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The detection of differential β-adrenoceptor blockade in the cat

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Summary

1. Tachycardia and bronchodilatation were produced by intravenous dimethylphenylpiperazinium iodide (DMPP) in the spinal or pithed cat. Responses other than those involving β-adrenoceptors were minimized by appropriate blocking agents.

2. With intact adrenals, both the tachycardia and bronchodilatation with 10 μg/kg DMPP were equal to those with 1 μg/kg adrenaline.

3. After adrenalectomy, tachycardia with 100 μg/kg DMPP was equal to that with 1·5 μg/kg adrenaline, but the bronchodilatation was equal to that with only 0·3 μg/kg. This difference may reflect the relative activity of the sympathetic supply to the organs.

4. After adrenalectomy, propranolol was 3 times as effective against bronchodilatation due to DMPP as against tachycardia. Practolol was inactive against bronchodilatation.

5. With intact adrenals, propranolol reduced both tachycardia and bronchodilatation due to DMPP equally. Propranolol also antagonized equally both effects of adrenaline in the pithed cat after adrenalectomy.

6. In the spinal cat, propranolol caused bradycardia and bronchoconstriction that persisted after adrenalectomy or pithing. It was reduced by pempidine and guanethidine and is attributed to spontaneous adrenergic neuronal activity.

7. The Appendix describes a device for cycle-by-cycle spirometry and correction for zero drift of a micromanometer, used in this study for the continuous recording of bronchial resistance.

Introduction

In a recent study of β-adrenoceptor blockade of the effects upon heart rate and airway resistance of thoracic cord stimulation in the guinea-pig (Burden, Parkes & Gardiner, 1971), it was found that propranolol appeared more active in blocking effects upon heart rate than upon airway resistance, while practolol was similarly effective against both effects. To test the possibility that this unexpected result might have been a characteristic of the species used, a similar comparison has been made in the cat.

As an alternative to electrical stimulation of the spinal roots to activate the sympathetic nerve supply to the organs studied, intravenous injections of the

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ganglionic stimulant dimethyl-phenylpiperazinium iodide (DMPP) were used. Responses other than those involving β-adrenoceptors were minimized by the use of appropriate blocking agents. In this way, the effects of β-adrenoceptor blocking agents on simultaneous responses of heart rate and bronchiolar resistance have been studied.

The preparation was demonstrated to the British Pharmacological Society in January 1972 as an example of the use of the device described in the Appendix (Gardiner & Parkes, 1972).

Methods

Male cats weighing 2·1 to 4·5 kg (2·83 ± S.E. 0·03) were anaesthetized with chloralose, to avoid exposing the lungs to volatile anaesthetics; 40 mg/kg was injected intraperitoneally, followed by 20 mg/kg into a cephalic or saphenous vein; hyoscine hydrobromide, 1 mg, was also injected subcutaneously.

Following the customary cannulation of the trachea, external jugular vein and the right common carotid artery, the left common carotid artery was tied, both vagi tied and cut, and the animals were spinalized by decapitation or by removing the roof of the axis vertebra and the length of spinal cord lying within; they were then maintained on artificial respiration. Some were additionally pithed in one of three ways: by passing a No. 8 knitting needle down the spinal canal from the axis, by complete removal of the cord by the similar passage of a half-inch tube brush or by its destruction by means of a PVC catheter passed down the spinal canal through which 3 ml of a 10% solution of silver nitrate was infused. Both adrenal glands were also ligated in many of the experiments.

These operative procedures were carried out with the repeated injection of the reversible ganglion blocking agent trimetaphan, 0·5 mg/kg intravenously, as required to prevent rise in blood pressure and tachycardia, in order to reduce bleeding and avoid excessive ganglionic activity. The animals were previously injected intravenously with mepyramine maleate, 0·5 mg/kg, and subsequently with hyoscine hydrobromide, 5 mg/kg, and sodium meclofenamate, 5 mg/kg, also intravenously. They were then allowed about an hour to reach a steady state and again injected with hyoscine and sodium meclofenamate and also with phenoxybenzamine, 2 mg/kg intravenously, before experiment. Where necessary to maintain adequate blood pressure, 20 ml of a 6% solution of polyvinylpyrrolidone in 0·9% w/v NaCl solution (saline) was also injected intravenously.

