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Investigation of changes in the lipid content of guinea-pig lung after anaphylaxis

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A detailed analysis of the lipid content of guinea-pig lung following anaphylaxis *in vivo* induced by aerosolized antigen showed a significant reduction in all fractions. Anoxia induced by nitrogen produced reductions in the partial glycerides and ethanolamine phospholipid. Exposure to an aerosol of histamine caused a reduction in all but the choline phospholipid and sphingolipid fractions. It was concluded that the losses of choline phospholipid and sphingolipid result from the anaphylactic reaction and not from subsequent changes.

A fall in the phospholipid content and a short-lived rise in the glyceride content of the lungs of guinea-pigs subjected to anaphylaxis by the microshock technique of Herxheimer (1952) was observed by Goadby & Smith (1962). The fall of phospholipid was prevented by a dose of hydrocortisone which reduced the amount of Slow Reacting Substance released by anaphylaxis. However, the methods of lipid analysis were simple and would only detect gross changes and it was also observed that the fall in phospholipid was greater in lungs removed 1 h after anaphylaxis than at earlier times. A more detailed analysis, using an extended time scale, has now been made.

A change in the lipid content of lungs could be caused directly by the antigen-antibody reaction, or alternatively, could result from events occurring subsequent to anaphylaxis. An attempt was made to elucidate the effects on the lipid content of lung of these secondary events.

The main symptom of anaphylaxis in guinea-pig lung is contraction of the bronchial muscles resulting in severe hypoxia (Auer & Lewis, 1910; Herxheimer, 1952). Therefore, the effect of anoxia on lung lipids was determined.

Histamine is released in guinea-pig lungs during anaphylaxis (Bartosch, Feldberg & Nagel, 1932), and it has been shown that administration of histamine, as an aerosol gives symptoms of dyspnoea and cough apparently similar to the effects of administering aerosolized antigen to sensitized guinea-pigs (Armitage, Herxheimer & Rosa, 1952). Thus, the effect of histamine aerosol on lung lipids was also investigated.

MATERIALS AND METHODS

Sensitization of guinea-pigs

Guinea-pigs (250–350 g) of either sex were sensitized by the intraperitoneal injection of 2 ml of 5% (w/v) egg albumen solution. Three weeks later, they were exposed to an aerosol of 1% (w/v) egg albumen until the onset of dyspnoea and cough as described by Goadby & Smith (1964). The exposure was repeated at weekly intervals for a further two weeks and the mean time to onset of dyspnoea and cough for each animal was calculated. This was termed the "collapse time" for the particular animal. The animals were arranged in groups of eight such that the group mean

"collapse time" was between 60 and 90 s and none of the animals exhibited a "collapse time" outside the range of 45 to 105 s.

Anaphylaxis in guinea-pigs

One week after the last exposure to antigen, groups of standardized sensitized animals were exposed to aerosolized antigen for their individual "collapse time" and were killed 0·5, 1, 2, 4 and 8 h later. Control animals did not receive antigen on this occasion. The lung lipids were extracted and analysed as described below.

Anoxia in guinea-pigs

Standardized, sensitized guinea-pigs were placed individually in a glass chamber and nitrogen was passed in at 5 lb inch^{-2} and a flow rate of 50 ml min^{-1}. After 2 min the animal became ataxic and after a further minute the animal was unable to stand. This phase was sometimes accompanied by clonic convulsions. After about 5 min the animals underwent severe convulsions and were promptly removed.

Groups of eight animals underwent this procedure and were killed 0·5, 1, 2 and 4 h later. Control animals were exposed to air under the same conditions. The lungs were excised, extracted and their lipid content determined as described below.

Histamine aerosol

Guinea-pigs (350–450 g) were exposed to an aerosol produced by applying air at 15 lb inch^{-2} to a 5% (w/v) solution of histamine (as acid phosphate) contained in a nebulizer (Wright, 1958). The animals were removed at the onset of dyspnoea and cough, and the time (collapse time) recorded. This treatment was repeated weekly for a further two weeks to enable the mean "collapse time" to be calculated.

