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A TERATOGENIC EFFECT OF A SULPHONAMIDE IN EXPERIMENTAL ANIMALS

BY

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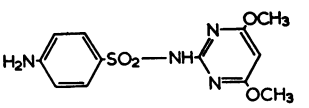
Sulphamoprine, when given in the diet at levels from 0.025% upwards to pregnant rats and mice, produced abnormalities of eruption of the incisor teeth and of the skull of the offspring. Rabbits are not susceptible to this teratogenic action.

The systematic examination of drugs to determine whether they have any adverse effect on the foetus of experimental animals has demonstrated that many drugs in common use do have such actions. Although the significance of the experimental findings for the human use of the drug for the most part cannot be ascertained, a number of warnings about the use of such drugs has been issued by various pharmaceutical firms. This paper records the experimental results on which such a warning was based (Green, 1963a, b). The experiments are of interest in that the effect encountered in animals appears highly specific and may be unique; they also demonstrate features of interest in the design of experiments in this field.

The sulphonamide which features in them is sulphamoprine (sulphadimethoxypyrimidine; 2-*p*-aminobenzenesulphonamido-4,6-dimethoxypyrimidine) which has the structural formula:



It is marketed in admixture with sulphadimidine (Sulphamezathine I.C.I.) as Bimez (I.C.I.).

METHODS

The rats, mice and rabbits used in these experiments were all bred in our own laboratories. The rats were a specific-pathogen-free (SPF) Wistar derived albino strain, Alderly Park Rat 1. The mice were a SPF albino strain, Alderley Park Mouse 1. The rabbits were New Zealand white.

Administration of drugs

Sulphamoprine was supplied from manufacturing stock intended for use in formulating the commercial product; when administered in the diet, the required amount of drug was well

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mixed in a powder diet and fed to the animals *ad libitum*. When oral dosing was used, the drug was made up as a 10% suspension in a Lissapol-Dispersol mixture and administered by gavage as indicated. All animals were provided with water *ad libitum*. Control animals received either unmedicated diet or oral doses of suspending fluid alone.

Breeding methods

Rats. Mature virgin females, weighing 200 g, were obtained from the breeding unit and mated in pairs with males of proven fertility. They were observed for pregnancy. Pregnant animals were allowed to bear and suckle a litter. Animals failing to become pregnant or failing successfully to bear and rear a litter greater in number than six were discarded. The remaining animals were allocated at random to experimental groups. They were then mated in harems of four females to one male with males of known fertility. A vaginal smear was taken daily and examined for the presence of spermatozoa at an appropriate stage of the oestrous cycle. The day on which spermatozoa were found was termed day 1 of pregnancy. The animals were then separated from the males and caged individually until the end of the experiment or until weaning of their young. Newborn rats were observed as soon as possible after birth for number, weight, the presence of abnormalities and the presence of stillborn animals. They were examined again at 7 days and again at the end of weaning. Every effort was made to preserve suckling rats that died during the course of the experiment.

Mice. The methods used for mice were similar to those used for rats except that the presence of a vaginal plug was taken as evidence of successful insemination and vaginal smears were not used.

Rabbits. Virgin does were mated with bucks of proven fertility and copulation was observed. This day was termed day 1 of pregnancy.

Examination of young animals

Stillborn animals, those that died during the suckling period, and survivors at 21 days after birth were examined. Dissections were made to demonstrate the presence and normal structure of the major viscera and the skeletons were then prepared by Dawson's method of alizarin staining (Gatenby & Beams, 1950).

RESULTS

Rats

Table 1 shows the results of experiments in which three levels of sulphamoprine were administered from day 1 of pregnancy through weaning. The highest level chosen had little effect on litter size, but there was a very high mortality during the early part of the suckling period. Lower levels permitted the survival of the young rats in greater numbers, but a high proportion of the young showed a characteristic

TABLE 1

EFFECTS OF DIET CONTAINING SULPHAMOPRINE GIVEN FROM DAY 1 OF PREGNANCY TO END OF LACTATION

* All abnormalities were of teeth and skull. † Includes results of two experiments

