J. Pharm. Pharmacol., 1964, 16, 241–249 Received October 28, 1963

Method for recording respiratory changes induced in guinea-pigs by aerosols of histamine or of specific antigen, and for assessing drugs which antagonise bronchoconstriction

A. BENNETT* AND MARY F. LOCKETT†

A lightly-anaesthetised guinea-pig is made to breathe an aerosol of histamine or of specific antigen pumped into a breathing chamber. The guinea-pig's respiration produces pressure changes which are recorded on a smoked drum. Isoprenaline and the antihistamine thenyldiamine, administered as aerosols or by injection, have been used to inhibit the reduction in "tidal volume" due to the bronchoconstrictor agents.

MANY investigations of the inhibition of bronchoconstriction produced by aerosols of histamine (Kallós & Pagel, 1937; Halpern, 1942; Bovet & Walthert, 1944; Loew, Kaiser & Moore, 1945) or specific antigen (Kallós & Pagel, 1937; Herxheimer, 1949; Feinberg, Malkiel, Bernstein & Hargis, 1950; Friebel, 1953) have been based on the ability of a drug to prevent anoxic death in guinea-pigs. In 1952, however, Herxheimer developed a "microshock" method for evaluating the effect of drugs on "asthma" in guinea-pigs. He showed that when a sensitised guinea-pig was exposed at intervals to an aerosol of the specific antigen, the time taken to induce dyspnoea (the "preconvulsion time") remained fairly constant when sufficient time elapsed between each exposure. Lengthening of the "preconvulsion time" by a drug has been used as an index of its anti-asthmatic activity by Herxheimer (1952, 1953, 1955, 1956), Armitage, Herxheimer & Rosa (1952), Herxheimer & Rosa (1953), Herxheimer & Stresemann (1960) and others. Spirometric techniques have been widely used in the examination of the relief of bronchospasm in man, but do not appear to have been used in guinea-pigs.

A reduction of tidal volume and of the force of inspiration and expiration, hereafter referred to collectively as "tidal volume", occur during bronchoconstriction. A technique for measuring changes in the "tidal volume" of guinea-pigs exposed to aerosols of histamine or specific antigen is described below. The method is used to assess the ability of drugs to inhibit bronchoconstriction.

Experimental

APPARATUS

In principle, the apparatus consists of a breathing chamber which accommodates the head of a lightly-anaesthetised guinea-pig. Aerosol is pumped into the chamber through an inlet tube and leaves by an outlet tube to which is connected a tambour and a frontal writing lever (see Fig. 1).

From the Department of Physiology and Pharmacology, Chelsea College of Science and Technology, London, S.W.3.

* Present address: Department of Surgery, King's College Hospital Medical School, London, S.E.5.

† Present address: Department of Pharmacology, University of Western Australia.

The breathing chamber consists of a glass cylinder 7 cm in diameter and 10 cm long sealed at one end. A rubber diaphragm 0·8 mm thick, with a central, slightly elliptical hole into which fits the head of a guinea-pig, is secured to the open end of the chamber. A cylinder of strong flexible polythene, into which are fixed a hand pump and a water manometer, is attached to the front of the chamber.

