antimuscarinic activity to gallamine was least affected by lowering of the temperature. The lower frequency of stimulation used at 22 °C was not responsible for variations in the dose-ratios as stimulating at either 1.8 or 3 Hz at 37 °C did not alter the effectiveness of homatropine or gallamine. Similarly, Reil[16] has recently reported that the affinity of benzetimide a competitive antimuscarinic drug is unaltered by varying the frequency of electrical stimulation of guinea-pig atria.

Some antimuscarinics also exhibit increased affinity in guinea-pig ileum as the temperature is lowered. The affinity of lachesine was increased 1.3fold with a decrease in temperature from 37.5 to 30.5 °C[17] and the affinity of atropine, hyoscine and hyoscine methiodide was increased 1.5 to 4fold with a reduction from 37 to 29 °C[18]. However, the affinity of atropine methiodide was unaffected by the temperature change and some compounds showed a decrease in affinity[18].

Belleau et al.[19] suggested that antagonists will produce a positive entropy change (+ ΔS) and agonists a negative change (− ΔS) on binding at the muscarinic receptor. In the atria the 3 antagonists produced + ΔS values. However, the change for gallamine is close to zero and the increase in affinity (− log K) with decrease in temperature was not large relative to the SE of the mean values. Furthermore, Barlow et al.[18] found negative as well as positive entropy changes for the binding of different antimuscarinics in guinea-pig ileum. It is of interest that Roufogalis et al.[20] found a linear Arrhenius plot for the interaction of gallamine with carbamylated acetylcholinesterase over the temperature range 10-30 °C and concluded that gallamine interacted with only 1 conformational state of the carbamylated enzyme. The results reported here for gallamine when plotted in the form a van't Hoff plot also produced a linear relationship.

In conclusion, the antimuscarinic characteristics of the 3 antagonists homatropine, gallamine and stercuronium are maintained over a wide temperature range. Also, no consistent difference was found for the effect of temperature changes on the affinity of the competitive antagonist homatropine and that of gallamine or stercuronium.

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Quantitation of nitroglycerin in human blood after administration by sustained release[1]

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Summary. Nitroglycerin was traced in the blood of 20 patients up to 4 h after oral administration of a sustained release preparation (Nitro-Mack Retard). The determination needs an extremely sensitive method using GLC-columns with 3% SE-30 (Packard) equipped with an electron capture detector.

The effectiveness of nitroglycerin retard preparations in angina pectoris has been clinically tested by means of subjective parameters, such as the patients' statements compared with placebos in double-blind cross-over trials, a far from reliable method. However, a recovery test of nitroglycerin, added to fresh blood, yields only 5–10% of the expected quantity[3]. This dilemma resulted in a controversy carried through the letter section of the American Journal of Cardiology in 1976. Needleman[4] claimed that orally administered nitrites and nitrates are rapidly and efficiently degraded by the liver, and therefore cannot exert a clinically useful effect since, essentially, none of the present compound arrives in the circulation. This seemed to be in good correlation with a statistical analysis covering the literature from 1952 to 1972, in which Stipe and Fink[5] found that fewer than 10% of patients with angina pectoris benefitted from the oral administration of organic nitrites. This view was contradicted by Krantz and Leake[6] and by Winsor and Berger[7]; the latter used orally administered sustained release nitroglycerin tablets and found that 47.2% of the patients had significantly fewer and less severe anginal attacks. Similar results were obtained by Jerie[8] with peroral isosorbide dinitrate.

The controversy can be only partly attacked by the determination of the 'escape' nitrates or nitrites detected in the blood within 1-4 h after administration[9,10]. Colorimetric methods cannot detect nitrate quantities below 1 µg/ml, nor do they enable us to distinguish between organic and inorganic nitrates. They are only sensitive enough for individual tablet analysis[11]. Recently it has been shown that nitrate esters can be separated on gas liquid chromatography (GLC) columns, an electron-capture detector being used for measuring the separated component, since it is much more sensitive and selective for nitrate esters than the flame-ionisation detector. It allows the detection of 0.1 ng nitrate ester[3].

Materials and methods. Our measuring method is based in principle on the Williams and Murray method[12] slightly modified for a quantitative micro-assay. 10 ml of heparinized blood is taken for a determination which is carried out in triplicates of 3 ml each. Immediately after blood withdrawal, 3 ml of 30% NaCl are added to the fresh blood sample and agitated to avoid enzymatic decomposition of nitroglycerin. The solution obtained is thoroughly shaken with 6 ml n-hexane, and the organic phase is separated after centrifuging. This extraction is repeated 3 times. After evaporation of the hexane, the residue is mixed with 0.1 ml of n-hexane and kept in a dried atmosphere. Then aliquots of 5 µl each of the concentrate are injected onto the GLC column. The gas chromatographic separation is carried out by using a 3% SE 30 gas chrome Q 100-200 mesh (Packard) on a spiral glass column 15 cm long and 6.5 mm in diameter (Packard Instrument Company). The column is heated in an oven at 150 °C and the column inlet at 200 °C. The electron capture detector which includes a tritium source (Packard) is heated to 175 °C. Nitrogen is used as carrier gas at a flow-rate of 80 cm/min. Under the above conditions, one can easily determine 0.05 ng (0.05×10^{-9}g) nitroglycerin (figure). Quantitative estimations using an internal standard were performed by measuring the height

