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Release of adrenaline by anaphylaxis in the guinea-pig: its effect on lung lipid content

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The study was undertaken to discover whether the catecholamines released as a result of the stress and hypoxia of anaphylaxis were responsible for the concomitant loss of lipid from the lung. A method is described whereby the respiratory rate and volume and heart-rate of conscious sensitized guinea-pigs were measured and the electrocardiogram recorded during anaphylaxis induced by aerosolized antigen. After 7 days or more, some animals were anaesthetized with pentobarbitone and respiration was artificially maintained at the level recorded in the conscious state, whilst the quantity of catecholamines liberated during anaphylaxis was assayed using an extracorporeal blood circulation to superfuse smooth muscle preparations. In other animals of the same group, it was shown that intravenous infusion of adrenaline in a similar quantity to that detected in the circulation following anaphylaxis (0·3 μg min^{-1} for 40 min) caused losses of triglyceride and partial glycerides from the lungs. Thus, the loss of choline-containing phospholipid was attributed to the direct effects of the anaphylactic reaction on the lung tissue.

Anaphylaxis causes a loss of lipid from guinea-pig lung *in vitro* (Smith, 1962) and *in vivo* (Goadby & Smith, 1962, 1966; Grünspan & Rusovici, 1967). The loss of many of the lipid components results from the anoxia and release of histamine caused by anaphylaxis (Grünspan & Rusovici, 1967). However, the loss of choline phospholipid and sphingolipid is independent of these events (Goadby, 1975).

Release of catecholamines into the circulation of guinea-pigs following anaphylaxis has been demonstrated by Piper, Collier & Vane (1967). Catecholamines can induce lipolysis in adipose tissue (Gordon & Cherkes, 1958; Steinberg, 1963), and therefore the possibility that release of catecholamines by anaphylaxis could be responsible for the decrease in lung lipids was investigated.

MATERIALS AND METHODS

Materials

Chemical reagents and drugs were as described by Goadby (1975) with the addition of (−)-noradrenaline and (−)-adrenaline (Koch-Light) which were used as solutions in 0·9% (w/v) saline stabilized with L-ascorbic acid.

Sensitization and standardization of guinea-pigs

Guinea-pigs (250–350 g) of either sex were sensitized to egg albumen as described by Goadby (1975). Three weeks later, two nickel silver electrodes were placed on shaved areas on each side of the thorax and held in place with a harness of soft elastic rubber. The tension in the harness was maintained constant for each animal on this

and all subsequent occasions. An earth electrode was placed on the shaved right leg. The electrodes were connected to an Impedance Pneumograph (E & M Instruments, Texas, USA) and also to a High Gain Amplifier (E & M), to record the electrocardiogram (ecg). The output from the amplifier was also passed to a Cardiotach (E & M) which was triggered by the QRS spikes of the ecg. All signals were displayed on a multichannel pen recorder (E & M).

The guinea-pigs were placed in a Weldmesh cylinder, 8 cm in diameter and 23 cm long, the ends of which were closed by easily removed large corks. The cylinder restricted movements of the animal, but allowed free access to the air and any vapour or droplets contained therein. The animal in the cylinder was placed in a large glass desiccator and the "normal" respiration was recorded. An aerosol of 1% (w/v) egg albumen was then passed into the desiccator until the onset of dyspnoea and cough. The time of exposure was noted, and the animal was quickly removed from the chamber. Animals which suffered severe dyspnoea were artificially respired with oxygen containing 5% carbon dioxide to assist their recovery. Recording was continued throughout the exposure to antigen.

The experiments were repeated for each animal at weekly intervals for three weeks and a mean "collapse time" (Smith, 1967) for each animal was calculated. Mean rate and depth of respiration and mean heart rate were calculated for the time during which the animals were exposed to antigen.

Estimation of circulating catecholamines

At least one week after the last exposure to antigen, the animals were anaesthetized with pentobarbitone sodium (60 mg kg^{-1}). Respiration was maintained by a Miniature Ideal pump (Palmer, London). A sidearm on the tracheal cannula was connected to an apparatus which measured the overflow volume of air (Lessin & Kramer, 1969). Carotid arterial pressure was monitored by a pressure transducer (Bell and Howell, 4-327-L 221) connected to a cannula, a sidearm of which allowed blood to be impelled by a roller pump (Watson-Marlow MRHE 200) through a thin-walled, small bore, polythene tube, maintained at 37° by water in an outer tube, to an oxygenator, from which the warmed blood superfused a section of chick rectum (Mann & West, 1950) and a rat stomach strip preparation (Vane, 1957) arranged in series (Vane, 1969). The blood then passed to a collecting reservoir at 37°, from where it was returned to the jugular vein.

