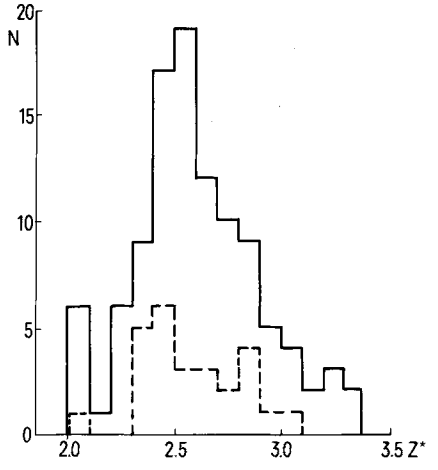
valence number than 3.20. On the other side, antimetabolites[8] are generally characterized by higher average quasi-valence numbers, close to or above the borderline value of 3.20, as can be seen from table 2.

The fact that 'Carcinogenic substances have also a paradoxical tumour-inhibitory action' (Sellei et al.[8], p. 197) according to the valence theory does not seem paradoxical. Alkylating cytostatics, which have average quasi-valence numbers below 3.20, should be treated as potential carcinogens, while the antimetabolic cytostatics generally should belong to the class of noncarcinogens. The action of alkylating cytostatics could be coupled with their potential carcinogenic activity, while the mechanism of the antimetabolic cytostatics might be of another type.



Number of investigated alkylating compounds vs. average quasi-valence number (full line - all investigated compounds, dashed line - compounds having good activity).

The average quasi-valence number criterion could be used for the selection of cytostatics of the above-mentioned 2 groups of substances. As can be seen from the figure, optimal antitumour action can be expected in the given group of substances only for alkylating cytostatics having some specific average quasi-valence numbers (2.3-2.9). Selection and 'design' of new alkylating cytostatics could, in this way, be guided by the choice of the proper average quasi-valence number.

We believe that the valence theory could be of benefit to the selection of alkylating compounds having good cytostatic activity, and that it might shed additional light on the problem of cytostatic action.

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Effect of variations in temperature on antimuscarinic activity in guinea-pig atria[1]

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Summary. The characteristics of the antimuscarinic activity of homatropine, gallamine and stercuronium in guinea-pig atria remained constant over the temperature range 22–37 °C in that a linear Arunlakshana-Schild plot was obtained with homatropine and nonlinear plots occurred with gallamine or stercuronium. A trend towards higher dose-ratios with reduction in temperature was only significant for gallamine.

Reduction of temperature causes marked changes in the interaction of atrial adrenoceptors with agonists and antagonists. There is an interconversion of β-adrenoceptors to the α-type[2,3], an increase in sensitivity to sympathomimetics[4,5] and partial agonists develop characteristics of full agonists[6]. The affinity of β-adrenoceptor antagonists in the atria may be decreased[3,7] or unaltered[4,5].

In contrast, the effect of temperature on caridia muscarinic receptors has been little investigated. Benfey[8] found that phenoxybenzamine was a less active antagonist of acetylcholine at 14 °C than at 24 °C in frog heart whereas atropine was equieffective at both temperatures. A decrease in effectiveness is also observed with phenoxybenzamine in guinea-pig atria on lowering the temperature from 31 to 14 °C[8] although the response to acetylcholine in this tissue is almost unchanged with a decrease from 32 to 18 °C[9].

Cardiac muscarinic receptors are selectively inhibited by gallamine[10,11] and the antimuscarinic action of gallamine can be differentiated from that produced by atropine in a number of ways[13]. For example, although gallamine produces parallel shifts of the concentration-response curve for the negative inotropic response to carbachol (CCh) in guinea-pig atria the degree of antagonism reaches a limiting value at high concentrations of gallamine resulting in an Arunlakshana-Schild (A-S) plot[12], which is nonlinear[13]. An allosteric mechanism was proposed to account for the antimuscarinic activity of gallamine.

An investigation of the effect of temperature on the interaction of gallamine and homatropine was undertaken to determine whether differences occur in the nature and extent of the blockade produced by the 2 antagonists with variations in temperature from 22 to 37 °C. Homatropine was chosen in preference to atropine because the former can be used over the same concentration range as that required to demonstrate the antimuscarinic activity of gallamine and the resulting nonlinear A-S plot.

Methods. Left atria of guinea-pigs in McEwen's solution[14] gassed with O_2/CO_2 (95:5) were stimulated electrically at 1.8 or 3 Hz with pulses of 2 msec duration and supramaximal voltage at 22, 25, 32 or 37 °C (±0.5 °C). At 22 °C atria were stimulated at 1.8 Hz as arrhythmias developed with stimulation at 3 Hz. Concentration-response lines to CCh were determined in the absence and presence of antagonists as described previously[13] except that the contact time for CCh was increased up to 8 min in some experiments at the lower temperatures to ensure full development of the response. Statistical comparisons were made using Student's t-test (2 tailed).