Drug concentration (%)	No. of dams	No. pregnant	Born			Weaned		
			Total	Average litter size	Total stillborn	Total	Average litter size at weaning	No. abnormal*
0·1	10	9	66	7·3	5	0	0	—
0·075	10	9	97	10·8	8	7	0·8	7
0·05	10	8	75	9·4	0	70	8·7	17
0·025†	23	19	200	10·5	1	144	7·6	7
Controls	8	8	94	11·7	1	91	11·4	0

abnormality of the jaws and teeth (Fig. 1). As the incisor teeth began to erupt at about day 7, there was failure of occlusion of the upper and lower dentition, resulting in the continued growth, particularly of the lower incisors which eventually impinged

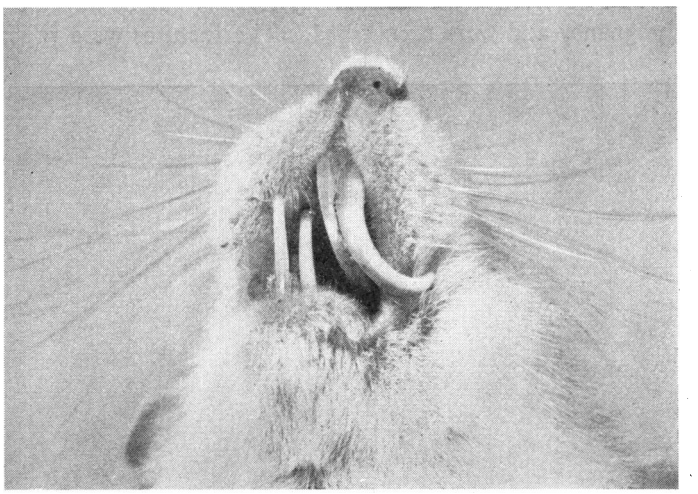

Fig. 1. Suckling rat aged 21 days, showing malocclusion of incisor teeth, due to administration of sulphamoprine to dam throughout pregnancy.

on the upper gum at one side or other of the mid-line. Skull preparations showed that this abnormality was associated with a deviation of the mid-line sutures (Fig. 2). In a proportion of cases there was a bilateral shortening of the nasal and premaxillary bones, resulting in a short-snouted rat in which malocclusion of the incisors did not occur (Fig. 3).

These abnormalities were not detected until the second week of life, and it seemed possible that they arose as a result of absorption of the drug during postnatal life. An experiment was therefore performed in which animals born to treated mothers were fostered on to untreated mothers and offspring of normal mothers were fostered on to mothers still receiving the drug. The results of this experiment are given in Table 2 and it can be seen that the occurrence of the abnormalities was associated

TABLE 2

EFFECT OF CROSS-FOSTERING OF OFFSPRING OF TREATED AND UNTREATED DAMS

Offspring of treated dams were fostered on untreated mothers and offspring of untreated dams were fostered on treated mothers. Note : all abnormalities seen in this experiment were skull and teeth defects

Drug (%) in diet form				Total number of			
Insemination to birth	Birth to weaning	No. of dams	No. of pregnant dams	Live born	Stillborn	Weaned	Abnormal
0·1	Control	10	10	82	10	4	1
0·075	Control	10	9	80	3	15	4
0·05	Control	10	10	98	3	67	22
Control	0·1	13	9	75	9	67	0
Control	0·075	8	8	80	8	79	0
Control	0·05	11	9	107	0	91	0

with drug treatment during pregnancy and not with the absorption of the drug from the mother during suckling.

To clarify the exact nature of the abnormality, an experiment was performed in which pregnant rats received a diet containing 0.075% sulphamoprine from day 1 to day 21 of pregnancy and were then killed. The foetuses were prepared by the

