J. Pharm. Pharmacol., 1964, **16**, 788–793 Received October, 12, 1964

The influence of diet on the toxicity of acetylsalicylic acid

G. B. WEST

The toxicity of acetylsalicylic acid in rats is greater when animals are fed a high carbohydrate diet than when they receive a high protein diet. Magnesium deficiency increases this difference, particularly in pregnant animals. The maximum B.P. human dose of acetylsalicylic acid is well tolerated by pregnant rats fed on the high protein diet but one quarter of this amount produced foetal resorption and deaths in rats fed on the high carbohydrate diet. Acetylsalicylic acid is not teratogenic under the conditions used and appears to be unlikely to be teratogenic in man since its dose-toxicity curve is steep. It appears essential to report the composition of the diet in toxicity tests.

THIS paper describes the toxicity of acetylsalicylic acid in rats fed on diets of different protein levels with and without added magnesium. Magnesium was chosen as the trace metal to be omitted from the diet since the urinary free histamine levels in rats have recently been shown to be markedly elevated both in magnesium deficiency (Bois, Gascon & Beaulnes, 1963) and in pregnancy (West, 1960). Besides, magnesium influences the ability of other agents to produce coronary lesions (Olsen & Parker, 1964) and is essential for the maintenance and growth of the soft tissues (Martindale & Heaton, 1964). Both non-pregnant and pregnant animals have been used in the present work as it was considered possible that the stress of pregnancy might magnify some of the alterations in growth produced by various conditions. A preliminary note on some of the results has already been published (Brown & West, 1964). Other authors (for example, Obbink & Dalderup, 1964) have recently studied the effect of acetylsalicylic acid on foetal animals using one standard diet.

Experimental

Hooded Lister rats (150–200 g) of either sex were reared on standard diet (London Flour Millers No. 41B). Males were left with females for 3 days after which the sexes were separated and fed on test diets. These were either a high carbohydrate diet (sucrose 65%, casein 24%) or a high protein diet (casein 89%), with corn oil (5%) and the vitamin and salt mixture as used by Colby & Frye (1951). Drinking water was allowed *ad lib*. For the deficiency experiments, magnesium sulphate (600 mg/100 g diet) was omitted from the salt mixture in each diet, whilst for the drug experiments acetylsalicylic acid powder was mixed with the diet beforehand. Each rat consumed 14–16 g food per day when the diet was made up into a thick paste with water. With the mating regimen adopted, 70–80% of the females were successfully mated. Most of the pregnant animals were killed on the 20th day of gestation, and the number of live and dead foetuses, in addition to the resorption sites, were counted. Foetal mortality, represented by the proportion of dead foetuses and

From the Department of Pharmacology, School of Pharmacy, University of London, Brunswick Square, W.C.1.

resorption sites to the total number of implantations, was then calculated for each dose of acetylsalicylic acid. A few pregnant animals were allowed to litter and to suckle their young until weaning time.

Results

Effect of diet on weight. The weight of both males and non-pregnant females continued to increase on both diets over a period of 90 days though it was always slower in the groups of animals on the high protein diet. The increase in weight of pregnant animals during the gestation period also was always less on the high protein diet (mean gain 16·6 g) than on the high carbohydrate diet (mean gain 45·3 g), and foetuses always weighed less at the 20th day of gestation. A similar result was found when rats were allowed to litter and suckle their young, the animals at weaning time being about 5 g less on the high protein diet than on the high carbohydrate diet (average weight 39 g).

Effect of diet on gastric ulceration produced by acetylsalicylic acid. When males and non-pregnant females were fed for 20 days on one of the two diets containing acetylsalicylic acid (500 mg/kg daily), the incidence of gastric ulceration was significantly greater ($P < 0{\cdot}01$) in those rats on the high carbohydrate diet (mean 45%) than on the high protein diet (mean 22%). In pregnant animals a similar result was obtained though the degree of ulceration was more extensive; in addition, no live foetuses were found in animals on either diet, although animals on the high protein diet continued to increase in weight during the gestation period (mean gain 10·0 g) and contained more foetuses (32 out of 96, or 33%), whereas those of the high carbohydrate diet steadily lost weight (mean loss 10·0 g) and contained less foetuses (6 out of 120, or 5%).

