AN INVESTIGATION INTO THE TOXICITY OF SULPHANILAMIDE IN ALBINO RATS AND THE PROTECTIVE EFFECT OF NICOTINIC ACID

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THE introduction of the sulphonamide group of drugs of which the parent substance is sulphanilamide (p-amino benzene sulphonamide) marked a renaissance in chemotherapy and these drugs have a wide clinical applicability to-day. As to every other chemotherapeutic remedy a number of toxic reactions has been ascribed to them. These reactions are many and varied and, in some instances, of a serious nature. Most of the symptoms, *e.g.*, headache, vertigo, anorexia, nausea, vomiting, general malaise, palpitation, prostration, cyanosis, etc., are relatively mild. More serious are skin reactions with the occasional occurrence of exfoliative dermatitis and lesions of the hæmopoietic system such as agranulocytosis, acute hæmolytic anæmias, jaundice, etc. A number of other symptoms has been reported which include stomatitis, diarrhœa, peripheral neuritis and psychoses.

Various measures have been advocated to guard against the danger of undesirable toxic effects following the administration of sulphonamides. Of these the following may be mentioned: avoidance of sulphur in any form and purgatives in general (except liquid paraffin) with the object of preventing the formation of sulphæmoglobinæmia; methylene blue (Wendel, 1939) has been of value in removing the cyanosis due to methæmoglobinæmia; the administration of sodium lactate (Hartmann, Perley and Barnett, 1938*a*) and sodium bicarbonate (Lucas and Mitchell, 1939) has been suggested to prevent fall in CO_2 capacity and to counteract acidosis and renal injury, but the use of the latter as a routine measure has been criticized (Hartmann *et al.*, 1938*b*); acute toxic symptoms produced by sulphanilamide have been found to be reduced by the simultaneous administration of sodium acetate (James, 1939, 1940); vitamin C (ascorbic acid) has also been reported to be effective in preventing undesirable reactions (Dainow, 1939); vitamin B_1 (thiamin chloride) has been suggested by Findlay (1939). According to McGinty, Lewis and Holtzclaw (1939) and Cottini (1940), nicotinic acid (β-pyridine carboxylic acid) has been successfully used for the same purpose.

The object of the present investigation was to observe the effect of the administration of nicotinic acid on acute toxic reactions produced by sulphanilamide in rats. It is now known that patients treated with sulphanilamide excrete porphyrin in the urine which has been found to be chiefly porphyrin of type III (Rimington and Hemmings, 1938). Rimington and Hemmings (1939) after further work have tentatively suggested that sulphanilamide, by causing methæmoglobinæmia and increased destruction of erythrocytes, brings about an increased excretion of type III porphyrin derived from the broken-down blood pigment, and have pointed out that the porphyrinuric action of drugs of the sulphonamide group appears to run parallel with their toxicity. The toxic reactions produced by sulphanilamide bear a certain resemblance to the manifestations—both the premonitory symptoms and the diagnostic syndrome—of pellagra. In pellagra there is also an increased excretion of porphyrin type III (Editorial, 1939) and nicotinic acid has a curative action in this disease. These facts indicate that nicotinic acid may have a protective effect against the toxic manifestations produced by sulphanilamide.

Experimental

Various doses of sulphanilamide were used to determine the lethal dose for rats. The 'characteristic mortality' produced by different doses was studied, and the median lethal dose (LD 50) was ascertained. The latter is the dose which will kill 50 per cent of a sufficient number of animals (Trevan, 1927). The possibility of modifying the mortality of rats receiving LD 50 by the administration of nicotinic acid was then studied. In one group of experiments, the median lethal dose was given as a single dose preceded and followed by the administration of nicotinic acid. The latter was given 2 to 3 hours before, and 4 to 6 hours after, the dose of sulphanilamide. In another group of experiments, the median lethal dose of sulphanilamide was given in three portions within a period of 24 hours, each administration being preceded by a dose of nicotinic acid given 2 to 3 hours before. The idea underlying the second group of experiments was that the sudden administration of a heavy dose of sulphanilamide might exert a toxic effect too rapidly for an antidote to exert a protective action.

The animals used were rats of the albino stock of the Nutrition Research Laboratories, Coonoor. Both male and female rats were used, the body weights varying from 60 to 150 grammes. The animals were fed on a diet consisting mainly of rice and poor in nicotinic acid. Sulphanilamide tablets (Parke, Davis & Co.—p-amino benzene sulphonamide) were pulverized, and the requisite quantity, calculated on the basis of body weight, was given orally

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to fasting animals in the form of a pellet made with a little sugar and water. There was no difficulty in giving the animals sulphanilamide in the manner described. The amount of nicotinic acid given daily as an antidote was 625 μg. throughout the investigation. This was administered in solution by mouth through a graduated pipette. The choice of this average dose was reached after a consideration of doses in cases of human pellagra as suggested by Spies (500 mg. daily) and also depended on the fact that preliminary observations showed a dose of this order to be innocuous.

Median lethal dose

The toxic effect produced by the oral administration of various doses of the drug was studied in 104 rats. The results are shown in table I and represented graphically in figure 1.