The tension of the smooth muscle preparations was recorded by two ST1 strain gauges (Ether Ltd., Welwyn, Herts.). The output from the gauges and transducers was simultaneously recorded on a Devices pen recorder. Body temperature was monitored by a rectal probe thermistor.

The harness and plate electrodes were applied, as previously, using the same tension as when the animal was conscious and respiration rate and depth, the ecg and the heart-rate were recorded as before. To ensure that the dose of antigen administered by aerosol to the anaesthetized animal was similar to that given to the conscious animal, the rate and depth of artificial respiration were adjusted to give a similar record to that of the unanaesthetized animal. An injection of adrenaline (0·1 μg) into the superfusion system was used to check that the tissues were responding to catecholamines. An aerosol of 1% (w/v) egg albumen was then passed to an expansion chamber before being administered to the guinea-pig at atmospheric pressure.

The animal received the aerosol for a time equal to its "normal collapse time". When the effects of anaphylaxis had abated, intravenous infusions of adrenaline and noradrenaline were administered from a constant speed injection apparatus (Braun, Germany) into the jugular vein.

Effect of adrenaline infusion on lung lipids

Guinea-pigs were anaesthetized with pentobarbitone (50 mg kg^{-1}) and the trachea and jugular vein cannulated. Artificial respiration was maintained by a Miniature Ideal pump. Fifteen minutes after cannulation, an infusion of adrenaline (15 μg ml^{-1}) in 0·9% (w/v) saline at a rate of 0·02 ml min^{-1} was commenced. The infusion was maintained for 40 min or until the animal was killed. Control animals were infused with saline at the same rate for the same time. Groups of four test and four control animals were killed 0·5, 1, 2 and 4 h after the commencement of the infusion. The lungs were excised and their lipid content was determined (Goadby, 1975).

RESULTS

The results of anaphylaxis in a restrained, conscious guinea-pig are shown as Fig. 1. The trace shows a slow decrease in the volume of each breath, with a cough occurring after 61 s. After 89 s there was a deep expiratory cough, after which the tidal volume showed a marked decrease. The animal was then removed from the aerosol chamber. The respiration depth and rate for each animal was calculated over a period of 10 s at the start of the aerosol, over 20 s in the middle of the recording and over 10 s before the end. The mean rate and depth of respiration used in the experiments under anaesthesia was the mean of these three observations.

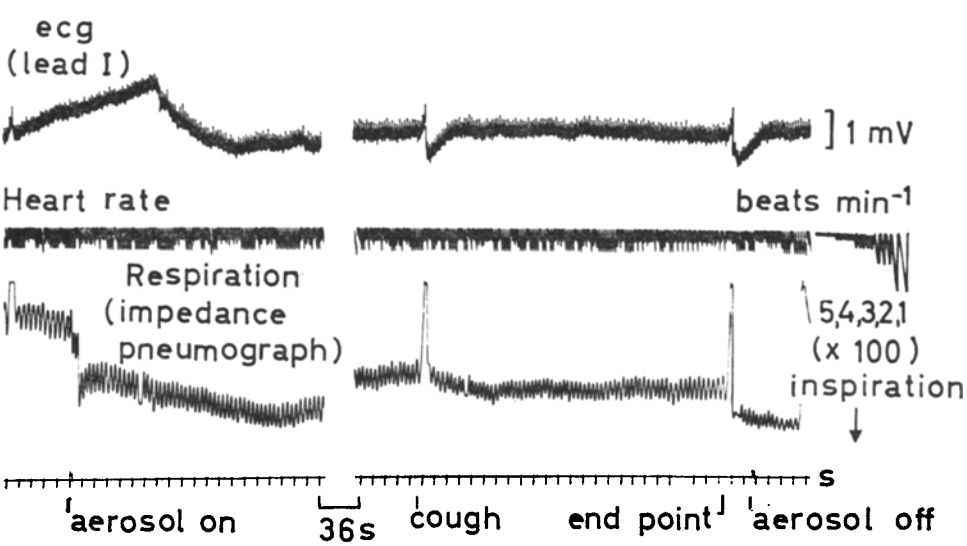

FIG. 1. Effects of anaphylaxis induced by aerosolized antigen on the respiration and cardiac function of the conscious guinea-pig. Aerosol produced by applying air at 15 pounds per square inch to a 1% (w/v) egg albumen solution in a nebulizer (Wright, 1958). Respiration rate and depth measured by impedance pneumograph. Time in seconds.

