14. PORPHYRINURIC ACTION OF 2-(*p*-AMINO-BENZENESULPHONAMIDO)PYRIDINE (M AND B 693). COMPARISON WITH SULPHANILAMIDE

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EXPERIMENTS previously reported [Rimington, 1939; Rimington & Hemmings, 1939] have shown that sulphanilamide and certain related nitro- and amino-compounds cause a marked increase in urinary porphyrin excretion when administered to rats for some days. A theory of pigment metabolism has been put forward to explain the mechanism of this toxic action [Rimington & Hemmings, 1939; Rimington, 1939].

The toxicity of the drug 2-(*p*-aminobenzenesulphanilamido)pyridine (M and B 693) has been examined by Wien [1938], who stated that it did not cause porphyrinuria in rats when administered at levels of 3 g./kg. and 6 g./kg. respectively for 15 days. It was claimed that M and B 693 offered a big advantage over sulphanilamide on account of lower toxicity. It may be here remarked that an examination of the figures published by Wien suggest that, in fact, a small and irregular increase of porphyrin was commencing. Sulphanilamide produced a much more striking result.

Since this early publication and following the introduction of the drug into clinical medicine, a number of reports have appeared which indicate that M and B 693 may cause more severe toxic symptoms than was at first suspected. Thus haematuria and haemoglobinuria have been repeatedly observed and not infrequently granulocytopenia. In addition, skin rashes, often showing an association with exposure to ultraviolet or sun light, have been reported [Hallam, 1939; Ker-Gray, 1939, etc.], similar to those which have been encountered during sulphanilamide therapy.

Detailed study of the rates of absorption and excretion of M and B 693 has been undertaken by Long & Feinstone [1938], Marshall *et al.* [1938], Baines & Wien [1939], Stokinger [1939], Powell & Chen [1939], and others.

Long & Feinstone stated that the drug is less readily and more irregularly absorbed by human beings than is sulphanilamide. Moreover, they consider that relatively more of the drug is conjugated in man than is the case when sulphanilamide is administered. An examination of their data hardly supports this conclusion. From the figures quoted, the mean value for the proportion acetylated is 22 %. Actually, valid comparison can only be made upon the same time basis, since the acetyl derivative and free base have different rates of elimination and the proportion of conjugated amine appears larger, therefore, the greater the time which has elapsed since administration. The findings of Baines & Wien [1939] are not in harmony with the view expressed by Long & Feinstone [1938] that M and B 693 is less readily absorbed in man than is sulphanilamide.

The comparatively high M.L.D. of M and B 693 has been ascribed largely to incomplete absorption [Marshall *et al.* 1938]. Using the soluble sodium derivative, intravenously injected into dogs, Marshall & Long [1939] reported toxic

effects after 0.2 g./kg. with a blood level of 22 mg./100 ml. Deductions cannot be safely made concerning the toxicity of the free base, when administered orally, from experiments upon the intravenously injected sodium derivative. In the present work the sodium derivative of M and B 693 has been compared with the sodium derivative of sulphanilamide when both are administered orally. Acting largely upon Long's report [1939] upon "Sulfapyridine", the term adopted in America for 2-(*p*-aminobenzenesulphanilamido)pyridine, the Council on Pharmacy and Chemistry of the American Medical Association commented adversely upon the drug (see also *Lancet*, 1939, i, 703).

For the above reasons it was felt that a more extended comparison was desirable of the effects of M and B 693 and sulphanilamide, and of their respective sodium derivatives upon pigment metabolism.

Rats, in groups of 3, were again selected for the work and the experiments carried out upon the same general plan as those formerly reported [Rimington & Hemmings, 1939]. The dose of M and B 693 ranged from 0.2 to 2.9 g./kg. and that of the sodium derivative from 0.2 to 0.9 g./kg. All substances were administered daily *per os* (the sodium derivatives in aqueous solution, the insoluble M and B 693 as a suspension in gum acacia). The controls received the same quantity of gum acacia solution alone. Urinary porphyrin excretion was determined over 3-day intervals during a preliminary control period lasting about 12 days and dosing was then commenced.

Upon the completion of the observation period of 12–15 days, the rats of each group were killed, 4 hr. after receiving their last dose, and the concentrations of free and total amine in the blood of each of the three animals determined by Marshall's method, as used by Baines & Wien [1939]. Specimens were also taken for histological examination.

RESULTS

The results are summarized in Table 1.

