that the drug, in concentrations of 3 mM and above, inhibits the activities *in vitro* of nucleic acid polymerases prepared from rat liver. In the initial experiments reported in the present paper a dose of 400 mg/kg body weight was chosen because the salicylate concentration in the liver and kidneys of mice 30 min after intraperitoneal injection of this dose was approximately 2 mM (Sturman, Dawkins & others, 1968). It is therefore possible that

salicylate inhibits these enzyme activities *in vivo* and therefore prevents the incorporation of the orotic acid. The failure of the salicylate injection to effect the 6 h incorporation of the orotic acid could have been due to the lower salicylate concentrations (0·5 mM) present in the organs at the longer time interval. However, the 6 h incorporation was not affected by a dose of 400 mg/kg salicylate being given 5½ h after the orotate injection when salicylate concentration of about 2 mM would have been present in the liver and kidney. It is possible that salicylate only inhibits the biosynthesis of certain types of nucleic acids, e.g., messenger RNA and not the formation of other RNA species, e.g., ribosomal RNA. A second mechanism is that salicylate could interfere with the series of phosphorylation reactions concerned in the conversion of orotic acid to UTP and related nucleotide triphosphates. The drug is known to uncouple oxidative phosphorylation reactions in respiring mitochondrial preparations in concentrations above 0·5 mM (Brody, 1956) and salicylate concentrations as low as 0·1 mM decrease the formation of ATP in the isolated rat diaphragm (Smith & Jeffrey, 1956). The present results do not exclude the possibility that the salicylate may have interfered with the transport of the labelled orotic acid from the injection site into the circulation and hence to the liver and kidney.

If salicylate inhibits the biosynthesis of nucleic acids *in vivo* this may explain, at least in part, the effects of the drug in retarding growth in young animals (see Limbeck, Conger & others, 1966) and in wheat coleoptiles (Reid, 1957) and its teratogenic effects in rodents (see Larsson, Bostrom & Ericson, 1963).

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