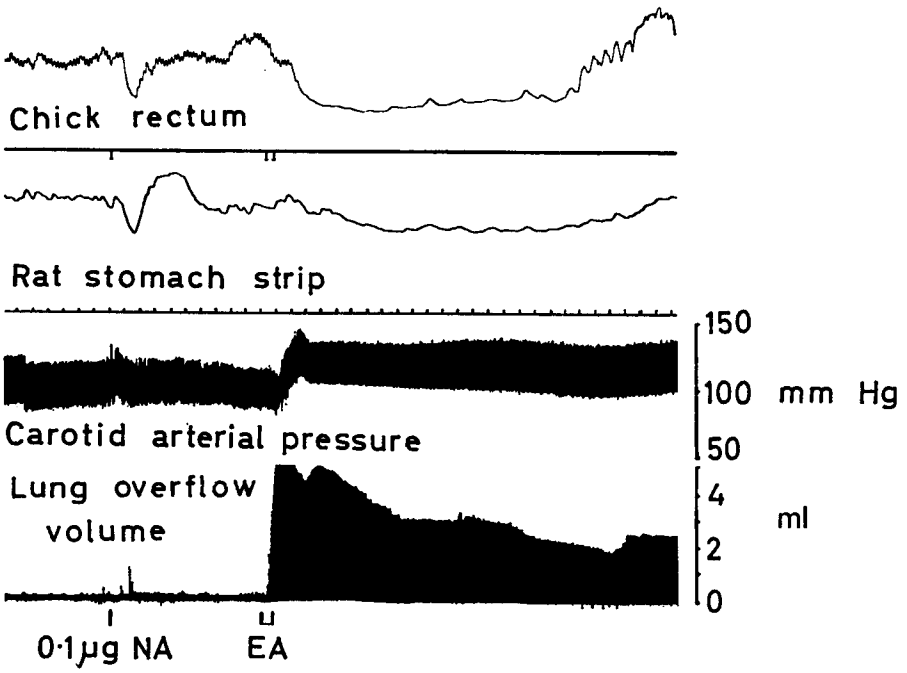

FIG. 2. Detection of catecholamines in the circulation of an artificially-respired, anaesthetized guinea-pig, subjected to anaphylaxis by aerosolized antigen, by means of an extracorporeal blood circuit superfusing smooth muscle tissues. NA - noradrenaline injected into the superfusion circuit. EA = 33 s aerosol of 1% egg albumen solution. Time in min.

Fig. 2 shows a record of the effects of anaphylaxis in the anaesthetized guinea-pig. During anaphylaxis there was a sharp reduction in respiratory volume which was followed by a rise in arterial blood pressure and this, in turn, was followed by a fall in the tension in the chick rectum and rat stomach preparations. The tissues had returned to their pre-anaphylaxis tensions after 28 min. The respiratory volume increased over the next 90 min, but did not return to the pre-shock value for the remainder of the experiment. The mean arterial pressure also continued above its original value for the remainder of the experiment.

When the smooth muscle preparations had regained their normal tensions, infusions of adrenaline and noradrenaline were administered. A fixed time of 5 min was used for the determination of catecholamine activity, since this was usually sufficient to establish a constant reduction in tension of the isolated tissues and longer infusions reduced their viability. The results of one such experiment are given in Table 1. The degree of reduction of tension in the smooth muscle preparations increased with increase in the rate of infusion of catecholamines. The time of depression of tension was partly influenced by the rate of infusion, but the major influence was the time of infusion.

The rates of release of noradrenaline and adrenaline following anaphylaxis were calculated from the relative reductions in tension of the two tissues using the method of Gaddum & Lembeck (1949) as used by Burn (1950). The duration of catecholamine release following anaphylaxis was calculated as the mean of the times during which the tensions in the smooth muscle preparations were reduced below normal.