Table 1

Substance	Dose g./kg.	Blood level mg. per 100 ml.		Porphyrin excretion × normal	Remarks
		Free	Total		
M and B 693	0.2	6.4	7.0	1.5	Loss of weight
		11.0	11.5		
		7.7	8.7		
	0.5	11.1	14.8	3.5	Loss of weight
		11.2	14.8		
	0.9	18.1	19.7	4	Marked loss of weight. One rat died on 15th day
		16.8	24.2		
		13.9	18.0		
	1.4	29	32	3	Bloods pooled before analysis
	2.9	38	41	5	Bloods pooled before analysis
M and B 693 Na derivative	0.2	—	—	No increase	Dosed for 12 days
	0.4	12.3	12.5	2	Same rats as above; dose raised to 0.4 g./kg. for 9 days. No loss of weight
		12.8	13.4		
		6.4	7.2		
	0.9	22.8	24.8	5.5	One rat died on 7th day. Bloods pooled before analysis
Sulphanilamide Na derivative	0.2	—	—	1.6	Dosed for 12 days
	0.4	10.8	13.2	2.4	Same rats as above; dose raised to 0.4 g./kg. for 9 days. No loss of weight
		4.7	6.5		
		5.0	6.8		

It will be seen from Table 1 that 0.2 g./kg. of M and B 693 daily for 12 days was practically without effect upon urinary porphyrin excretion, but that at higher dosage levels increases up to five times the normal quantity were recorded. In general, the severity of porphyrinuria developing corresponded to the blood concentration of the drug attained. Judged by this standard, the sodium derivative given by mouth appeared about as toxic as M and B 693 itself. The blood concentration was slightly lower at the lower dosage levels, due, no doubt, to more rapid elimination. Between the sodium derivatives of M and B 693 and of sulphanilamide there appears to be little, if any, difference, the latter compound producing slightly greater porphyrinuria. Considerable variation was noticed in drug levels in the bloods of different individual rats receiving the same dose, an observation in accordance with that of other workers. Nervous instability and restlessness were commonly observed in animals receiving the large doses of M and B 693. The urine of the group receiving 2.9 g./kg. contained haemoglobin from the 7th day onwards, and one rat dosed with 0.9 g./kg. of the sodium derivative died some hours after the 7th dose.

Histological examination of the livers, spleens and kidneys revealed congestion of the spleen in nearly all cases, but otherwise little evidence of abnormality. It is worthy of note that deposits of stainable iron in the spleens were only slightly increased, whereas such deposits are a characteristic feature after sulphanilamide administration.

CONCLUSIONS AND SUMMARY

There is no doubt that M and B 693 administered to rats under the conditions of these experiments causes an increase in urinary porphyrin excretion. In this respect it resembles sulphanilamide and the other amines previously studied [Rimington & Hemmings, 1939]. Viewed from the quantitative standpoint, the degree of porphyrinuria developing as a response to dosage levels of 0.2–0.9 g./kg. is approximately the same for each substance, but whereas porphyrinuria increases at higher levels of sulphanilamide roughly in proportion to the dose, this relationship does not hold in the case of M and B 693. This may be due in part to the less complete absorption following larger doses, but, even in relation to the blood concentration levels, it appears that the porphyrinuria is relatively less marked at the higher than at the lower levels.

Whilst M and B 693 certainly possesses properties which render it potentially toxic, the study of its porphyrinuric action does not indicate that it is essentially any more toxic than is sulphanilamide in this same respect, even allowing for the less ready absorption of the pyridine derivative.

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REFERENCES

- Baines & Wien (1939). *Quart. J. Pharm.* **12**, 4.
 Hallam (1939). *Brit. med. J.* **i**, 559.
 Ker-Gray (1939). *J.R. nav. med. Serv.* **25**, 422.
 Long (1939). *J. Amer. med. Ass.* **112**, 538.
 — & Feinstone (1938). *Proc. Soc. exp. Biol., N.Y.*, **39**, 486.
 Marshall, Bratton & Litchfield (1938). *Science*, **88**, 597.
 — & Long (1939). *J. Amer. med. Ass.* **112**, 1671.
 Powell & Chen (1939). *J. Pharmacol.* **67**, 79.
 Rimington (1939). *Proc. R. Soc. Med.* **32**, 1268.
 — & Hemmings (1939). *Biochem. J.* **33**, 960.
 Stokinger (1939). *Proc. Soc. exp. Biol., N.Y.*, **40**, 61.
 Wien (1938). *Quart. J. Pharm.* **11**, 217.