Department of Biological Sciences,
Ewell County Technical College,
Ewell, Surrey, England.

REGINA M. BOTTING
D. BURDEN

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Inhibition of nucleic acid polymerases by salicylate *in vitro*

The incorporation of labelled amino-acids into the proteins of rat isolated diaphragm and of microsomal preparations from rat liver is inhibited by salicylate in concentrations of 0·3 mM and above (Dawkins, Gould & Smith, 1966). The initiation and maintenance of protein synthesis requires RNA and DNA and we have therefore studied the effects of salicylate on the activities of nucleic acid polymerases prepared from rat liver.

Liver nuclei were isolated from male rats (300–450 g) of the Wistar strain by the method of Widnell & Tata (1964) with the following modifications. A 10% (w/v) homogenate was centrifuged at 700 *g* for 10 min. The pellet was suspended in 5 vol of 2·4 M sucrose containing 1 mM $MgCl_2$, centrifuged at 50,000 *g* for 1 h and the purified nuclear pellet washed twice in 0·25 M sucrose containing 1 mM $MgCl_2$, before final suspension. RNA polymerase activity was estimated at 17°, to minimize interference from ribonuclease, by measuring the incorporation of radioactivity from ATP-α^{32}P into RNA in a Beckman LS 200B liquid scintillation system, using glass fibre discs. The RNA product from the mixtures incubated at 17° and 37° had a DNA-like base composition [(G + C)/(A + U) = 0·75]. DNA polymerase was purified up to the ammonium sulphate fractionation stage (Mantsavinos, 1964) and desalted by dialysis. Calf thymus DNA was used as a primer and the activity measured by the incorporation of radioactivity from dATP- ^{3}H into DNA.

Salicylate, in concentrations of 3 mM and above, significantly decreased the incorporation of radioactive ATP into RNA in the rat liver preparation (Table 1). DNA polymerase activity is inhibited by salicylate concentrations of 1 mM and above (Table 2).

These preliminary observations suggest that salicylates may interfere with the biosynthesis of nucleic acids and hence of proteins. A further implication of the present results is that an inhibitory action of salicylates on nucleic acid biosynthesis may be one of the factors concerned in the teratogenic effects of the drugs. It has been reported that the injection of the drugs in pregnant rats and mice not only produces premature birth and foetal death (Eriksson & Larsson, 1968) but also several congenital malformations of the litters carried to full term (Warkany & Takacs, 1959; Larsson, Bostrom & Ericson, 1963).

Table 1. *Effect of salicylate on the nuclear* RNA *polymerase*

Salicylate (mM)	No. of expts	pmol nucleotide incorporated per mg DNA	*P*
0	8	2001 ± 288	
0·3	7	1807 ± 153	0·2
0·6	7	1717 ± 259	0·1
1·0	8	1699 ± 273	0·05
2·0	6	1692 ± 141	0·05
3·0	7	1638 ± 112	0·005
6·0	8	1613 ± 199	0·005
10·0	6	1503 ± 243	0·005
15·0	7	1355 ± 254	0·001
20·0	7	1212 ± 267	0·001

Incubation mixtures contained in a total volume of 0·5 ml; GTP, CTP and UTP, 0·5 μmol each; ATP α-^{32}P, 0·5 μmol (specific activity 2·5 μCi/μmol); $MnCl_2$, 2 μmol; saturated $(NH_4)_2SO_4$, pH 7·5, 0·05 ml; Tris-HCl Buffer, pH 7·5, 50 μmol; 0·1 ml of nuclear preparation in 0·25M Sucrose containing 200 μg of DNA. Incubation is for 1 h at 17°; the reaction stopped by the addition of 3 ml of cold N $HClO_4$ containing 0·02 M $Na_4P_2O_7$. After standing at 0° for 30 min, the precipitate was collected by centrifugation, washed twice with 0·2 N $HClO_4$ containing 0·02 M $Na_4P_2O_7$, once with cold ethanol and twice with cold ethanol:ether mixture (3:1). RNA was extracted according to the directions of Widnell & Tata (1964). The results, given as means and standard deviations, have been analysed by the Students *t*-test; the minimal acceptable level of significance being taken as $P = 0{\cdot}005$.

Table 2. *Effect of salicylate on the incorporation of* dATP-^{3}H *into* DNA in vitro.

Salicylate (mM)	No. of expts	Specific activity	*P*
0	5	63·2 ± 9·7	
0·3	5	57·4 ± 3·2	0·3
0·6	5	59·5 ± 8·7	0·6
1·0	5	41·6 ± 4·4	0·005
2·0	5	38·3 ± 5·5	0·001
3·0	5	33·6 ± 3·9	0·001
6·0	5	27·7 ± 9·8	0·001
10·0	5	25·5 ± 7·5	0·001
15·0	5	24·2 ± 5·8	0·001
20·0	5	18·5 ± 1·7	0·001

Incubation mixture contained in a total volume of 0·5 ml: 50 nmol of dCTP, dGTP and TTP; dATP-^{3}H, 3 nmol (specific activity 70 μCi/μmol), 4·0 μmol, $MgCl_2$; 0·5 μmol,2-mercaptoethanol; 50 μg calf-thymus DNA; 50 μmol, Tris-HCl buffer; pH 8·0, sodium salicylate, 0–10 μmol, 0·1 ml enzyme solution containing 2·95 mg/ml of protein. The specific activities, given as means and standard deviations, are expressed as pmol of dAMP-^{3}H incorporated into DNA per mg of enzyme.

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Department of Biochemical Pharmacology,
King's College Hospital Medical School,
Denmark Hill,
London, S.E.5.

K. JANAKIDEVI
M. J. H. SMITH

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