beginning of a still higher polymer. When the concentration is increased even more to 0.05% the curve shows two peaks (Fig. 4, curve 1), which are greatly flattened and shifted from those of the monomeric and other lower forms in both the major and minor peak regions. The density is reduced from that of the 0.045% solution, indicating the formation of one or more new polymeric forms with lower absorption coefficients.

The data obtained from this study clearly indicate that Vasoflavine is capable of forming polymers, dependent upon the dye concentration. The dye apparently exists in polymeric forms at concentrations greater than 0.025%, and even higher polymers appear to exist above 0.04%.

Upon calculation of the possible concentration of Vasoflavine in the blood of injected rabbits according to our regular technic, it was found that the best results, with less diffusion out of the vessels, were obtained when the dye concentration in the blood was greater than 0.03%, assuming that the dye dispersed rapidly throughout the blood stream. It seems therefore reasonable to believe that the dimeric or polymeric form of the dye is the more desirable, for it diffuses from the blood vessels less readily and apparently imparts a more brilliant yellow-green fluorescence to the blood vessel walls.

Summary and conclusions. A more fluorescent and less rapidly diffusible dye with a strong affinity for the walls of the blood vessels has been described. This dye is a purified, water soluble fraction of Thioflavine-S and has been named Vasoflavine. Studies leading to a more accurate characterization of this dye are described. These include paper chromatography, dialysis, and spectrophotometry.

The following conclusions are offered: 1. Vasoflavine approaches the ideal dye for the visualization of blood vessels under ultraviolet light and is much superior to the Thioflavine-S previously used. 2. The solution of dye probably exists as an equilibrium between monomeric and various polymeric forms. 3. The higher the concentration of an aqueous solution of the dye the more of the polymeric forms exist. 4. The yellow-green fluorescence is apparently produced by the higher form and the bluish by the monomeric form. 5. The polymers are probably too large in molecular size to pass the barrier of the vessel walls readily, while the monomeric form quickly diffuses out of the vessels, thus explaining the yellow-green fluorescence of vessels and the bluish fluorescence of the surrounding tissue. It should be noted that Vasoflavine exhibits no apparent toxic effects in experimental animals (rats, rabbits, and dogs) in blood concentrations as high as 275 mg %, but extensive toxicity studies have not been carried out.

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Mechanism of Action of Mercurial Diuretics. (18735)

GEORGE FAWAZ AND EVA N. FAWAZ.

From the Department of Pharmacology, American University of Beirut, Beirut, Lebanon.

Gremels(1) observed that mercurial and xanthine diuretics increase the oxygen consumption of the isolated dog kidney perfused with blood from a heart-lung preparation.

1. Gremels, H., *Arch. Exp. Path. u. Pharmakol.*, 1929, v140, 205.

Farah and Maresh(2) have shown that the diuretic action of mersalyl is inhibited by the administration of half a mole of dimercaptopropanol (BAL) for every mole of mersalyl. This finding suggested to these authors "that the possible mechanism of action of the mercurial diuretics is the inactivation of one or more SH-containing enzyme systems responsible directly or indirectly for reabsorption of salt and water from the lumen of the kidney tubules."

Among the SH-containing enzymes the succinic oxidase system has been studied extensively and methods for its determination are available(3). It appeared desirable to study the respiration of tissue slices as well as the activity of the succinic oxidase and other SH-containing enzyme systems in the kidney of the rat at the height of mercurial diuresis. Although our studies are still in progress we feel it is desirable to submit this preliminary report, particularly in view of the recent publication of Handley and Lavik(4), in which they state that "it may well be that the succinic dehydrogenase system of the kidney is involved in the active reabsorption of water and sodium chloride by the cells of the renal tubule."

Procedure. Mersalyl* was used as the mercurial diuretic for the experiments described below.

a. *Pre-diuretic control experiment.* Albino rats weighing 150-250 g were used. Food and water were withheld from all animals for 15 hours prior to the time they were used for an experiment (5 p.m. to 8 a.m.). The left kidney was removed under ether anesthesia and determinations were made of the respiration of cortical tissue by the conventional Warburg technic, and of the succinic oxidase activity by the method of Schneider and Potter(3). A week later, when the abdominal wound was healed, the right kidney was removed under the same conditions and the same determinations were made. It was found that the respiration as well as the succinic oxidase activity of the right kidney remained the same and thus each animal could be used as its own control.

b. *Diuretic experiment.* The respiration and succinic oxidase activity of the left kidney was determined as above. A week later 10 mg per kg body weight of mersalyl (*i.e.* 4 mg Hg/kg) dissolved in 2 cc saline were injected into the tail vein. Simultaneously 50 cc saline per kg body weight were injected intraperitoneally and the kidney was removed three hours later. The control rats were injected only with the same volume of saline, and the right kidney was also removed after 3 hours. The details of this method of assay will be published separately. At present it can be said that this method of administration of the mercurial results in profuse diuresis in almost every single rat as compared to the controls receiving only saline. The diuresis reaches its maximum in 2 to 3 hours. Most of the fluid is excreted within 8 hours although the effect of the diuretic is still apparent after 10 hours. Lipschitz *et al.*(5) devised a method of assay giving the mersalyl intraperitoneally and they observed a diuretic effect only after 5 to 10 hours even with the simultaneous administration of ammonium chloride. Dicker (6) administered mersalyl intramuscularly to rats and observed the diuretic effect 10 hours after the injection.

