Relative standard deviations varied from 3.8 to 26.5% without improvement by inclusion of an internal standard. The calculated sensitivity was 6.4 µg/ml (azlocillin), 4.2 µg/ml (mezlocillin) and 3 µg/ml (Bay k 4999) using 1 µl injection volume (Table 3). Since up to 10 µl aliquots could be injected the sensitivity reached 0.7 µg/ml (azlocillin), 0.5 µg/ml (mezlocillin) and 0.3 µg/ml (Bay k 4999). The accuracy of the assay was determined by analysing spiked plasma unknowns under 'blind' conditions. The results (Table 4) gave good agreement between spiked plasma concentration and concentration found.

Plasma levels of the antibiotics were determined during and after cessation of an intravenous infusion of the drug in a therapeutic dose range of 10 mg/min to human volunteers. The plasma levels reached a steady state within 3 h of infusion and could be detected up to 3 h after termination of the infusion, i.e. 4 to 6 half-lives of the drug. The results of the study will be reported in detail in a subsequent publication.

The method can therefore be used for specific measurement of plasma levels of the ureidopenicillins in pharmacokinetic studies and to monitor plasma levels for therapeutic advice.

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INTERACTION BETWEEN SALICYLATE AND TWO LOOP DIURETICS

In cardiology and nephrology, the concomitant use of frusemide and anti-inflammatory drugs is frequent. It was demonstrated that indomethacin partially inhibits the diuretic effect of frusemide (Patak, Mookerjee, Bentzel, Hysert, Baby & Lee, 1975). Salicylate has been shown to be a prostaglandin synthesis inhibitor *in vitro* (Damas & Mousty, 1978) and in man (Hamberg, 1972; Samuelsson, 1974; Smith & Willis, 1971; Vane, 1976). Many studies have shown a direct effect of frusemide on renal prostaglandin metabolism (Bailie, Crosslan & Hook, 1976; Williamson, Bourland & Marchand, 1975). Piretanide, a new high ceiling diuretic, seems to have a similar action to that of frusemide (Merkel, Bormann, Mania, Muschaweck & Hropot, 1976; Lawrence, Ansari & Elliot, 1978; Roberts, Homeida, Roberts & Bogie, 1978; Valette, Duhaze & Apoil, 1979). This study was undertaken to investigate a possible interaction between diuretics

and lysin acetylsalicylate, with particular reference to water and sodium excretion, and venous compliance.

Four healthy subjects, three males and one female (not taking oral contraceptives) aged from 25 to 28 years, familiar with plethysmographic experiments, volunteered after the study had been explained to them.

The diet had a normal salt intake (10 g NaCl/day) and a fluid intake of 1000 to 1500 ml/day.

Each drug was given intravenously: lysin acetylsalicylate: 1 g (Egic Laboratories); frusemide 20 mg; piretanide 12 mg. An i.v. continuous perfusion of saline (30 ml into fore-arm) carried the bolus injection.

Urinary electrolytes (sodium, potassium) were evaluated by a spectrophotometric method (240-Mark 2 Atomic absorption spectrophotometer Corning EEL Scientific Instruments).

The results for urinary values are shown in Table 1.

Table 1 Urine volumes, sodium and potassium excretion, and venous compliance (C) (in % of control values) after intravenous administration of lysin acetylsalicylate (LAS), frusemide (F) and piretanide (P) alone, and in combination [mean values, $n=4$]

Drugs	ΔC (%)	Diuresis (ml)	Na^+ (mEq)	K^+ (mEq)
LAS	-2	58	10	4
F	+47*	NS $\begin{bmatrix} 650 \\ 445 \\ 611 \end{bmatrix}$ *	NS $\begin{bmatrix} 79 \\ 50 \\ 70 \end{bmatrix}$ *	NS $\begin{bmatrix} 11 \\ 24 \\ 13 \end{bmatrix}$ *
LAS-F	+1			
F-LAS	+47*			
P	+28*	NS $\begin{bmatrix} 550 \\ 347 \\ 587 \end{bmatrix}$ *	NS $\begin{bmatrix} 71 \\ 32 \\ 63 \end{bmatrix}$ *	NS $\begin{bmatrix} 12 \\ 8 \\ 10 \end{bmatrix}$ *
LAS-P	0			
P-LAS	+23*			

NS = non significant

* $P=0.05$

There was a significant difference between the 7 sequences.

Lysin acetylsalicylate, when given before the diuretics, significantly decreased the diuresis and sodium excretion, and appeared to increase potassium excretion, when given before frusemide but not before piretanide.

