(Figure 1). The basal (0.9% NaCl) holoenzyme was less than half the total activity. The induction of TP by cortisol (and stressful agents that release corticosteroids) involves increased apo-TP synthesis (Schimke, 1969). Here, the increase in the total TP activity is matched by a proportionate rise in that of the holoenzyme. A relatively larger rise in the holoenzyme is produced by tryptophan, which acts mainly by activating pre-existing apoenzyme. 5-Aminolaevulinate, acting via haem (Badawy & Evans, 1973), causes a strong saturation of apo-TP; the majority of activity being as holoenzyme.

Fe-deficient rats lost weight. The basal holoenzyme and total enzyme activities (after 0.9% NaCl, 'B' groups in Figure 1) rose in Fe deficiency by 93-110% ($P = 0.02$-0.001). Similar rises have been reported (Badawy & Evans, 1973) in starved rats which also exhibited weight loss. The stress condition of Fe-deficient rats, and the qualitative resemblance of the rise in their TP activities to that by cortisol suggest that corticosteroids may be involved in the effect of Fe deficiency. Cortisol, tryptophan and 5-amino-laevulinate were equally effective in increasing TP activities in Fe-deficient ('B' groups) and control ('A' groups) rats. This suggests that TP induction by hormones (cortisol), and its activation by substrate (tryptophan) or cofactor (haem) are not impaired in Fe deficiency.

The present and other results (Symes, Missala & Sourkes, 1971; Callender, Grahame-Smith, Woods & Youdim, 1974; Bailey-Wood, Blayney, Muir & Jacobs, 1975) suggest that enzymes requiring haem- or non-haem-Fe may be increased, unaltered or decreased in Fe deficiency. The activities of liver TP, brain tryptophan hydroxylase and monoamine oxidase(s) may all control the level of brain 5-HT in Fe-deficient rats. Work on these aspects is planned.

References

BADAWY, A.A.-B. & EVANS, M. (1973). The effects of chemical porphyrogens and drugs on the activity of rat liver tryptophan pyrrolase. *Biochem. J.*, **136**, 885-892.

BADAWY, A.A.-B. & EVANS, M. (1974). Alcohol and tryptophan metabolism–a review. *J. Alcohol.*, **9**, 97-116.

BAILEY-WOOD, R., BLAYNEY, L.M., MUIR, J.R. & JACOBS, A. (1975). The effects of iron deficiency on rat liver enzymes. *Br. J. exp. Path.*, (in press).

CALLENDER, S., GRAHAME-SMITH, D.G., WOODS, H.F. & YOUDIM, M.B.H. (1974). Reduction of platelet monoamine oxidase activity in iron deficiency anaemia. *Br. J. Pharmac.*, **52**, 447P-448P.

CURZON, G. (1969). Tryptophan pyrrolase–a biochemical factor in depressive illness. *Br. J. Psychiat.*, **115**, 1367-1374.

McCALL, M.G., NEWMAN, G.E., O'BRIEN, J.R.P., VALBERG, L.S. & WITTS, L.J. (1962). Studies on iron metabolism–the experimental production of iron deficiency in the growing rat. *Br. J. Nutr.*, **16**, 297-304.

SCHIMKE,R.T. (1969). On the role of synthesis and degradation in regulation of enzyme levels in mammalian tissues. *Curr. Top. cell. Regul.*, **1**, 77-124.

SYMES, A.L., MISSALA, K. & SOURKES, T.L. (1971). Iron and riboflavin-dependent metabolism of a monoamine in the rat *in vivo*. *Science*, **174**, 153-155.

The measurement of serum morphine levels by radioimmunoassay following oral administration of diamorphine or morphine

G.W. AHERNE, V. MARKS, B.A. MORRIS, EVELYN M. PIALL, J.D. ROBINSON & R.G. TWYCROSS*

Division of Clinical Biochemistry, Department of Biochemistry, University of Surrey, Guildford, and St. Christopher's Hospice, Lawrie Park Road, London SE26 6DZ

The development of radioimmunoassay for morphine and related substances has resulted in greater opportunities for studying such compounds (Spector & Parker, 1970; Catlin, Cleeland & Grunberg, 1973; Morris, Robinson, Piall, Aherne & Marks, 1974). We report a method currently in use at the Department of Biochemistry, University of Surrey, and present preliminary data relating to the measurement of serum morphine in patients with advanced malignant disease receiving either diamorphine or morphine by mouth.

