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TOXICITY OF CERTAIN PROPOSED ANTILEPROSY DYES: FLUORESCEIN, EOSIN, ERYTHROSIN, AND OTHERS *

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Gordon Ryrie's stimulating preliminary note (10) on the use of dyes for intravenous therapy in leprosy has aroused much interest in the further clinical application of these agents. The recent editorial comment in the JOURNAL (15) has greatly clarified the present status of this form of treatment, and together with Ryrie's published correspondence has tacitly advised caution in administration. We feel that a more general appreciation of the widely varying toxicities of different dyes in laboratory animals might lead to more rational use of these potentially dangerous compounds in humans, and have accordingly reported our findings (1, 2) in this regard on those agents which had been considered favorable from the clinical results obtained with lepers. Since Ryrie now more highly recommends the use of fluorescein, toxicity results with fluorescein-group dyes are included in the present paper.

Trypan blue, methylene blue, gentian violet and brilliant green were previously examined and complete toxicity figures for these

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dyes given by various routes of administration in different experimental animals are available (1, 2). Intravenously it was found that single doses killing half of all animals, thus representing the mid-points of the lethal ranges, were respectively 200 to 300, 40, 20, and 3 mgm. per kilogram. Deaths occur below these dosages, the tolerated dose being generally somewhat less than half the lethal doses cited above. Amounts that, according to reports seen, have been used clinically in humans have in some instances been well within the lethal range in animals, and special significance should be attached to the untoward effects noted by Ryrie (10) on injection of higher doses.

Existing toxicity data for fluorescein and eosin are scanty. Jodlbauer and Busek (7) found 600 mgm. per kilogram in mice and 400 mgm. per kilogram in guinea pigs to be the fatal subcutaneous doses of fluorescein when the animals were kept in the dark. Eosin is more toxic since subcutaneous doses of 450 mgm. per kilogram in mice and 300 mgm. per kilogram in guinea pigs are fatal, according to these authors. Erythrosin is generally stated to be still more toxic, but definite values could not be found in the literature available to us; its high iodine content makes erythrosin of particular interest for leprosy therapy. Photodynamic action is a complicating factor, and must be remembered both in regard to toxicity determinations and the clinical use of these drugs. Both the patient and the solution of the dye to be injected should be kept from direct sunlight, a point of special importance in the tropics.

In rabbits we have found the mid-points of the lethal range of single intravenous doses of fluorescein, eosin and erythrosin to be 300, 350, and 200 mgm. per kilogram respectively. Intraperitoneally in rats, deaths occur at 600, 500, and 300 mgm. per kilogram. Tolerated doses are again about half of these amounts. Complete toxicity figures may be found in Table 1.

Methylene green, a nitro-derivative of methylene blue, was of interest to us because of the high activity of the methylene blue nucleus against cultivated leprosy bacilli in vitro. It was found to be lethal at 125 mgm. per kilogram on intraperitoneal injection in rats, and at 150 mgm. per kilogram on intravenous administration in rabbits. The lethal dose intravenously in anesthetized cats is 50 to 75 mgm. per kilogram. The small difference in toxicity of methylene blue and methylene green is to some extent compensated by the difference in molecular weights, pointing to a similar mechanism of action.

TABLE 1.—*Toxicity of three fluorescein dyes and methylene green in watery solutions.*

Dye	Animals	Mode of administration	Solution per cent	Dose mgm./kg.	Mortality ratio [a]
Fluorescein [b]					
	Rats	Intraperitoneal	1 to 3	500	0/3
				600	2/5
				750	4/5
				1000	3/3
		Oral	5	1000	0/5
	Rabbits	Intravenous	2	200	0/3
				300	2/3
				400	2/2
Eosin [c]					
	Rats	Intraperitoneal	2	250	0/3
				500	2/5
				600	5/5
		Oral	5	1000	0/5
	Rabbits	Intravenous	5	200	0/3
				300	1/5
				350	2/3
Erythrosin [d]					
	Rats	Intraperitoneal	2	200	0/3
				300	3/5
				400-500	8/8
		Oral	5	1000	0/5
		Intravenous	2	150	0/3
				200	2/3
Methylene green [e]					
	Rats	Intraperitoneal	1	50	0/3
				100	1/3
				125	3/5
				150	3/3
		Oral	5	400	0/3
				500	2/5
	Rabbits	Intravenous	5	50-100	0/6
				150	2/3
				175	2/2
	Cats [f]	Intravenous	5	50	1/3
				75	3/3

[a] Mortality Ratio = no. of animals dying / no. of animals used.