Effect of magnesium deficiency. When magnesium was omitted from the salt mixture in both diets, non-pregnant rats after a few days excreted large quantities of free histamine in the urine (estimated on the isolated guinea-pig ileum). At the same time, erythema of the ears developed and was particularly noticeable when the histamine excretion became maximal (after about 10 days). Continued feeding of the deficient diets resulted in excessive histamine excretion for about the next 10 days, after which the values returned to control levels. These results agree with those reported by Bois & others (1963). At this stage, many of the tissue mast cells in the subcutaneous connective tissue were grossly degranulated but not disrupted (stained with nuclear fast red), yet the dextran anaphylactoid reaction was still obtainable. When magnesium was restored to the diets for 15 days or more and then omitted for the next 30 days, the histamine excretion again increased, this process being repeatable many times (see Fig. 1). Replacement of the magnesium possibly enabled the store of histamine to be replenished and this was then released when magnesium was again withdrawn from the diets. It is now well known that magnesium is essential for the uptake of catecholamines and 5-hydroxytryptamine by adrenal medullary granules, and the same may be true for the uptake of histamine by mast cell granules.

Whereas the effects of magnesium deficiency were similar in non-pregnant rats fed on either diet, there was a marked difference in pregnant animals. Magnesium lack was so detrimental in animals on the high carbohydrate diet that there was no gain in weight during the gestation period (mean loss 3·4 g), and a large number of resorption sites and dead foetuses (116 out of 148, or 79%) were found when these rats were killed. On the high protein diet, however, magnesium deficiency did not markedly alter the gain in weight during pregnancy (mean gain 12·2 g) although at the 20th day of gestation the foetal mortality rate was 46% (see Tables 1 and 2). It is clear that magnesium deficiency in pregnant rats fed a high carbohydrate diet is more detrimental than in those on a high protein diet.

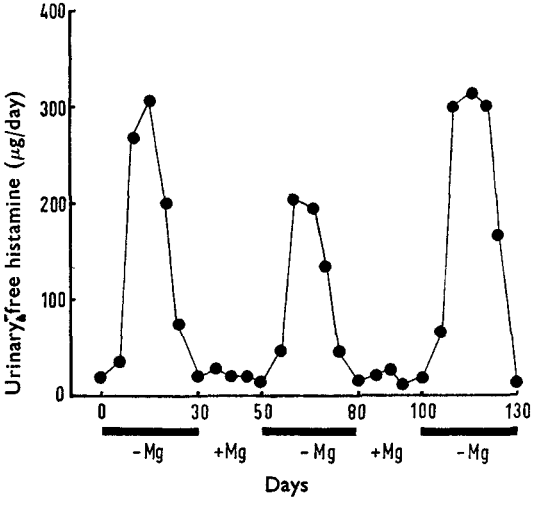

FIG. 1. Excretion of urinary free histamine (μg/day) by a non-pregnant rat when fed for 130 days the high carbohydrate diet in the presence and absence of magnesium. Note that the increase in histamine excretion occurs very soon after the deficient diet is fed but it is not maintained and does not re-occur until after magnesium is restored to the diet.

TABLE 1. TOXICITY OF ACETYLSALICYLIC ACID IN PREGNANT RATS FED A HIGH CARBOHYDRATE DIET WITH OR WITHOUT MAGNESIUM

Magnesium in diet	Acetylsalicylic acid in diet (mg/kg)	No. of rats	Mean weight gain (g) Day 6–20	No. of implants		No. of foetuses			No. of resorption sites	Mortality (%)
				Total	Per litter	Total	Live	Dead		
Present ..	0	11	+45·3	95	8·6	94	94	0	1	1
	12·5	4	+44·9	43	10·8	39	35	4	4	19
	25	6	+30·7	83	13·8	71	49	22	12	41
	50	6	+41·9	74	12·3	64	49	15	10	34
	250	4	+31·0	46	11·5	23	10	13	23	80
	500	10	−10·0	120	12·0	6	0	6	114	100
Absent ..	0	14	− 3·4	148	10·6	48	32	16	100	79
	12·5	3	+ 3·0	32	10·7	14	4	10	18	88
	50	7	+10·6	72	10·3	57	1	56	15	99
	250	6	+ 0·7	60	10·0	5	2	3	55	96
	500**	11	−15·5	128	11·6	21	0	21	107	100

