EFFECTS ON THE COPROPORPHYRIN TEST OF DRUGS USED IN THE TREATMENT OF PARASITIC DISEASE

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The effects of the following drugs on urinary coproporphyrin estimations were studied: foudadin, tartar emetic, coramine, emetine hydrochloride, carbon tetrachloride, hexylresorcinol, and atebirin. None of these substances interferes directly with the fluorimetric determination of urinary porphyrin if porphyrin is extracted from urine by ether or ethyl acetate and subsequently re-extracted into the aqueous phase with hydrochloric acid.

Persons under treatment with tartar emetic showed a significantly higher coproporphyrin excretion. For this reason the coproporphyrin test for abnormal lead absorption is likely to be unreliable in persons treated with tartar emetic.

The excretion of coproporphyrin in urine is increased in persons exposed to lead who in consequence absorb higher amounts of lead than normal. The determination of urinary coproporphyrin has therefore been used very extensively as one of the most reliable tests of abnormal lead absorption. The test is comparatively simple and fast, and is therefore used particularly frequently in the early diagnosis of occupational lead poisoning.

The methods mainly used for the quantitative determination of coproporphyrin fall into two groups: fluorimetric (De Langen and ten Berg, 1948; Fikentscher, 1932; Harrold, Meek, and Padden, 1952; Johnson and Whitman, 1950; Maloof, 1950; Parkinson and Cholak, 1952; Schwartz, Hawkinson, Cohen, and Watson, 1947; Schwartz, Zieve, and Watson, 1951) and, less frequently, spectrophotometric (Sveinsson, Rimington, and Barnes, 1949). Practically all the fluorimetric methods described in the literature are based on the extraction of coproporphyrin from acidified urine by means of ether or ethyl acetate and its subsequent further extraction from the organic solvent with hydrochloric acid. The fluorescence intensity of either the solvent extract or the final hydrochloric acid extract is used for the determination of the urine coproporphyrin concentration.

In our laboratories, when applying the fluorimetric coproporphyrin test, difficulties have been experienced with a number of urine samples taken

from persons under treatment for parasitic disease. It was often observed that the concentrations measured were not correlated with the exposure to lead. This investigation was therefore undertaken to find out whether these unreliable results might be due to some of the drugs most frequently used in the treatment of parasitic disease.

Methods and Instruments

The two most frequently used fluorimetric methods for urinary coproporphyrin determination were checked: that of Fikentscher (1932) and that of Schwartz *et al.* (1951). In the first method, urine is acidified with acetic acid and the coproporphyrin is separated by repeated extractions with ether. The ethereal extracts are treated with hydrochloric acid, whereby the coproporphyrin is again transferred into the aqueous phase in which its fluorescence is measured. The coproporphyrin concentration is read from a calibration curve.

In the second method, the urine sample is acidified with buffered acetic acid, the coproporphyrin is extracted with ethyl acetate, the extract is washed with sodium acetate and iodine solutions, and the coproporphyrin is finally extracted by several successive portions of hydrochloric acid. The coproporphyrin concentration is estimated as before.

All fluorescence measurements were performed by means of a Lumetron photoelectric fluorescence meter, model 402-EF, with a set of filters consisting of a primary filter for the near-ultraviolet exciting radiation and two secondary filters for orange-red fluorescence.

All the fluorescence measurements were carried out against a haematoporphyrin standard (haematoporphyrin from Fluka, A.G., Chemische Fabrik, Buchs, 1 mg./l. in 1.5 N HCl).

Since preliminary experiments had shown that the fluorescence quenching of coproporphyrin and haematoporphyrin were very similar, and the latter was commercially available, all the experiments on fluorescence quenching were carried out with haematoporphyrin (300 µg. haematoporphyrin/l. in 1.5 N HCl).

The influence of the following drugs was studied: foudadin (pentasodium antimonybiscatechol-3:5-disulphonate), tartar emetic (antimony potassium tartrate), coramine (diethylamide of pyridine-3-carboxylic acid), emetine hydrochloride, carbon tetrachloride, hexylresorcinol, and atebtrin (dihydrochloride of 2-chlor-5-(4-diethyl-amino-1-methylbutylamino)-7 methoxyacridine).*

Results and Discussion

In Fig. 1 the results of the experiments on the quenching of haematoporphyrin fluorescence with tartar emetic (I), emetine hydrochloride (II), foudadin (III), and atebtrin (IV) are presented. In the figures ϕ represents the values of intensity relative to the intensity of the same substance ($\phi_0 = 100$) in the absence of any interfering substance. It is clear from the respective quenching curves that atebtrin