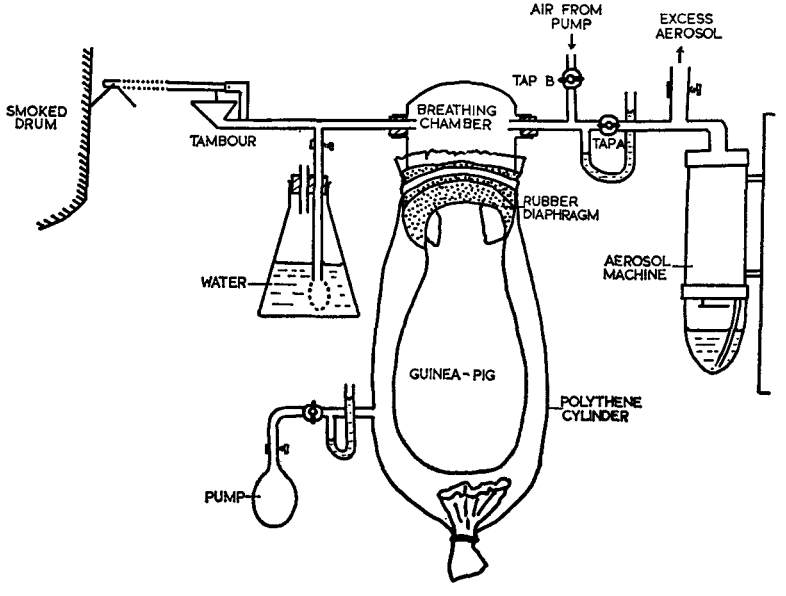

FIG. 1. Diagram of the apparatus used to measure changes in the 'tidal volume' of a guinea-pig exposed to broncho-constrictor aerosols.

The aerosol is produced by a model SB4 Aerolyzer (Aerosol Products Limited). The volume of liquid in the reservoir is adjusted to a fixed amount so that the weight of liquid vapourised per min is kept constant. The aerosol is led into a T-junction and forced through a glass tap (A) at a pressure of 5 cm of water above atmospheric pressure and enters the breathing chamber. The excess aerosol not forced through tap A passes through the other arm of the T-junction, and is removed from the laboratory by a wide-bore rubber tube controlled by an adjustable screw clip.

As shown in Fig. 1, a small tambour covered with a loose, thin rubber membrane is connected to the outlet from the breathing chamber. The respiration of the guinea-pig alters the pressure and causes changes in the distension of the tambour which are recorded on a smoked drum by means of a light frontal writing lever (magnification 40:1).

The aerosol leaving the breathing chamber passes through a rubber tube controlled by an adjustable screw clip and bubbles into water through a perforated rubber teat. The perforations in the teat ensure the formation of small bubbles which do not interfere with the movement of the tambour.

Air, instead of aerosol, can be blown into the breathing chamber through tap B at a pressure of 5 cm of water above atmospheric pressure, by means of an Es-Es pump.

DRUGS

Histamine acid phosphate, isopropylnoradrenaline sulphate and thenyldiamine hydrochloride were dissolved in 0·9% w/v aqueous NaCl before each experiment. Doses have been expressed as the weights of the salts.

METHOD

Guinea-pigs were sensitised by injecting 100 mg egg albumin intraperitoneally and 100 mg subcutaneously, at least 14 days before the experiments.

A guinea-pig was weighed and anaesthetised lightly with sodium pentobarbitone (0.5 ml/kg) injected intraperitoneally. Occasionally more anaesthetic was required. The aerosol delivery tube was closed (tap A) and air was pumped into the breathing chamber through tap B. Five to 6 min later, the head of the guinea-pig was pushed through the hole in the rubber diaphragm, and the polythene cylinder closed and inflated to give a pressure of 5 cm of water above atmospheric pressure. Ten min after the injection of pentobarbitone the kymograph and the aerosol machine containing 0·9% w/v aqueous NaCl were switched on. Tap B was closed, tap A opened, and a recording made of the respiratory pattern of the animal exposed to the saline aerosol. Five min later the guinea-pig was removed from the apparatus and allowed to recover from the anaesthetic. Any aerosol remaining in the breathing chamber was removed by air blown through tap B.

Subsequently, the normal guinea-pigs were anaesthetised lightly with pentobarbitone sodium and exposed to an aerosol of 0·5% histamine acid phosphate on alternate days. Sensitised guinea-pigs were anaesthetised and exposed to an aerosol of a centrifuged solution of 1% egg albumin every five days. These procedures were continued until in each animal the time taken to induce a marked reduction in "tidal volume" was approximately constant. When a severe, unrelieved bronchoconstriction occurred, or after 4–5 min when bronchoconstriction was slight or absent, the aerosol machine was switched off and the guinea-pig removed quickly from the apparatus. Guinea-pigs were artificially respirated if necessary and all exposed to aerosols of antigen were then made to breathe an aerosol of 1% isoprenaline sulphate. Animals not responding satisfactorily to the bronchoconstrictor aerosols were discarded.

