Fig. 2 Standard curve for the g.l.c. estimation of theophylline. The reproducibility is indicated by the s.e. means.

Standard solutions were prepared by injecting known quantities of a stock solution of theophylline into human plasma samples. After extraction in the usual manner and developing the chromatograms, a standard curve of ratio of the recorded area under the curve of theophylline to internal standard against concentrations of theophylline in serum was a straight line (Figure 2). The reproducibility of the procedure is indicated by the standard errors presented in that figure.

The method is technically simple, suitable for clinical use and sufficiently sensitive ($1 \mu\text{g/ml}$). In a number of patients the theophylline plasma levels were determined by the described method and good results were obtained. Several drugs present at the time of blood sampling did not interfere with the determination of theophylline. Caffeine, almost constantly present in human

plasma, was well separated from theophylline at all concentration levels.

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INTERACTIONS OF AMITRIPTYLINE WITH GUANETHIDINE AND THYMOXAMINE IN THE HUMAN IRIS

It is established that the antihypertensive effect of guanethidine, an adrenergic neurone blocking agent, is reversed by tricyclic antidepressant drugs (Mitchell, Cavanaugh, Arias & Oates, 1967).

Guanethidine produces miosis in the normal

human eye when instilled locally in the conjunctival sac (Sneddon & Turner, 1969). To establish if this miotic effect of guanethidine is also reversed by tricyclic drugs we carried out investigations on depressed patients receiving tricyclic antidepressant drugs under inpatient

supervision. Seven patients were studied who had received amitriptyline (150 mg daily) for at least 14 days. Their pupil diameters were measured by a photographic method as described by Sneddon & Turner (1967) before and at 24 h after instillation of guanethidine (5% eye drops) into the conjunctival sac of one eye. The responses were compared with those of five normal control subjects, matched for eye colour. There was no significant difference in the initial mean pupil diameters between the control and amitriptyline treated group ($P > 0.50$). In the patients receiving amitriptyline, guanethidine produced an insignificant miosis of 3.5% (± 2.4 , s.e. mean) while controls showed a highly significant miosis of 20.0% (± 4.1 , s.e. mean). The control miosis is similar to that previously reported by Sneddon & Turner (1969). The difference between the responses of the control and amitriptyline-treated patients was highly significant ($P < 0.01$). It appears, therefore, that the miotic effect of guanethidine was reversed by tricyclic drugs. However, this could have been due to their anticholinergic effect rather than to uptake inhibition of guanethidine.

In order to differentiate between the above two mechanisms we further studied the miotic effect of thymoxamine, a selective α -adrenoceptor blocking drug. Eight patients receiving amitriptyline in the same dose as before were studied under similar conditions. Pupil diameters were measured before and at 30 min and 60 min after instillation of thymoxamine (0.5%) into the conjunctival sac of one eye. These responses were compared with those of five control subjects matched for eye colour. There was no significant difference in the initial mean pupil diameters between the control and amitriptyline group ($P > 0.10$). In the patients receiving amitriptyline, thymoxamine produced a miosis of 8.3% (± 3.67 , s.e. mean) at 30 min and 12.7% (± 4.1 , s.e. mean) at 60 minutes. The control miosis was similar to that previously reported by Sneddon & Turner (1968), 33.8% (± 4.7 , s.e. mean) at

30 min and 27.3% (± 2.55 , s.e. mean) at 60 minutes. The difference between the responses of the controls and amitriptyline treated patients was highly significant ($P < 0.002$ at 30 min and $P < 0.05$ at 60 minutes).

From these investigations we conclude that the reversal of the miotic effect of guanethidine is more likely to be due to the anticholinergic effect of the tricyclic drugs rather than due to inhibition of uptake of guanethidine, because the miotic effects of both the adrenergic neurone and the α -adrenoceptor blocking drug were inhibited.

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