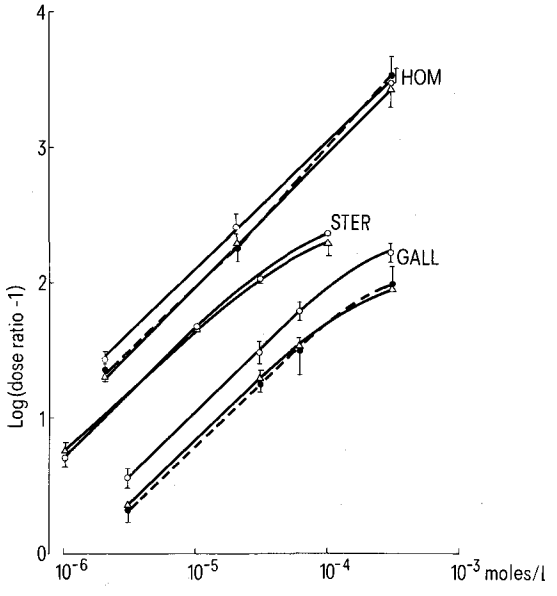
Dose ratios produced by antagonists against carbachol at various temperatures in electrically stimulated guinea-pig atria

Antagonist concentration (μmoles/l)	Temperature (°C)	Frequency (Hz)	Dose ratio[a]	−log (±SEM)	ΔH^d (kcal/mole)	ΔS^d (e.u.)	$\Delta G(22\,°C)^d$ (kcal/mole)
Homatropine (20)	22	1.8	255.3 (199.8-326.3) (7)	7.06±0.03	−1.26	+28.00	−9.53
	32	3	208.5 (166.6-260.8) (7)	7.01±0.04			
	37	1.8	214.3 (182.5-251.7) (11)	7.03±0.03			
	37	3	210.1 (161.5-273.9) (9)	7.02±0.06			
Gallamine (30)	22	1.8	32.2 (18.0-57.8) (4)	6.02±0.16 (6.10)	−1.99	+0.23	−8.70
	25	3	30.3 (2)	5.99			
	32	3	21.0 (13.9-31.7) (4)	5.82±0.12 (5.90)			
	37	1.8	17.5 (11.4-27.0) (4)[c]	5.74±0.13			
	37	3	17.8 (12.7-24.8) (4)[c]	5.75±0.09 (5.74)			
Stercuronium (10)	22	1.8	48.5 (42.8-55.2) (4)	6.71±0.02 (6.71)	−1.26	+27.02	−9.23
	32	3	47.9 (39.8-57.5) (12)	6.68±0.04 (6.68)			

[a]Geometric mean dose ratio (95% confidence limits), (n); [b]mean dissociation constant±SEM for homatropine, gallamine and stercuronium estimated from the relationship K=concentration of antagonist/(dose ratio−1). Figures in parenthesis for gallamine and stercuronium are graphical estimates of −logK from the figure; [c]significantly different from corresponding dose ratio obtained at 22°C ($p<0.05$); [d]ΔH and ΔS estimated from van't Hoff relationship $-\log K = -\Delta H/2.3\ RT + \Delta S/2.3\ R$, ΔG estimated from $\Delta G = \Delta H - T\Delta S$ (22°C).

Results. The sensitivity of the atria to CCh was significantly lower ($p < 0.05$) at 22°C than at either 32 or 37°C. The geometric mean EC_{50} values for CCh being at 22°C (1.8 Hz): 0.27 μmoles/l (95% confidence limits, 0.21-0.35, 15 experiments); 32°C (3 Hz): 0.13 (0.11-0.16, 28); 37°C (1.8 Hz): 0.09 (0.08-0.10, 15) and at 37°C (3 Hz): 0.19 (0.15-0.24, 17).

The effectiveness of homatropine (20 μmoles/l) was not significantly different ($p > 0.05$) at any of the 3 temperatures (table) nor was the A-S plot altered (figure). The slopes of the resulting regressions for homatropine (2-300 μmoles/l) at any temperature were not significantly different ($p > 0.05$) from a theoretical slope of 1.0 for a competitive antagonist. Gallamine (30 μmoles/l) produced a significantly greater mean dose ratio at 22°C than at 37°C ($p < 0.05$) (table). The degree of antagonism produced by the highest concentration of gallamine (300 μmoles/l) was always less than expected for a competitive antagonist and the slope of a linear regression fitted through the A-S plot obtained at any temperature was significantly less than 1.0 ($p < 0.01$) (figure). Stercuronium (1-100 μmoles/l) another compound with cardioselective antimuscarinic activity[15] also produced a nonlinear A-S plot at either 22 or 32°C ($p < 0.001$) (figure). No significant difference was found in the dose-ratios produced by stercuronium (10 μmoles/l) (table) at the 2 temperatures. Values of ΔG, ΔH and ΔS from a van't Hoff plot are also shown in the table.



The antimuscarinic activity of homatropine (HOM) (2-300 μmoles/l), stercuronium (STER) (1-100 μmoles/l) and gallamine (GALL) (3-300 μmoles/l) at 22°C (O——O), 32°C (△——△) or 37°C (●---●). Each point represents the mean (±SEM) of a number of experiments, the effect of each concentration being determined in every experiment. For homatropine at 22°C n=3, 32°C n=4, 37°C n=4; for gallamine at 22°C n=4, 32°C n=3, 37°C n=3 and for stercuronium at 22°C n=4, 32°C n=10. For clarity lines linking points at any one temperature are shown. Where error bars are not shown they lie within the dimension of the symbol.

Discussion. The finding that gallamine and stercuronium continue to produce nonlinear A-S plots over a wide temperature range indicates that the nonlinearity cannot be due to a temperature-sensitive alteration in receptor conformation. Despite a similar trend for an increase in affinity with reduction in temperature for homatropine and gallamine the increase was only marked for gallamine while stercuronium although producing a similar pattern of

1 Supported by a grant from the NHMRC, Australia. Gifts of gallamine (May & Baker Australia Pty. Ltd.) and stercuronium (Gist-Brocades Australia Pty.Ltd.) are gratefully acknowledged.

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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