When each animal showed a constant respiratory pattern, thenyldiamine hydrochloride or isoprenaline sulphate were either mixed in solution with the bronchoconstrictor substance or were injected intramuscularly into the conscious animals 30 min before exposure to aerosol. After each experiment in which a guinea-pig was protected by a drug, the animal was next time exposed to the bronchoconstrictor agent alone so that any change in

the sensitivity of the animal could be recognised and not erroneously attributed to a drug. In the few instances where the unprotected guinea-pig produced a control pattern significantly different from before, exposures to histamine or antigen alone were repeated until two consistent responses were obtained.

Results

Aerosols of histamine acid phosphate (0·5% w/v) and of specific antigen (1 % w/v egg albumin) each reduced the "tidal volume" of the guinea-pigs (see Fig. 2). Fairly constant reductions of "tidal volume" were produced in 15 of 21 guinea-pigs exposed to aerosols of histamine and in 11 out of

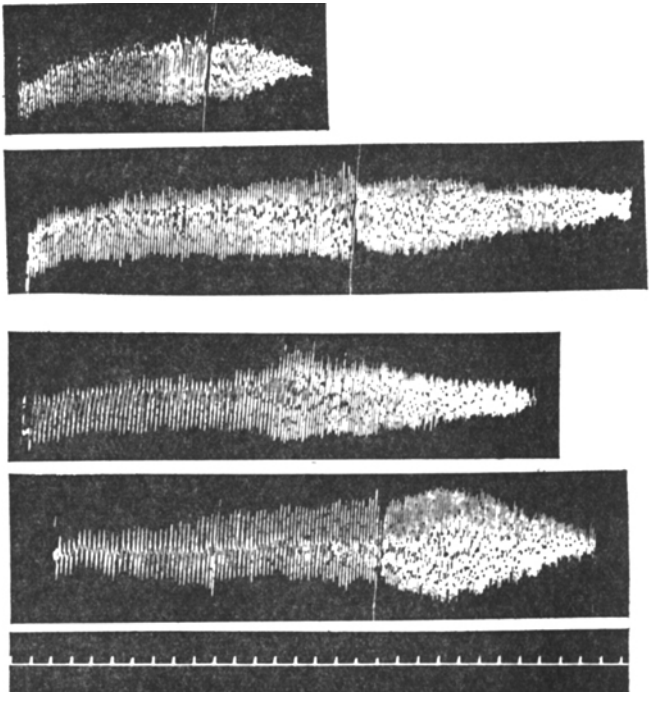

FIG. 2. Typical records of the respiratory patterns of guinea-pigs exposed to aerosols of histamine (top 2 traces) and of specific antigen (lower 2 traces). Time trace, 5 sec.

16 guinea-pigs exposed to aerosols of specific antigen. The remaining animals gave inconsistent responses and were discarded. When isoprenaline sulphate or thenyldiamine hydrochloride were present in the aerosols or had been injected intramuscularly into the guinea-pigs, the reduction in "tidal volume" was either postponed or did not occur at all (Table 1 and Fig. 3). In some animals the length of postponement varied on different occasions.

