(the total of which would equal a frequently used single intravenous dose of tryparsamide, a closely related compound), careful watch for eye symptoms would be a necessary phase of the treatment. No symptoms of any sort were noted in our normal subjects.

Summary. 3-acetylamino-4-hydroxyphenylarsonic acid (acetarsone N.N.R. or "stovarsol") and 4-carbaminophenylarsonic acid ("carbarsone") are rather slowly excreted in the urine after oral administration. In one normal human subject 20% of the ingested arsenic in 0.5 gm. acetarsone was recovered in the urine in 72 hours, but only 8% of the arsenic of the same dose of "carbarsone" after 52 hours. In another normal human, 7% of the arsenic received in the same dose of acetarsone was found in the urine in 24 hours, while 13% was recovered from the same amount of "carbarsone" in 42 hours. Even though relatively small absorption from the bowel is indicated by these experiments, "carbarsone" should be used cautiously in human therapy until it is established whether or not it is liable to injure the optic tract.

5202

Iodoxybenzoate as a Test Reagent for Free Phenolic Hydroxyl Groups in Organic Compounds.

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While attempting to study the pharmacological action of oxidation products of morphine, I tried to induce rapid oxidation of the drug by means of ammonium iodoxybenzoate. The salts of iodoxybenzoic acid have been studied by Loevenhart and his associates[1] and their oxidizing properties are well known. Upon treating morphine salts with an aqueous solution of ammonium iodoxybenzoate the straw to garnet color characteristic of a morphine solution on eremacausis develops within a few moments. No other opium alkaloid, except apomorphine, seems to yield color with this reagent. Codeine is monomethyl morphine, with the methyl group replacing the hydrogen of the phenolic hydroxyl group of morphine. This

[1] Loevenhart, A. S., and Grove, W. E., *J. Pharmacol. Exp. Therap.*, 1911, **3**, 101; Arkin, A., *Ibid.*, 1911, **3**, 145; Young, A. G., and Yeomans, J. B., *J. Am. Med. Assn.*, 1926, **87**, 746.

"muzzling" of the phenolic hydroxyl group (which may explain the difference in action between the drugs), and the resulting inhibition of the oxidation color development on treatment with ammonium iodoxybenzoate, suggested that the reagent might be used for the detection of "free" phenolic hydroxyl groups in various other aromatic organic compounds.

This was found to be the case. The reactivity of the phenols in color development, especially in contact with aldehydes and inorganic acids, has been recently reviewed by Levine and Magiera.[2] The reaction system, phenol-aldehyde-acid, is so powerful, however, that "muzzled" phenolic hydroxyl groups, such as found in codeine or acetyl-salicylic acid, are opened up so that color develops. Hence such tests as Pettenkofer's[3] and Marquis'[4] for the opium alkaloids, for instance, do not differentiate morphine from its associated alkaloids most of which seem to contain "muzzled" phenolic hydroxyl groups. The advantage, then, of iodoxy benzoate as a test reagent in connection with phenolic compounds lies in the fact that it does not decompose the compounds with which it comes in contact, but merely reveals, by an oxidizing color reaction, the presence of a "free" phenolic hydroxyl group. Thus, like Lautenschlager's diazonium reagent,[5] it will not react with any opium alkaloid except morphine and apomorphine. Again, in comparison with an unneutralized arsphenamine solution, it reacts only with extreme slowness with a completely neutralized one, although a precipitate forms at once in both.

Obviously, this oxidation reaction between iodoxybenzoate and "free" phenolic hydroxyl groups in aromatic organic compounds may be made quantitative, and applied to the estimation of the concentration of a solution of unknown strength of a reacting substance by colorimetric comparison with a standard. The test, by virtue of its simplicity, should be useful for such purposes as differentiating morphine and apomorphine from each other and from other opium alkaloids, epinephrine from ephedrine, and acetarsone from other pentavalent arsenicals. It might also be useful for detecting the presence of phenol or cresol as preservatives in organic fluids. For these reaction purposes, a 1% freshly prepared aqueous solution of ammonium iodoxybenzoate allowed to act directly on the material, or in known concentration in solution, is satisfactory.

[2] Levine, V. E., and Magiera, E. A., *J. Lab. Clin. Med.*, 1927, **12**, 743.

[3] Pettenkofer, M., Medical Jurisprudence, Ed. 2, New York, 1911.

[4] Marquis, E., *Arb. Pharmakol. Inst. Dorpat*, 1896, **15**, 117.

[5] Lautenschlager, L., *Arch. d. Pharm.*, 1919, **257**, 13.

It does not matter whether or not the substance or solvent is miscible with the water solution of the iodoxybenzoate as long as thorough shaking is employed. Carvacrol and eugenol, for example, give powerful color reactions under these conditions, as does thymol in an organic solvent.

Of general biological significance in connection with this "free" phenolic hydroxyl oxidation reaction, is the fact that the pharmacological effect of compounds so reacting does not seem to be altered quantitatively or qualitatively. Thus, as far as I could determine in rabbits and dogs, the effects of freshly made colorless solutions of morphine sulphate and of morphine sulphate solutions in equal concentration oxidized to a deep garnet color by ammonium iodoxybenzoate were the same on subcutaneous injection of equivalent amounts. On intravenous injection the acute reactions peculiar to ammonium iodoxybenzoate occur, of course, with the solutions in which this agent is present. This interfered somewhat with the comparison between unoxidized and oxidized epinephrine in the manner indicated but did not seem greatly to inhibit the blood pressure raising effect characteristic of the drug. In the case of the arsphenamines, Voegtlin and Smith[6] suggest that oxidation of these arsenicals is a necessary preliminary step to the manifestation of their typical biological activity. On the other hand, the "muzzling" of the "free" phenolic hydroxyl group greatly reduces the toxicity and general pharmacological action of the parent substance. Thus codeine, phenetidin, and completely neutralized arsphenamine are less toxic and less "active" than morphine, p-amidophenol, and unneutralized arsphenamine respectively. Two reasons, then, indicate that the characteristic pharmacological actions of aromatic organic compounds containing "free" phenolic hydroxyl groups are mediated by oxidative processes in the body involving these groups; (*a*) oxidation outside the body previous to administration does not interfere with their typical effects; (*b*) "muzzling" of these groups and thus inhibiting their oxidative reactivity does alter their characteristic action, as appears for example in the differences in pharmacological activity between morphine and codeine, or between salicylic acid and acetyl salicylic acid.

[6] Voegtlin, C., and Smith, H. W., *J. Pharmacol. Exp. Therap.*, 1920, **15**, 475.