The calculated rates of release of adrenaline and noradrenaline in eight guinea-pigs subjected to anaphylaxis in similar experiments are shown in Table 2. The rate of adrenaline release was always greater than that of noradrenaline and showed less variability, i.e. mean adrenaline $= 0{\cdot}30 \pm 0{\cdot}05\ \mu g\ min^{-1}$, whilst mean noradrenaline $= 0{\cdot}05 \pm 0{\cdot}02\ \mu g\ min^{-1}$. The duration of release of both amines showed wide differences and these contributed to the large variations in total adrenaline release (1·53 to 23·65 μg) and total noradrenaline release (0·11 to 8·99 μg).

The results of the lipid analyses are shown in Table 3. Adrenaline infusion caused a marked fall in triglyceride content after 0·5 h and a fall in partial glycerides after 1 h. However, all of the animals killed at 1 h or later showed a reduced content of partial glyceride when compared with the animals so treated at 0·5 h.

The only other significant changes occurred in the sphingolipid content at 2 h and in the cholesterol ester content at 1 h. Both results pose problems in evaluation. The sphingolipid content of the control lungs at 2 h was greater than that found in any other group of lungs and therefore, the difference between it and the test group was

Table 1. *Estimation of catecholamines released after anaphylaxis in the anaesthetized guinea-pig from data obtained by superfusion of arterial blood over smooth muscle preparations.* Guinea-pig: Albino R_0L_1, 650 g, ♂, mean "Collapse Time" = 58 s.

Treatment	Rat stomach strip		Chick rectum	
	Relaxation (mm)	Time (min)	Relaxation (mm)	Time (min)
Anaphylaxis	9	44	20	51
Adrenaline infusions (10 μg ml^{-1} in normal saline)				
5 min, 0·15 μg min^{-1}	4	7	11·5	8
5 min, 0·3 μg min^{-1}	7	6·5	17	7·5
5 min, 0·6 μg min^{-1}	10	6·5	22·5	8·5
5 min, 1·2 μg min^{-1}	12	6·5	21	9·5
Noradrenaline infusions (10 μg ml^{-1} in normal saline)				
5 min, 0·6 μg min^{-1}	8	6·5	1·5	2·5
5 min, 1·2 μg min^{-1}	11	8	4	3·5
5 min, 2·25 μg min^{-1}	13	7·5	7·5	5·5

Rat stomach strip.
By calculation 0·75 μg min^{-1} adrenaline $\equiv$ 1 μg min^{-1} noradrenaline and
anaphylaxis releases $\equiv$ 0·52 μg min^{-1} adrenaline
Thus, if anaphylaxis releases A μg min^{-1} adrenaline and N μg min^{-1} noradrenaline then

$$A + 0{\cdot}75N = 0{\cdot}52 \quad .. \quad .. \quad .. \quad (1)$$

Chick rectum
By calculation 0·042 μg min^{-1} adrenaline $\equiv$ 1 μg min^{-1} noradrenaline and
anaphylaxis releases $\equiv$ 0·48 μg min^{-1} adrenaline
therefore

$$A + 0{\cdot}042N = 0{\cdot}48 \quad .. \quad .. \quad .. \quad (2)$$

From (1) and (2)

$$N = \frac{0{\cdot}52 - 0{\cdot}48}{0{\cdot}75 - 0{\cdot}042} = 0{\cdot}056\ \mu g\ min^{-1}$$

$$A = \frac{(0{\cdot}75 \times 0{\cdot}48) - (0{\cdot}042 \times 0{\cdot}52)}{(0{\cdot}75 - 0{\cdot}042)}$$

$$= 0{\cdot}478\ \mu g\ min^{-1}$$

Table 2. *Catecholamine content of arterial blood of anaesthetized guinea-pigs after anaphylaxis induced by aerosolized antigen.*

Animal	Collapse time (s)	*Adrenaline release (ng min^{-1})	*Nor-adrenaline release (ng min^{-1})	Mean time of release (min)	Total adrenaline release (μg)	Total nor-adrenaline release (μg)
A1	58	478	56	47·5	23·65	2·66
A2	75	301	4	28	8·43	0·11
C	64	188	37·5	24	4·51	0·90
B1	60	383	155	58	22·21	8·99
B2	75	200	36	43	8·60	1·55
B3	58	450	13	41·5	18·68	0·54
G	67	153	15	10	1·53	0·15
S	77	186	44	62	11·35	2·73

Mean release of adrenaline = 294·5 ± 47·5 ng min^{-1}.
Mean release of noradrenaline = 45 ± 17 ng min^{-1}.
Mean time of release of catecholamines = 39·25 ± 6·2 min.
* Calculated from superfusion data by the method of Gaddum & Lembeck (1949) as described by Burn (1950).