Artificial respiration was provided by a Starling pump, a side-arm on the tracheal cannula leading to a water valve, 13 cm in depth, as used in the Konzett-Rössler method for recording resistance of the lungs to inflation. The air overflow was detected by a pneumotachograph (Fleisch tube) connected to a differential micromanometer as described in a modification of that method (Burden *et al.*, 1971). The signal from the micronomanometer was processed by the device described in the Appendix to give an output to a pen recorder representing the volume of overflow per respiratory cycle. In addition, carotid blood pressure was recorded from a Statham transducer and heart rate from a ratemeter triggered by the pulse wave from the transducer or by the QRS complex of the E.C.G. detected by chest leads.

In some animals, bronchiolar tone developed during the period between preparation and experiment with reduction in sympathetic dilator activity, so that dilatation

could be recorded on injection of adrenaline or DMPP. Where this was not so, constriction was induced by the infusion, via a saphenous vein, of 5-hydroxytryptamine creatinine sulphate, 0·05 mg/ml ; usually, an infusion rate of 0·3 ml per min was adequate to maintain a steady level of air overflow that could be reduced by dilatation. Responses of both heart rate and air overflow were taken as the area enclosed by the excursion of the record from the base line. All drugs used in the experiments described were injected intravenously, dissolved in saline, except where otherwise specified.

Drugs

The sources of drugs were: choralose (British Drug Houses), hyoscine hydrobromide (Macfarlane Smith), mepyramine maleate (May & Baker), trimetaphan (Roche), sodium meclofenamate (Parke Davies), phenoxybenzamine (Smith, Kline & French), polyvinylpyrrolidone (Koch–Light), 5-hydroxytryptamine creatinine sulphate (Koch–Light), (−)-adrenaline (British Drug Houses), (−)-isoprenaline (Burroughs Wellcome), dimethylphenylpiperazinium iodide (Aldrich Chemical Co.), pempidine tartrate (May & Baker), guanethidine sulphate (Ciba), propranolol hydrochloride and practolol (Imperial Chemical Industries).

Results

Cats with intact adrenals

DMPP, in doses of the order of 10 μg/kg, caused reversible tachycardia and reduction in air overflow, with regression of both effects upon dose (Figure 1). Removal of the adrenals from the circulation (see below) suggested that the effects of these doses were due to the stimulation of adrenal medullary secretion, as reported by Chen, Portman & Wickel (1951). In confirmation of this, the effect of a dose of DMPP on the lung could be matched by the injection of a similar dose of adrenaline to that required to match the effect on the heart (Figure 1).

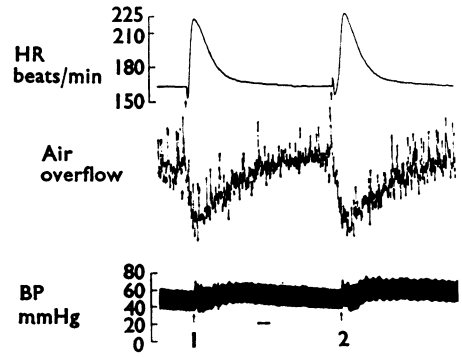

FIG. 1. Portion of a record from a pithed cat 2·5 kg, after pretreatment with hyoscine hydrobromide, 1 mg, s.c. and 2×5 mg/kg i.v., sodium meclofenamate, 2×5 mg/kg i.v., and phenoxybenzamine, 2 mg/kg i.v. From above downwards, heart rate, air overflow and blood pressure. At arrows: 1—Adrenaline, 1 μg/kg, i.v. ; 2—DMPP, 12·5 μg/kg, i.v. Time scale, 1 minute.