TABLE I

Mortality resulting from various doses of sulphanilamide

Dose per kg. of body weight.	Number of rats used	Number dying	Percentage mortality
3.5 grammes	10	0	0.0
4.5 „	24	5	20.8
5.5 „	30	15	50.0
6.5 „	25	19	76.0
7.0 „	15	13	86.6

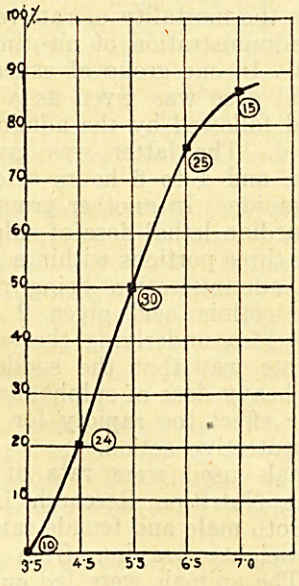

Fig. 1

The expected mortality from any given dose, and vice versa, can be ascertained from the figure. The LD 50 was 5.5 grammes per kilo of body-weight. Statistical calculations indicate that the actual range of the number of expected deaths at this level of dosage was 15 ± 8.22, or, expressed as a percentage, 50 ± 27.4, with an approximate probability of 0.9973. Conversely, the range of possible doses which give rise to a mortality of 50 per cent is 5.5 ± 18.15 per cent, *i.e.*, from 4.6 to 6.6 g. per kilo of body-weight.

These figures for lethal doses are not in agreement with those obtained by other workers for rats. Halpern and Mayer (1937) reported LD 90 to be 4 g./kg. In the experiments of Murayama and Leake (1938) doses of 4, 5, 6, 8 and 10 grammes per kg. produced mortality rates of 20, 29, 36, 40 and 50 per cent respectively. The results of the present investigation correspond, in general, to those of Molitor and Robinson (1939), who found LD 50 to be 6.2 g. It is difficult to explain the cause of these wide variations. Age has been mentioned by Molitor and Robinson (*loc. cit.*) as a possible factor in variation. It is possible that the strain of rats used and their diet may have some significance. In one experiment (not reported in this series) it was found that a group of rats receiving a diet of wheat and milk was definitely less susceptible to the toxic action of the drug than rats fed on the rice diet.

Acute toxic reaction.—The signs of the acute toxic reaction leading to death were as follows :—

The effects of poisoning appeared in 2 to 8 hours, usually between 3 and 4 hours. The animals became less active, but showed suggestions of ' agitation '. They refused food. Thirst developed. Even in the early stages the rate of respiration was obviously increased. Later, the animals became more restless, showing diverse purposeless incoordinated movements. Weakness and paralysis of the limbs followed. At this stage severe clonic convulsions lasting a few seconds usually occurred. The convulsions gradually diminished in frequency, and the animals passed into a comatose state with limbs extended and rigid, with very rapid respirations. Death ensued. Some animals showed bleeding from the gums and the nose. Most of the deaths occurred between 24 and 48 hours after administration of the drug. The earliest time was 12 hours and the latest 74 hours.

The protective effect of nicotinic acid in rats receiving 5.5 g./kg. of sulphanilamide (LD 50)

Further experiments were carried out in which the protective effect of nicotinic acid was investigated. In these experiments, groups of animals similar in age and sex composition were observed, one group receiving 5.5 g./kg. of sulphanilamide only and another group nicotinic acid in addition to this dose of sulphanilamide. The results obtained in experiments in which sulphanilamide was administered as a single dose are shown in table II.

TABLE II

Effect of nicotinic acid on mortality from sulphanilamide poisoning

Group	Number of animals	Number of deaths	Percentage mortality
Receiving sulphanilamide only.	30	15	50.0
Receiving sulphanilamide + nicotinic acid.	30	7	23.3

The difference in the percentage mortality in the two groups is statistically significant at a 0.03 level of significance.

In another group of experiments, the median lethal dose of sulphanilamide was given in three portions. Here a distinction was made between severe and mild toxic reactions and the groups were compared on this basis. The absence of convulsions and recovery was taken as the criterion of a mild reaction. The results are shown in table III. Animals showing mild manifestations include those showing no toxic signs at all, while a number of animals dying (figures in parenthesis in table III) is included in the group showing severe reactions.

TABLE III

Effect of nicotinic acid on the exhibition of mild and severe reactions to sulphanilamide poisoning—LD 50 administered in divided doses

Group	Number of animals	Mild reaction	Severe reaction	Percentage of severe reactions
Receiving sulphanilamide only (divided doses).	30	9	21 (6)	70.0
Receiving sulphanilamide + nicotinic acid.	30	16	14 (3)	44.6

N.B.—Figures in parenthesis indicate deaths.

Statistical analysis showed the difference in the percentage of severe reactions in the two groups to be statistically significant, at 0.01 level of significance.