Table 3. *Lipid content of lungs of anaesthetized guinea-pigs after infusion of adrenaline (0·3 μg min^{-1} for 30 or 40 min).*

Lipid fraction	Lipid content (mg lipid g^{-1} dry lung) Time after infusion (h) 0·5 Control	0·5 Test	1 Control	1 Test	2 Control	2 Test	4 Control	4 Test**
Cholesterol ester	0·2 ± 0·1	0·2 ± 0·1	0·4 ± 0·1	*0·3 ± 0·1	0·9 ± 0·1	0·7 ± 0·1	0·6 ± 0·1	0·5 ± 0·1
Cholesterol	19·3 ± 1·5	19·5 ± 0·8	23·1 ± 1·1	19·6 ± 1·7	25·8 ± 1·3	23·8 ± 1·8	20·2 ± 1·1	20·5 ± 0·4
Triglyceride	6·2 ± 1·1	*2·9 ± 0·8	6·8 ± 0·9	5·1 ± 1·8	10·2 ± 1·8	13·4 ± 3·1	4·5 ± 0·7	7·3 ± 0·4
Partial glycerides	4·9 ± 0·4	4·8 ± 0·6	4·1 ± 0·5	*2·2 ± 0·2	3·0 ± 0·3	4·5 ± 1·3	3·9 ± 0·9	3·2 ± 0·4
Choline phospholipid	48·6 ± 6·1	39·3 ± 2·1	61·6 ± 1·6	55·1 ± 3·7	56·2 ± 7·8	53·5 ± 2·6	56·7 ± 2·0	58·6 ± 3·7
Ethanolamine phospholipid	36·1 ± 2·4	40·1 ± 1·1	35·9 ± 2·9	32·0 ± 1·9	34·3 ± 0·6	31·8 ± 0·3	33·2 ± 0·3	34·8 ± 0·8
Sphingolipid	3·2 ± 0·2	2·7 ± 0·4	4·3 ± 0·5	3·5 ± 0·1	7·0 ± 1·3	*3·2 ± 0·2	4·1 ± 0·4	3·8 ± 0·2

Results are expressed as mean ± standard error of the mean (n = 4); **n = 3.
* Significantly different from controls ($P < 0·05$).

Table 4. *A summary of lipid changes occurring in guinea-pig lungs after several* in vivo *experimental procedures.*

Lipid fraction	Anaphylaxis*	Histamine* aerosol	Nitrogen* anoxia	Adrenaline infusion
Cholesterol ester	Prolonged fall	Prolonged fall	No change	No change
Cholesterol	Delayed fall	Delayed fall	Delayed rise	No change
Triglyceride	Delayed transient fall	Delayed transient fall	No change	Transient fall
Partial glyceride	Prolonged fall	Fall	Fall	Delayed transient fall
Choline phospholipid	Fall	No change	No change	No change
Ethanolamine phospholipid	Transient fall	Fall	Transient fall	No change
Sphingolipid	Fall	No change	No change	Delayed transient fall?

* Data from Goadby (1975).

treated with caution. The cholesterol ester fraction results also require cautious evaluation because, although there is a difference between the test and control groups at 1 h, this difference is much smaller than the differences between control groups.

The results for 4 h are for a group of three animals only because of difficulty in maintaining the animals under anaesthesia for 4 h after the adrenaline infusion.

DISCUSSION

The superfusion of smooth muscle preparations by an extracorporeal blood circuit was used by Piper & others (1967) to show that catecholamines were released into the circulation of the guinea-pig infused with the agents released in anaphylaxis. The experiments reported here extend these findings and show that anaphylactic shock causes the release of catecholamines. The amount of adrenaline released was much greater than that of noradrenaline. Hypoxic blood caused a reduction in the tension of the smooth muscle preparations and hence an oxygenator was placed between the guinea-pig and the tissues.