FIG. 1.—Quenching of porphyrin fluorescence (300 µg. haematoporphyrin/l. 1.5 N HCl) with tartar emetic (▲), emetine hydrochloride (○), foudadin (●), and atebtrin (×).

quenched the fluorescence of haematoporphyrin most effectively, that the effects of foudadin and emetine hydrochloride were less pronounced, and that tartar emetic had practically no quenching effect on haematoporphyrin fluorescence. Even smaller quenching effects were observed in the experiments with coramine, carbon tetrachloride, and hexylresorcinol. For this reason tartar emetic, coramine,

FIG. 2.—Quenching of porphyrin fluorescence with foudadin: ● aqueous solution; ○ ether extract; × ethyl acetate extract.

carbon tetrachloride, and hexylresorcinol were ruled out as interfering substances in the fluorimetric porphyrin determination.

Figure 2 shows the results of further quenching experiments with foudadin. Curve II (ether extracts) and curve III (ethyl acetate extracts) clearly showed that foudadin, which is probably not extracted by ether or ethyl acetate, did not affect the results of porphyrin determination by either the Fikentscher or the Schwartz, Zieve, and Watson (1951) method.

Figure 3 gives the quenching effects of emetine hydrochloride. As to the probability of the practical interference of this drug in fluorimetric porphyrin determination, the same conclusions can be drawn as with foudadin. Its quenching effects were already negligible after extraction of porphyrin in ether or ethyl acetate, and they practically disappeared in the final hydrochloric acid extract.

Figure 4 shows the results of the experiments with atebtrin. Curve I shows that atebtrin first increased the fluorescence intensity of the solution, and only in higher concentrations did its quenching effect on the fluorescence of porphyrin prevail. This unexpected shape of the curve is probably due to the fact that atebtrin is also a fluorescent compound. At low concentrations its new fluorescence was superim-

FIG. 3.—Quenching of porphyrin fluorescence with emetine hydrochloride: ● aqueous solution; ○ ether extract; ▲ ethyl acetate extract; × final hydrochloric acid extract.

*The chemical terminology is taken from the *British Pharmacopoeia*, General Medical Council, London, The Pharmaceutical Press, 1958.

FIG. 4.—Quenching of porphyrin fluorescence with atebtrin: ● aqueous solution; ○ ether extract; ▲ ethyl acetate extract; × final hydrochloric acid extract.

posed onto the fluorescence of porphyrin. At higher concentrations quenching by atebtrin started and its effects on the porphyrin fluorescence became increasingly evident. In agreement with this view, the fluorescence intensity of the atebtrin and haematoporphyrin solution coincided with the maximum fluorescence of atebtrin alone. Atebtrin does not seem to be extracted by ether, as shown by curve II, and is not therefore likely to interfere with the Fikentscher method, but the shape of the fluorescence curve of the ethyl acetate extract (III) remains to be explained. When, however, porphyrin was extracted from ethyl acetate by means of hydrochloric acid, there was no longer any interference of practical significance.

Although the above results show that the drugs used most frequently in the treatment of parasitic disease are not likely to interfere with the fluorimetric determination of porphyrin, it has been noticed that in persons under treatment with tartar emetic the possibility of a higher porphyrin excretion cannot be excluded. Table 1 gives the results of porphyrin determinations in 109 urine samples collected from persons without a history of parasitic disease, from persons infected with bilharzia, from people infected with bilharzia and under treatment with tartar emetic, and from persons with a history of bilharzia

TABLE 1
EFFECT OF TARTAR EMETIC ON THE COPROPORPHYRIN LEVELS IN URINE

	No.	Mean Concentration of Urinary Coproporphyrin (μg./l.)
I Persons without a history of parasitic disease	10	130.5
II Persons infected with bilharzia without treatment	39	146.5
III Persons infected with bilharzia under treatment with tartar emetic	40	329.4
IV Persons with a history of bilharzia but cured	20	137.3

but cured. The mean coproporphyrin concentrations of groups I, II, and IV do not differ significantly (t test). However, the mean coproporphyrin concentration of group III (patients under treatment with tartar emetic) is significantly higher than the mean urinary coproporphyrin concentration of all other groups (groups I, II, IV) tested by the t test ($P < 0.01$). These results call for a cautious interpretation of the results of determinations of coproporphyrin in the urine used as a test of lead absorption in persons under treatment with tartar emetic.

Conclusions

The drugs used in the treatment of parasitic disease are not likely to interfere directly with the coproporphyrin test for lead absorption, but the possibility of a higher urinary coproporphyrin excretion as the consequence of treatment with tartar emetic cannot be excluded.

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