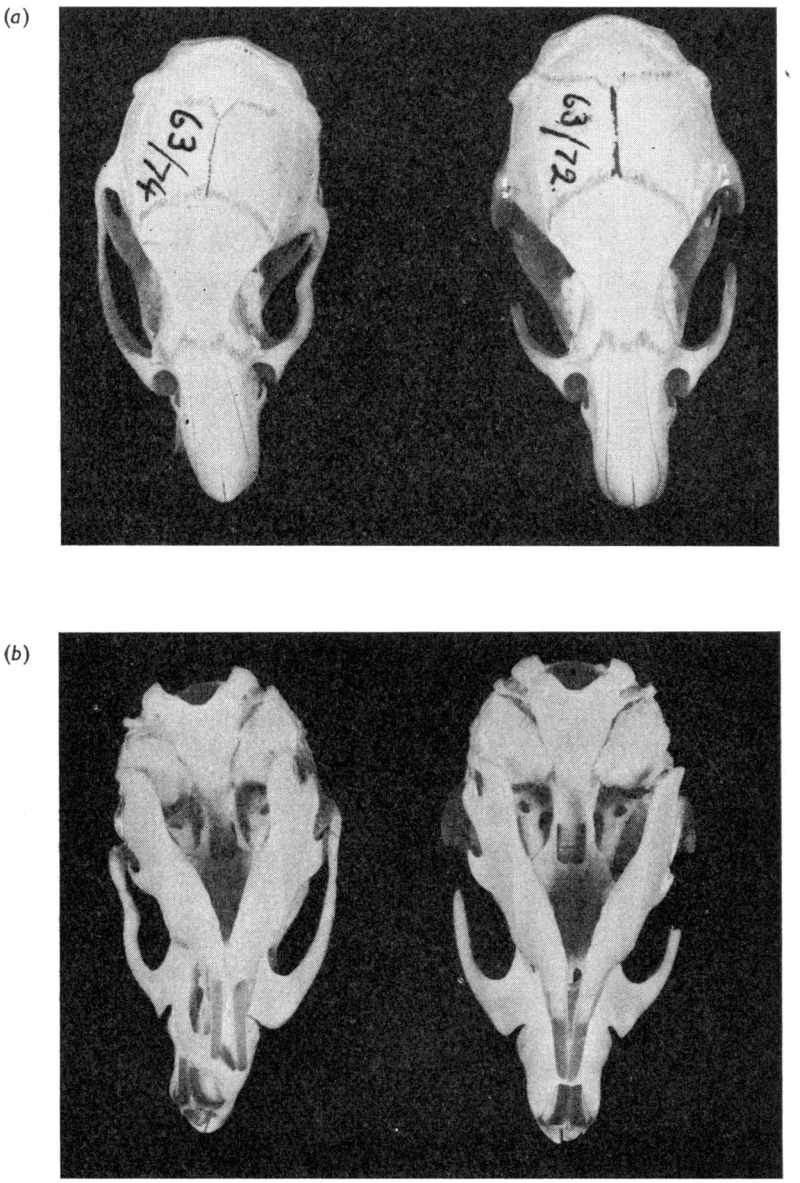

Fig. 2. The effect of administration of sulphamoprine to rat dam throughout pregnancy. Views of cranium (*a*) and base of skull (*b*) of control rat (on right, numbered 63/72) and affected rat (on left, numbered 63/74). Note considerable deviation of all midline sutures in skull of affected rat.

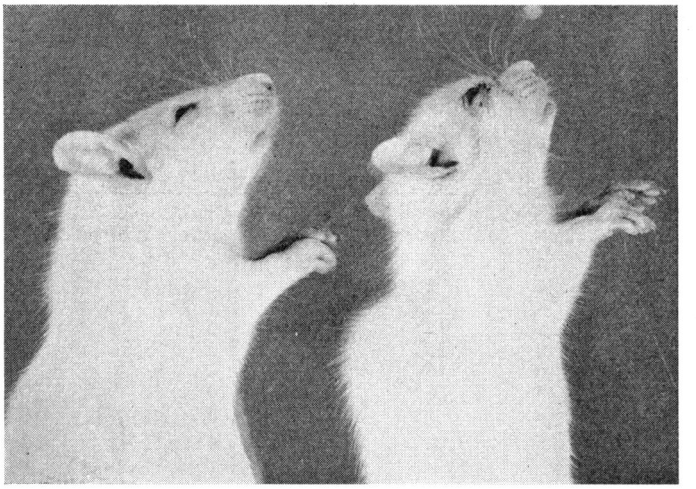

Fig. 3. Effect of administration of sulphamoprine to dam throughout pregnancy. Control rat on left, "short-snouted" rat on right.

alizarin technique. Table 3 gives the results of this experiment. Despite the fact that the foetuses were examined meticulously and in the knowledge that a high proportion of them must have an abnormality, and that such an abnormality most