Table 1. Nitroglycerin blood levels after oral administration of 7.5 mg Nitro Mack Retard in 10 patients

Time after administration (h)	Blood levels ± SEM (ng/ml)
½	0
1	0.2* ± 0.02
2	0.3* ± 0.03
3	0.6* ± 0.05
4	0.3* ± 0.03
5	0

* $p<0.05$ from 10 patients, each one undergoing 3 parallel assays.

Table 2. Nitroglycerin blood levels after oral administration of 10 mg Nitro Mack Retard in 10 patients

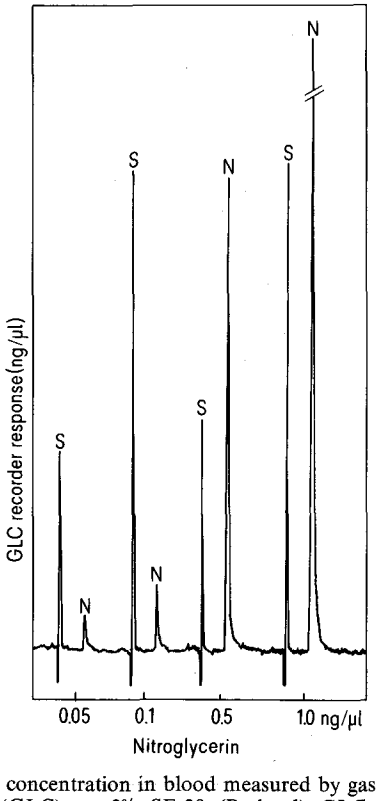
Time after administration (h)	Blood levels ± SEM (ng/ml)
½	0.1* ± 0.01
1	5.0* ± 0.2
2	5.0* ± 0.25
3	7.0* ± 0.3
4	10.0* ± 0.4
5	0

* $p<0.05$ from 10 patients, each one undergoing 3 parallel assays.

of the recorder responses, compared to adequate standard responses. Results of 3 parallel estimations and their SEM were evaluated statistically by Student's t-test.

Results. Oral administration of 2.5 mg or 5.0 mg (1 or 2 capsules of Nitro Mack Retard) of the sustained release form of nitroglycerin did not show any detectable levels of nitroglycerin. However, after oral administration of 7.5 mg or 10 mg (3-4 capsules) of the sustained release form of nitroglycerin the blood levels of nitroglycerin per ml (absorption curve) could be determined at hourly intervals in 20 patients suffering from coronary insufficiency. Average results are given in tables 1 and 2. They show that sustained nitroglycerin release can be detected in blood up to 4 h after peroral administration, then it disappears completely.

Discussion. Quantitative determinations of nitroglycerin by the GLC method using 1%, 5%, or 10% SE-30 were not very accurate due to the fact that electron capture detector responses are not linear (figure), but the extreme sensitivity of the present analytical method with 3% SE-30 allowed detection of very small quantities of nitroglycerin.



Nitroglycerin concentration in blood measured by gas liquid chromatography (GLC) on 3% SE-30 (Packard) GLC columns. S, Solvent (hexane), N, nitroglycerin.

The blood level values (tables 1 and 2) represent only 5-10% of the expected recovery due to the rapid decomposition of nitroglycerin in blood[3]. For the same reason, nitroglycerin recovery tests after addition to fresh blood showed only 5-10% recovery of the added quantity of nitroglycerin. Detailed metabolic studies of nitroglycerin in vivo showed that the molecule is rapidly denitrated and does not appear in significant quantities in the urine after 24 h[3].

The curves of our tests proved that nitroglycerin and no metabolite was measured in the blood of the patients. Thus, it seems that it is the direct action of the intact molecule of nitroglycerin and other nitrate esters which is responsible for the pharmacological effects. The oil-water-partition coefficient also plays a part in differentiating the pharmacological potency of a nitrate ester[4]. Hence, lipid-soluble nitrate esters, such as nitroglycerin, have a high pharmacological potency, due to their easy penetration through the cell membrane.

Sustained release nitroglycerin preparations have until 1972 been studied only in experimental animals because methods for detecting nitroglycerin in human blood were not sensitive enough to allow determination after administration of doses allowed in man. Such determinations carried out in rats showed recovery of sustained release nitroglycerin preparations for 5 h after administration[13]. The latter study is in good agreement with our results.

Recently the first study of plasma levels in man after buccal administration of nitroglycerin was published[14]. The method using gas chromatography has only 1/10 of the sensitivity of the method described here. Still it shows that nitroglycerin can be assayed in human blood.

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