# Sodium salicylate toxicity and pregnancy in mice

Foetal malformations after salicylate administration during pregnancy have been reported (Warkany & Takacs, 1959; Larsson, Ericsson & Boström, 1964; Akiyama, 1967; Takacs & Warkany, 1968; Larsson, 1970). Recently foetal death after high doses of salicylates has been described (Eriksson, 1969, 1970, 1971a,b,c). We have examined the toxicity of salicylates to mice during and up to 14 days after pregnancy.

Pregnant and control mice of the Konárovice strain, 30–50 animals in each group, were used to assess the acute toxicity (LD50) of sodium salicylate. The animals were observed for a week after intraperitoneal drug administration on the 7th, 14th or 20th day of pregnancy. The LD50 values (mg $kg^{-1}$) were:

| Control | Day of pregnancy | | | Day after parturition | |
|---|---|---|---|---|---|
| | 7 | 14 | 20 | 7 | 14 |
| 760 | 760 | 535+ | 520+ | 700 | 780 |
| (679–851) | (658–868) | (476–567) | (428–619) | (636–770) | (711–849) |

There was an increase in the toxicity of sodium salicylate in pregnant mice from day 14 of pregnancy onwards, then the LD50 values returned to the control values of non-pregnant animals by 7 days after parturition.

A possible explanation of the increased toxicity of salicylates in the later stages of pregnancy could be the hormonal changes in pregnant animals, therefore, four groups of 40 non-pregnant mice were pretreated for four days with a total of 2 $\mu$g oestradiol propionate or 0·4, 0·8 or 1·6 mg progesterone. The LD50 of sodium salicylate in these animals was compared with control animals. A significant increase in sodium salicylate toxicity was observed in the groups treated with oestradiol propionate or 1·6 mg progesterone—the oestrogen reducing the LD50 dose to $75 \pm 12\%$ of the control and progesterone to $80 \pm 6\%$ of the control. We then examined the possible changes in the ratio of free salicylic acid to other metabolites during pregnancy. When salicylate was labelled with $^{14}C$, no changes in the total excretion of $^{14}C$ activity were found, but on day 19 the amount of free salicylic acid in urine had increased approximately twofold and the values for glucuronides decreased significantly.

The increased toxicity of sodium salicylate in pregnant mice may be explained in several ways. Firstly, toxicity correlates with the higher amounts of free salicylic acid. The increased free levels are probably due to decreased conjugation. Yoshinaga, Hawkins & Stocker (1969) have found that the amount of oestrogens increases in late pregnancy and that they are excreted partly as glucuronides. Also, the amount of the glucuronide metabolite of progesterone, i.e. pregnanediol, is increased in the urine during pregnancy (Marrian, 1949) or after exogenous administration of progesterone. Our finding that the oestrogen and progesterone (1·6 mg) increased the toxicity of salicylates in non-pregnant mice suggests that the increased toxicity of salicylates in late pregnancy could be due to an increased amount of free salicylic acid because the sex hormones compete for the glucuronidation mechanism. Female sex hormones may depress enzyme activities in the liver (Gram & Gilette, 1971) and this cannot be excluded as a reason for the increased salicylic acid level.

Eriksson (1969) described late embryotoxic effects of salicylates without changes in the total elimination of [$^{14}C$] from labelled salicylate. She also found that there were significant differences in strain susceptibility to embryotoxic salicylate effect in late pregnancy. In agreement with her, we did not find any changes in the total $^{14}C$ activity in the urine of pregnant females, but higher levels of free salicylic acid

in late pregnancy could explain the embryotoxicity characterized in Eriksson's experiments by haemorrhages.

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## Factors influencing the response of the mouse vas deferens preparation to noradrenaline

Jones & Spriggs (1971) reported the relative insensitivity of the mouse isolated vas deferens preparation to most common agonists though the tissue responded well to transmural electrical stimulation. This surprised us as for some time we have been satisfactorily determining noradrenaline dose-response curves on mouse vas deferens. The experimental technique used by Jones & Spriggs (1971) differed in a number of respects from ours e.g. they stripped off the serous coat—we did not; they worked at 32°, we at 35·5°, but probably the most significant difference was their use of Huković solution (NaCl 114, KCl 4·70, $CaCl_2$ 2·54, $MgSO_4$ 1·19, $NaHCO_3$ 25·00, $KH_2PO_4$ 1·19, glucose 11·5 mM: Huković, 1961) while we preferred McEwen solution (NaCl 130, KCl 5·65, $CaCl_2$ 2·16, $NaHCO_3$ 25·0, $NaH_2PO_4$ 0·92, glucose 11·1, sucrose 13·1 mM: McEwen, 1956). The most noticeable difference between these two physiological saline solutions is the absence of $MgSO_4$ from McEwen solution and we have therefore investigated the effect of this salt on the response of the mouse isolated vas deferens preparation to noradrenaline.

Vasa deferentia were removed from freshly killed mice (Tuck No. 1 strain, 12 weeks old, 20–30 g weight) and suspended in an organ bath at 35·5° in either Huković or McEwen solution gassed with 5% carbon dioxide in oxygen. Changes in length of the tissues in response to (—)-noradrenaline (Koch-Light, Ltd.) and to transmural stimulation (5 s trains of 0·2 ms duration 40 V rectilinear pulses applied at 50 Hz through parallel platinum wire electrodes) were recorded isotonically (load 150–200 mg).

In McEwen solution the tissues responded well to both transmural stimulation and exogenous noradrenaline (Fig. 1) and since the length of the vas deferens was usually between 20 and 30 mm, the maximal response of 8–14 mm represents a considerable shortening. In Huković solution the tissues responded less well and this difference is probably due to the presence of $MgSO_4$ since tissues suspended in McEwen solution