[b] Fluorescein, Eimer and Amend, neutralized with $NaHCO_3$.

[c] Eosin Y, Coleman and Bell.

[d] Erythrosin B. Iodine-Eosin B, Schultz No. 592, Lot 4154, National medicinal product, National Aniline and Chemical Co.

[e] Methylene green, Coleman and Bell.

[f] Anesthetized with sodium pentobarbital (Lilly), 30 mgm./kg., I. P.

When given orally all of these dyes which we have tested with the exception of gentian violet are well tolerated in animals. Total doses of 150 mgm. per kilogram of gentian violet given in a period of 13 days proved fatal to dogs, according to Santori (12). Clinical experience of one of us (H. H. A.) demonstrated gentian violet to be unsuited for human oral administration because of gastro-enteric irritation, even when salol coatings were used.

Lethal doses of the other dyes given orally have been collected by Heffter (5). All are fairly high. Since fluorescein, eosin and methylene blue are rapidly absorbed from the gut and excreted in the urine, unchanged or with but slight modification as in the case of the conversion of methylene blue to methylene azure, oral administration of these three dyes should be as effective as intravenous therapy and considerably safer. Trypan blue is not absorbed on oral administration in rats. Rats tolerate doses of 1.0 gm. per kilogram of fluorescein, eosin and erythrosin given orally, while 2 of 5 rats died after oral administration of 500 mgm. per kilogram of methylene green. Sollmann (14) records the highest recommended therapeutic doses for humans, for oral administration in capsules, of the following dyes: fluorescein, 6.0 gm.; methylene blue, 0.6 gm.; and gentian violet, 0.065 gm. in capsules.

Our therapeutic experiments on leprous rats afford some chronic toxicity data for several of the more important antileprosy dyes. The drugs were given intraperitoneally in 0.5 to 1.0 per cent aqueous solution twice a week, in amounts equivalent to one-fifth of a single lethal dose. The average cumulative lethal doses for those rats dying under treatment were: trypan blue, 1.75 gm. per kilogram; gentian violet, 17 mgm. per kilogram; and brilliant green, 12 mgm. per kilogram. Of these treated groups, all animals treated with gentian violet and brilliant green have died, while 6 of 8 leprous rats treated in this way with trypan blue are still alive.

The effects of administration by the subcutaneous route were determined in groups of infected rats treated weekly with one-fifth of the single subcutaneous lethal dose. With trypan blue 6 of 12 animals died after receiving 0.64 gm. per kilogram, and with mercurochrome 1 of 12 after 35 mgm. per kilogram. All of 12 leprous rats died after a single subcutaneous injection of 200 mgm. per kilogram of methylene blue.

The chronic toxicity of trypan blue given intravenously was also studied in normal rabbits. Six rabbits ranging from 1.5 to 2.5 kilo-

grams in weight were treated twice a week with one-fifth of a single intravenous lethal dose. In a previous experiment 3 of 5 rabbits died after a single intravenous dose of 150 mgm. per kilogram; in the present one 2 of the 6 animals were killed by 120 mgm. per kilogram in divided doses, and another by 150 mgm. per kilogram. Of the 3 surviving animals, 2 had tolerated 450 mgm. per kilogram and 1 of them 240 mgm. per kilogram before treatment was stopped. The susceptibility to repeated doses of trypan blue thus appears to vary widely, but total amounts equivalent to the single lethal dose may kill, which indicates a rather complete fixation of the dye in the tissues.

