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The influence of maternal protein deficiency on the placental transfer of salicylate in rats

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1 The influence of a low protein diet (5% as compared with a control 21% protein diet) on the placental transfer of sodium salicylate was investigated in Sprague-Dawley rats on day 20 of gestation.

2 Maternal plasma salicylate concentrations (assayed by high pressure liquid chromatography) were generally lower in protein-deficient than in control animals at a wide range of times (0.25–12 h) and dose levels (2–250 mg/kg, i.v.); however, foetal plasma salicylate levels in the two groups of animals did not differ.

3 The placental transfer of salicylate as indicated by the ratio of foetal plasma or foetal liver to maternal plasma salicylic acid concentration was consistently and significantly greater in the protein-deficient group than in the control group of animals following the administration of the drug to the mother as well as to the foetus.

4 A decrease in calorie without a concomitant decrease in protein intake (pair-fed controls) did not alter the placental transfer of salicylate.

5 The increased placental transfer of salicylate in protein-deficient animals could not be attributed to changes in serum protein-salicylate binding.

6 It is suggested that the pharmacokinetic factors responsible for maintaining a lower level of salicylate in the foetus than in the mother are impaired by maternal malnutrition, and this may increase the foetal effects of maternally ingested salicylate.

Introduction

Although salicylates are relatively safe agents they can cause adverse effects, including foetal toxicity (Turner & Collins, 1975; Flower, Moncada & Vane, 1980). Ingestion of salicylate by pregnant women has been found to deliver potentially toxic levels of the drug to the foetus (Garrettson, Procknal & Levy, 1975; Gronroos, Haataja, Honkonen & Anttila, 1981). Salicylates can interfere with the physiological role of prostaglandins in the foetus (Sharpe, Larsson & Thalme, 1975), and West (1964) reported that the foetal toxicity of salicylate in rats was enhanced by a protein-deficient diet. Obviously the foetal effects of salicylate will be modified by any changes in its placental transfer. In view of the prevalence of protein-calorie malnutrition, especially in developing countries, and the widespread use of salicylates, it was of interest to determine the influence of maternal protein deficiency on the placental transfer of salicylate in rats as the experimental model. Results of this study suggest that protein-deficiency in rats is associated with an increase in the transfer of salicylate across the placenta.

Methods

Animals and diet

Virgin Sprague-Dawley female rats weighing between 200–225 g (10–11 weeks old) were housed with fertile males during the night. The following morning was designated as day zero of pregnancy for animals with evidence of sperm in the vaginal washings. These animals were housed individually and had free access to tap water. Starting from the day zero of gestation, animals were either fed *ad libitum* diets containing 21% (control) or 5% (deficient) protein or a restricted quantity (10 g rat, daily) of the control diet (Yue & Varma, 1982); the latter group of animals constituted the pair-fed controls. Experiments were done on day 20 of gestation.

The composition of the control 21% protein diet was as follows (g/kg): vitamin-free casein 231, sucrose 519, corn starch 150, corn oil 50, mineral mixture (Williams-Briggs) 40 and vitamin mixture (Teklad) 10. The 5% protein diet contained 55 g/kg

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vitamin-free casein and 695 g/kg sucrose; all other constituents were identical to those in the control diet. Both diets were isocaloric in composition and were purchased in a pellet form from Teklad Test Diets, Madison, Wisconsin, U.S.A.

Injections and collection of samples

Sodium salicylate was dissolved in water and injected into the tail vein under light ether anaesthesia. For injection into the foetal peritoneal cavity, animals were anaesthetized and the uterus exposed by means of an abdominal incision; sodium salicylate (3 mg) was injected through the uterine wall into foetal peritoneal cavities (0.5 mg × 6) and the abdomen was sutured. For the collection of the blood and tissue samples, animals were re-anaesthetized; maternal blood was collected by means of cardiac punctures and foetal blood was collected into 1.5 ml Eppendorf test tubes after decapitation. Maternal and foetal livers and placentae were removed for the extraction and assay of salicylic acid and any metabolites.

Extraction and assay of salicylate

Salicylate from plasma, liver and placenta was extracted in benzene: ethyl acetate (1:1, v/v) after acidification of the samples with one drop of 85% H_3PO_4 and assayed in salicylic acid equivalents by means of high-pressure liquid chromatography (Altex) (Peng, Gadalla, Smith, Peng & Chiou, 1978). The chromatographic conditions were as follows: 3.9 × 100 mm reverse-phase column (μBondapak-C_{18}, Waters, Milford, Mass., U.S.A.); a mobile phase of 30% acetonitrile in 0.05% H_3PO_4 (pH) 2.5) at a flow rate of 1 ml/min; the absorbance was measured at 237 nm and phthalic acid served as the internal standard (Yue & Varma, 1982).

