

These experiments were conducted upon 4 dogs having gastric pouches of the Pavlov type, and one having the Heidenhain pouch.

5226

The Toxicity and Balantidicidal Action of "Di-Hydranol"
in Guinea Pigs.*

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The frequent natural infestation of guinea pigs with such an easily observed intestinal parasite as *Balantidium coli* offers an excellent opportunity for a carefully controlled study of the parasitidal action *in vivo* of new drugs of possible use in this regard. With proper toxicity studies of the same drugs in healthy uninfected guinea pigs, one may thus obtain quantitative data of considerable value in estimating the relative chemotherapeutic indices of a series of compounds, and thus the more readily estimate their worth for clinical trial in humans. Sweeney¹ has already nicely demonstrated the possibilities of this technique. We determined to use this method as an adjunct to our general investigation of the chemotherapy of amebiasis, especially to assist in determining whether or not a compound is worth the time and expense of study in natural or induced amebic infestation in monkeys or other animals.

Such studies of toxicity and experimental activity, together with quantitative data on the rate of secretion, we consider absolutely necessary before one may be justified in attempting the clinical evaluation of a new drug in human therapy.² New drugs are continually being offered for clinical evaluation in amebiasis and we are amazed that so many come to clinical trial without reference to quantitative data either on toxicity and therapeutic activity in experimental

* This note is part of an extended cooperative study of the chemotherapy of amebiasis conducted by the Pacific Institute of Tropical Medicine of the Hooper Foundation for Medical Research and the Pharmacological Laboratory of the University of California Medical School, San Francisco, and supported in part by Eli Lilly and Co., Indianapolis, and the Ciba Co., Inc., New York City.

¹ Sweeney, M. A., *Am. J. Hyg.*, 1929, **9**, 544. We desire to express our thanks for advice from Dr. E. L. Walker and Miss M. A. Sweeney in connection with this study, and to Miss Dorothy A. Koch for aid in stool examinations.

² Leake, C. D., *J. Am. Med. Assn.*, 1929, **90**, 1632.

animals or rate of excretion in normal humans. Thus Faust has recently reported³ on the clinical evaluation of 2-4 dihydroxy-phenyl-n-heptane ("di-hydranol") in humans before the publication of a proper evaluation of the relationship between the toxic and parasiticidal dosage of this compound as it may be estimated in experimental animals without involving danger to human patients. It is to supply this essential information that the present report is made.

Crystalline "di-hydranol" was generously supplied by Dr. Veader Leonard. Its toxicity on single oral administration to guinea pigs was estimated by a general technique described previously,⁴ with results as indicated in Table I. At the average lethal dose of 400

TABLE I.
Toxicity of "Di-hydranol" on single oral administration in guinea pigs.

Dose	Number used	Number died	Time of death
600 mgm./kg.	10	10	All died within 4 days of administration.
400 " "	10	5	3, 3, 3, 11, 14 days.
200 " "	5	0	—

mgm. per kg. or higher, the guinea pigs lost markedly in weight, became lethargic, suffered from diarrhea, shivering and anorexia, and usually died within 4 days. On postmortem examination congestion of the small intestines, liver and kidneys was noted, and microscopic study of tissues obtained at such an examination revealed cloudy swelling of the kidney and liver. Some animals after a display of the symptoms above noted tended to recover somewhat, but a weakening of resistance was indicated by pneumonic sequelae terminating in death 10 days or more after the administration of the drug.

In estimating the therapeutic value of "di-hydranol" in *Balan-tidium coli* infestations in guinea pigs, we applied the same rigorous criteria of "cure" as insisted upon by Sweeney.¹ We found that stools could be more readily secured in a satisfactory condition for examination by daily feeding of greens, about an hour after which individual stool samples were obtained by manual expression. Each animal was isolated in a separate cage with a wide mesh false bottom, and with food and water containers arranged to prevent contamination. The cages were cleaned and scalded daily. The animals selected for therapeutic study were heavily infested. Stools

³ Faust, E. C., PROC. SOC. EXP. BIOL. AND MED., 1930, 27, 905.

⁴ Anderson, H. H., and Leake, C. D., PROC. SOC. EXP. BIOL. AND MED., 1930, 27, 267; *Am. J. Trop. Med.*, 1930, 10, 249.

were examined daily during treatment and for 2 weeks after treatment ceased, when the "cured" animals were sacrificed in order to determine whether or not parasites might be found in the caecum or elsewhere in the intestinal tract. A control group of 5 heavily infested guinea pigs were followed for a 2 week period under the same conditions of hygiene as the treated animals. Treatment was continued in all pigs at the dosage indicated for 5 doses, whether the stools became negative or not, in order to estimate the possible danger of cumulative toxicity on continued administration of sublethal doses. The data presented in Table II indicate the wisdom of this procedure.

TABLE II.
Curative power of "Di-hydranol" on repeated sublethal oral doses in *Balanitium coli* infestations in guinea pigs.

Dose mgm./kg.	No. of animals	No. cured	Total curative dose mgm./kg.	Time of cure days	No. not cured	Total do- sage not curing mgm./kg.	No. died	Lethal dosage mgm./kg.
100 every 2 days	5	4	200	4	1*	100*	3	100, 200, 500
75 every 2 days	5	4	225	6	1	225	3	225, 300, 375
50 daily	5	2	150	4	3	1 at 150 2 at 250	1	accidentally at 150
No drug	5	0	—	—	5 at 14 days	—	0	—

* Died 20 hours after initial administration of drug; evidence of tuberculosis found at postmortem.

Summary. 2-4 dihydroxy-phenyl-n-heptane ("di-hydranol") causes death in guinea pigs in about 4 days with symptoms of loss

of weight, diarrhea, and lethargy at an average dose of 400 mgm. per kg. on single oral administration. In natural *Balantidium coli* infestations in guinea pigs, it seems to be curative at a total of 200-225 mgm. per kg. on continued administration at daily or two daily intervals of 75 to 100 mgm. per kg. Toxicity may be encountered, however, at a total dosage only slightly higher than this indicated curative range on continued administration of the sublethal doses proposed.

5227

Ammonium Metavanadate as a Reagent for Reducing Carbohydrates.

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A reagent consisting of 2% ammonium metavanadate in 10% sodium carbonate solution has proved effective in the detection of reducing carbohydrates or of non-reducing carbohydrates after hydrolysis. To make a test we heat on the water-bath 2 cc. of a 0.25% or 0.50% solution of the compound under consideration with 5 cc. of the reagent. A brownish to yellow color develops with a slight precipitate. On the addition of 1% hydrochloric acid, the mixture becomes clear and assumes a characteristic green color, due to the reduction of the metavanadate to a compound in which vanadium holds a lower valence. The acid must be added in quantities just sufficient to neutralize the reaction mixture, the point of neutralization being determined with litmus paper.

The following compounds reduced with the formation of the characteristic color: pentoses (xylose, arabinose, rhamnose); hexoses (glucose, glucosamine, levulose, galactose, mannose); disaccharides (lactose, maltose, cellobiose). The following compounds reduced only after preliminary subjection to hydrolysis followed by neutralization: disaccharides (sucrose, trehalose); trisaccharides (raffinose, melizitose); polysaccharides (dextrin, starch, inulin, glycogen, cellulose); glucosides (aesculin, amygdalin, arbutin, phloridzin, strophantin, picrotoxin, salicin, saponin); gums (arabic, acacia, tragacanth); glycoproteins (mucin, ovomucoid); glycolipins (phrenosin); nucleic acid.

Compounds other than carbohydrates also reduced. Cysteine gave