TABLE 1. PROTECTION AGAINST BRONCHOCONSTRICTION PRODUCED BY AEROSOLS OF HISTAMINE AND EGG ALBUMIN, USING ISOPRENALINE AND THENYLDIAMINE

Bronchoconstrictor aerosol	Protecting drug	Dose*	Degree of protection†
egg albumin 1%	Isoprenaline sulphate	25 μg/ml	o
		50 "	+
		100 "	+++
		200 "	+++
		0·1 μg i.m.	o
		5 "	+
		20 "	+++
	Thenyldiamine hydrochloride	1·0 mg/ml	*o* to ++
		2·0 "	+ to ++
		4·0 "	+++
Histamine Acid Phosphate 0·5%	Isoprenaline sulphate	25 μg/ml	o
		50 "	+
		100 "	+++
		200 "	+++
		0·1 μg i.m.	o
		0·2 "	o
		2·0 "	+
		20·0 "	+++
		50 "	+++
	Thenyldiamine hydrochloride	0·5 mg/ml	o to ++
		1·0 "	+ to ++
		1·5 "	++
		2·0 "	+++
		5 μg i.m.	+
		20 "	+++
	5 μg Thenyldiamine i.m. + 2 μg Isoprenaline i.m.		+++
	0·5 mg/ml Thenyldiamine + 50 μg/ml Isoprenaline		+++
	5 μg Thenyldiamine i.m. + 50 μg/ml Isoprenaline		+++

* Doses are expressed as μg or mg/ml in the bronchoconstrictor solution aerosolised, or as μg injected intramuscularly 30 min before exposure to aerosol.
† Protection ranges from nothing (o) to a maximum at +++.

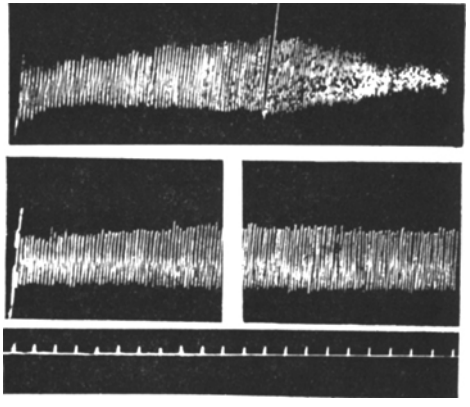

FIG. 3. The reduction in the "tidal volume" of a guinea-pig exposed to an aerosol of histamine (top trace), and the protection afforded to the same guinea-pig two days later by 2 mg/ml thenyldiamine in the histamine aerosol (lower trace). Time trace, 5 sec.

The reductions of "tidal volume" were plotted against time taking the measurement at 20 sec as 100% with the histamine aerosol, and the measurement at 40 sec as 100% with the antigen aerosol (Figs 4–7). In several experiments the rate and depth of respiration were also measured

but showed no relationship to the time taken to induce bronchoconstriction in the same animal on different occasions. Table 1 shows that 50 μg/ml of isoprenaline sulphate or 0·5–1 mg/ml of thenyldiamine hydrochloride in the aerosol retarded the onset of bronchoconstriction induced by histamine or antigen, while 100 μg/ml of isoprenaline sulphate or 2–4 mg/ml of thenyldiamine hydrochloride completely prevented it. The intramuscular injection of 2 μg isoprenaline sulphate or of 5 μg thenyldiamine hydrochloride retarded the onset of histamine-induced bronchoconstriction while 20 μg of either drug completely protected guinea-pigs from the effects of histamine; 5 μg and 20 μg isoprenaline sulphate injected into