The animals were then arranged in groups of eight such that the group mean "collapse time" was between 55 and 75 s. One week later they were exposed to histamine aerosol for their collapse time and were killed 0·5, 1, 2 and 4 h later. Control animals were killed without exposure to histamine. The lungs were then extracted and analysed as described below.

Extraction of lung lipids

Guinea-pigs were killed by a blow on the occiput and their lungs were excised. They were perfused free of blood via the pulmonary circulation with aerated Tyrode solution at 37° for 10 min at a rate of 2 ml min^{-1}, whilst being artificially respired with 4·5 ml of air 58 times min^{-1}. The lung tissue was then removed from the trachea and major bronchi and chopped into pieces 1 mm^3 before being freeze-dried.

The freeze-dried lung was powdered, weighed and the lipids were extracted for 18 h at room temperature (*ca* 18°) with chloroform-methanol (2:1). The solvent solution was filtered off and its volume recorded before it was dried *in vacuo*. The residue was taken up in the same volume of chloroform, 5 g of activated silicic acid added and stirred for 10 min and the silicic acid then filtered off, aliquots of the filtrate were analysed for neutral lipid. The silicic acid was shaken with the same volume of methanol as the original volume of extract and, after filtration, the methanol eluate was analysed for phospholipid.

Neutral lipid analysis

Silicic acid (Mallinkrodt, 100 mesh) was activated at 115° for 12 h and 1 g samples were slurried in 10 ml diethyl ether and poured into glass columns (15 × 0·8 cm) as

described by Lis, Okey & Tinoco (1961). The silicic acid was allowed to settle under gravity and the column was conditioned by washing with 3 ml acetone–diethyl ether (1:1), 5 ml diethyl ether and 20 ml light petroleum (60°–80°) successively.

Each lipid sample was taken to dryness *in vacuo*, redissolved in light petroleum and placed on a conditioned column. The column was eluted with (1) 30 ml 1% (v/v) diethyl ether in light petroleum (60°–80°); (2) 30 ml 4% (v/v) ether in light petroleum, (3) a further 10 ml of this mixture of solvents and (4) 40 ml 100% diethyl ether. The volume of each recovered fraction was measured and duplicate aliquots were used to determine the glyceride content by the method of Van Handel, Zilversmit & Bowman (1957) and the cholesterol content by the method of Hanel & Dam (1955).

Phospholipid analysis

Samples were dried *in vacuo* below 40° and redissolved in 50 ml of chloroform–methanol (1:1). Aluminium oxide (8 g, chromatographic grade, BDH) was added and stirred with the solution for 10 min. The powder was allowed to settle and filtered off; the filtrates were pooled.

The lipid phosphorus in the filtrate (choline phospholipid) was determined by the method of Bartlett (1959). The aluminium oxide was transferred to 50 ml of chloroform–ethanol–water (2:5:2) and stirred for 10 min before being filtered off and washed with 50 ml of solvent which after filtration, was mixed with the former filtrate. The phospholipid contained in this fraction was termed ethanolamine phospholipid. The lipid phosphorus content was determined as above.

A portion of the choline phospholipid fraction (80 ml) was taken to dryness *in vacuo* and 20 ml of 0·1M alcoholic sodium hydroxide solution was added and incubated at 37° for 30 min. The solution was then neutralized with 0·2 ml of concentrated hydrochloric acid and dried *in vacuo*. The residue was dissolved in 3 ml of upper phase and 6 ml of lower phase of a mixture of water–isobutanol–chloroform (3:2:4) with vigorous shaking. The phases were cleared by centrifugation (1000 *g* for 10 min) and the upper layer was removed and discarded. The lower layer was dried *in vacuo* and redissolved in 20 ml chloroform to which 1 g of activated silicic acid was added with stirring. After 10 min, the silicic acid was filtered off, dried at low temperature (<40°) and shaken with 20 ml of methanol. The methanol contained sphingomyelin and its lipid phosphorus content was determined.

Materials

Solvents were of analytical grade (British Drug Houses, Poole, Dorset) and were redistilled before use.

