

Southern Branch

Tulane University, May 23, 1929.

4493

Parasitocidal Potencies of the Tri- to Hepta-Methyl Derivatives of Rosanilin.

ERNEST CARROLL FAUST.

From the Laboratories of Parasitology, Peking Union Medical College, and Tulane University.

Faust and Yao¹ have shown that gentian violet, either by oral or by intravenous injection, is parasitocidal for *Clonorchis sinensis*, a fluke residing in the biliary passages of man, dogs and cats in the Far East. Although in chronic cases of long standing the flukes are at times so effectively walled off by the thickening of the biliary epithelium that it is impossible for the dye to reach them in lethal amounts, from 60 to 95% of the flukes can be destroyed by this dye, thus producing material improvement in the patient. Olivier and Kandou,² utilizing gentian violet therapy on 18 *Clonorchis*-infected patients in Java, have been able to confirm the results of Faust and Yao. It has seemed desirable, therefore, to follow the experimental work a step further in order to determine (1) which homologs in the methylated rosanilin series possess the parasitocidal properties and, if possible, (2) the portion of the potent homologs which is responsible for the results. With this in view the following dyes were obtained and the bactericidal and clonorchicidal potencies tested.

1. Dahlia crystals, *e. g.*, tri-methyl rosanilin (National Aniline and Chemical Co., and Grübler).

2. Gentian violet crystals, *e. g.*, a half and half combination of tetra-, penta-, and hexa-methyl pararosanilins with dextrin. (N.A. C.C.).

¹ Faust, E. C., and Yao, K. F., *Arch. für Schiffs- u. Tropen-Hygiene*, 1926, xxx, 383.

² Olivier, P. H., and Kandou, R., *Geneeskundig Tijdschrift voor Nederlandsch-Indië*, 1927, lxxvii, 59.

3. Enteric coated gentian violet pills (N.A.C.C.).
4. Methyl violet B crystals, *e. g.*, about 8% tetramethyl, 80% penta-methyl and 12% hexa-methyl pararosanilin. (N.A.C.C.).
5. Crystal violet crystals, *e. g.*, pure hexa-methyl para-roosanilin. (N.A.C.C.).
6. Methyl green, *e. g.*, hepta-methyl pararosanilin. (Grübler.)

Table I shows the relative bactericidal potencies of the several dyes at different dilutions.

TABLE I.
Growth of B. subtilis in the following media.

Dilution	1 Dahlia Nat. An. Chem. Co.	2 Dahlia Grübler	3 Gentian Violet Tablets Nat. An. Chem. Co.	4 Gentian Violet Crystals Nat. An. Chem. Co.	5 Methyl Violet B. Nat. An. Chem. Co.	6 Crystal Violet Nat. An. Chem. Co.	7 Methyl- Green Grübler
1/100,000	—	—	+	—	—	Not tried	+++
1/200,000	—	—	++	—	—	Not tried	+++
1/500,000	++++	+++	++++	+	+	—	++++
1/1,000,000	++++	+++	++++	+	+	—	++++

It will be seen that crystal violet is the only member of the series which conforms to the biological specification of the Commission on the Standardization of Biological Stains (Conn³), namely that it should have "sufficient bacterostatic power so that, when added to nutrient agar in the proportion of one part to a million, it will entirely prevent the growth of *Bacillus subtilis* when this organism is streaked over the surface of the hardened agar."

Guinea pigs and puppies artificially infected with *Clonorchis* and cats naturally infected with the fluke were used in testing the clonorchicidal potency of the dyes. Both oral and intravenous routes of administration were utilized, although the latter (in one-half per cent solution) was better tolerated by the animals. The experiments, which extended over a period of nearly a year, may be summarized as follows: 1. Dahlia was found to be without measurable parasiticidal potency or without any toxic effects on the host. 2. Gentian violet, methyl violet and crystal violet were equally potent (being 50 to 100% clonorchicidal, depending on whether the infection was chronic or of recent origin). These dyes were well tolerated by the infected animals, particularly when intravenous injection was utilized. 3. Methyl green was found to be without measurable clonorchicidal potency. However, it had a definite toxic effect on all animals to which it was administered even in small

³ Conn, H. J., *Biological Stains*, Geneva, N. Y., 1925, 151 pp.

amounts. The symptoms which developed some days after administration consisted of spastic paralysis and epileptiform seizures, terminating fatally.

The results of these experiments seem to indicate that the tetra-, penta-, and hexa-methyl pararosanilins are equally clonorchicidal, although theoretically, as well as by the bactericidal test, crystal violet, the pure hexa-methyl pararosanilin, should be the most potent member of the series. On the other hand the lower and higher homologs of the series, tri-methyl and hepta-methyl pararosanilins, are non-clonorchicidal, while the latter is definitely responsible for fatal disturbances of the central nervous system. The structural formulae of these dyes indicates that the compound is lethal for *Clonorchis* when there are no fewer than 4 and no more than 6 methyl groups. Apparently the potency is as great with 4 as with 5 or 6 such substitutions.

4494

Ineffectiveness of Radiant Energy on Hemorrhagic Anemia in Dogs.*

HENRY LAURENS AND H. S. MAYERSON.

From the Laboratory of Physiology, School of Medicine, Tulane University of Louisiana, New Orleans.

Twenty white animals in which the Hb level had been reduced by 3 or 4 successive hemorrhages, at from 2 to 3 days intervals to between 35 and 50%, were fed the standard Whipple (salmon bread) diet and irradiated with the flaming C ("Pan Ray") arc. The interval between successive exposures, as well as their number and intensity, was varied in different experiments and in successive periods of the same experiment. The efficacy of the experimental procedure in effecting regeneration was measured by the amount of Hb which had to be removed during the experimental period, as compared with that removed during earlier and later control periods, in order to keep the Hb at the chosen reduced level.

Such irradiation produces a marked increase in the number of erythrocytes which progressively decrease in size but maintain a normal saturation, as was earlier reported for "normal" animals.

* Aided by a grant from the David Trautman Schwartz Research Fund of Tulane University.