Differential inhibition of RNA polymerase activities by salicylate *in vitro*

The final stage in the biosynthesis of RNA is the polymerization of nucleoside triphosphates by a polymerase enzyme (nucleoside triphosphate: RNA nucleotidyltransferase EC 2.7.7.6.). It was reported by Widnell & Tata (1966) that the activity of the mammalian nuclear polymerase, with respect to the types of RNA synthesized, depended on whether the assay system contained either magnesium ions or ammonium sulphate plus manganese ions. Several hypotheses have been formulated to explain this observation (see Stirpe & Fiume, 1967) but recent work strongly suggests that there are two forms of RNA polymerase with different specificities towards the DNA templates and different requirements for divalent cations (Liao, Sagher, & others, 1969). Base composition analysis of the reaction products showed that the Mn^{2+}: ammonium sulphate-activated enzyme synthesized DNA-like RNA whereas the Mg^{2+}-activated enzyme caused the formation of ribosomal RNA (Widnell & Tata, 1966).

Salicylate, in concentrations of 3mM and above, significantly inhibits the activity of rat liver nuclear RNA polymerase in the presence of Mn^{2+} and ammonium sulphate (Janakidevi & Smith, 1969). The present results (Table 1) confirm this finding but also show that inhibition does not occur with the Mg^{2+}-activated enzyme in rat liver nuclei either incubated with 3 mM salicylate *in vitro* or obtained from rats killed 30 min after injection with 400 mg/kg body weight of sodium salicylate. Similar results were obtained in the mouse and with a nuclear fraction obtained from whole 13 day and 16 day rat foetuses.

Table 1. *Effects of salicylate on the nuclear RNA polymerases*

Treatment	p mol nucleotide incorporated per mg DNA	
	Mn^{2+}: $(NH_4)_2SO_4$ activated enzyme	Mg^{2+}-activated enzyme
Incubated with 3 mM salicylate	890 ± 38 (5)	166 ± 30 (5)
Control	* 991 ± 42 (4)	161 ± 18 (5)
Obtained from animals injected with 400 mg/kg body weight salicylate	505 ± 62 (3)	177 ± 22 (3)
Control from animals injected with saline	* 1052 ± 152 (3)	174 ± 20 (3)

Incubation mixtures according to the directions of Widnell & Tata (1966) except that the nucleoside triphosphate concentrations were 0·4 mM, ATP-8-^{14}C being used as the labelled precursor, the Mn^{2+}: $(NH_4)_2SO_4$ activated enzyme being incubated for 1 h at 17° and the Mg^{2+}-activated enzyme for 15 min at 37°. The polymerase activities were assayed as described previously (Janakidevi & Smith, 1969). Each value is given as the mean ± standard deviation, the number of experiments being given in parentheses. The results have been analysed by the *t*-test and * indicates a statistically significant difference ($P < 0.05$) between the control and salicylate values.

These observations support the view that there are two forms of RNA polymerase in rodent tissues. Salicylate resembles α-amanitin in only inhibiting the Mn^{2+}: ammonium sulphate enzyme and differs from other inhibitors of RNA polymerase, such as actinomycin D and aflatoxin B_1, which are more inhibitory for the Mg^{2+}-activated enzyme (Stirpe & Fiume, 1967). They also suggest that salicylate preferentially interferes with the biosynthesis of some species of RNA. The formation of DNA-like RNA, including messenger RNA, but not of ribosomal RNA would be expected to be inhibited by the drug both *in vitro* and *in vivo*. This could explain the finding that the intraperitoneal injection of salicylate into adult mice decreases the incorporation of radioactive orotic acid in the RNA of liver and kidney at 30 min but not at 6 h (Janakidevi & Smith, 1970).

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

K. JANAKIDEVI
M. J. H. SMITH

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The effect of oxotremorine on the acetylcholine content of different parts of cat brain

Tremorine (Pepeu, 1963; Holmstedt, Lundgren & Sundwall, 1963) and also its active metabolite oxotremorine (Holmstedt & Lundgren, 1966) cause an increase of brain acetylcholine and tremor in rats. A causal relation between the two effects was proposed (Holmstedt & Lundgren, 1966) but a number of substantial objections have been recently presented (Cox & Potkonjak, 1969a).

We have now investigated whether oxotremorine also increases brain acetylcholine in rats and whether the increase occurs uniformly in all brain regions.

Of eight young cats, each weighing about 1 kg, four received an intraperitoneal injection of saline and four 1·0 mg/kg of oxotremorine. Within a few minutes of giving the drug the cats showed intense tremor, salivation, miosis and behaviour similar to false rage. Fifteen min after the injection the cats were killed under light halothane anaesthesia, the skull opened, the brain removed and placed on ice. From each brain 3 samples were prepared: (I) about 500 mg of cortex were excised from the frontal lobes; (II) the diencephalon and the upper part of the midbrain were dissected following the lateral ventricles, the head of the caudate nucleus and a plane from the posterior colliculi to the rostral border of the pons. The weight of this sample was about 2·0 g; (III) the caudal part of the brain stem including the pons and the medulla oblongata; its weight was about 1·5 g.

Acetylcholine was extracted by the method of Smallman & Fisher (1958) modified by Bartolini & Bedarida-Jarach (1965), and assayed on the dorsal muscle of the leech. Recovery of added acetylcholine was 90%.

The results are reported in Table 1. The values of acetylcholine content found in the control cats are in good agreement with previous observations (Macintosh, 1941; Pepeu, 1966).

Table 1. *The influence of oxotremorine* (1 *mg/kg, i.p.*) *on the acetylcholine content of the cat cortex* (*I*), *diencephalon and upper part of midbrain* (*II*) *and the caudal part of the brain stem* (*III*). (Means ± s.e. of four experiments)

	Acetylcholine (μg/g)			
	Controls	After oxotremorine	% Increase	*P*
I	1·12 ± 0·26	1·43 ± 0·33	27	N.S.
II	2·53 ± 0·28	4·79 ± 0·49	89	<0·01
III ..	3·32 ± 0·61	3·45 ± 0·58	4	N.S.