Serum protein-salicylate binding

In order to determine the binding under *in vivo* conditions, 10 mg/kg sodium salicylate was injected intravenously and unheparinized maternal and foetal blood was collected 15 min later as described above. Blood samples were allowed to stay in test tubes for 15–30 min and then centrifuged at 800 *g* for 5 min to yield the serum. In other experiments, sodium salicylate solution (36 mM or 18 mM in 0.9% w/v sodium chloride solution) was added to the maternal and foetal serum in a ratio of 1:100 (v/v) to yield a final concentration of 360 μM and 180 μM sodium salicylate, respectively. The fractional binding was determined by the ultrafiltration procedure (Yue & Varma, 1982) by means of Amicon Centriflow membrane cones (CF 50A). In additional experiments on 3 control rats, oxyphenbutazone, which binds extensively with rat serum proteins (Varma, 1980), was injected intraperitoneally 30 min before the administration of 10 mg/kg sodium salicylate (i.v.). Three hours after the injection of salicylate, animals were killed and the maternal and foetal blood collected.

Serum proteins were measured according to Lowry, Rosebrough, Farr & Randall (1951) with bovine serum albumin as the standard.

Chemicals

Sodium salicylate, salicylic acid and phthalic acid (BDH, Montreal, Canada), and acetonitrile, ethyl acetate and benzene of high purity grade (Fisher Scientific, Montreal, Canada) were all purchased. Oxyphenbutazone was a gift from Ciba-Geigy, Montreal, Canada.

Statistics

Statistical significance between two means was ascertained by Student's *t* test and a probability of less than 0.05 was assumed to denote a significant difference. Data are presented as the mean ± s.e.

Results

General effects of maternal protein deficiency

A low protein diet caused a significant decrease in maternal body weight gain, foetal body weight, maternal and foetal liver weights and serum proteins, maternal serum albumin and placental weight; the litter size was not affected (Table 1).

Table 1 General effects of protein deficiency in rats on day 20 of gestation

Parameters	n	*Dietary protein*† 21% (control)	5% (deficient)
Maternal			
body weight (g)	46	345 ± 3.7	251 ± 4.0*
liver weight (g)	25	12.6 ± 0.4	6.9 ± 0.2*
serum proteins (g dl)	12	7.4 ± 0.3	4.7 ± 0.1*
serum albumin (g/dl)	6	2.4 ± 0.1	1.8 ± 0.1*
Foetal			
body weight (g)	468	3.9 ± 0.05	3.1 ± 0.09*
liver weight (mg)	276	262 ± 9.0	181 ± 11.0*
serum proteins (g dl)	11	2.3 ± 0.06	2.0 ± 0.0*
serum albumin (g/dl)	6	1.6 ± 0.1	1.3 ± 0.1
Placental weight (mg)	324	496 ± 14.0	351 ± 9.0*
Litter size	46	11.5 ± 0.3	11.5 ± 0.3

† Diets fed *ad libitum* for 20 days.
* denotes significant ($P < 0.05$) difference when compared with the corresponding control value; data are expressed as mean ± s.e.

Plasma salicylate levels

Plasma salicylic acid concentrations were generally higher in control than in protein-deficient pregnant animals at different times after a dose of 10 mg/kg sodium salicylate (Figure 1a) and at 3 h after different doses (Figure 1d). These differences were significant at 0.25, 0.5, 1, 3 and 12 h after the drug but not at 6 h, and at dose levels of 2, 10 and 50 mg/kg but not at 250 mg/kg. Despite this difference in the ma-

