where Y_1, Y_2 and Y_3 are the successive group inflammatory scores at X time intervals. In the anti-inflammatory test described here, X = 1 (hr) and the formula simplifies to

$$\text{Area} = Y_1 + Y_2 + \frac{Y_3}{2} \qquad (2)$$

For each dose of test compound the integral is calculated and expressed as a percentage of that of control animals. Using probit-logarithmic graph paper this percentage plotted against the dose gives a linear relation from which the dose which reduces the integral of the control response by half (ED50) can be determined by inspection.

Table 1 summarises the results obtained with six known anti-inflammatory drugs. These results are in good agreement with the known therapeutic value of these anti-inflammatory drugs.

Formula (1) is applicable to an experiment in which three observations are made at constant intervals, but the formula can be easily adapted to an experiment in which n observations are made at X time intervals. The integration of the resulting time-response curves can be calculated from the general expression,

$$\text{Area} = XY_1 + XY_2 + XY_3 + \ldots \ldots XY_{n-1} + \frac{X}{2} Y_n \qquad (3)$$

If the time interval between observations increases geometrically, the log interval is constant and is used for X in formula (3) in the calculation of the response integrals. In fact the general formula (3) applies to all time response relationships commencing at the origin irrespective of slope. It is concluded that the analysis has wide application in pharmacological test systems.

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Effects of ascorbic acid on the catecholamine content of guinea-pig myocardium

SIR,—When studying the effect of inhibitors of catechol-*O*-methyltransferase such as pyrogallol, and monoamine oxidase inhibitors such as nialamide or iproniazid, on the concentration of adrenaline or noradrenaline in auricles and ventricles of the guinea-pig heart, we injected ascorbic acid before the pyrogallol to prevent the latter causing a possible methaemoglobinaemia even at low doses (10 mg/kg, i.p.).

Adrenaline and noradrenaline were measured by the method of Bertler, Carlsson & Rosengren (1958). In this way we found that 10 min after an intraperitoneal injection of 500 mg/kg of ascorbic acid there was a significant decrease of adrenaline in both auricles and ventricles in unanaesthetised animals, there being no appreciable modification of the noradrenaline (Table 1).

This significant decrease of adrenaline in auricles and ventricles effected by ascorbic acid was also seen in guinea-pigs anaesthetised with urethane (1·2 g/kg, i.p.), when there was also a decrease of noradrenaline in auricles. Urethane alone

TABLE 1. CONTENT OF ADRENALINE AND NORADRENALINE IN HEART

Drugs	Auricles ± s.e.		Ventricles ± s.e.		Total heart ± s.e.	
	Adrenaline	Noradrenaline	Adrenaline	Noradrenaline	Adrenaline	Noradrenaline
Control (8)* ..	0·566 ±0·074	4·237 ±0·241	0·474 ±0·039	2·170 ±0·156	0·520 ±0·070	3·203 ±0·363
Ascorbic acid (6)	0·209 ±0·037 P<0·001 (*d*)	4·112 ±0·664	0·101 ±0·046 P<0·001 (*d*)	2·378 ±0·293	0·155 ±0·025 P<0·001 (*i*)	3·245 ±0·465
Pyrogallol (12) ..	0·391 ±0·091	4·059 ±0·353	0·308 ±0·055	1·849 ±0·116	0·349 ±0·063	2·954 ±0·198
Ascorbic acid + pyrogallol (6) ..	0·667 ±0·063	3·549 ±0·152	0·458 ±0·020	2·093 ±0·274	0·522 ±0·036	2·820 ±0·199
Pyrogallol ..	P<0·01 (*i*)	P<0·02 (*d*)	P≪0·001 (*i*)		P<0·01 (*i*)	
Ascorbic acid ..	P<0·01 (*i*)		P≪0·001 (*i*)		P≪0·001 (*i*)	P<0·001 (*d*)
Urethane (6) ..	0·396 ±0·080	4·548 ±0·325	0·422 ±0·127	1·641 ±0·361	0·409 ±0·103	3·094 ±0·091
Urethane + ascorbic acid (6) ..	0·205 ±0·027 P<0·001 (*d*)	3·110 ±0·283 P<0·02 (*d*)	0·210 ±0·061 P<0·01 (*d*)	1·913 ±0·178	0·207 ±0·031 P<0·001 (*d*)	2·511 ±0·213
Urethane + pyrogallol (6) ..	0·467 ±0·043	3·348 ±0·295	0·388 ±0·024	1·493 ±0·169	0·427 ±0·023	2·420 ±0·124
Pyrogallol ..			P<0·02 (*i*)		P<0·02 (*i*)	P<0·01 (*d*)
Urethane ..		P<0·02 (*d*)				P<0·01 (*d*)
Urethane + ascorbic acid + pyrogallol (6) ..	1·316 ±0·191 P<0·01 (*i*)	3·135 ±0·344	0·450 ±0·040 P<0·01 (*i*)	2·895 ±0·414	0·883 ±0·086 P<0·001 (*i*)	3·040 ±0·521

* Number of animals in parentheses.
(*i*) Increase.
(*d*) Decrease.

did not modify the myocardial catecholamine concentration. Pyrogallol alone, 20 min after intraperitoneal injection, also did not modify the catecholamine concentration of guinea-pig heart, but in animals treated with ascorbic acid it produced a significant increase of adrenaline in auricles and a highly significant increase of adrenaline in ventricles. These results occur both in unanaesthetised animals and in others anaesthetised with urethane.

According to McLean & Cohen (1963), ascorbic acid liberates adrenaline from the adrenal medulla granules *in vitro*, and this is antagonised by chelating agents. If this effect also occurs *in vivo*, and this has yet to be shown, the ascorbic acid-induced depletion of adrenaline in auricles and ventricles might be explained, as also might the increase of adrenaline in auricles and ventricles when both ascorbic acid and pyrogallol are given, as a consequence of an increase in extracellular catecholamines by inhibition of catechol-*O*-methyltransferase.

At the doses used, it is also possible that ascorbic acid could prevent the *in vivo* oxidation of pyrogallol, which would prolong its effects compared with the conditions when pyrogallol is given alone.

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