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Colorimetric Study of Absorption and Excretion of 4-(p-Dimethylaminostyryl) Quinoline Methiodide and Related Compounds.* (21961)

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Since 4-(p-dimethylaminostyryl) quinoline methiodide(1) and several related dyes, when administered in the diet, have been found to cause regression of Lymphoma 8 tumors in rats(2-4), the fate of the unchanged dye was studied colorimetrically. The results of this study have led to finding a compound that appears to be unusually active in bringing about regression of Lymphoma 8(3,4).

Methods. The compounds used were prepared in the chemical laboratories of Carson-Newman College by Wilfred Lyke and Joan Wilson, with the financial assistance of a Frederick Gardner Cottrell Grant from the Research Corporation and a grant from the Medical Research Foundation: 4-(p-Dimethylaminostyryl) quinoline methiodide (4M 2M). 4-(p-Dimethylaminostyryl) quinoline methochloride (4M2MCl). 2-(p-Diethylaminostyryl) quinoline ethiodide (2E2E). 2-(p-Dimethylaminostyryl) pyridine methiodide (2M2PM)‡, and 2-(p-Dimethylaminoanil) pyridine methiodide (2M2NPM). The usual method of administering the dyes was to mix them with powdered food of animals, by grinding in a ball mill 8 hours or longer. After offering dyed food for 2 days the animals were given untreated food *ad libitum*, beginning the evening of second day. In a few instances a solution of a dye in a 0.9% NaCl or 5% glucose was administered by a stomach tube or was injected subcutaneously in the flank or intravenously via a tail vein. Urine and feces were collected separately, by conventional apparatus, until 2 days after last dose of dye was administered. The dye was

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‡ This compound was included because of the observation by workers at the Midwest Research Institute that it seemed to cause damage to Sarcoma 180 in mice used in tumor necrosis screening tests, although it did not cause conspicuous inhibition of the growth of tumors. Goodson, Lewis H., personal communication.

recovered from feces by hot 95% ethanol in a Soxhlet extractor and measured by Beckman Type B spectrophotometer. The absorption peak at 540 mμ was used for quinolinium compounds and the 469 mμ peak was used for 2M2PM. Absorption due to normal fecal pigment was taken into account. To measure the dye content of a tissue, such as liver, the sample was washed quickly with alcohol, then minced under a small amount of alcohol. If the pink color of dye appeared in the alcohol, the dye was extracted with the aid of a Soxhlet extractor. A sensitive test for traces of dye in urine and other aqueous solutions was carried out by cutting a thin wick from a disc of filter paper(5) and allowing the solution to rise through this wick and spread through the entire filter paper. Any 4M2MCl, or 2E2E would color the wick. Free dye was removed from urine by passing it through a tube packed with absorbent cotton or by stirring the sample with filter paper. After the dyed cellulose was washed with water and dried, the dye was eluted with boiling alcohol and the quantity measured colorimetrically. In some instances the decolorized urine gradually turned purple on standing, and could be recovered and measured as described. The appearance of pink or purple color could be hastened by heating the sample in a boiling water bath for about 20 minutes, provided the sample was slightly basic. Before heating, therefore, a base was added to urine samples that were acid. Appearance of pink color when a basic decolorized solution was heated, is referred to in this paper as a "heat-pink" test.

Results. Fecal excretion. From 36 to 47% of the 4M2M fed, was recovered from feces of Wistar rats, hooded rats, black mice, and brown mice. Practically the same results were obtained whether this dye was administered at concentration of 0.3% or 0.1% in Rockland Mouse Ration or concentration of 0.3% in starch, but a higher excretion was noted when food and dye were ground only 45 minutes. Recovery of 4M2MCl from the feces was about 58%, whereas recovery of 2E2E and 2M2PM was 13 to 27%. No 2M2NPM was recovered, perhaps because of hydrolysis in the stomach. The anil pyridines are usually readily hydrolyzed by acids, in contrast to the styryl compounds.