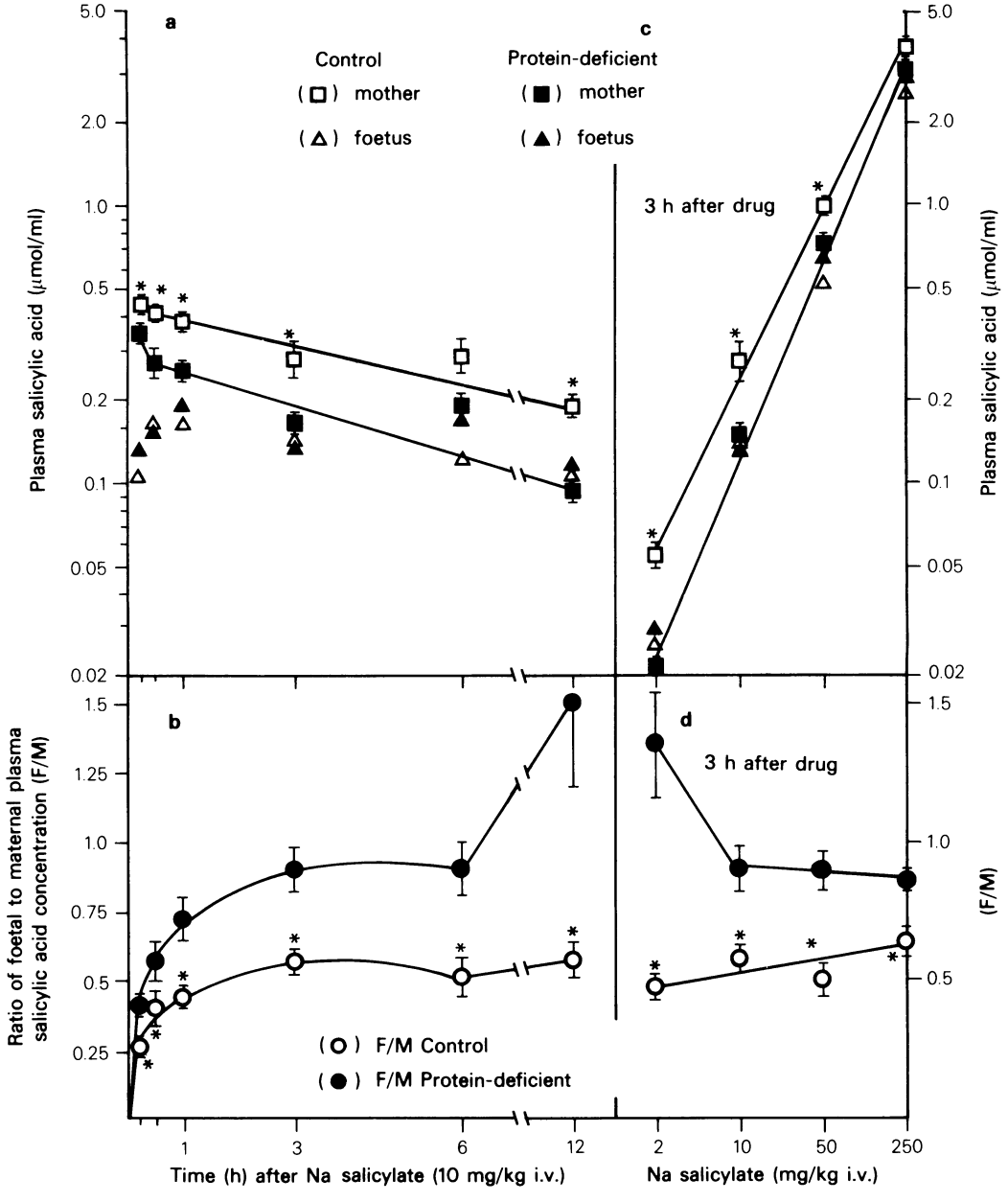

Figure 1 Plasma levels and the ratios of foetal to maternal plasma salicylate concentrations as a function of time and dose. Rats were fed *ad libitum* a 21% (control) or a 5% (deficient) protein diet and experiments were done on day 20 of gestation. Asterisks denote significant ($P < 0.05$) differences from the corresponding values in the plasma of protein-deficient mothers in (a) and (c) and the corresponding F/M ratios in (b) and (d); each value is the mean ± s.e. of 3 to 6 experiments.

ternal plasma salicylate levels, concentrations in the foetal plasma of the two groups of animals did not differ significantly at any time-period (Figure 1a) or dose level (Figure 1c).

When a fixed dose of sodium salicylate (a total of 3 mg which on the maternal body weight basis was approximately 8 mg/kg in the case of controls and 12 mg/kg in the case of protein-deficient animals) was injected into foetal peritoneal caivities, there was no significant difference in the maternal plasma salicylate levels in the two groups of rats but the concentration in the foetal plasma of protein-deficient animals was greater than that in control foetal plasma (Table 2).