Lawrence *et al.* (1978) and Roberts *et al.* (1978) have shown, in healthy volunteers, that piretanide has a diuretic effect similar to that of frusemide; one of our previous studies (Valette *et al.*, 1979) demonstrated that frusemide and piretanide significantly increase venous compliance. Lysin acetylsalicylate inhibits diuresis and the effect on venous compliance when given before both diuretics. Berg (1977) found impaired frusemide-induced natriuresis in dogs pretreated with 70 to 90 mg/kg of acetylsalicylic acid, but he failed to show a similar interaction in healthy volunteers given 2 g orally of acetylsalicylic acid. In our study, plasma concentration of lysin acetylsalicylate could be extrapolated from Warembourg, Delbeque & Fruchart's study (1975) as about 200 $\mu\text{g/ml}$. In Berg's (1977) experiment, it was measured and found to be 130 $\mu\text{g/ml}$. This interaction might be due to two different mechanisms; a) pharmacokinetics related to the transport and renal fixation of diuretics, or b) prostaglandin synthesis inhibition. The initial high concentration of lysin acetylsalicylate could saturate the protein binding sites and/or renal receptors. Biamino, Wessel & Schuren, (1974) in healthy volunteers, and Dikshit, Vyden & Prahash (1971) in patients with congestive heart failure, have demonstrated the venodilator effect of frusemide. Biamino *et al.* (1974) also suggested a direct action on membrane sodium transport. Bourland, Day & Williamson (1977) have shown that the kidney is necessary for this venous dilatation which could be impaired by indomethacin. Our experiment also

suggest that prostaglandins, renal or extra renal, may be involved in some way as mediators of this early non-diuretic action of frusemide and piretanide.

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α -ADRENOCEPTOR MEDIATED INHIBITION OF HUMAN FAT CELL ADENYLATE CYCLASE

In human adipose tissue α -adrenergic receptor stimulation inhibits the formation of cAMP and lipolysis whereas β -adrenergic receptor agonists stimulate the synthesis of cAMP and lipolysis (Burns, Langley & Robison, 1972; Rosenquist, 1972). The relationship between α - and β -adrenergic responsiveness of human adipose tissue has been shown to be profoundly influenced by dietary conditions (Arner, Engfeldt & Ostman, 1979) or disease such as hypothyroidism (Rosenquist, 1972) or untreated juvenile onset diabetes mellitus (Arner *et al.*, 1979). β -adrenoceptor sites appear to be linked to the membrane-bound adenylate cyclase in most tissues including human fat tissue (Kather & Geiger 1977; Kather & Simon, 1977). As opposed to β -adrenoceptors, α -adrenergic excitation is probably mediated via distinct effector systems depending on the cellular origin of the preparation (Kunos, 1978).

The apparent effect of the naturally occurring catecholamines adrenaline and noradrenaline on the human fat cell adenylate cyclase is a stimulatory one displaying the characteristics of β -adrenoceptor mediated excitation (Kather & Simon, 1977). When added alone these agonists caused a dose-dependent increase of enzymic activity with a maximum of 50%–300% at 5×10^{-4} mol/l (Kather & Simon, 1977). Upon complete β -adrenergic receptor blockade using 0.05 mmol/l propranolol, however, an inhibitory

component of the naturally occurring mixed agonists adrenaline and noradrenaline became apparent which was not shared by the almost pure β -adrenergic receptor agonist isoprenaline (Figure 1). Under these conditions the mixed agonist noradrenaline caused a dose-dependent inhibition of basal enzymic activity with a maximum of 30%–40% at 10^{-4} mol/l. The noradrenaline-induced inhibition could be reversed by simultaneous α -adrenergic receptor blockade with 10^{-5} mol/l phentolamine. These results show that the inhibitory α -adrenergic component, like β -adrenergic stimulation, is mediated via the membrane-bound adenylate cyclase in human adipose tissue. The results of the present study thus confirm the concept of antagonistic α - and β -adrenergic receptor sites coexisting as regulatory subunits of the human fat cell adenylate cyclase as suggested by Rosenquist (1972) and by Burns *et al.* (1972).

The mutual interrelationship of α - and β -adrenergic responsiveness is poorly understood in biochemical terms. It is of special interest, however, to clarify the mechanisms and interrelationship of α - β -adrenergic responsiveness. This aspect involves questions as to the modulation of the response to drugs by diet, age or disease and is pertinent to the actual discussion about the use of β -adrenoceptor blocking agents in diabetics. Studies utilizing the human fat cell adenylate cyclase, therefore, may be