ation it is seen that despite a marked variation in water solubility the chronic toxicity of the compounds, expressed as parts of fluorine per million parts of food, is remarkably constant. For all practical purposes, it may be stated that in the compounds studied fluorine is capable of producing chronic intoxication, as indicated by bleaching of the rat incisors, in concentrations as low as 14 to 16 parts per million regardless of the solubility of the compound containing the fluorine. Depending upon the amount of food consumed by the albino rat, this means that from one-half to 1 mg. of fluorine per day per kilo of body weight is capable of producing definite signs of injury to the rat incisors.

Since 2, and possibly one, part of fluorine per million of drinking water is capable of producing mottled enamel in children, and since the average water intake is approximately one liter, it is evident that an intake of one to 2 mg. of fluorine per diem is toxic to a child. Accordingly, human subjects appear to be more susceptible to fluorine poisoning than do rats, and the data obtained on rats become all the more significant from the public health standpoint.

7337 C

Acute Toxicity of Trypan Blue, Gentian Violet and Brilliant Green.*

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Difficulties encountered in administering sufficient amounts of the usual chaulmoogra oil derivatives uniformly to effect a cure in leprous subjects have led to the attempted application of other forms of therapy. Thus, recent clinical developments[1, 2] again have aroused interest in the efficacy of certain non-chaulmoogryl drugs, synthetic dyes in particular, against leprosy. In the course of examining the possible antileprotic activity of certain such compounds

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1 Ryrie, G. A., *Trans. Roy. Soc. Trop. Med. and Hyg.*, 1933, **27**, 85.

2 Ryles, C. S., *Leprosy Review*, 1933, **4**, 90.

by a standardized technique,[3] determination of their toxicity in the test animals was a necessary preliminary. As trypan blue, gentian violet, and brilliant green have been used in an empirical manner in the clinic before a critical pharmacological evaluation of their worth has been made, it is advisable to present the results of this toxicity study on experimental animals, before toxic doses may unavoidably be given to human leprous subjects. The intravenous dosage of these dyes, as reputedly used by Ryrie[1] and Ryles,[2] is within the possible lethal range as estimated from experimental animals. While these workers do not give the sources of the dyes used by them, nor their standards of purity, so that it cannot be judged whether or not their dyes are comparable in purity to those used by us, it is significant that Ryrie[1] admits the occurrence of dangerously severe acute toxic effects in his patients.

Previously reported toxicity data on these 3 dyes are so inadequate and variant that a recent encyclopedic work on chemotherapy[4] omits all reference to their toxicity. Discrepancies in some cases may be explained by variations in purity and by the use of old solutions. Photodynamic action is not a significant factor in the toxicity of these dyes as far as we could determine. We will later make a more extended report surveying the therapeutic effectiveness as well as the chronic and acute toxicity of these agents. Such a study is indicated in view of enthusiastic endorsement of dye therapy in leprosy on grounds that it is as good as treatment with hydnocarpus oil derivatives, *safe*, cheap and not unduly painful.

In Table I may be found our toxicity observations. The variation in toxicity with mode of administration emphasizes the difficulty of absorption of compounds of such high molecular weight. Particularly marked is the absence of toxic effects on oral administration due to negligible assimilation from the gut. On the other hand, when given by other routes some excretion of the dyes into the gut has been noted. Examination of the data in Table I discloses the wide toxic range of all these agents, which suggests caution in human therapy. Also, considerable species variation is encountered, with the guinea pig being generally more susceptible. Special interest may be attached to the variation in intravenous toxicities of trypan blue in different species, since it is by this route that it is recommended for human therapy.

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3 Anderson, H. H., Emerson, G. A., and Leake, C. D., *Internat. J. Lep.*, in press.

4 Fischl, V., and Schlossberger, H., *Handbook of Chemotherapy*, Vol. 1, Baltimore, 1933.

TABLE I.

Acute Toxicity of Trypan Blue, Crystal Violet, and Brilliant Green in Various Species of Animals by Various Routes of Administration.