Table 1 shows results of comparative assays in 9 cats and gives the doses of adrenaline equivalent to 10 μg/kg DMPP for effects on heart rate and air overflow. It will be seen that these doses did not differ significantly.

TABLE 1. *Doses of adrenaline required to match the responses of heart rate and air overflow to doses of dimethyl-phenylpiperazinium iodide (DMPP).*

State of Adrenals	Dose DMPP (μg/kg)	Equivalent dose of adrenaline (μg/kg) for effect on Heart rate	Air overflow	*P* for diff.
Intact	10	0·97±S.E.0·14 (*n*=55)	0·88±S.E.0·2 (*n*=52)	Not Sig.
Tied	100	1·41±S.E.0·22 (*n*=37)	0·3±S.E.0·04 (*n*=33)	<0·001

The activity of propranolol, in doses of 1–200 μg/kg, in reducing the responses to DMPP was similar for both tachycardia and bronchodilatation, as shown by the doses required to reduce these responses by 50% ; given in Table 2.

TABLE 2. *Activity of propranolol in reducing the effects of dimethyl-phenylpiperazinium iodide (DMPP) or adrenaline upon heart rate and air overflow.*

Stimulant	Heart rate			Air overflow		
Drug	ED_{50}	b±S.E.	*n*	ED_{50}	b±S.E.	*n*
DMPP	13(9–18)	45±5	20	15(7–31)	41±10	20
Adrenaline	37(24–54)	58±6	5	30(17–55)	33±5	8

The figures in brackets give 95% confidence limits. b is the computed regression coefficient of response, measured as the area below the recorded excursion, on log dose.

Cats with tied adrenals

The activity of propranolol in reducing the corresponding effects of injected adrenaline upon heart rate and air overflow was determined in cats after adrenal ligation, as this gave more reliable results. The doses of propranolol required for 50% reduction of these effects are shown in Table 2 and are seen also to be similar for tachycardia and bronchodilatation.

After ligation of the adrenals, DMPP caused increase in heart rate and reduction of air overflow, as it did before adrenalectomy, but doses of the order of 100 μg/kg were now required. Moreover, as shown in Fig. 2, the effect upon air overflow was now less, relative to the effect upon heart rate, than that of injected adrenaline. This was confirmed by the doses of adrenaline required to match each of these effects of DMPP. Reasonably parallel log dose-response relations could be obtained for DMPP and adrenaline in their effects on air overflow al-

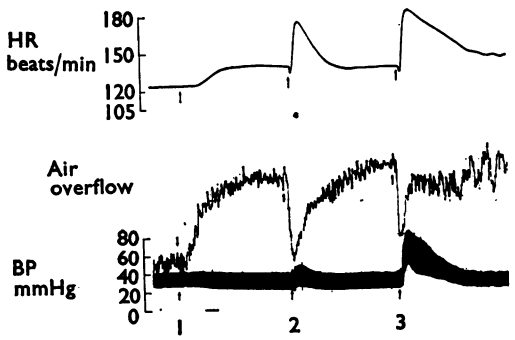

FIG. 2. Portion of a record from a pithed cat 3·3 kg, with both adrenals tied, after pretreatment with hyoscine hydrobromide, 1 mg, s.c., and 2×5 mg/kg i.v. sodium meclofenamate, 2×5 mg/kg i.v., and phenoxybenzamine, 2 mg/kg i.v. From above downwards, heart rate, air overflow and blood pressure. At arrows: 1—Start of i.v. infusion of 5-hydroxytryptamine creatinine sulphate, 50 μg/ml at 0·05 ml/min ; 2—adrenaline, 1 μg/kg i.v. ; 3—DMPP, 100 μg/kg i.v. Time scale, 1 minute.

though, in its effect upon heart rate, DMPP often showed a steeper dose-response relation than that for adrenaline. Acceptable comparisons in 13 cats gave the doses of adrenaline equivalent to 100 μg/kg DMPP shown in Table 1, where it is seen that the dose matching the effect on the heart rate greatly exceeds that for air overflow. The difference was highly significant by t test ($P<0{\cdot}001$).