** At this dose, 4 other pregnant rats died after 12 days.

TABLE 2. TOXICITY OF ACETYLSALICYLIC ACID IN PREGNANT RATS FED A HIGH PROTEIN DIET WITH AND WITHOUT MAGNESIUM

Magnesium in diet	Acetylsalicylic acid in diet (mg/kg)	No. of rats	Mean weight gain (g) Day 6–20	No. of implants		No. of foetuses			No. of resorption sites	Mortality (%)
				Total	Per litter	Total	Live	Dead		
Present ..	0	10	+16·6	102	10·2	100	100	0	2	2
	50	9	+18·0	129	14·3	120	120	0	9	7
	250	8	+20·2	84	10·5	64	43	21	20	49
	500	9	+10·0	96	10·7	32	0	32	64	100
Absent ..	0	11	+12·2	99	9·0	83	52	31	16	46
	50	8	+10·6	93	11·7	78	58	20	15	38
	250	8	− 2·6	89	11·1	52	11	41	32	82
	500	7	− 7·1	60	8·6	10	0	10	50	100

Effect of diet and magnesium deficiency. The foetal toxicity of acetylsalicylic acid in pregnant rats on the two diets is shown in Tables 1 and 2. Although the weight gain was maintained when rats on the high carbohydrate diet received doses of acetylsalicylic acid up to 250 mg/kg, the foetal mortality rate steadily increased up to 80%. On the high protein diet, however, the toxic effect was less, 250 mg/kg yielding a 49% foetal mortality and 50 mg/kg showing no adverse effects. Thus the foetal toxicity of acetylsalicylic acid in animals on the high carbohydrate diet was much greater than in animals on the high protein diet (see Fig. 2).

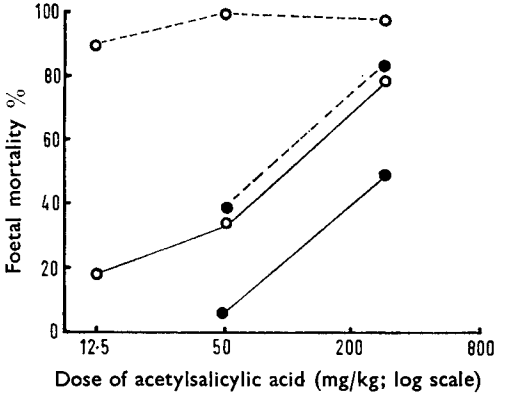

FIG. 2. Toxicity of acetylsalicylic acid in pregnant rats fed a high protein diet (●—●) or a high carbohydrate diet (○—○). The effects of magnesium deficiency are shown by the broken lines. Note that magnesium deficiency and the high carbohydrate diet markedly increase the toxicity.

When magnesium was omitted from the diet, the foetal toxicity of acetylsalicylic acid in pregnant rats again increased. Thus, a dose of 50 mg/kg killed 99% of foetuses when the high carbohydrate diet was used, and 250 mg/kg on the high protein diet produced over 80% mortality. It is interesting to note from Table 2 that a dose of 50 mg/kg acetylsalicylic acid did not increase the toxic effects of magnesium deficiency in pregnant

rats fed the high protein diet. Four out of 15 pregnant rats receiving the highest dose of acetylsalicylic acid mixed with the high carbohydrate magnesium-deficient diet died after about 12 days on this diet (see Table 1).