Animal	% Aqueous Solution	Method of Administration	Dosage (mg./Kg.) and Mortality Ratio	
Trypan Blue: National Aniline Chemical Co., 90% C. P.				
Mouse			350	2/5
	1	Intraperitoneal	400	5/5
			100	1/5
	1	Intravenous	200	3/5
Rat			Not absorbed	
	5-10	Oral	(1 gm./Kg. tolerated)	
			300	2/5
	2	Subcutaneous	400	4/5
			300	2/5
	2	Intraperitoneal	350	5/5
			200	0/5
	2	Intravenous	300	5/5
Rabbit			300	0/3
	5	Intraperitoneal	400	3/3
			100	1/5
	5	Intravenous	150	3/5
Guinea Pig			200	0/5
	2	Subcutaneous	300	2/3
			200	1/5
	2	Intraperitoneal	250	3/6
Crystal Violet: Coleman and Bell, 80% C. P.				
Mouse			10	1/5
	0.1	Intraperitoneal	20	5/5
			10	0/3
	0.1	Intravenous	20	2/3
Rat			250	0/3
	1	Oral	—	—
			10	1/5
	0.5	Intraperitoneal	15	3/5
Guinea Pig			5	0/3
	1	Intraperitoneal	10	3/3
Brilliant Green: Coleman and Bell, Medicinal Grade				
Mouse			3	0/3
	0.1	Intraperitoneal	5	4/5
			1	0/3
	0.1	Intravenous	3	4/4
Rat			5	0/5
	1	Intraperitoneal	8	4/5
Guinea Pig			1	0/5
	1	,,	3	4/5

Pathological examination of animals dying of fatal doses has not elucidated the mechanism of toxic action. After such doses the dye is regularly distributed throughout the tissues, with some segregation in the reticulo-endothelial system noted only with trypan blue. Brilliant green and gentian violet appear to damage the liver relatively more than does trypan blue, which has been found in considerable quantity in the kidneys especially intracellularly in the descending loop. In all animals dying of toxic doses, marked congestion of the lungs was noted. Lengthening of the coagulation

time of the blood of treated animals has been reported for trypan blue,[5] while on prolonged administration of this agent to rabbits we have noted grossly a shortening of coagulation time after 2 acute toxic doses are administered in divided amounts over a period of 3 weeks. Certain observations lead us to suspect central nervous system depression in trypan blue treated animals; the brain and spinal fluid are reported to be impermeable to gentian violet, which is rapidly fatal in small amounts intracisternally.[6]

Experimental therapy on leprous rats has shown gentian violet and brilliant green to be too toxic for continued administration, while trypan blue, and other dyes which seemed preferable to us[7] because of their high leprocidal activity, are better tolerated.

7338 C

An Improved Method for In Vitro Cultivation of B. Leprae.*

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Many attempts have been made by various investigators to culture Hansen's bacillus of human leprosy. Undoubtedly, in a number of these attempts the investigators have mistaken the increase in number of acid-fast rods, which occurs in removed autolysing pieces of leprous tissue independent of any specially supplied cultural conditions, for multiplication *in vitro* upon artificially prepared nutrient medium. The failure to appreciate the facts that *B. leprae* continues to multiply and that growth ceases when the leprous tissue autolysate is no longer available, explain the increasing difficulty to obtain macroscopic growth of the specific microorganism in transplants through successive generations, and accounts for the absence of growth in subculture in or upon a medium that does not contain the split protein products (amino-acids).

One of us (Duval[1]) reported that *B. leprae* seemed unable to

[5] Hugget, A. St. G., and Rowe, F. M., *J. Physiol.*, 1933, **80**, 82.

[6] Kolmer, J. A., *Principles and Practice of Chemotherapy*, Philadelphia, 1926, p. 94.

[7] Emerson, G. A., and Salle, A. J., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 428.

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[1] Duval, C. W., *J. Exp. Med.*, 1910, **12**, 649.