COMPARATIVE TOXICITY STUDIES ON TEN ANTIBIOTICS IN CURRENT USE

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The acute toxicities of ten currently used antibiotics have been compared on two strains of mouse by four different routes.

On several occasions we have needed to know the toxicity of one or other of the antibiotics currently in clinical use. Information on the subject is widely scattered and therefore hard to collect, though this difficulty was largely met by Spector's publication[1], which deals with some 340 antibiotics, including all of those in which we were interested.

Study of its contents revealed clearly what we had already good grounds for suspecting, namely, that exactly to compare the toxicities of different antibiotics, and especially of the dozen or so commercially available, was not possible on the basis of available information. Often insufficient details were given in the original papers of the type (and strain) of animal used, and seldom, if ever, were simultaneous comparisons, by identical routes, made on more than two substances. We therefore decided to conduct a more strictly controlled study of antibiotic toxicity than we believe previously to have been made or at any rate to have been recorded in the literature. We present here the results of this study, which began in February 1956 and was concluded in September of that year, except for a small supplementary test in August 1958.

We are fully aware that relative toxicities by the same route and on the same strain of the same species of laboratory animal do not necessarily indicate the relative toxicities to any other species of animal, including man. Nevertheless, there is a wealth of experience to confirm the view that such studies, which can at worst supply negative evidence, may provide positive leads to therapeutic practice.

Materials and Methods

Antibiotics

Ten antibiotics were studied. Full details of them, with batch or code number when available, are given in Appendix I.

Animals

In our experiments we used two different strains of highly inbred mice, both males and females. The original experiments involved the use of male albino mice (A26 strain)* and female fawn mice (GFF strain)*.

* Some details of these strains are to be found in the *Catalogue of Uniform Strains of Laboratory Animals maintained in Great Britain* (Laboratory Animals Bureau, 1959).

When marked differences in toxicity appeared between the two strains, further experiments were made on male mice of the fawn strain and female mice of the albino strain. All the results are included in Table I, which gives the median lethal doses for the ten antibiotics. All mice weighed between 14 and 22 g. at the beginning of the experiment. The LD50 values were calculated by the method of de Beer[2] from the number of deaths occurring within seven days of treatment.

Methods of Administration

The different antibiotics were administered in graded doses to groups of ten mice by one of four different routes—intravenous, intraperitoneal, subcutaneous and oral. The intravenous injections were made at the rate of approximately 1 ml. per 30 seconds into the tail veins. All antibiotics were administered as aqueous solutions or suspensions, but in some instances, as indicated in Table I, a small amount of gum tragacanth was added to aid suspension. All solutions or suspensions were prepared immediately before use.

The range of doses was chosen so as to lie on each side of the expected LD50 when an approximate value for this was available: otherwise a probe test was undertaken to supply such a value.

Results

Penicillin remains the least toxic of all currently used antibiotics, by either intraperitoneal or intravenous route. Streptomycin, neomycin and penicillin have one-eighth to one-quarter the oral toxicity of the other seven substances tested. Polymyxin appeared to be the most toxic by any route.

Generally the differences in toxicity to the two strains of mice were small; in the few instances when marked differences occurred, it was between strains rather than between sexes.

For the most part it seems to us that the figures speak for themselves. It may, however, be pointed out that the subcutaneous toxicity of tetracycline hydrochloride is greatly decreased when it is administered as a suspension neutralised with sodium hydroxide. At pH 1·8 tetracycline hydrochloride produces severe necrosis at the site of injection, but at neutral pH it produces none; the necrosis is almost certainly due to the relatively high acidity of the unneutralised solution.

Discussion

Certain other differences seem to us to call for comment and in some instances to raise questions of interest, to most of which, however, we do not know the answers. For example, toxicity by the intraperitoneal route is, as might have been expected, shown to have been almost always less than by the intravenous route, but the difference seems doubtfully significant for chlortetracycline and, curiously, is reversed for bacitracin. On taking the means for the two strain-sex groups of mice, it is seen that intravenous injections of chloramphenicol, neomycin and streptomycin were 6 or more times as toxic, at the LD50 level, as intraperitoneal

TABLE I

ACUTE SYSTEMIC TOXICITIES OF TEN ANTIBIOTICS IN CURRENT USE

(Mouse LD50 values in mg./kg. per body weight)

Antibiotic	Intravenous		Intraperitoneal		Subcutaneous		Oral			
	A2G(M)	GFF(F)	A2G(M)	GFF(F)	A2G(M)	GFF(F)	A2G(M)	A2G(F)	GFF(M)	GFF(F)
Bacitracin	356 (2·5 per cent solution)	313	219 (2·5 per cent solution)	300	538 (5 per cent solution)	660	3375 (15 per cent solution)	—	—	3375
Chloramphenicol	180 (2 per cent suspension[T])	210	1225 (10 per cent suspension[T])	1300	2300 (10 per cent suspension[T])	2585	3150 (20 per cent suspension[T])	2600	5500	5300
Chlortetracycline hydrochloride	102 (0·75 per cent solution)	108	128 (0·75 per cent solution)	168	5500 (25 per cent suspension)	8250	3350 (20 per cent suspension)	—	—	4200
Erythromycin	120 (0·5 per cent suspension)	138	655 (2·5 per cent suspension)	720	4700 (20 per cent suspension)	6800	4050 (20 per cent suspension[T])	3500	5800	7100
Neomycin sulphate	32 (0·2 per cent solution)	33	213 (per cent solution)	180	240 (1 per cent solution)	310	14,500 (50 per cent solution)	—	—	14,000
Oxytetracycline hydrochloride	154 (0·75 per cent solution)	189	285 (1·5 per cent solution)	420	243 (1·5 per cent solution)	330	3600 (20 per cent solution)	—	—	4400
Sodium benzylpenicillin	1800 (15 per cent solution)	1913	3880 (20 per cent solution)	4120	6000 (50 per cent solution)	8000	12,750 (50 per cent solution)	13,000	15,750	16,500
Polymyxin B sulphate	5 (0·03 per cent solution)	5	24 (0·1 per cent solution)	24	84 (0·5 per cent solution)	103	713 (5 per cent solution)	—	—	1050
Streptomycin sulphate	85 (0·75 per cent solution)	111	610 (5 per cent solution)	575	500 (5 per cent solution)	550	15,550 (75 per cent solution)	16,125	30,000	30,000
Tetracycline hydrochloride	145 (1 per cent solution pH 2·6)	170	390 (1·5 per cent solution pH 2·6)	495	1125 (15 per cent partial suspension pH 1·8)	1950	3375 (15 per cent partial suspension pH 1·8)	—	—	3550
	80 (0·5 per cent partial suspension[N])	75	230 (2 per cent partial suspension[N])	440	8640 (24 per cent partial suspension[NT])	8880	Not tested			