Discussion

This work was initiated during the testing of drugs for those most likely to exhibit teratogenic activity in man, but it was soon evident that dietary factors play important roles in the toxicity of drugs and this is seen also in non-pregnant animals. For example, the incidence of gastric ulceration, as a result of feeding acetylsalicylic acid to non-pregnant rats, was found to be much greater on the high carbohydrate diet than on the high protein diet. The result was similar in pregnant animals though here, in addition, the toxicity of acetylsalicylic acid to the foetus was increased. The highest dose of acetylsalicylic acid used in the present experiments (500 mg/kg or about 10 times the maximal B.P. human dose) produced death of all the foetuses though it was of interest that pregnant animals continued to increase in weight during gestation when fed the high protein diet but lost weight on the high carbohydrate diet.

When the daily dose of acetylsalicylic acid was reduced to 50 mg/kg (equivalent to 3 × 5 grain tablets four times a day in man), a relatively large number of dead foetuses and resorption sites were found in those animals fed the high carbohydrate diet. On the other hand, the foetal mortality rate in animals given this dose on the high protein diet remained well within the range of values found in control animals (maximum 10% in 82 rats studied). Only when the dose in the protein diet was increased five times did the toxic action of acetylsalicylic acid become prominent. It should be noted that the diet for optimal growth of the young rat has always been accepted as about 14% protein and 75% carbohydrate.

When the effects of magnesium deficiency were assessed, gastric ulceration occurred in nearly all the non-pregnant rats fed acetylsalicylic acid in the high carbohydrate diet but only in half of those on the high protein diet. In pregnant animals, even without acetylsalicylic acid, magnesium deficiency resulted in a foetal mortality on the high carbohydrate diet which was about twice that on the high protein diet, and when the acid was included, the mortality rates were correspondingly increased. Thus, magnesium plays an important role in the developing rat embryo; the full diet contained about 600 ppm magnesium whereas the deficient diet had only about one-tenth this amount. Magnesium deficiency has been extensively studied in young rapidly-growing animals by other workers who have reported skin lesions, hyperexcitability and convulsions, but in the present experiments these effects were not found. It is possible that the magnesium content of the deficient diet was higher than that in diets used by other workers or that the experiments were not carried on for a long enough period. Other factors such as the dietary cholesterol (Olson & Parker, 1964) may be involved. The importance of using foods containing adequate amounts of absorbable magnesium during pregnancy is emphasised.

This preliminary study thus shows that acetylsalicylic acid, when incorporated in a diet high in carbohydrate, is substantially more toxic to foetal rats than when it is given in a diet high in protein. The foetal toxicity also increases when magnesium is omitted from both diets. The toxicity of acetylsalicylic acid in the first week of pregnancy in rats is now being tested. Animal tests cannot prove a drug to be non-teratogenic to man, but it is useful to note that the steep slope of the dose-response curves of acetylsalicylic acid is similar to that of reserpine and unlike that of thalidomide (West, 1963). Consequently, acetylsalicylic acid appears to be less likely to produce congenital malformations in the young (and it did not in the present experiments), since a 10-fold increase in dose produces a large increase in toxicity. The present work, however, indicates that one of the important factors which must be taken into account in assessing the toxicity of a drug is the diet.

References

Bois, P., Gascon, A. & Beaulnes, A. (1963). *Nature, Lond.*, **197**, 501–502.
Brown, R. A. & West, G. B. (1964). *J. Pharm. Pharmacol.*, **16**, 563–565.
Colby, R. W. & Frye, C. M. (1951). *Amer. J. Physiol.*, **166**, 209–212.
Martindale, L. & Heaton, F. W. (1964). *Biochem. J.*, **92**, 119–126.
Obbink, H. J. K. & Dalderup, L. M. (1964). *Lancet*, **1**, 565.
Olsen, E. J. & Parker, H. E. (1964). *J. Nutrition.*, **83**, 73–78.
West, G. B. (1960). *Int. Arch. Allergy*, **16**, 39–48.
West, G. B. (1963). *J. Pharm. Pharmacol.*, **15**, 63–64.