apparent conjugation defect we observe in these patients. Other possible explanations for our findings are a deficit in the absorption or transport of tyramine (Bonham Carter *et al.*, 1978b) or poor absorption of dietary cysteine and other sulphur containing amino acids. We are planning to investigate some of these possibilities in the near future.

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FATTY ACIDS MODULATION OF MEAL-INDUCED VARIATIONS IN DIAZEPAM FREE FRACTION

Diazepam is highly bound to human serum albumin. Only $1.33 \pm 0.21\%$ (mean $\pm$ s.d.) of diazepam in plasma is in the unbound form over the range of concentrations normally encountered in therapy (Abel, Sellers, Naranjo, Shaw, Kadar & Romach, 1979). Since drug elimination is dependent on free fraction (Klotz, Antonin & Bieck, 1976) changes in diazepam free fraction may, therefore, cause important variations in disposition and effect. Meals induce a significant decrease in diazepam free fraction associated with reciprocal changes in total plasma

diazepam concentrations (Naranjo, Sellers, Giles & Abel, 1980). Meals decrease free fatty acids (FFA) (Barter, Carroll & Nestel, 1971; Schlierf & Dorow, 1973) and FFA modify the binding of many drugs (Birkett, Myer & Hagedorn, 1979). Therefore, FFA could be the modulators of meal-induced variations in diazepam free fraction. We studied the effect of standard breakfast on diazepam free fraction and the correlation of these changes with concomitant variations in FFA.

Eight healthy subjects (7 males, 1 female, aged

Figure 1 Decrease in total free fatty acids (a) and concomitant decrease in diazepam free fraction (b), in eight healthy volunteers after a standard breakfast.

21–42 years) fasted 14 h overnight. Blood samples (20 ml) were taken in glass syringes by venipuncture from an antecubital vein. Blood was collected in non-heparinized tubes at 0800 and 2 h after a standard breakfast. The breakfast provided at total of 661.4 kcal (carbohydrates 83 g, fats 25 g and proteins 26.1 g). No subject received tea, coffee, tobacco or alcohol 14 h prior to and during the experiment. The tubes were covered with Teflon tape, stored in a refrigerator and the blood was allowed to coagulate for 30 min. Serum was separated by centrifugation at 5000 rev/min for 10 min in a refrigerated Sorvall centrifuge at 0°C. Diazepam free fraction was determined in triplicate by equilibrium dialysis (Abel *et al.*, 1979). [^{14}C]-diazepam (Amersham Corp; 97% radiochemically pure, specific activity 200 $\mu\text{Ci}/\text{mg}$ was added mixed with cold drug, to 0.5 ml aliquots of serum as a tracer to achieve a concentration of 600 ng/ml, and was dialyzed against a physiological saline phosphate buffer (pH 7.4), at 37°C for 6 h to reach equilibrium. A 2% coefficient of variation was obtained for diazepam free fraction determinations. The concentration of total free fatty acids in serum was determined by the method of Trout, Estes & Friedberg (1960). The coefficient of variation of the duplicate FFA determinations was 5%. The statistical analysis of data was performed using two-tailed paired *t*-test and standard linear regression analysis. Figure 1 shows that FFA (paired *t*-test, $t=4.99$, $P<0.01$) and diazepam free fraction (paired *t*-test, $t=4.13$, $P<0.01$) decreased after a meal. The mean $\pm$ s.d. percent decrease in FFA was $45.40 \pm 17.18\%$ and in diazepam free fraction

$18.03 \pm 10.68\%$. Figure 2 shows that variations in FFA and diazepam free fraction were highly correlated ($r=0.87$, $P<0.001$).

This study shows that meals induced a decrease in FFA and that these variations were correlated with changes in diazepam free fraction. Recently,

Figure 2 Correlation of variations in total free fatty acids (mEq/l) and diazepam free fractions (%) in eight healthy volunteers. $y=0.78+0.21x$, $r=0.87$, $P<0.001$.

variations in diazepam free fraction associated with reciprocal changes in total plasma diazepam concentrations were described (Naranjo *et al.*, 1980). However variations in FFA were not determined and the full mechanism of the fluctuations was not elucidated. Diazepam is highly bound to human

serum albumin and this study suggests that endogenous displacers such as FFA which vary with food ingestion and fasting could modulate variations in diazepam free fraction. In fact, several investigators have showed that FFA decrease diazepam binding to albumin *in vitro* (Sjödén, 1977; Tsutsumi, Inaba, Mahon & Kalow, 1975; Sellers, Naranjo, Abel, Khouw, Sandor & Alexander, 1980) and *in vivo* (Colburn & Gibaldi, 1978) even at FFA/albumin molar ratio as low as 1:1 (Sjödén, 1977). FFA levels in humans are highest during night and early morning (Barter *et al.*, 1971; Schlierf & Dorow, 1973) when total diazepam levels are lowest and diazepam free fraction highest (Naranjo *et al.*, 1980). Therefore, it is probable that when FFA decrease after a meal the binding of diazepam increases (i.e. free fraction decrease) causing diazepam in the tissues to enter the intravascular compartment and consequently total plasma diazepam concentrations raise. This study shows that FFA account for 75% of the variation in diazepam free fraction and probably are the major determinant of meal-induced changes in diazepam free fraction and total plasma diazepam concentrations. The important role of FFA is also supported by the fact that they modulate changes in diazepam free fraction in other situations, such as after heparin administration (Naranjo, Sellers, Khouw, Alexander, Fan & Shaw, 1980).

Previous investigations failed to detect meal-related variations in diazepam free fraction (Klotz, Antonin & Bieck, 1977; Kortilla & Kangas, 1977). The discrepancies in the results may be related to the sensitivity and precision of the free fraction determinations, since measured free fractions were unusually high in those studies. In addition, the results obtained are also highly dependent on the time-course of FFA and diazepam free fractions changes. Previous studies with frequent sampling showed that the maximum effect occurs 2 h after breakfast (Naranjo *et al.*, 1980), which is the sampling time selected in this study. Our findings and other investigations (Sellers *et al.*, 1980), suggest that FFA are important modulators of diazepam binding.

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