In pair-fed controls ($n = 3$), maternal and foetal plasma salicylic acid concentrations (means ± s.e.) 3 h following sodium salicylate (10 mg/kg, i.v.) were 312 ± 43 amd 125 ± 5 nmol/ml, respectively; these values did not differ from those in controls fed *ad libitum* (Table 3).

Table 2 Maternal and foetal plasma salicylate 3 h after the administration of 3 mg sodium salicylate into foetal peritoneal cavities on day 20 of gestation

	Dietary protein†	
	21% (control)	5% (deficient)
MC_P (nmol/ml)	274 ± 24	263 ± 17
MC_p (nmol/ml)	144 ± 10	236 ± 9*
FC_p/MC_p (ratio)	0.5 ± 0.1	0.9 ± 0.1*

† Diets fed *ad libitum* for 20 days.
MC_p: maternal plasma salicylic acid concentration; FC_p: foetal plasma salicylic acid concentration.
* Significantly ($P < 0.05$) different from control; values are mean ± s.e. of 3 animals each group.

Tissue distribution of salicylate

These were determined only at 3 h after a 10 and 250 mg/kg dose of sodium salicylate. There was no significant difference in the placental and foetal liver concentrations of salicylic acid in the two groups of rats at any of the two dose levels; however, maternal liver salicylate at a 250 mg/kg dose level was higher in protein-deficient than in control rats (Table 3). No metabolite of salicylate was detected in any of the maternal and foetal tissues studied.

Placental transfer of salicylate

Foetal to maternal plasma salicylate concentration ratios (F/M) were consistently greater in protein-deficient than in control rats at all times (0.25–12 h) (Figure 1b) and dose levels (2–250 mg/kg, i.v., Figure 1d) studied. F/M ratios were approximately 0.5 in control animals and tended to approach unity in protein-deficient rats. Indeed, 12 h after a 10 mg/kg dose and 3 h after the small dose of 2 mg/kg, F/M ratios were greater than unity in protein-deficient animals. Also, the F/M ratio was greater in protein-deficient than in control rats following intrafoetal injection of sodium salicylate (Table 2).

The F/M ratio was 0.41 ± 0.06 in pair-fed controls after a dose of 10 mg/kg sodium salicylate and this value did not differ from the F/M ratio of 0.57 ± 0.05 in controls fed *ad libitum*.

Oxyphenbutazone (50 mg/kg, i.p.) was administered 30 min before the injection of sodium salicylate (10 mg/kg, i.v.) into 3 control pregnant rats; in these animals, maternal and foetal plasma salicylate concentrations were 310 ± 30 and 140 ± 10 nmol/ml respectively, and the F/M ratio was 0.46 ± 0.04; these values were not significantly different from untreated controls.

Table 3 Distribution of salicylic acid in maternal and foetal tissues 3 h after the administration of sodium salicylate (i.v.) on day 20 of gestation

	21% protein diet		*5% protein diet*	
Na salicylate dose	10 mg/kg	250 mg/kg	10 mg/kg	250 mg/kg
MC_p (nmol/ml)	275 ± 39	3898 ± 145	163 ± 16*	3420 ± 171
FC_p (nmol/ml)	146 ± 9	2507 ± 131	139 ± 7	2877 ± 81*
MC_L (nmol/g)	17 ± 1	2145 ± 72	18 ± 1	2572 ± 68*
FC_L (nmol/g)	17 ± 1	2130 ± 145	18 ± 1	2580 ± 202
Placenta (nmol/g)	122 ± 16	2696 ± 101	131 ± 6	3072 ± 181
FC_L/MC_p (ratio)	0.06 ± 0.01	0.54 ± 0.04	0.12 ± 0.02*	0.78 ± 0.08*

Salicylic acid concentration: MC_p, maternal plasma; FC_p, foetal plasma; MC_L, maternal liver; FC_L, foetal liver.
* Significantly ($P < 0.05$) different from control values at the corresponding dose level; mean ± s.e. of 4–6 experiments.

Table 4 Influence of dietary protein on the binding of salicylic acid (SA) *in vivo* and *in vitro* to maternal and foetal serum proteins on day 20 of gestation

Serum source	Condition	n	SA (μM)	Dietary protein† 21% Bound SA	5% % of total
Mother	*in vivo**	4	413±41	87±1.6	—
			319±13	—	77±5.4
Foetus	*in vivo*	4	116±16	80±3.1	71±3.5
Mother	*in vitro*	7	360	81±3.8	74±4.6
Foetus	*in vitro*	7	180	66±7.4	60±8.4

† Diets were fed *ad libitum* from day 0 to day 20 of gestation.