Results. The results of our experiments are summarized in Table I. It will be seen that the standard diuretic dose of 10 mg mersalyl (4 mg Hg/kg) had no significant effect either on the respiration of cortical tissue slices or on the succinic oxidase activity. Handley and Lavik, using a technic different from ours reported a 40% decrease in the succinic oxidase activity after injecting 8 mg of Hg or double the dose we used. We have tried to repeat Handley's experiments(4) using mersalyl, but as can be seen from the last two columns of

2. Farah, A., and Maresh, G., *J. Pharmacol.*, 1948, v92, 73.

3. Schneider, W. C., and Potter, V. R., *J. Biol. Chem.*, 1943, v149, 217.

4. Handley, C. A., and Lavik, P. S., *J. Pharmacol.*, 1950, v100, 115.

* Mersalyl was generously supplied by the British Drug Houses Ltd. and the Sterling-Winthrop Research Institute.

5. Lipschitz, W. L., Hadidian, Z. and Kerposar, A., *J. Pharmacol.*, 1943, v79, 97.

6. Dicker, S. E., *Brit. J. Pharmacol.*, 1946, vI, 194.

TABLE I. Effect of Mersalyl on Respiration of Rat Kidney Slices and on Succinic Oxidase Activity of Homogenates at Height of Diuresis. (For Explanation See Text.)

No. of animals	Mersalyl, mg/kg	Qo_2 L. kidney Mean	Qo_2 L. kidney Stand. dev.*	Qo_2 R. kidney Mean	Qo_2 R. kidney Stand. dev.	Qo_2 (succin.) L. kidney Mean	Qo_2 (succin.) L. kidney Stand. dev.	Qo_2 (succin.) R. kidney Mean	Qo_2 (succin.) R. kidney Stand. dev.
13	—	19.1	1.1	19.1	1.3				
19	10	19.8	1.04	18.8	1.1				
10	—					187	7.6	188	8.7
15	10					183	5.1	188	8.2
4	—					196	8	190	9.6
10	20					182	14.2	163	16.1

* $\sqrt{\frac{\Sigma d^2}{n-1}}$

Table I, the decrease in the succinic oxidase activity observed was statistically insignificant. The maximum inhibition obtained in one case was 22%. It has been observed that after injection of the mercurial the kidney swells and if no dry-weight determination is done on that particular kidney misleading results could be obtained.

In our experience, a dose of mersalyl representing 8 mg Hg per kg is not a diuretic dose for the unilaterally nephrectomized rat. Lipschitz *et al.* have called attention to the narrow dosage range for the diuretic activity of mercurials. The technic followed by Handley and Lavik would not permit them to ascertain whether the dose they used produces diuresis. When we gave the high dose of 8 mg Hg/kg to unilaterally nephrectomized rats under the conditions reported above, we found that at least half of the animals showed oliguria as compared to the controls. The kidneys were removed a day after the administration of the mercurial and sent for histopathological examination. It was found that with this high dose of mercury all rats showed extensive necrosis of the proximal convoluted tubules, involving about 60% of the cells. Even with the standard dose of 4 mg Hg/kg some animals showed definite irreversible damage which was much milder than with the greater dose. A dose of 5 mg mersalyl (2 mg Hg/kg) produced no pathological change in unilaterally nephrectomized rats and no diuresis was observed.

Discussion. The fact that diuresis caused by mercurials can be inhibited by dimercaptopropanol (BAL) is no proof that mercurial diuretics act by inhibiting SH-containing enzymes. It only means that BAL binds the mercury more firmly than do the tissues. Mercurials can react with proteins other than through SH-groupings, and even if certain SH-containing enzymes are inhibited by therapeutic doses of mercury it does not necessarily follow that SH-containing enzymes are involved in the reabsorption of salt and water from the renal tubules. The question as to whether or not a certain enzyme, be it SH-containing or not, is involved in the reabsorption of salt and water can best be answered by observing whether a known specific inhibitor of that enzyme can produce diuresis. It would then be necessary to reach a high concentration of that inhibitor in the kidney tissue. The heart-lung kidney preparation of the dog is convenient for this purpose and work is proceeding in this laboratory to test the effect of such inhibitors on diuresis.

Summary. 1. Mersalyl given intravenously in therapeutic doses has no influence on the respiration of kidney cortical slices of the rat at the height of diuresis. 2. Under the same conditions mersalyl has no effect on the succinic oxidase activity of cortical homogenates. 3. The question of participation of enzymes in the reabsorption of salt and water from the renal tubules is discussed.

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