A2G(M)—Male mice of A2G strain GFF(M)—Male mice of GFF strain
A2G(F) —Female mice of A2G strain GFF(F) —Female mice of GFF strain
T—suspension aided by trace of gum tragacanth
N—neutralised with NAOH

injections, the corresponding ratios falling for erythromycin, polymyxin, tetracycline, penicillin, oxytetracycline and chlortetracycline, in that order, to the reversed ratio for bacitracin.

Strain differences unconfounded with sex differences can only be established from our results for four of the antibiotics tested by the oral route. The GFF mice were markedly less susceptible than the A2G mice to the oral toxic effects of chloramphenicol, erythromycin, benzylpenicillin, and streptomycin. The confounded strain-sex differences by the oral route showed the GFF females to be somewhat less susceptible than the A2G males to chlortetracycline, oxytetracycline and polymyxin, but about equally susceptible to bacitracin, neomycin, and tetracycline.

The confounded strain-sex differences by any of the parenteral routes were never marked; they tended to be greatest by the subcutaneous and least by the intravenous route. Almost always the GFF females were less sensitive than the A2G males when there was any apparently significant difference at all. Intraperitoneally the females of strain GFF seemed more sensitive than the males of strain A2G only to neomycin and streptomycin, the difference being so small, however, as possibly to have been fortuitous. This is also true of bacitracin only for the intravenous route, all the eight other substances being either equally lethal, or non-lethal, to both groups of mice (chlortetracycline, neomycin, polymyxin) or slightly less lethal to the GFF females (chloramphenicol, erythromycin, oxytetracycline, benzylpenicillin, streptomycin, tetracycline) after intravenous injection. By the subcutaneous route toxicities were either the same for both strain-sex groups or slightly to distinctly greater for the A2G males. The evidence of the oral tests, the only ones permitting a distinction between strain and sex effects, shows the difference between strains to have been much greater than the difference between sexes for four of the antibiotics, namely, chloramphenicol, erythromycin, benzylpenicillin and streptomycin, though for benzylpenicillin the difference was small, giving ratios of 1·24 and 1·27 for males and females respectively.

There is increasing evidence in the literature of interstrain differences between the responses of animals of the same species to the same stimulus or stress. Examples of this are differences between the two strains used by us in susceptibility to infection by *Bordetella pertussis* and *Mycobacterium tuberculosis* seen by our colleague, Dr. Ungar. At a recent symposium it was reported that mice of different strains showed an enormous difference in susceptibility to the toxic action of histamine hydrochloride[3]. These are only three of many examples that might be cited. The same phenomenon, though less marked, has appeared again in the course of the work reported here. Nor is its occurrence an occasion for surprise. "Pure lines" of mice were originally produced for the use of cancer investigators because of the wish to take advantage of differences in susceptibility to spontaneous or implanted tumours. It is hardly surprising that these differences, largely or entirely genetic, manifest themselves also in other types of investigation. The existence of these intraspecific differences between animals of nominally the same species makes it all the more desirable that investigators should invariably give the fullest possible

details, by mention or by reference, not only of the species, but also of the strain, of animal used in any work they report.

Acknowledgements

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Appendix I

The preparations used.

1. Bacitracin (production batch BAC–0001, 76 U/mg., Glaxo Laboratories).
2. Chloramphenicol (Chloromycetin, batches LT 831A, LS 366M and LU 181A, Parke Davis and Co.).
3. Chlortetracycline hydrochloride (Aureomycin, batch 7–8281, Lederle Laboratories).
4. Erythromycin (Erythrocin, batches 21057, 900 μg./g. and 24170, Abbot Laboratories).
5. Neomycin sulphate (production batch 3PB/2/47, 600 U/mg., Glaxo Laboratories).
6. Oxytetracycline hydrochloride (Terramycin, Pfizer).
7. Sodium benzylpenicillin (production material, 1680 U/mg. and 1670 U/mg., Glaxo Laboratories).
8. Polymyxin B sulphate (Aerosporin, batch AN 49960, 4949 U/mg., Burroughs Wellcome).
9. Streptomycin sulphate (production batches B-1333 and SSU 2353, 740 U/mg., Glaxo Laboratories).
10. Tetracycline hydrochloride (Tetracyn, 967 U/mg., 968 U/mg. and 965 U/mg., Pfizer.

References

1. Spector, *Handbook of Toxicology*, Vol. II, Saunders Co., Philadelphia and London, 1957.
2. de Beer, *J. Pharmacol.*, 1945, **85**, 1.
3. Brown, *Quality in Laboratory Animals.* Papers read at Laboratory Animals Symposium, London, 1959 (in the press).