Iron deficiency anaemia and drug metabolism

From animal work it is known that the levels of a number of haemoproteins in addition to haemoglobin are reduced in iron deficiency e.g. cytochrome C, cytochrome oxidase, aconitase, succinic hydrogenases and myoglobin (Sagone & Balcerzac, 1970). Iron deficiency causes an increase in the rate of microsomal drug metabolism. Catz, Juchau & Jaffe (1970) using mouse liver microsomal preparations found an increase in the rate of metabolism of hexobarbitone and aminopyrine and in cytochrome b_5 content, but no change in the concentration of the haemoprotein cytochrome P_{450}, an essential component of the liver microsome system for metabolizing drugs. Becking (1972) found an increase in rate of aminopyrine and aniline metabolism in the rat both *in vitro* and *in vivo*, but again no change in cytochrome P_{450} concentration.

The metabolism of many drugs takes place in liver microsomes and we examined the possibility that severe iron deficiency anaemia affects the rate of drug metabolism in man.

Seven patients (3 females and 4 males; mean age 44 years) who satisfied the following criteria were included in the study: 1, hypochromic, microcytic anaemia; 2, haemoglobin less than 70%; 3, serum iron less than 50 μg per 100 ml; 4, saturation of iron binding capacity less than 16%; 5, normal vitamin B_{12} and folate levels; 6, appropriate haematological response to iron therapy.

All patients had a potentially remedial cause for iron deficiency viz, three patients had peptic ulcers, one had menorrhagia, one patient had aspirin-induced anaemia and in the remaining two the cause was related to deficient diet.

Plasma antipyrine half-life was used as the index of drug-metabolizing capacity. The procedure followed was as previously described (O'Malley, Crooks & others, 1971), half-life estimations being carried out before iron therapy was instituted and again 1 and 3 months later.

Table 1 shows the mean values for the haematological parameters before and during treatment and the corresponding mean plasma antipyrine half-life. While there is a steady improvement in iron and haemoglobin status the plasma half-life of the drug remains unaltered, there being no significant difference between the 3 means (Students *t*-test, $P > 0{\cdot}05$). Similarly, there is no significant difference between the mean half-life values and our overall control value for 61 control subjects (mean $\pm$ s.d., 12·0 $\pm$ 2·5 h, O'Malley & others, 1971).

It would seem therefore that the effect of iron deficiency is not to reduce levels of cytochromes involved in drug metabolism. As Becking (1972) suggests, iron deficiency in the rat either increases the rate of binding of drug to cytochrome P_{450} or increases the rate of reduction of the P_{450}-substrate complex.

While this preliminary study, in seven patients, shows a slight tendency for the test drug half-life to lengthen with treatment it seems unlikely that in the clinical situation one gets the dramatic alterations in drug-metabolizing capacity that occur in experi-

Table 1. *Effect of iron therapy on haematological parameters and plasma antipyrine half-life.*

Anaemic patients	Antipyrine half-life (h)	Haemoglobin %	Serum iron (μg per 100 ml)	% Saturation iron-binding capacity
Before treatment	11·3 $\pm$ 4·2	52·0 $\pm$ 1·08	24·4 $\pm$ 13·7	5·9 $\pm$ 3·3
1 month iron therapy	13·2 $\pm$ 4·1	82·6 $\pm$ 10·7	64·3 $\pm$ 36·2	17·5 $\pm$ 10·0
3 months iron therapy	12·3 $\pm$ 4·0	97·4 $\pm$ 10·8	91·6 $\pm$ 31·9	27·7 $\pm$ 9·8

Results are means $\pm$ s.d. for 7 patients.

mental iron deficiency anaemia in animals. Alterations in sensitivity to drug effect are consequently unlikely in anaemic patients. Presumably in anaemia the amount of liver cytochrome P_{450} is unchanged or at least not altered sufficiently to affect the overall rate of drug metabolism.

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REFERENCES

BECKING, G. C. (1972). *Biochem. Pharmac.*, **21**, 1585–1593.
CATZ, C. S., JUCHAU, M. R. & JAFFE, S. J. (1970). *J. Pharmac. exp. Ther.*, **174**, 197–205.
O'MALLEY, K., CROOKS, J., DUKE, E. & STEVENSON, I. H. (1971). *Br. med. J.*, **3**, 607–609.
SAGONE, A. L. & BALCERZAC, S. P. (1970). *Am. J. med. Sci.*, **259**, 350–357.

Iminostilbene—a metabolite of carbamazepine isolated from rat urine

The metabolites of the anti-epileptic drug carbamazepine (I) that have been found so far in the urine of man and rat are carbamazepine-10, 11-epoxide (II) (Frigerio, Fanelli & others, 1972; Frigerio, Biandrate, & others, 1972) and 10,11-dihydro-10, 11-dihydroxy-5*H*-dibenz-[b,f]azepine-5-carboxamide (III) (Goenechea & Hecke-Seibicke, 1972; Baker, Csetenyi & others, 1972), the latter appearing also as its glucuronide. We now report the isolation of another metabolite, iminostilbene (IV) from the urine of rats.

Two male Sprague Dawley rats (200g) were injected intraperitoneally with a propylene glycol solution (0·5 ml) of 10,11-[$^{14}C_2$]carbamazepine diluted with cold carrier. The dose given was 10 mg kg^{-1} with a specific activity of 0·56 μCi mg^{-1}. 24 and 48 h later, the urine was collected, pooled, adjusted to pH 4, and extracted successively with 1,2-dichloroethane, ethyl acetate, and after incubation with Ketodase at pH 4·5, again extracted with ethyl acetate. Each of the extracts was evaporated under nitrogen at 60° and investigated by thin-layer chromatography (Woelm precoated plates, F256/366) in the solvent system benzene–ethanol–diethylamine (8:1:1). A non-polar radioactive peak was found at R_F 0·70 in both ethyl acetate extracts, but not the 1,2-dichloroethane extract, corresponding to the R_F of authentic iminostilbene (IV). The peak corresponded to about 2% of the excreted activity. The material was scraped from the plate, eluted with ethyl acetate, and investigated by direct injection mass spectrometry at a probe temperature of 70°. A mass spectrum with an intense molecular ion at *m/e* 193 and little further fragmentation was obtained, identical to the spectrum of authentic iminostilbene.

The iminostilbene was obtained in greatest quantity after incubation with Ketodase and hence is present in urine as the glucuronide. Glucuronides of amines are much more susceptible to acid hydrolysis than hydroxyl glucuronides, and the finding of iminostilbene in the ethyl acetate extract before Ketodase incubation could be accounted for by hydrolysis due to acidification of the urine before extraction. As a proof that iminostilbene did not arise through acid hydrolysis of carbamazepine, labelled 10,11-[$^{14}C_2$]carbamazepine was added to urine, incubated with Ketodase overnight at 37°, and subjected to the same extraction procedure as used before.