* Blood was collected 15 min following the administration of 10 mg/kg sodium salicylate; aliquots of serum were used for the determination of total salicylic acid concentration and fractional binding.

Serum protein-salicylate binding

A low protein diet did not significantly alter the binding of salicylate to maternal or foetal serum proteins both under *in vivo* and under *in vitro* conditions (Table 4).

Discussion

The general effects of a marginal protein deficiency on the mother and the foetus (Table 1) are in accordance with previous reports (Nelson & Evans, 1953) and extend our data corresponding to day 21 of gestation (Mulay, Varma & Solomon, 1982).

A lower plasma salicylate level in protein-deficient than in control rats is similar to the findings in male rats (Yue & Varma, 1982) and can be attributed to an increase in glycine conjugation and urinary excretion in protein-deficient animals (Yue & Varma, 1982). The absence of a significant difference in the plasma salicylate concentrations in the two groups of animals at the high dose level of 250 mg/kg is perhaps due to a saturation of metabolic and excretory processes (Nelson, Hanano & Levy, 1966); in male rats also the higher the dose of salicylate the smaller was the difference in plasma salicylic acid concentration in the two groups of rats (Yue & Varma, 1982).

Despite differences in maternal plasma salicylate levels, concentrations in the foetal plasma of control and protein-deficient animals were not significantly different. This implies that at any time after the administration of the drug and over a wide range of dose levels, the foetal compartment contains a greater proportion of salicylate in the protein-deficient than in control rats. This could result from increased foetal accumulation (due to changes in volume of distribution, metabolism or delivery into the maternal circulation) or increased rate of placental transfer or both. The present data are insufficient to provide a definitive answer with regard to these possibilities. However, the lack of a significant difference in the absolute salicylate concentrations in the foetal plasma and tissues of the two groups of rats points against cumulation being an important factor for a relatively high foetal liver and plasma to maternal plasma salicylate concentration ratios in protein-deficient animals.

In man, foetal tissue concentrations are considerably lower than maternal serum concentrations (tissues obtained following legal abortions) (Gronroos *et al.*, 1981) although salicylate levels in the neonate of a mother who had ingested the drug just before delivery was higher than in the mother (Garrettson *et al.*, 1975). Concentrations of indomethacin ($T_{\frac{1}{2}}$ 15–60 min) in the foetal plasma of ewes and rabbits have also been found to exceed those in their mothers (Harris & Van Petten, 1981). However, in the present study an F/M ratio (foetal to maternal plasma salicylate concentration ratio) of greater than unity was found only in protein-deficient rats (Figure 1b and d). This cannot be attributed entirely to a relatively short half life of salicylate in protein-deficient rats (Yue & Varma, 1982). An F/M ratio of greater than unity was also found in protein-deficient rats at a 2 mg/kg dose although at this dose level plasma half-lives of salicylate in control and protein-deficient animals do not differ (Yue & Varma, 1982). It would seem that at low maternal plasma salicylate levels the foetus of protein-deficient animals behaves as a more 'deep' compartment (Levy & Hayton, 1973) than the control foetus.

Salicylates cross the placenta by passive diffusion (Flower *et al.*, 1980). It would appear that the equilibration of the foetal salicylate at approximately the maternal plasma salicylate levels in protein-deficient animals and only at approximately half the maternal plasma salicylate levels in the controls is caused by an increase in the placental permeability or a breakdown in the so-called placental 'barrier' in the former group of animals. Such an interpretation would be consistent with the reported increase in the placental transfer of amino-isobutyric acid (a non-metabolizable amino acid) in protein-deficient guinea-pigs (Young & Widdowson, 1975).

These studies suggest that the increased overall transfer of salicylate in protein-deficient animals is caused by a deficiency of proteins rather than that of calories because no such increase was observed in pair-fed controls. The inability of oxyphenbutazone, which is likely to influence the binding of salicylate to serum proteins, to increase the placental transfer of salicylate in control animals to the level found in

protein-deficient rats as well as a lack of significant difference in the binding of salicylate to the maternal and foetal serum proteins of the two groups of animals suggest that differences in protein binding are not important factors in the observed increase in the placental transfer of salicylate in protein-deficient animals.