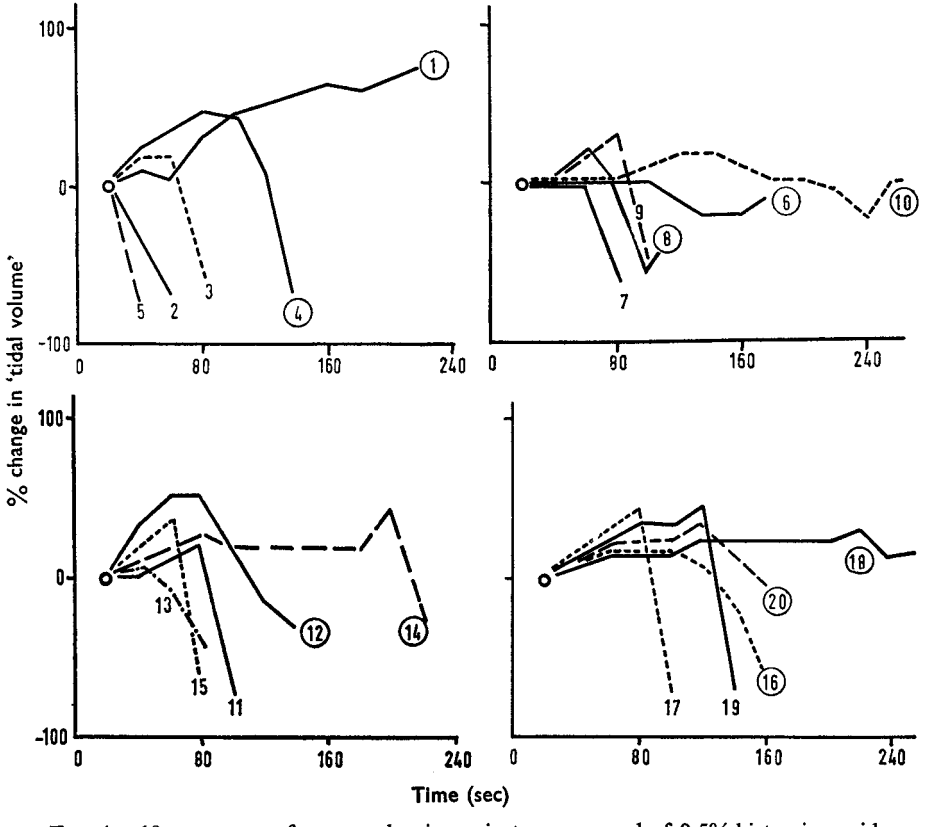

FIG. 4. 19 exposures of a normal guinea-pig to an aerosol of 0·5% histamine acid phosphate on every second day. The sequence of experiments is indicated by a number. When another drug is administered in addition to histamine, the number is ringed and the explanation is given below:

1. 0·9% NaCl aerosol only.
4. Thenyldiamine, 100 μg/ml.
6. Thenyldiamine, 2 mg/ml.
8. Thenyldiamine, 1 mg/ml.
10. Thenyldiamine, 2 mg/ml.
12. Thenyldiamine, 1 mg/ml.

Break of 12 days between 12 and 13.

14. Thenyldiamine, 5 μg i.m. + Isoprenaline 2 μg i.m.
16. Thenyldiamine, 5 μg i.m.
18. Thenyldiamine, 5 μg i.m. + Isoprenaline 2 μg i.m.
20. Isoprenaline 2 μg i.m.

sensitised guinea-pigs afforded similar protection against aerosols of egg albumin. These results show that thenyldiamine is almost as active as isoprenaline when injected intramuscularly, but is much less active when administered as an aerosol. Low doses of isoprenaline and thenyldiamine administered together markedly inhibit histamine-induced broncho-constriction.

Discussion

The method records changes of pressure produced in a breathing chamber by the respiration of a guinea-pig. The measurements obtained are a function of the tidal volume and of the force of inspiration and