It will be observed that the mortality in the 30 animals receiving sulphanilamide in divided doses (table III) was 6, while that in the 30 animals receiving the same amount in single dose without any nicotinic acid (table II) was 15. The difference is striking and shows that the administration of a single heavy dose of the drug is more dangerous than the same dose given in divided portions. In clinical practice, the drug is usually given in small and divided doses, and deaths during therapy are rare. In the second group of experiments, in which sulphanilamide was given in divided doses, conditions thus correspond more closely with those obtaining in clinical practice, and here the efficacy of nicotinic acid in preventing severe toxic reactions was strikingly apparent.

Discussion

The following lethal doses of sulphanilamide for different animals have been reported by various observers :—

Animals	LD 50 g./kg. (oral)	Authors
Rabbit ..	2.0	Raiziss *et al.*, 1937.
Guinea-pig ..	2.5	Domagk, 1935.
Mice ..	3.5	Marshall *et al.*, 1938.
Rats ..	6.2	Molitor and Robinson, 1939.

A similar figure (LD 50) for dogs is not available; the number of dogs used in experiments in sulphanilamide poisoning has been small. Halpern and Mayer (1937) found the oral toxic dose to be 1 g./kg. Molitor and Robinson (1939) reported that 2 g./kg. by mouth produced severe symptoms in all animals, but mentioned the important fact that after large doses the dogs usually vomited early and the experiments had to be discontinued. In general it may be stated that dogs are quite susceptible.

It is an interesting fact that rats should have a tolerance for the drug which other animals, especially dogs, have not. The fact that rats can synthesize nicotinic acid (Shourie and Swaminathan, 1940) may be of significance.

Reference has already been made to clinical observations on the beneficial effects of nicotinic acid on acute sulphanilamide poisoning. The results reported in the present investigation, as far as they go, support these clinical findings.

Summary

1. In oral toxicity experiments the LD 50 of sulphanilamide was found to be 5.5 (± 18.15 per cent) grammes per kilogram in rats.

2. Nicotinic acid when administered along with this dose of sulphanilamide (LD 50) to rats reduced mortality to a significant extent and limited the occurrence of severe reactions.

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A CLINICAL SIGN IN SANDFLY FEVER

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THE purpose of this paper is to describe a clinical sign which is easily elicited, and which appears to be present in the majority of cases of sandfly fever, and which, as far as I am aware, has not been recorded before. The sign consists of choking of the optic discs, which varies in its slighter manifestations from blurring of the edges of the discs, with distention of the retinal veins, up to a papillœdema with a swelling of about 2-2.5 D. Exudates or retinal hæmorrhages have not been observed.

The sign was observed in cases occurring in a non-immune group of British troops recently arrived from Britain, amongst whom the disease was of frequent occurrence.

Cases.—Of a total number of thirty cases which presented the clinical criteria of sandfly fever, the sign was present in twenty-seven. The three cases in which it did not occur were very mild with only very slight headache.

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The clinical criteria applied were (1) sudden onset often with rigor, (2) flushing of the face limited by the collar line, (3) injection of the conjunctivæ, (4) supra-orbital or post-orbital headache, with tenderness on pressure over the globes, (5) backache and stiffness in the back of the neck, (6) pyrexia of from 48 to 96 hours' duration, (7) bradycardia, (8) slight leucopenia, or normal white cell count (4,000-7,000 cells per cubic mm.), (9) Vesication of palate and fauces, thought to be inconstant and rather unreliable, (10) slow convalescence seen in some cases, (11) exclusion of any other infection, especially malaria.

Cases were seen within 6 to 48 hours of onset; the sign was present in the earliest cases seen, although it sometimes became more marked on the second day. The swelling of the disc was usually most obvious in the eye in which the globe was most tender, or on the side with the most severe supra-orbital or post-orbital pain. In the slighter cases the swelling subsided rapidly; in the more severe cases it was still apparent on the day after the patient had become apyrexial.

One case not included in the 30, which was considered clinically to be sandfly fever, and which showed papillœdema after a protracted convalescence, developed catarrhal jaundice on the 15th day. Another case also not included in the series, which showed the typical clinical picture with papillœdema, tenderness of the globes and a negative blood film, and in which the temperature showed a tendency to rise rather than settle, was then found on the third day to have subtertian ring forms in the blood. These cases I consider to have been possible examples of sandfly fever, with a concurrent or consecutive other disease, although cerebral malaria has been described as sometimes showing papillœdema associated with amaurosis.

Discussion

Visual acuity tests were not carried out in any case, but it was noticed that sandfly-fever cases were photophobic and generally averse to using their eyes, as in reading.

The papillœdema is probably due to the associated rise in cerebro-spinal fluid pressure which has been reported in this disease by Le Gac and Albrand (quoted in Manson's Tropical Diseases, 11th edition, 1941). Owing to the conditions (Indian Frontier) under which I saw the cases, cerebro-spinal manometry was not practicable, and as morphia gr. 1/6 hypodermically was invariably found to relieve the headache, lumbar puncture was not instituted in any case for the relief of this symptom.

Summary

(1) Some degree of papillœdema was observed in twenty-seven out of thirty cases of clinical sandfly fever.

(2) This sign taken in conjunction with the other clinical findings should be of assistance in the early diagnosis of the disease.