TABLE 3

EFFECTS IN DAMS KILLED ON DAY 21 AND FOETUSES EXAMINED AFTER ALIZARIN STAINING

There were six rats in each group. Abnormalities : *short mandible and pair of fused ribs; † one rat had two centres of ossification in sternebrae 2, 3, and 4, the other had asymmetrical parietal bones

Drug concentration	No. of pregnant dams	Foetuses			
		Total	Average litter size	Average foetal weight (g)	No. abnormal
0·075% from days 1 to 21	5	66	13·2	3·14	1*
Control	6	76	12·6	3·09	2†

probably concerned the skull bones, only one abnormal foetus was found in the treated group. This had an abnormal skull and a pair of fused ribs. Thus, if the skull bones were abnormal, the abnormality must have been so slight that it could escape even informed scrutiny until the teeth had erupted.

The effect of very large single doses at various stages of pregnancy was also investigated. Table 4 shows that these large doses are virtually destructive to the foetus and those which do survive suffer from a large variety of severe abnormalities (Table 5). Because sulphamoprine is highly persistent, too much stress should not be placed on these results as a basis for timing of the teratogenic effect.

TABLE 4

THE EFFECT OF VERY LARGE SINGLE DOSES OF SULPHAMOPRINE ON PREGNANT RATS

All treated animals received a single dose of 1 g/kg of sulphamoprine. The abnormalities are listed in Table 5. * Excluding twelve foetuses from a dead rat ; † excluding four foetuses from a sick rat ; ‡ one stillborn and five weanlings

Day of administration to dam	No. of dams	No. of pregnant dams	Total number of				Mean litter size at birth	No. abnormal
			Stillborn	Live born	Post-natal deaths	Weaned		
8	5	4	1	12	6	6	3·0	6‡
9	5	4	3	10	10	0	2·5	3
10	5	4	22	1	1	0	0·25	22
11	5	4	12	3	3	0	0·75	12
12	5	5	25	2	2	0	0·40	25
13	5	5	16	0	0	0	0	16
14	5	5	26*	0	0	0	0	26*
15	5	4	1†	3	3	0	0·75	1†
Control	5	5	0	47	4	43	8·6	0

TABLE 5

ABNORMALITIES FOUND IN OFFSPRING FROM MOTHERS GIVEN SINGLE MASSIVE DOSES OF SULPHAMOPRINE

Days refer to time of administration to dam

Day 8 Weanlings : skull defects. Stillborn : irregular sternabrae.

Day 9 Skull defects, shortening, thickening and bending of humerus, radius, ulna, femur, tibia and fibula. Irregular curvative of ribs, irregular sternabrae. One with no fusion of mandibular symphysis.

Day 10 Wide skull sutures, short nasal bones, abnormal sternabrae, scoliosis, some with abnormal radius, ulna, humerus, femur, tibia and fibula. Two with brachygnathus and microstomata. Cleft palates in some.

Day 11 Wide skull sutures. Limb long bones shortened, thickened, and some bent. Scoliosis. Abnormal numbers of digits or paws. Irregular sternabrae. Brachygnathus, microstomata and cleft palates.

Day 12 Skull defects. Irregular size and shape of sternabrae. Scoliosis in some. Abnormal fore and hind limbs. Some with irregular clavicles and scapulae. All had brachygnathus, microstomata, and moderate to very widely cleft palates.

Day 13 Defects of skull and lower jaw. Abnormal fore and hind limbs. Clavicles small or nonexistent. Sternabrae irregular in size and shape. Some with rib defects. All had brachygnathus and microstomata. Moderate to very widely cleft palates. Scoliosis.

Day 14 Sternabrae irregular in size, shape and number ; some completely fused together. Rib defects, skull defects. Abnormal fore and hind limbs. Clavicles small or nonexistent. All had brathygnathus, microstomata and slightly to moderately cleft palates.

Day 15 Irregularities in shape and size of sternabrae. Some with abnormal ribs. Some with irregular tibia, radius and ulna. Four with brachygnathus and microstomata. One with slightly cleft palate.

Mice

Table 6 shows the effects of the drug on mice when administered throughout pregnancy and lactation. It can be seen that the mouse was no less susceptible than the rat to the effects of sulphamoprine on the foetus and that the abnormalities produced were of a similar kind to those found in the offspring of treated rats.