The effect of DMPP on both heart rate and air overflow, as with those of adrenaline, could be reduced by previous intravenous injections with β-adreno-

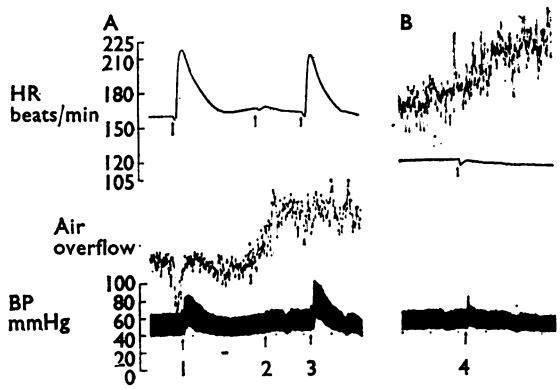

FIG. 3. Portion of a record from a pithed cat, 4·0 kg, with both adrenals tied, pretreatment with hyoscine hydrobromide, 1 mg, s.c. and 2×5 mg/kg i.v., sodium meclofenamate, 2×5 mg/kg i.v., and phenoxybenzamine, 2 mg/kg i.v. and i.v. infusion with 5-hydroxytryptamine creatinine sulphate (5-HT), 50 μg/ml at 0·15 ml/minute. From above downwards, heart rate, air overflow and blood pressure. At arrows: 1 and 3 DMPP, 100 μg/kg i.v.; 2—Propranolol, 50 μg/kg i.v.; 4—Propranolol, 100 μg/kg i.v. Between panels A and B 55 mg/kg pempidine was injected intravenously in one dose of 10 mg/kg and 9 doses of 5 mg/kg and the infusion of 5-HT was stopped. Time scale, 1 minute.

TABLE 3. *Characteristics of dose-response relations for β-adrenoceptor blockade on the effects of dimethyl-phenylpiperazinium iodide* 100 μg/kg, *on heart rate and air overflow in pithed adrenalectomized cats.*

	Heart rate			Air overflow		
	ED_{50} μg/kg	n	b±S.E.	ED_{50} μg/kg	n	b±S.E.
Propranolol	77(47–130)	33	36(22–49)	19(13–30)	17	48(32–63)
Practolol	56 (8–111)	15	35(14–57)	Inactive at 5 mg/kg		

The figures in brackets give the 95% confidence limits. Values for b are the regression coefficients of percentage reduction in response, measured as the area below the recorded excursion, on log dose.

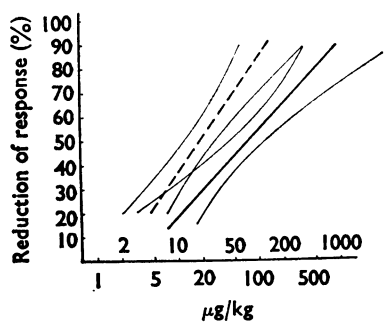

FIG. 4. Relations between dose of propranolol and percentage reduction of the response of heart rate (unbroken line) and air overflow (broken line) of pithed, adrenalectomized cats to intravenous injection with DMPP, 100 μg/kg. The envelopes enclose the 95% confidence limits. For heart rate, b=35·6±13·5; n=31; for air overflow, b=47·6±15·8; n=17.

ceptor blocking agents, though the doses now required for a given reduction differed for the two effects (Figure 3). The relations between dose of propranolol and responses of heart rate and air overflow to DMPP in spinal or pithed cats that were also adrenalectomized are shown in Figure 4. Doses of propranolol and practolol computed from these relations to reduce these responses to 50% are given in Table 3.