In all the animals, the increases in respiratory overflow volume and arterial blood pressure caused by anaphylaxis persisted after the tissues in the extra-corporeal circuit had returned to their original tension. One explanation is that substances released by anaphylaxis (e.g. bradykinin and prostaglandins) continued to be released into the circulation and caused the bronchoconstriction (Piper & Vane, 1969), and also increased the tone in the smooth muscle preparations. This could mean that there was still an increased level of circulating catecholamines when the tissues returned to their original tension which would explain the increased arterial pressure and heart rate. Alternatively the relaxation of the tissues could have been brought about by agents other than catecholamines. However, all the other chemical substances reported as being released by anaphylaxis in guinea-pig lung, contract or have no effect on these smooth muscle preparations (Piper & Vane, 1969). It is unlikely that the relaxation of the tissues was due to hypoxia since the oxygenator maintained the resting tone in the preparations even when the artificial respiration of the donor guinea-pig was removed.

The standard infusions of adrenaline and noradrenaline were administered only after anaphylaxis because catecholamines administered before antigen challenge decrease the anaphylactic response (Smith, 1967; Assem & Schild, 1971). This would also take account of any change in sensitivity of the detecting tissues.

Infusion of adrenaline in a similar quantity to that detected in the superfusion experiments and under similar conditions (e.g. same anaesthetic; same rate and depth of respiration) produced transient decreases in the triglyceride and partial glyceride fractions. The sphingolipid content of the lungs 2 h after anaphylaxis was significantly lower than that of the controls. However, since the control value for this group was significantly greater than the control values at all other times, the result was treated with caution. The data show that release of adrenaline into the circulation could account for the decrease in lung glycerides seen in guinea-pigs subjected to anaphylaxis, histamine aerosol and nitrogen anoxia (Goadby, 1975).

A summary of the effects on lung lipids of anaphylaxis and experiments to simulate events occurring subsequent to anaphylaxis is shown as Table 4. Only two changes in the lung lipids do not appear to be the result of secondary events. These are the

loss of choline phospholipid and the loss of sphingolipid. It is concluded that these and previous experiments (Goadby, 1975) provide evidence that loss of choline-containing phospholipids from guinea-pig lungs occurs as a direct result of anaphylaxis and not as a result of events occurring subsequently.

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REFERENCES

ASSEM, E. S. K. & SCHILD, H. O. (1971). *Int. Archs Allergy Appl. Immun.*, **40**, 576–589.
BURN, J. H. (1950). *Biological Standardisation*, Oxford University.
GADDUM, J. H. & LEMBECK, F. (1949). *Br. J. Pharmac. Chemother.*, **4**, 401–408.
GOADBY, P. & SMITH, W. G. (1962). *J. Pharm. Pharmac.*, **14**, 739–745.
GOADBY, P. & SMITH, W. G. (1966). *Biochem. J.*, **100**, 30–31P.
GOADBY, P. (1975). *J. Pharm. Pharmac.*, **27**, 248-253.
GORDON, R. S. Jnr. & CHERKES, A. (1958). *Proc. Soc. exp. Biol. Med.*, **97**, 150–151.
GRUNSPAN, M. & RUSOVICI, L. (1967). *Archs int. Physiol. Biochim.*, **95**, 277–282.
LESSIN, A. W. & KRAMER, R. L. (1969). *J. Pharm. Pharmac.*, **21**, 309–313.
MANN, M. & WEST, G. B. (1950). *Br. J. Pharmac. Chemother.*, **5**, 173–177.
PIPER, P. J. COLLIER, H. O. J. & VANE, J. R. (1967). *Nature, Lond.*, **213**, 838–840.
PIPER, P. J., & VANE, J. R. (1969). *Ibid.*, **223**, 29–35.
SMITH, W. G. (1962). *Biochem. Pharmac.*, **11**, 183–185.
SMITH, W. G. (1967). *Canad. J. pharm. Sci.*, **2**, 55–64.
STEINBERG, D. (1963). *Biochem. Soc. Symp.*, **24**, 11–143.
VANE, J. R. (1957). *Br. J. Pharmac. Chemother.*, **12**, 344–349.
VANE, J. R. (1969). *Ibid.*, **23**, 360–375.
WRIGHT, B. M. (1958). *Lancet*, **2**, 24–25.