Standard tripalmitin, 1,3-dipalmitin, and monopalmitin (all 99%) were generously donated by Unilever Research Laboratories, Port Sunlight, Cheshire. L-α Lecithin, phosphatidylethanolamine, phosphatidyl-l-serine and sphingomyelin were obtained from Koch-Light Laboratories Ltd., Colnbrook, Bucks., and were of chromatographic grade (>95%). Cholesterol (99%) and cholesterol oleate (reputed 99%) were obtained from Sigma Chemical Co., London.

RESULTS

The analytical method was devised to provide information on the maximum number of fractions whilst at the same time allowing the multiple simultaneous determinations necessary to take account of animal to animal variation in response.

When tested with a mixture of known lipids, recoveries greater than 95% were obtained for triglyceride, partial glycerides, ethanolamine phospholipid and sphingolipid. However, recovery of cholesterol ester was low (60%), particularly with small column loads.

Analysis of guinea-pig lung lipids by this method and by the methods of Hirsch & Ahrens (1958) for neutral lipid, and Hanahan, Dittmer & Waraschina (1957) for phospholipid, produced results which were not significantly different.

Table 1. *Lipid content of guinea-pig lungs after anaphylaxis* in vivo *induced by aerosolized antigen.*

Lung lipid fraction	Control	Lipid content (mg g^{-1}) Time after anaphylaxis				
		0·5 h	1 h	2 h	4 h	8 h
Cholesterol ester	1·1 ± 0·2	0·8 ± 0·1	*0·5 ± 0·1	*0·5 ± 0·1	*0·5 ± 0·1	*0·6 ± 0·1
Cholesterol	19·8 ± 1·0	*13·3 ± 0·6	16·1 ± 0·9	16·8 ± 1·0	20·4 ± 1·0	20·0 ± 0·7
Triglyceride	12·0 ± 1·5	7·6 ± 1·0	*4·7 ± 0·5	8·9 ± 1·1	8·8 ± 1·3	9·9 ± 0·4
Partial glyceride	6·7 ± 0·8	*3·8 ± 0·3	5·7 ± 0·4	*4·0 ± 0·3	4·7 ± 0·6	*4·2 ± 0·2
Choline phospholipid	63·8 ± 2·8	59·1 ± 3·1	*50·1 ± 2·7	61·9 ± 3·1	61·1 ± 1·7	57·6 ± 2·0
Ethanolamine phospholipid	50·6 ± 1·4	*43·3 ± 1·8	52·9 ± 2·4	53·7 ± 1·8	51·5 ± 5·4	52·1 ± 4·0
Sphingolipid	5·8 ± 0·5	*4·1 ± 0·5	*4·1 ± 0·6	5·6 ± 0·2	5·7 ± 0·6	5·1 ± 0·3

Values expressed as mg lipid per g dry lung
The mean and its standard error are shown. n = 8 for test groups. n = 16 for controls.
* Significantly different from control ($P < 0·05$).

The results of the analysis of guinea-pig lungs after anaphylaxis induced by aerosolized antigen are shown in Table 1 where they are compared to a group comprising all the control animals. There was a loss of lipid in all fractions. The falls in the phospholipid fractions had all returned to control level within 2 h. The decrease in free cholesterol had returned to normal after 4 h. The mean triglyceride content was not significantly different from the control value at 2 h, but the partial glyceride and cholesterol ester fractions were still significantly lower than the controls after 8 h.

Nitrogen-induced anoxia did not significantly affect the lung content of cholesterol ester, triglyceride, choline phospholipid or sphingolipid (Table 2). The mean content of free cholesterol 0·5 and 1 h after anoxia was elevated above that of the controls, but the difference was not significant until 2 h after anoxia; it had returned to normal at 4 h. There was a significant fall in partial glyceride content at 0·5 and 1 h and, though the mean value was still below that of the controls at 4 h, the difference was not significant. The ethanolamine phospholipid content 0·5 h after anaphylaxis was significantly reduced and remained below the control level at 1 h but the difference was not significant.

Exposure of animals to histamine aerosol resulted in a reduction in the mean content of ethanolamine phospholipid at 0·5, 1 and 2 h which was significantly different from the control value at 1 h (Table 3). There was an increase in free cholesterol at 1 and 2 h and a reduction in triglyceride at 1 h. The mean content of partial glyceride and cholesterol ester was reduced below control values in all lungs analysed after exposure to histamine aerosol.