It was regularly observed that after giving even very small doses of propranolol to spinal cats the air overflow increased to a higher level (Fig. 3) indicating bronchoconstriction. This was accompanied by bradycardia and could repeatedly be elicited by successive doses of propranolol. Since this suggested blockade of an existing sympathetic tone, cats were additionally pithed but this effect persisted and was not abolished by complete eradication of the spinal cord.

Injections of trimetaphan or pempidine also caused bradycardia and bronchoconstriction and blocked the effects of DMPP in pithed cats. After the repeated injection of 10 mg/kg doses of pempidine until a total of at least 50 mg/kg had been given, propranolol caused only a small fall in heart rate and rise in air overflow (Figure 3). These residual effects could be further reduced by injecting 1 mg/kg doses of guanethidine.

Discussion

The object of this work was to compare the effectiveness of β-adrenoceptor blocking agents for the responses of the heart and bronchioles to sympathetic nerve stimulation. As an alternative to electrical stimulation of the thoracic spinal outflow, as used in the guinea-pig by Burden & Parkes (1972), injections of the ganglionic stimulant DMPP were used for this purpose, cholinoceptor and α-adrenoceptor responses being blocked by injections of hyoscine and phenoxybenzamine.

DMPP is known to release adrenaline from the adrenal medulla (Chen *et al.*, 1951) and, in the presence of intact adrenals, the β-adrenoceptor stimulant actions of DMPP reported above have been shown to be due largely, if not entirely, to the adrenaline released. A given dose of DMPP was found to be equivalent to amounts of injected adrenaline that were identical, whether assessed by either tachycardia or bronchodilatation; moreover, the activity of propranolol in blocking both effects was similar, as it was also found to be in blocking both effects of injected adrenaline.

In contrast, after bilateral adrenal ligation, the effects of the larger doses of DMPP required are less on air overflow than on heart rate, relative to those effects before adrenal ligation (compare Figs. 1 and 2). Moreover, they are now matched by doses of injected adrenaline that are much smaller when assessed by their effects on air overflow than when assessed by tachycardia. The use of injected adrenaline to assess the relative effectiveness of DMPP for the two responses has permitted its comparison before and after adrenal ligation, although the effects of DMPP in the latter case are due to noradrenaline released as the consequence of ganglionic stimulation. This is shown by their abolition by ganglion blocking agents, in confirmation of the reports of Chen *et al.* (1951). It may be suggested, therefore, that this ganglionic stimulant action is relatively less effective for bronchodilatation than for tachycardia.

A similar finding resulted from the electrical stimulation of the thoracic spinal outflow in the guinea-pig (Burden & Parkes, 1972), where there was also a disparity in the consequent tachycardia and bronchodilatation, compared to the effects of injected catecholamines. There is thus no reason to suppose that DMPP exerts a differential stimulant action on the sympathetic ganglion cells concerned in the two effects. DMPP has been shown to release transmitter from sympathetic nerve endings in the heart (Brus & Jacobowitz, 1970) but it is not known if this may not also occur in the lung.

It may be suggested that the differences in the intensity of the effects of DMPP on heart and lung reflect the relative richness of effective sympathetic innervation, or of β-adrenoceptor sites, or of the amount of transmitter released, in the two organs. Direct evidence on the relative intensity of innervation of the heart and bronchi is not available; both are described as richly innervated from fluorescence microscopic studies (Falck, Häggendal & Owman, 1963; Angelakos, Fuxe & Torchiana, 1963; Dahlstrom, Fuxe, Hökfelt & Norberg, 1966).