Antiserum was raised in a goat to a conjugate of 6-succinylmorphine-bovine serum albumin. Because the antiserum cross reacts almost equally with diamorphine, monoacetylmorphine, morphine and codeine, results are expressed as 'morphine equivalents'. Tritiated dihydromorphine was used as the radioactive label in the assay which has been described elsewhere (Aherne, Piall, Robinson, Morris & Marks, 1974). There is no cross reactivity for normorphine and only some 10% for morphine-3-glucuronide. Other drugs being taken by the patients did not cross react.

Quantities as small as 300 pg/ml can be measured by this method.

Twenty patients received diamorphine hydrochloride and twenty-four morphine sulphate, administered orally every 4 h in an elixir. The dose of diamorphine hydrochloride ranged from 5-60 mg (median 10 mg) and the dose of morphine sulphate from 3.75-90 mg (median 19 mg). The two groups were comparable in terms of sex, age, primary site and survival. Although most blood samples were obtained before or shortly after the 9 a.m. drug round, five specimens were obtained more than 40 min later. At the time of venepuncture all the patients had received an opiate regularly for more than a week.

The serum concentrations showed a significant correlation with the dose administered. Linear correlation coefficients for the two groups were: oral diamorphine, $r = +0.91$ ($p < 0.001$), oral morphine, $r = +0.73$ ($p < 0.001$) and for both groups, $r = +0.79$ ($p < 0.001$). There was no apparent difference between the two groups in the serum concentrations measured at comparable dose levels.

Our results demonstrate that it is possible to measure unconjugated morphine in serum from patients who have been receiving either diamorphine or morphine by mouth regularly for a number of days. It is detectable both at the beginning and the end of the 4 h interval between administrations. The positive correlation between dose and serum concentration supports recently published data showing a similar association between the dose administered and urinary recovery (Twycross, Fry & Wills, 1974). Further experiments to determine rates of absorption and elimination for the two drugs are planned in both volunteers and patients.

References

AHERNE, G.W., PIALL, E.M., ROBINSON, J.D., MORRIS, B.A. & MARKS, V. (1974). Two applications of a radioimmunoassay for morphine. *Radioimmunoassay and Related Techniques in Clinical Biochemistry.* London: Heydon (in press).

CATLIN, D., CLEELAND, R. & GRUNBERG, E. (1973). A sensitive, rapid radioimmunoassay for morphine and immunologically related substances in urine and serum. *Clin. Chem.*, **19**, 216-220.

MORRIS, B.A., ROBINSON, J.D., PIALL, E., AHERNE, G.W. & MARKS, V. (1974). The development of a radioimmunoassay for morphine having minimal cross-reactivity with codeine. *J. Endocr.*, **64**, 6-7P.

SPECTOR, S. & PARKER, C.W. (1970). Morphine: radioimmunoassay. *Science*, **168**, 1347-1348.

TWYCROSS, R.G., FRY, D.E. & WILLS, P.D. (1974). The alimentary absorption of diamorphine and morphine in man as indicated by urinary excretion studies. *Br. J. clin. Pharmac.*, **1**, 491-494.

A microsomal oxidase system in rat intestinal mucosa

S.H. CURRY & G.P. MOULD*

Department of Pharmacology and Therapeutics, The London Hospital Medical College, Turner Street, London E1 2AD

Orally administered drugs may be metabolized before reaching the general circulation. For example, L-dopa is decarboxylated in the human gastric tissue (Rivera-Calimlin, Dujovne, Morgan, Bianchine & Lasagna, 1971); clorazepate is converted to N-desmethyldiazepam, also in the human stomach (Boehringer Ingelheim Ltd., personal communication); orally administered isoprenaline is conjugated in the gut wall during absorption (Dollery, Davies & Connolly, 1971) and propranolol has been suggested to be extensively metabolized during the first passage through the liver (Shand, Nuckolls & Oates, 1970).

In an earlier report we showed that chlorpromazine is destroyed in rat intestinal tissue *in vitro* and *in vivo* (Curry, D'Mello & Mould, 1971); this reaction is believed to occur in man (Sakalis, Curry, Mould & Lader, 1972). It is possible that a mixed-function oxidase system similar to that in liver may exist in the rat intestine, as Takesue & Sato (1968) have already demonstrated the existence of enzymes associated with such a system in the microsomal fraction isolated from the mucosa of another species, rabbit. In contrast to this, Chhabra, Pohl & Fouts (1974) were unable to detect one of the enzymes concerned, cytochrome P-450, in the mucosa of rat small intestine. We would like now to report on the separation and partial evaluation of a microsomal fraction from the mucosal cells of rat small intestine, containing cytochrome P-450.

The mucosa was scraped off 10 cm lengths of proximal rat small intestine and homogenized in 0.05 M phosphate buffer pH 7.4 containing 1.15%