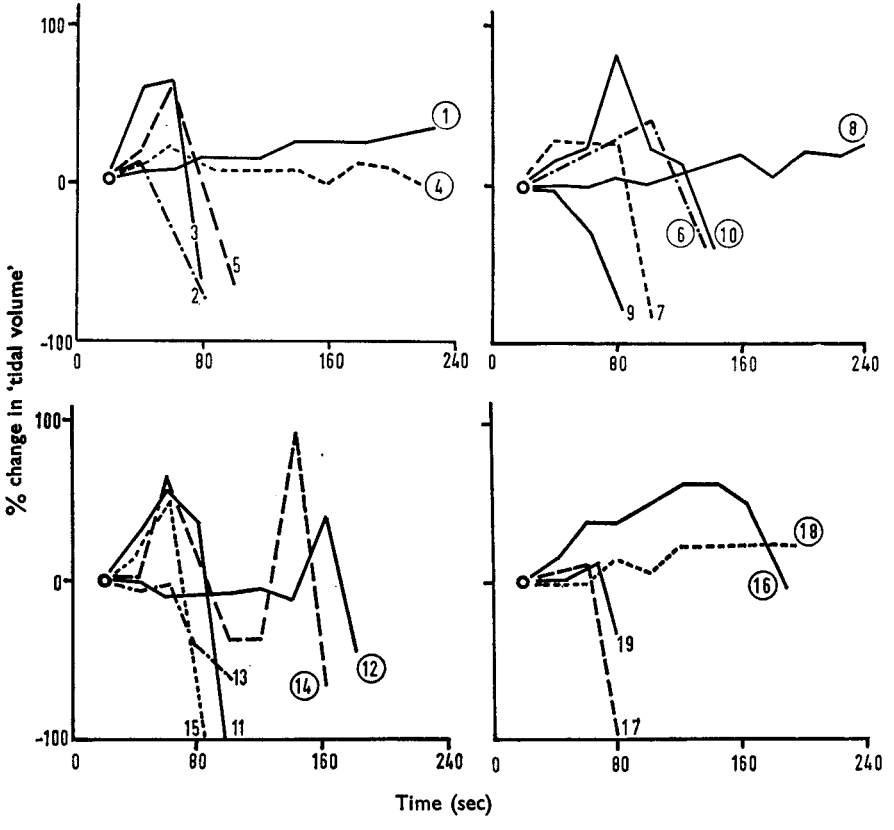

FIG. 5. Eighteen exposures of a normal guinea-pig to an aerosol of 0·5% histamine acid phosphate on every second day, as in Fig. 4.

1. 0·9% NaCl aerosol only.
4. Thenyldiamine, 20 μg i.m.
6. Thenyldiamine, 5 μg i.m.
8. Thenyldiamine, 20 μg i.m.
10. Thenyldiamine, 5 μg i.m.
12. Isoprenaline, 50 μg/ml.
14. Isoprenaline, 50 μg/ml + thenyldiamine, 5 μg i.m.
16. Isoprenaline, 50 μg/ml.
18. Isoprenaline, 50 μg/ml + thenyldiamine, 5 μg i.m.

expiration. The apparatus, which is easy to construct and use, can be employed to examine the effects of aerosols on the respiration of a guinea-pig and to assess the ability of drugs to inhibit bronchoconstriction induced by aerosols of histamine or specific antigen.

The advantages of this method for assessing anti-asthmatic drugs are that each animal can be used many times and each acts as its own control.

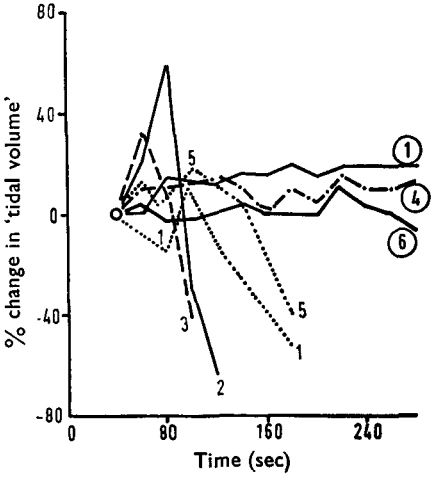

FIG. 6. Six exposures of a sensitised guinea-pig to an aerosol of a 1% solution of egg albumin every fifth day. The sequence of experiments is indicated by a number. When a drug is administered in addition to the egg albumin, the number is ringed and the explanation is given below: 1. 0·9% NaCl aerosol only. 4. Isoprenaline, 20 μg i.m. 6. Isoprenaline, 200 μg/ml.