Further confirmation of the difference in intensity of sympathetic stimulation in the heart and lungs evoked by a dose of DMPP may be deduced from the greater activity of propranolol in reducing the effect on air overflow than that on heart rate after adrenalectomy. This is in agreement with the difference in dose of injected adrenaline to which the two effects of DMPP are equivalent since propranolol antagonizes both effects of the amine equally. There is no basis here for concluding a difference in β-adrenoceptor sensitivity to propranolol in the two organs, since this agent was shown to block with equal effectiveness not only the effects of injected adrenaline but also those of DMPP in cats with intact adrenals. Rather, it may be suggested, the intensity of sympathetic stimulation due to DMPP, and hence the amount of transmitter liberated, against which the blocking action of propranolol is exerted, is less in the lung than in the heart. In other words, because the effect of DMPP on air overflow represents a lower point on the dose-response relation for catecholamine than does the effect on heart rate, propranolol may be expected to block the former more easily than the latter. A similar suggestion was made by Burden & Parkes (1972) from their findings in the cord-stimulated guinea-pig.

An alternative explanation of this that cannot be excluded is that DMPP causes an equivalent intensity of sympathetic stimulation in both heart and lung whereas adrenaline, with which the effects of DMPP were compared, is relatively more effective as a bronchodilator than in provoking tachycardia. This explanation, however, requires also that sensitivity to the blocking action of propranolol should be relatively greater in the lung than in the heart, to just the extent of the relative activities of adrenaline in the two organs.

Whichever of these alternatives may be correct, it suggests that the relative effectiveness of β-adrenoceptor blocking agents for sympathetically innervated organs may not be the same for the effects of procedures that elevate sympathetic tone as for those of injected amines, a conclusion also drawn from their work in the cord-stimulated guinea-pig by Burden & Parkes (1972). But whereas in that preparation, careful quantitative assessment was necessary to establish the selective action of practolol between effects on heart rate and air overflow, in the adrenalectomized cat practolol was quite ineffective in blocking the bronchodilator effects of DMPP.

The observation of bradycardia and bronchoconstriction with small doses of propranolol suggests that even in the pithed cat residual sympathetic tone remains in both heart and bronchial tree; indeed, the persistence of the effects in appearing after repeated doses of propranolol suggests that it is extensive. Mostly it seems to be of ganglionic origin, since it is eventually reduced by large doses of ganglion blocking agent, though some appears resistant to this. This may be due to activity at the adrenergic nerve endings, since it was abolished by guanethidine.

The possibility of such spontaneous activity of sympathetic ganglion cells and nerve endings may be deduced from the recorded observations of spontaneous activity in decentralized or isolated ganglia (Brown & Feldberg, 1936; Blackman, Ginsborg & Ray, 1963) or sympathetic nerve endings (Burnstock & Holman, 1962) and has been suggested by Burn & Rand (1959) and Emmelin (1961). The experimental conditions used in this work have apparently permitted the appearance of such activity.

I wish to record my indebtedness to Mrs. Janet S. Norton (née Duncan) for invaluable technical assistance.

Appendix

A device for cycle-by-cycle spirometry

D. G. GARDINER

The Appendix to a recent paper (Burden *et al.*, 1971) described an arrangement for recording changes in resistance to inflation of the lungs *in vivo*, by the measurement of air overflow with a pneumotachograph and differential manometer, which depends on continuous integration of the manometer signal. The present device displays the integrated signal over each cycle successively. At the same time, the drift in the manometer zero is continuously corrected; both operations are achieved by the use of 'track and store' circuits. The advantage of this procedure is the achievement of rapid response, irrespective of a low frequency of operation as, for example, when inflating cat lungs.

The device is the subject of a current patent application (Gardiner, 1971) and was demonstrated to the British Pharmacological Society in January 1972 (Gardiner & Parkes, 1972).

Description

Figure 5 shows the stages in the cycle of air inflation of the lungs, by means of a pump, to constant pressure using a water-valve in parallel (Burden *et al.*, 1971). It will be seen that the positive-going output of the manometer during the overflow period is followed by a negative-going signal as the pressure in the system falls to atmospheric, due to the backflow of air into the water-valve and return of the water level in the tube to the resting level. During this phase, a trigger is actuated to generate two pulses, A and B (Fig. 5) by means of flipflop circuits. These pulses are made to perform two operations, one concerned with recording and the other with correcting the manometer zero. These operations are indicated in the block diagram (Figure 6).