Disappearance of dye. The color of dye sometimes persisted 2 to 5 days at sites of subcutaneous injections of 4M2MCl solution in rats. This is in contrast with permanent staining reported to result from subcutaneous injections of 2-(p-acetaminostyryl)-6-(p-acetamidobenzamido) quinoline methoacetate (Styryl 430), reported to produce sarcomata (6). When rats were sacrificed after several days of eating food containing styryl dyes, their tissues did not appear to be stained. However, when a mouse was killed 20 minutes after receiving the last of three 1 ml oral doses of a solution containing approximately 3 mg of 4M2MCl/ml of 5% glucose at 20-minute intervals, 0.8% of the dye was recovered by extraction from the liver, and small quantity was extracted from kidneys. Similar results were obtained at the end of 3 hours, but no dye was found in kidneys and liver of 2 mice and a rat killed 1 day after receiving the solution by stomach tube. The color of saturated solution of 4M2M in 0.9% NaCl became lighter as it passed down the digestive tract of a rat or mouse, until it was yellow in the small intestine.§ This yellow solution brought about bleaching of dilute solutions of 4M2M or 2E2E within 4 hours. Liquid from the stomach did not produce this effect, nor did liquid from stomach, gall bladder, or intestine of a dog.

Urinary excretion. Rarely a faint trace of dye was observed in urine of animals fed dyed food, but stronger traces were noted following subcutaneous injections. When a rat ate 24.3 mg of 4M2MCl in his food during 2 days, only 0.033 mg of the free dye was found in urine of the first 24 hours and 0.023 mg in the next 24 hours. The additional dye recovered after decolorized urine had stood, was 0.05 mg for each day. After subcutaneous injection of 0.6 mg of 4M2MCl in 5% glucose, 5% of the dye was found in urine. The substance responsible for the color observed after the decolorized dye had stood for some time, had

§ This experiment was planned and executed with the assistance of Dr. Robert A. Woodbury, University of Tennessee, Memphis.

very nearly the same absorption maximum in alcohol as that of a known sample of 4M2M. A negative heat-pink test was obtained with urine of rats, rabbits, hamsters, and a dog that received subcutaneous injections of 4M2MCl solution, but the test was positive after eating of food containing 4M2MCl. The inference that the substance responsible for the heat-pink test was formed in the digestive tract was supported by further observations: A faint pink color was produced when the heat-pink test was applied to samples of 4M2M solution in 0.9% NaCl that had been decolorized in the digestive tract of a rat. A sample of 4M2M solution in 0.9% NaCl, kept over night at room temperature, after addition of a few drops of the liquid from the lower small intestine, then decolorized by passing through cotton, gave a strong heat-pink test. The nature of the substance responsible for the heat-pink test is being investigated further.

Summary. 1. A substantial portion, usually less than half, of the 4M2M, 4M2MCl, 2E2E, and 2M2PM administered to rats and mice in the diet, was found by colorimetric measurements, in the feces. 2. Only a trace of dye color was detected in urine of rats that had received 4M2M or 4M2MCl orally or subcutaneously. 3. Heating, or prolonged exposure to air, of samples of urine from animals that received 4M2M, 4M2MCl or 2E2E orally, not subcutaneously, produced a pink color. The trace of material responsible for this color seemed to be formed from the dye in the digestive tract. 4. Very little unchanged dye was found in the tissues of rats and mice that had received 4M2M or 4M2MCl orally. 5. A large part of the 4M2M or 4M2MCl administered orally is evidently converted into other substances.

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Tissue Abnormalities in Newborn Rats from Vitamin B_{12} Deficient Mothers. (21962)

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Reproductive failure in female rats deficient in vit. B_{12} has been reported by Dryden, Hartman and Cary(1), Lepkovsky *et al.* (2), Richardson, Witten and Couch(3). Thirty to 60% of the offspring died within 3 days and those which survived were underweight and unhealthy. Ferguson and Couch (4) observed degeneration in heart, liver, thyroid and kidney in chick embryos from hens deficient in B_{12}. This suggested that similar changes might be found in rat embryos and might account for their early mortality.

Methods. Weanling female rats were placed on B_{12} low diet of O'Dell *et al.*(5) until 4 weeks before mating when they were transferred to experimental diets. Basal diet was synthetic and contained 25% commercial soybean protein* extracted 6 times by boiling

* The Drackett soybean protein C-1, the Drackett Co., Cincinnati.