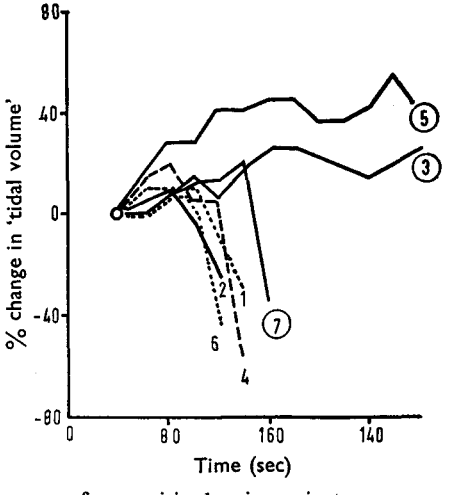

FIG. 7. Seven exposures of a sensitised guinea-pig to an aerosol of a 1% solution of egg albumin every fifth day, as in Fig. 6. 3. Thenyldiamine, 4 mg/ml. 5. Thenyldiamine, 2 mg/ml. 7. Thenyldiamine, 1 mg/ml.

Further, the onset of bronchoconstriction is clearly seen and easily measured. It is possible, however, that the light anaesthesia which is necessary may affect the response of the guinea-pig to the bronchoconstrictor aerosol. Koontz & Shackelford (1940) and Katz (1940) found that ether anaesthesia afforded some protection against anaphylactic shock in guinea-pigs, but Loew, Kaiser & Moore (1946) found that 10–20 mg/kg pentobarbitone sodium had little effect on the mortality of guinea-pigs exposed to aerosols of histamine. On the other hand, conscious animals may become conditioned by repeated experimentation whereas anaesthesia would prevent this.

The results obtained with this method are usually consistent and reproducible but large variations in the response of the animals sometimes occur. Therefore, in common with other methods of screening and assessing anti-asthmatic compounds, it enables only approximate estimates of drug potency to be made. In contrast to these other methods, however, changes in respiration are recorded and the onset of a marked bronchoconstriction can be measured directly.

References

Armitage, P., Herxheimer, H. & Rosa, L. (1952). *Brit. J. Pharmacol.*, **7**, 625–636.
Bovet, D. & Walthert, F. (1944). *Ann. pharm. franç.* **2**, Suppl. to No. 4, pp 3-43.
Feinberg, S. M., Malkiel, S., Bernstein, T. B. & Hargis, B. J. (1950). *J. Pharmacol.*, **99**, 195–201.
Friebel, H. (1953). *Arch. exp. Path. Pharmak.*, **217**, 35–42.
Halpern, B. N. (1942). *Arch. int. Pharmacodyn.*, **68**, 339–409.
Herxheimer, H. (1949). *Brit. med. J.*, **2**, 901.
Herxheimer, H. (1952). *J. Physiol.*, **117**, 251–255.
Herxheimer, H. (1953). *Ibid.*, **122**, 49P–50P.
Herxheimer, H. (1955). *Brit. J. Pharmacol.*, **10**, 160–162.
Herxheimer, H. (1956). *Arch. int. Pharmacodyn.*, **106**, 371–380.
Herxheimer, H. & Rosa, L. (1953). *Brit. J. Pharmacol.*, **8**, 177–180.
Herxheimer, H. & Stresemann, E. (1960). *Arch. int. Pharmacodyn.*, **125**, 265–271.
Kallós, P. & Pagel, N. (1937). *Acta. med. scand.*, **91**, 292–305.
Katz, G. (1940). *Amer. J. Physiol.*, **129**, 735–43.
Koontz, A. R. & Shackelford, F. T. (1940). *Curr. Res. Anesth.*, **19**, 196–201.
Loew, E. R., Kaiser, M. E. & Moore, V. (1945). *J. Pharmacol.*, **83**, 120–219.
Loew, E. R., Kaiser, M. E. & Moore, V. (1946). *Ibid.*, **86**, 1–6.