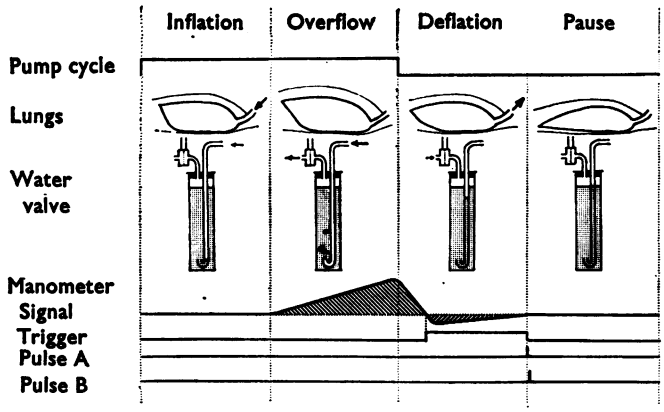

FIG. 5. Diagram showing events during one cycle of inflation of the lungs by a respiration pump. The lower traces refer to operations of the circuit shown in Figure 6.

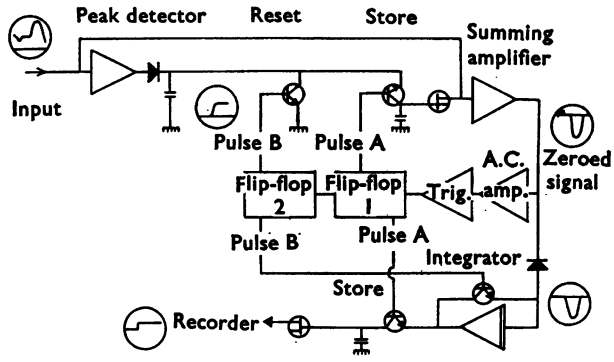

FIG. 6. Block diagram showing the features of the device described in the text.

The voltage of the negative-going portion of the manometer output waveform is inverted to become the positive output of a peak detector and transferred to a storage capacitor by pulse A. By means of an adding amplifier, the potential of the storage capacitor is added to the original signal, thus bringing its most negative-going portion to zero voltage (Figure 7). Meanwhile, pulse B resets the peak detector in readiness for the next cycle.

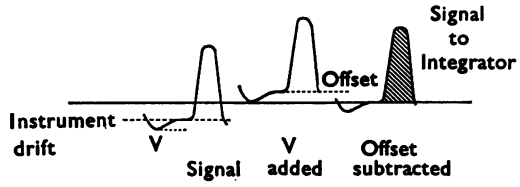

FIG. 7. Representation of the output of the differential manometer during one cycle of air inflation, showing the principle of correction for drift.

To allow for positive as well as negative drift of the manometer, a potential of −5 V is added to the manometer signal at the input of the peak detector. An adjustable d.c. offset at the input of the adder circuit is used both to remove this

potential and to set the wave-form at the desired level, with reference to zero. This is shown diagrammatically in Fig. 7, in which the initial offset of −5 V is omitted for simplicity of presentation.

The signal, now inverted and correctly referred to zero potential, is passed to the integrator circuit (Fig. 6), the output of which is transferred to a storage capacitor by pulse A. The potential of this capacitor actuates, via a FET output stage, one pen circuit of a multi-channel recorder, which displays this value until the storage capacitor receives that for the next cycle. In preparation for this, pulse B has meanwhile reset the integrator circuit.

The record of air overflow thus consists of a series of readings for each successive cycle (Figs. 1–3) which can show changes in resistance to air inflation, as well as cycle-by-cycle fluctuations.

Details of the circuit can be obtained from the author.

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