Table 2. *Lipid content of guinea-pig lungs after severe anoxia induced by nitrogen.*

Lung lipid fraction	Control	Lipid content (mg g^{-1}) Time after anaphylaxis			
		0·5 h	1 h	2 h	4 h
Cholesterol ester	1·1 ± 0·2	0·8 ± 0·3	0·9 ± 0·2	1·3 ± 0·4	0·7 ± 0·1
Cholesterol	19·8 ± 1·0	25·3 ± 1·1	25·2 ± 1·4	*28·2 ± 0·4	22·2 ± 1·1
Triglyceride	12·0 ± 1·5	9·5 ± 2·4	15·6 ± 2·3	8·8 ± 0·7	10·7 ± 1·9
Partial glycerides	6·7 ± 0·8	*2·9 ± 0·3	*2·9 ± 0·2	3·7 ± 0·4	5·0 ± 0·4
Choline phospholipid	63·8 ± 2·8	64·1 ± 2·9	59·5 ± 3·2	62·1 ± 0·9	61·3 ± 2·0
Ethanolomine phospholipid	50·6 ± 1·4	*39·9 ± 0·8	44·5 ± 0·6	51·3 ± 0·8	47·1 ± 1·1
Sphingolipid	5·8 ± 0·5	6·2 ± 0·4	5·1 ± 0·2	7·3 ± 0·1	5·4 ± 0·2

Values are expressed as mg lipid per g dry lung.
The mean and its standard error are shown n = 8 for test groups, n = 16 for controls.
* Significantly different from control ($P < 0·05$).

Table 3. *Lipid content of guinea-pig lungs after exposure to histamine aerosol (50 mg ml^{-1}).*

Lung lipid fraction	Control	Lipid content (mg g^{-1}) Time after anaphylaxis			
		0·5 h	1 h	2 h	4 h
Cholesterol ester	1·1 ± 0·2	*0·4 ± 0·1	*0·2 ± 0·1	*0·2 ± 0·1	*0·3 ± 0·2
Cholesterol	19·8 ± 1·0	22·8 ± 0·4	*25·1 ± 0·9	*26·5 ± 0·7	22·1 ± 0·6
Triglyceride	12·0 ± 1·5	12·8 ± 1·8	*4·0 ± 0·4	5·9 ± 0·7	21·8 ± 2·6
Partial glycerides	6·7 ± 0·8	*3·2 ± 0·2	*3·6 ± 0·2	*1·9 ± 0·4	4·2 ± 0·5
Choline phospholipid	63·8 ± 2·8	61·8 ± 2·1	58·6 ± 3·0	59·3 ± 2·4	62·3 ± 2·2
Ethanolamine phospholipid	50·6 ± 1·4	46·3 ± 2·0	*41·9 ± 0·8	43·8 ± 2·3	53·1 ± 1·5
Sphingolipid	5·8 ± 0·5	6·2 ± 0·5	6·0 ± 0·5	4·9 ± 0·3	5·6 ± 0·3

Values are expressed as mg lipid per g dry lung
The mean and its standard error are shown n = 8 for test groups, n = 16 for controls.
* Significantly different from control ($P < 0·05$).

DISCUSSION

This study provided information on a greater number of lipid fractions than previously (Goadby & Smith, 1962). The methods used permit the processing of multiple samples. Reliable results were obtained for all fractions except the cholesterol ester, recovery of which was poor, and therefore the results obtained for it were treated with caution.

Anaphylaxis caused a decrease in lung content of all lipid fractions studied. This contrasted with an earlier study (Goadby & Smith, 1962) which had indicated a rise in the glyceride fraction following anaphylaxis. A study of the earlier results revealed that certain animals had high (2–5 times normal) contents of lung glyceride and although all the groups were comparable in age, sex and "collapse times", a number of the animals having abnormal glyceride levels had been included in the groups killed 30 min after anaphylaxis. Experiments to determine whether this high glyceride content was associated with age, sex or diet were inconclusive, but did reveal that the wide differences in glyceride content probably resulted from differences in the

triglyceride fraction. All the animals in the present study were standardized for age and diet. The only changes which persisted beyond 2 h were decreases in cholesterol ester and partial glyceride and these were still evident 8 h afterwards. Consequently, all subsequent experiments were terminated after 4 h.