TABLE 6

EFFECT OF SULPHAMOPRINE ON THE MOUSE FOETUS

Abnormalities of weanling mice were confined to skull and lower jaw. Abnormalities in stillborn mice : some with slightly cleft palate and brachygnathus. Irregularities in size and shape of sternabrae. Eye abnormalities

Drug concentration (%)	No. of dams	No. of pregnant dams	Total number of				
			Stillborn	Live born	Post-natal deaths	Weaned	Abnormal
0·1	7	6	19	18	18	0	13
0·075	7	7	5	74	62	12	9
0·05	6	5	11	46	22	24	10
Control	6	6	2	43	6	37	0

Rabbits

Table 7 shows the results of an experiment on rabbits. This species appeared completely insusceptible to the teratogenic action of sulphamoprine, although some foetal loss had probably been caused by the compound and the surviving offspring were smaller than the controls, presumably as a result of the toxic action of the drug.

TABLE 7

EFFECT OF SULPHAMOPRINE ON THE RABBIT FOETUS

No significant abnormalities were detected in animals in any group. In (*B*) "stillborn" refers to reabsorbed foetuses, and "Average weight of offspring" refers to average foetal weight. Average weight of offspring, in (*A*), was determined at 7 days after birth

(*A*) *Rabbits allowed to litter*

Drug concentration (%)	No. of dams	No. of pregnant dams	Total number of			Average litter size at		Average weight of offspring (g)
			Live born	Weaned	Stillborn	Birth	Weaning	
Control	4	4	33	29	0	8·3	7·3	82·3
0·05	2	2	11	11	0	5·5	5·5	75·2
0·1	2	2	6	6	2	3·0	3·0	91·0
0·2	3	3	22	18	1	7·3	6·0	94·9
0·4	3	3	15	15	3	5·0	5·0	105·2
1·0	2	2	3	3	0	1·5	1·5	120·7
(*B*) *Rabbits killed at* 28 *days*								
Control	4	3	19	—	2	6·3	—	33·7
0·05	2	2	9	—	1	4·5	—	33·8
0·1	2	2	12	—	3	6·0	—	32·0
0·2	3	3	20	—	2	6·7	—	32·9
0·4	3	3	24	—	0	8·0	—	32·7

DISCUSSION

It is clear that sulphamoprine is a potent teratogen for the rat and mouse. Very large doses at various periods of early pregnancy are capable of producing a wide variety of abnormalities. Under conditions of continuous administration, however, these severe effects are not seen, presumably because, under these conditions, the vast majority of animals with severe defects die *in utero*. Doses which permit a high percentage of survival in the offspring of dosed mothers still, however, cause structural abnormalities in the young. The abnormalities appear to be highly specific and, in the most characteristic form, consist of a mal-occlusion of the incisor

teeth. There is an associated deviation of the mid-line sutures but the evidence does not enable us to decide which of these defects is primary.

The abnormalities are only detected easily when treated animals are allowed to litter and rear their young. This points to the importance of designing experiments in such a way that some, at least, of the animals will litter normally. The common experimental design is for pregnant animals to be killed on the expected day of delivery. Under these circumstances, an abnormality such as that described here would certainly not be detected.

Mice appear approximately as susceptible as rats to the teratogenic action of the drug and rabbits appear completely insusceptible. The reasons for this difference between species have not been elucidated. Differences in rates of absorption and excretion, degrees of protein-binding and paths of metabolism may all occur between species, but their relative importance is difficult to determine. It may be of significance that we have found that at least one other highly protein-bound sulphonamide similar to sulphamoprine would produce similar abnormalities, while a less protein-bound sulphonamide, sulphadimidine, was without effect on the foetus in experiments exactly similar to those reported here.

As with all toxicity experiments, it is difficult to assess the importance of these findings for the human use of the drug. No congenital abnormalities associated with the administration of this drug during pregnancy have ever been reported, nor has the publication of a warning on this subject resulted in the recollection of any cases that might be attributed to it. Nevertheless, the clear demonstration of potent teratogenic action of a drug such as this in a mammalian species at therapeutically relevant doses such as those used in these experiments must clearly indicate that it is unwise to use the drug in women who are, or may be, pregnant.

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