A NOTE ON THE INFLUENCE OF THE MEDIUM ON THE TOXICITY OF ANTIBIOTICS

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The strain of mice used has been found to affect the results when testing antibiotics for freedom for undue toxicity. The results confirm those of Maffii and colleagues in some instances, but not in others, being related to the strain of mice used. No protective action was seen with cortisone.

ANTIBIOTICS are tested for freedom from undue toxicity by intravenous injection in five mice. The sample passes the test if no mouse dies within 24 (B.P.) or 48 (U.S.P.) hours. The United States Pharmacopeia advises saline as the medium for bacitracin, chlortetracycline or procaine penicillin, but recommends the solution of penicillin or streptomycin in water. Water or saline is official for the antibiotics in the B.P. Recently, however, Maffii, Semenza and Soncin[1] have found that BZL mice tolerated the test dose of tetracyclines and of streptomycin only in solution of saline but died after receiving this dose in water. Water alone was not toxic.

In view of the practical significance of these findings we describe tests, some of which confirm the work of Maffii and his colleagues while others differ. Male mice of $23 \pm 2{\cdot}18$ g. body weight were taken at random from two mixed batches (MLI, ML2) and from three pure strains. Among the latter were two sublines of RIII, a "high cancer" strain known also for its high sensitivity to stress. A fresh 0·2 per cent solution of the sample under test was made up and 0·5 ml. injected intravenously within 5 seconds. The mice were observed for 48 hours. Fragility tests were done according to Harris[2]; the concentration of sodium chloride in which moderate haemolysis occurred was recorded.

The results in Table I show that no mouse died after injection of antibiotics in solution of saline, while the response to water alone or to antibiotics in aqueous solution varied with the stock or strain or even with the subline of the test animal. The findings obtained with strain ML1, for instance, agreed with those reported previously[1] on streptomycin and tetracyclines. Penicillin had the least and procaine penicillin the most toxicity. ML2 strain mice, however, died after injection of water alone, and to these mice aqueous solutions of penicillin or dihydrostreptomycin appeared to be more toxic than to the strain ML1.

There was no evidence that the toxicity of solutions in water as contrasted with solutions in saline was due to deviation from the optimal osmotic pressure. A rise in concentration of an aqueous solution of dihydrostreptomycin from 1 : 500 to 1 : 250 did not reduce but apparently increased the toxicity. Both these concentrations were tolerated when given in saline solution. Moreover, the toxicity of penicillin was not directly related to the fragility of mouse red blood cells, or to the animal's

senstitivity to water alone. One of the RIII sublines was very sensitive to water alone but surprisingly resistant to penicillin in solution of water.

No protective action was seen with cortisone. There was no difference in the toxicity of an aqueous solution of procaine penicillin to 13 ML2 mice pretreated with 0·25 mg. of cortisone acetate in alcoholic saline on

TABLE I

DEATHS AFTER INTRAVENOUS INJECTION*

Mouse strain	Average R.B.C. fragility NaCl per cent	Water	Saline	Penicillin G		Dihydrostreptomycin				Chlortetracycline		Procaine penicillin G	
				in water	in saline	in water		in saline		in water	in saline	in water	in saline
				1 mg.	1 mg.	1 mg.	2 mg.	1 mg.	2 mg.	1 mg.	1 mg.	1 mg.	1 mg.
ML1	0·39 ± 0·030	0/10	0/10	1/12	0/12	2/10	—	0/10	—	4/10	0/10	4/5	0/5
ML2	0·44 ± 0·028	3/5	0/5	6/12	0/12	7/10	8/9	0/10	0/6	3/5	0/5	14/15	0/15
	ML2—ML1: P 0·03												
suisse	0·41 ± 0·023	3/5	—	2/5	0/5	—	—	—	—	—	—	—	—
	suisse—ML1: P 0·3												
RIII	0·41 ± 0·020	4/5	0/5	0/5	—	—	—	—	—	—	—	—	—
	RIII—ML1: P 0·2												
RIII/a	0·40 ± 0·023	0/5	—	1/5	—	—	—	—	—	—	—	—	—
	RIII/a—ML1: P 0·6												
C57 black	0·43 ± 0·020	2/5	—	3/5	—	—	—	—	—	—	—	—	—
	C57—ML1: P 0·04												

* All injections were in 0·5 ml.

3 days preceding the test and 13 mice pretreated with the medium alone. The mortality after injection of water alone was only 33 per cent less in 13 mice pretreated with cortisone than in 13 control mice.

It would appear that different factors are involved in determining the animal's resistance to water alone on the one hand and to aqueous solutions of antibiotics on the other. Prescott, Kaufmann and James[3] have shown recently that the toxicity of isoniazid to DBA mice is less when it is in solution with glycerol than in solution with water. This result and the work referred to and presented here would suggest the necessity for detailed specification in the standardisation procedure by the pharmacopoeias stating the appropriate medium for each drug requiring tests for undue toxicity.

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REFERENCES

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2. Harris, *Experimental Physiology*, Churchill, London, 1941.
3. Prescott, Kaufmann and James, *Proc. Soc. exp. Biol., N.Y.*, 1957, **94**, 272.