Whatever the mechanism is for the increased placental transfer of salicylate, the foetus of a protein-deficient mother in the rat model is exposed to a relatively high concentration of the drug over a wide range of maternal plasma salicylate levels. It has also been reported that protein deficiency is associated with an increase in the pharmacological effects of salicylate (Yue & Varma, 1981) and this includes the foetus (West, 1964). It is difficult to extrapolate these findings in rats to human pregnancy. However, the present data do indicate that in so far as studies in the rat model reflect the human situation, ingestion of salicylate during maternal malnutrition poses an increased risk to the foetus.

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References

FLOWER, R.J., MONCADA, S. & VANE, J.R. (1980). Analgesic-antipyretics and anti-inflammatory agents; drugs employed in the treatment of gout. In *The Pharmacological Basis of Therapeutics*, ed. Gilman, A.G., Goodman, L. & Gilman, A. pp. 682–728. New York: Macmillan.

GARRETTSON, L.K., PROCKNAL, J.A. & LEVY, G. (1975). Fetal acquisition and neonatal elimination of a large amount of salicylate. *Clin. Pharmac. Ther.*, **17**, 98–103.

GRONROOS, M., HAATAJA, M., HONKONEN, E. & ANTTILA, M. (1981). Seral, uterine and fetal salicylate concentrations after oral administration. *Eur. J. Obstet. Gynec. reprod. Biol.*, **12**, 167–170.

HARRIS, W.H. & VAN PETTEN, G.R. (1981). Placental transfer of indomethacin in the rabbit and sheep. *Can. J. Physiol. Pharmac.*, **59**, 342–346.

LEVY, G. & HAYTON, W.L. (1973). Pharmacokinetic aspects of placental drug transfer. In *Fetal Pharmacology*, ed. Boreus, L. pp. 29–39. New York: Raven Press.

LOWRY, O.H., ROSEBROUGH, H.H., FARR, A.L. & RANDALL, R.J. (1951). Protein measurement with Folin phenol reagent. *J. biol. Chem.*, **193**, 265–275.

MULAY, S., VARMA, D.R. & SOLOMON, S. (1982). Influence of protein deficiency in rats on hormonal status and cytoplasmic glucocorticoid receptors in maternal and fetal tissues. *J. Endocr.*, **95**, 49–58.

NELSON, M.M. & EVANS, H.M. (1953). Relation of dietary protein levels to reproduction in the rat. *J. Nutr.*, **51**, 71–84.

NELSON, E., HANANO, M. & LEVY, G. (1966). Comparative pharmacokinetics of salicylate elimination in man and rats. *J. Pharmac. exp. Ther.*, **153**, 159–166.

PENG, G.W., GADALLA, M.A.F., SMITH, V., PENG, A. & CHIOU, W.L. (1978). Simple and rapid high-pressure liquid chromatographic simultaneous determination of aspirin, salicylic acid, and salicyluric acid in plasma. *J. Pharm. Sci.*, **67**, 710–712.

SHARPE, G.L., LARSSON, K.S. & THALME, B. (1975). Studies on closure of ductus arteriosus. XII. *In utero* effect of indomethacin and sodium salicylate in rats and rabbcts. *Prostaglandins*, **9**, 585–596.

TURNER, G. & COLLINS, E. (1975). Foetal effects of regular salicylate ingestion in pregnancy. *Lancet*, **ii**, 338–339.

VARMA, D.R. (1980). Anti-inflammatory and ulcerogenic effects and pharmacokinetics of oxyphenbutazone in protein deficient rats. *Indian J. Med. Res.*, **72**, 426–433.

WEST, G.B. (1964). The influence of diet on the toxicity of acetylsalicylic acid. *J. Pharm. Pharmac.*, **16**, 788–793.

YOUNG, M. & WIDDOWSON, E.M. (1975). The influence of diets deficient in energy, or in protein, on conceptus weight, and the placental transfer of a non-metabolisable amino acid in the guinea pig. *Biol. Neonate*, **27**, 184–191.

YUE, T.L. & VARMA, D.R. (1981). Influence of protein deficiency on lysosome stabilizing and paw edema suppressant activity of steroidal and nonsteroidal anti-inflammatory agents in rats. *J. Pharmac. exp. Ther.*, **217**, 776–783.

YUE, T.L. & VARMA, D.R. (1982). Pharmacokinetics, metabolism and disposition of salicylate in protein-deficient rats. *Drug Metab. Dispos.*, **10**, 147–152.

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