The recommendation that dyes be used in conjunction with other modes of therapy, if found insufficiently active in themselves to arrest leprosy, appears dangerous unless consideration is given previous experimental work in regard to possible synergistic toxic effects of such combinations. Light may synergize with the photodynamic dyes; the barbiturates synergize with trypan blue (3); and potassium cupro tartrate has been shown by Sellei (13) to synergize with minute amounts of eosin. It is not improbable that other such couples may exist. Until more data is available on the mechanism of the toxic and therapeutic actions of dyes, investigations of particular combinations considered for use in human lepers should be carried out on animals, and special care should be taken in the administration of combination therapy. This is particularly true with the dyes which are used at present in amounts just below the toxic range.

The possibility of enhancing the action of macrophages against *Mycobacterium leprae* by such dyes as trypan blue in selectively stained lepromata appears remote in view of the findings of Oliver (8). This observer noted in leprous rats that engorged lepra cells take up little or none of systemically administered trypan blue, while on the other hand those macrophages which became full of dye did not contain lepra bacilli. Therefore, even if trypan blue were highly leprocidal, which it is not, no beneficial action could be expected through fortifying the reticulo-endothelial system. It seems probable also that the concentration in lepromata of certain dyes which may act as redox indicators, such as methylene blue, is more apparent than real, since these dyes would remain in the colored form longer in such regions of decreased oxidation.

Some concept of the leprocidal activity of dyes in vitro may be helpful in interpreting subsequent clinical findings. We have

found (4) that the triphenylmethane dyes and methylene blue are far more active in vitro against the leprosy bacillus than are the phthalein dyes or trypan blue, which are generally inactive even in concentrations of 0.1 per cent. The bactericidal effectiveness of the dyes varies in the same direction as the toxicity to animals, but to a relatively greater extent. The mechanism of the toxic action in animals has been discussed by Salant and Bengis (11), del Zoppo (16) and the authors (1). Trypan blue may act through reticulo-endothelial blocking, liver damage, gastro-enteritis, capillary blocking or by reducing the coagulability of the blood, as found by Hugget and Rowe (6). The fluorescein dyes may act through decreasing intracellular oxidation (del Zoppo, 16), or by depressing blood pressure and respiration (Salant and Bengis, 11), or through photodynamic action. Methylene blue may act through gastro-enteric irritation or methemoglobin formation, and methylene green through a nitrite effect. Further work along this line is needed. Certainly the conclusion of Rost (9) that fluorescein group dyes have no toxic action appears unjustified.

The experimental work shown in Table 1 is only sufficient to indicate relative acute toxicities of the dyes studied. Therapeutic work with leprous rats, now in progress, will yield more significant data on the cumulative toxic actions of repeated doses. So far we have noted in rats no positive therapeutic action on the part of any of the dyes considered with the possible exception of mercurochrome, although sloughing lesions in rats treated with trypan blue tend to heal more cleanly than in controls, without intercurrent infections.

SUMMARY

Fluorescein, eosin, erythrosin and methylene green were found to be lethal at 300, 350, 200 and 150 mgm. per kilogram, respectively, when administered intravenously to rabbits, and at 600, 500, 300 and 125 mgm. per kilogram intraperitoneally in rats. Methylene green is lethal for anesthetized cats in doses of 50 to 75 mgm. per kilogram. Orally in rats these dyes are tolerated in doses of 1.0 gm. per kilogram with the exception of methylene green, which killed 2 of 5 rats at 500 mgm. per kilogram. Data are presented on the chronic toxicity of trypan blue, gentian violet, brillant green and mercurochrome. Three of 6 rabbits dying under repeated intravenous administrations of trypan blue had received a total cumulative dose, approximately equivalent to but one acute lethal dose, i.e., 120 to 150 mgm. per kilogram. The dangers of repeatedly using high doses in

human lepers, the superiority of oral administration over intravenous, and the danger of certain synergizing agents, including photodynamic effects, are discussed.

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