Similar changes in the lipid content of guinea-pig lung after anaphylaxis were reported by Grünspan & Rusovici (1967) who analysed lungs of animals that had been subjected to anoxia or exposure to histamine. Their conclusion was that the changes in lung lipid content were the result of events occurring subsequent to anaphylaxis. In the more detailed analysis reported here, this statement is shown to be valid for all but the choline phospholipid and sphingolipid fractions.

Histamine given by aerosol caused changes in the neutral lipids similar to those caused by anaphylaxis, but with little loss of phospholipids. Cuvelier, Andraud & others (1967) showed that histamine, given by aerosol or injection, caused similar losses of guinea-pig lung lipids. They found a larger decrease in total phospholipids than might have been expected from our findings. However, they did not further separate this fraction and it is possible that the changes which they report could be due to a decrease in ethanolamine phospholipid (compare Table 3).

Hypoxia and hypercapnia occur during anaphylaxis in the guinea-pig. Attempts to induce anaphylactic-type symptoms by administering increasing concentrations of carbon dioxide in air to guinea-pigs were unsuccessful, but symptoms apparently similar to those of anaphylaxis were produced when the air in the chamber was progressively replaced by nitrogen. This treatment produced very few changes in lung lipid content, decreases in the partial glyceride and ethanolamine phospholipid fractions and a slight increase in cholesterol being the only effects noted.

These findings indicate that, whilst many of the changes in lung lipid content following anaphylaxis could be the result of the action of released histamine and/or anoxia, the losses of choline phospholipid and sphingolipid appear to be independent of these actions. Thus, the experiments provide evidence of the activity of phospholipase during anaphylaxis. This conclusion derives support from the findings of Högberg & Uvnäs (1960) and Giacobini, Sedvall & Unväs (1965) that an enzyme of this sort is concerned in the release of histamine.

REFERENCES

ARMITAGE, P., HERXHEIMER, H. & ROSA, L. (1952). *Br. J. Pharmac. Chemother.*, **7**, 625–636.

AUER, J. & LEWIS, P. A. (1910). *J. exp. Med.*, **12**, 151–175.

BARTLETT, G. R. (1959). *J. biol. Chem.*, **234**, 466–468.

BARTOSCH, R., FELDBERG, W. & NAGEL, E. (1932). *Arch. ges. Physiol., Pflüger's*, **230**, 129–153.

CUVELIER, R., ANDRAUD, G., TRONCHE, P. & DOREL, M. (1967). *Compt. rend. Soc. Biol.*, **161**, 315–317.

GIACOBINI, E., SEDVALL, G. & UVNÄS, B. (1965). *Exp. Cell Res.*, **37**, 368–375.

GOADBY, P. & SMITH, W. G. (1962). *J. Pharm. Pharmac.*, **14**, 739–745.

GOADBY, P. & SMITH, W. G. (1964). *Ibid.*, **16**, 108–114.

GRÜNSPAN, M. & RUSOVICI, L. (1967). *Archs int. Physiol. Biochem.*, **75**, 277–282.

HANEL, H. K. & DAM, H. (1955). *Acta chem. scand.*, **9**, 677–682.

HANAHAN, D. J., DITTMER, J. C. & WARASCHINA, E. (1957). *J. biol. Chem.*, **228**, 685–700.

HERXHEIMER, H. (1952). *J. Physiol., Lond.*, **117**, 251–255.

HIRSCH, J. & AHRENS, A. H. (1958). *J. biol. Chem.*, **233**, 311–320.

HÖGBERG, B. & UVNÄS, B. (1960). *Acta physiol. scand.*, **48**, 133–145.

LIS, E. W., OKEY, R. & TINOCO, J. (1961). *Analyt. Biochem.*, **2**, 100–106.

VAN HANDEL, E., ZILVERSMIT, D. B. & BOWMAN, K. (1957). *J. Lab. clin. Med.*, **50**, 152–157.

WRIGHT, B. M. (1958). *Lancet*, **2**, 24–25.