markedly dependent on changes in urinary pH (Beckett, Rowland & Turner, 1965). The duration of action of dexamphetamine in opposing depressant actions of amylobarbitone is probably closely related to urinary pH.

Roback, Krasno & Ivy (1952) found that dexamphetamine sulphate was effective in preventing the depression of c.f.f.f. produced by antihistamine drugs. It is equally effective in combination with amylobarbitone.

I am grateful to Mr. J. V. Smart for statistical help in this experiment which was carried out during the tenure of a Wellcome Senior Research Fellowship in Clinical Science. Smith Kline and French Laboratories Ltd. provided tablets of amylobarbitone, dexamphetamine and placebo.

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April 6, 1965.

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Use of time-response relationships in assessing pharmacological activity

SIR,—In many pharmacological experiments it is possible to obtain a continuous record of drug activity. Time-response curves provide an ideal basis for determining quantitative estimates of potency since they include a consideration of onset, peak and duration of action of the compounds under investigation. However, the use of time-response curves to obtain such estimates is seldom made. We would like to describe a quick and easy means of processing data for estimating the potency of compounds from integration of time-response curves.

There are two types of curve: one in which an effect is elicited in a single group of animals and its intensity assessed at several different times later, and an alternative in which different groups of animals are each examined once but at a variety of times, a relationship being built up between drug effect and time. For the purpose of this letter the method is applied to the assessment of anti-inflammatory activity of compounds in the guinea-pig ultraviolet erythema test described by Winder, Wax, Burr, Been & Rosiere (1958) but could be equally applied to many other pharmacological test procedures.

Male albino guinea-pigs of 250–400 g had an area 3 inches square on one dorsal flank depilated. Next day the guinea-pigs, in groups of 5, had test compounds administered orally, either dissolved or suspended in 5% w/v gum acacia, in a volume of 5 ml/kg. Control animals received gum acacia only. Two hr after drug administration, the flank of each animal was exposed to ultraviolet light for 30 sec. The head of the lamp was covered with a mask in which 3 holes of 6 mm diameter had been cut. The average intensity of the three resulting erythematous circles was estimated 1, 2 and 3 hr later using an arbitrary scoring system (slight erythema, 1; moderate erythema, 2; severe erythema, 3). For control animals and with each dose of test compound the responses from the group of 5-guinea-pigs were summed (group inflammatory score) and plotted against time. The relationship between group inflammatory score and time for a typical group of control animals is illustrated in Fig. 1A.

The area under the graph (integral) represents the continual level of inflammation over the 3 hr period of the test and is given by the formula overleaf, obtained from Fig. 1B.

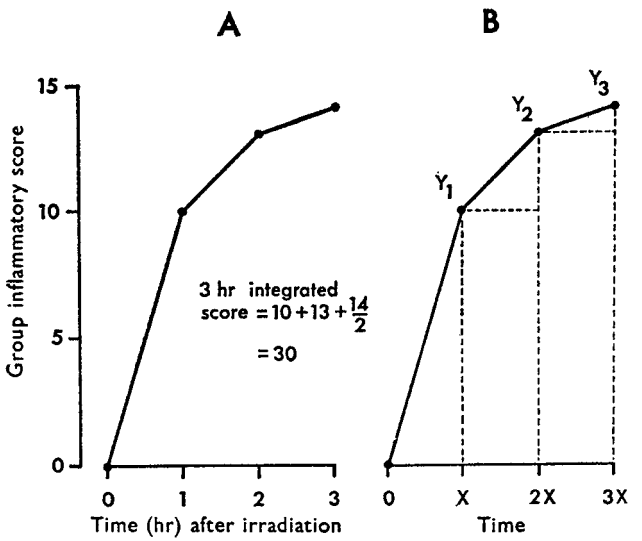

FIG. 1. Development of erythema in the guinea-pig. A, control animals. B, identical graph to show derivation of formulae for calculating area under the graph.

$$\text{Area} = \frac{XY_1}{2} + XY_1 + \frac{X(Y_2 - Y_1)}{2} + XY_2 + \frac{X(Y_3 - Y_2)}{2}$$

$$= XY_1 + XY_2 + \frac{XY_3}{2} \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

TABLE 1. ACTIVITY OF ANTI-INFLAMMATORY DRUGS AGAINST ULTRAVIOLET-INDUCED ERYTHEMA IN THE GUINEA-PIG

Drug	Oral dose mg/kg	Group inflammatory scores at 1 hr	2 hr	3 hr	3 hr integrated score*	ED50 mg/kg
Phenylbutazone ..	0	12	14	14	33	10·8
	8	5	9	11	19·5	
	15	4	5	8	13	
	30	0	4	6	7	
Oxyphenbutazone	0	11	14	14	32	15·6
	8	6	9	13	21·5	
	15	6	6	10	17	
	30	2	5	7	10·5	
Aspirin	0	10	12	14	29	139
	50	6	10	13	22·5	
	100	5	8	12	19	
	200	2	4	9	10·5	
Sodium salicylate ..	0	11	14	15	32·5	244
	100	7	12	14	26	
	200	7	9	12	22	
	400	2	4	5	8·5	
Aminopyrine ..	0	9	12	15	28·5	105
	50	7	11	13	24·5	
	100	4	7	11	16·5	
	200	0	2	5	4·5	
Phenazone ..	0	10	13	14	30	265
	50	9	13	12	28	
	100	8	10	11	23·5	
	200	6	7	11	18·5	

* See Formula (2)

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