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Effects of anaphylaxis *in vivo* on the lipid and protein content of guinea-pig serum and extracellular fluid of lung tissue

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Determinations of total cholesterol, phospholipid and glyceride in serum samples obtained from guinea-pigs by cardiac puncture before and after anaphylaxis *in vivo* indicated reductions occurred in all three fractions after anaphylactic shock. The fall in glyceride was preceded by a rise. Anaphylaxis also induced an accumulation of lipid in oedema fluid in the lungs. These changes were reduced in animals protected from anaphylaxis by pretreatment with a combination of mepyramine and ethanolamine or mepyramine and hydrocortisone.

CHANGES in the lipid content of isolated sensitised guinea-pig lungs after anaphylaxis *in vitro* have been previously reported (Smith, 1962). Ethanolamine, which posssesses anti-anaphylactic activity (Smith, 1961) prevented some but not all of these changes. Similar changes in the lung lipids were noted *in vivo* by Goadby & Smith (1962) who showed that the exposure of sensitised guinea-pigs to an aerosol of antigen solution caused alterations in the lipid metabolism (see Smith, 1964). The major change was a substantial loss of phospholipid from the lungs. This could be prevented by pretreatment of the animals with hydrocortisone sodium hemisuccinate. A loss of phospholipid from isolated perfused guinea-pig lungs, after injection of histamine-releasing agents, has also been reported (Marquis & Smith, 1963). We have examined guinea-pig serum and the extracellular fluid in the lungs for changes which might indicate the route by which lipid is lost from guinea-pig lungs after anaphylaxis *in vivo*.

Experimental

EFFECTS OF ANAPHYLAXIS

Guinea-pigs of 200–350 g were fed on Diet 18 (Oxo), and received 50 mg of ascorbic acid each day in their drinking water. There were sensitised to egg albumin (BDH) by the intraperitoneal injection of 100 mg as a 5% solution in water. Three to five weeks after sensitisation, 3 ml of blood was removed by cardiac puncture from each of a group of 4 animals, and 5 min later each animal was exposed to an aerosol of 1% egg albumin until it experienced severe anaphylaxis (Herxheimer, 1952). A further 3 ml of blood was removed, and at a selected time interval after the shock the animal was killed by dislocation of the neck, and the heart and lungs excised and perfused through the pulmonary artery with aerated Tyrode solution at 37° to remove the blood (Brocklehurst, 1960). Perfusion was then stopped and the lungs left inflating for 30 sec to allow as much residual Tyrode's solution as possible to drip out of the pulmonary system. The lung lobes were then chopped into small pieces, centrifuged

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for 3 min at 2,000 rpm and the extracellular fluid, which collects below the lung tissue, aspirated. The lung tissue was centrifuged for another 2 min and any further fluid collected was added to that previously aspirated. The fluid from four sets of lungs was pooled and centrifuged at 2,500 rpm for 15 min to remove any tissue debris.

The blood samples were allowed to clot for 1 hr, after which the clot was broken, the samples centrifuged for 15 min at 2,500 rpm and the serum removed. These procedures were repeated with a group of four sensitised control animals which were not exposed to antigen. The same time interval, with an additional 2 min to compensate for the aerosolisation time of the shocked group, was allowed between blood sampling. The control lungs were similarly chopped, centrifuged, and the fluid removed. Using four control and four shocked groups, each of four animals, in this way, the cholesterol, phospholipid, glyceride and protein composition of serum and extracellular fluid was estimated immediately, 15, 30 and 60 min after anaphylactic shock.

EFFECTS OF PRETREATMENT WITH ETHANOLAMINE AND HYDROCORTISONE

A further four groups (I-IV) of four animals were exposed to 1% egg albumin and their collapse times determined (Smith, 1961). One week later the animals in Group I were given 2 mg/kg ethanolamine (as the hydrochloride) and 1 mg/kg mepyramine (as the maleate) by intramuscular injection. After 55 min, 3 ml of blood was removed by cardiac puncture from each animal and 5 min later the animal exposed to aerosolised antigen until either it had reached a state of collapse or had been exposed to antigen for a period of twenty times its previous collapse time. Fifteen min after the collapse point a further 3 ml of blood was removed, the animal was killed, the lungs removed and the extracellular fluid collected as described above. The same course was followed using Group II as treatment controls, except that this group was not exposed to antigen. The same time intervals between removal of blood samples were observed, substituting a period equal to the mean collapse time of Group I for the aerosolisation time. Hydrocortisone sodium hemisuccinate (50 mg/kg) was given to Group III and Group IV by intramuscular injection followed 17 hr later by 1 mg/kg mepyramine. The first blood sample was taken 55 min after the antihistamine. From this point the same procedure used for Group I was followed with Group III, while Group IV was used as hydrocortisone control in the same way that Group II was used as ethanolamine control. The amounts of cholesterol, phospholipid, glyceride and protein in the serum and extracellular fluid samples were then estimated.

BIOCHEMICAL ESTIMATIONS

Phospholipid in the serum and extracellular fluid was extracted with chloroform: methanol solution (Dawson, 1960) and estimated as inorganic phosphorus (Bartlett, 1959). The cholesterol and glyceride fractions were extracted by the procedure of Mendelsohn & Antonis (1960) and estimated by the methods of Hanel & Dam (1955) and Van

Handel & Zilversmit (1957) respectively. The protein composition was estimated by cellulose acetate membrane filter electrophoresis using the stain Ponceau S according to Kohn (1960).

Reagents. Hydrogen peroxide, a 30% solution, phosphorus free was kindly donated by Laporte Chemicals, Luton. Other reagents and solvents were analar grade, except zinc chloride which was reagent grade.

Results

LIPIDS IN SERUM

Although all the guinea-pigs used in the present study were of the same age and were maintained on the same diet, wide differences in the normal values for serum lipids were noted. The figures for cholesterol ranged from 25·7 to 188 mg % over 48 samples of control serum. The phospholipid range was 39·5 to 113·5 mg %, and that for glyceride was 27·4 to 111·0 mg %. Consequently, changes induced by anaphylaxis were

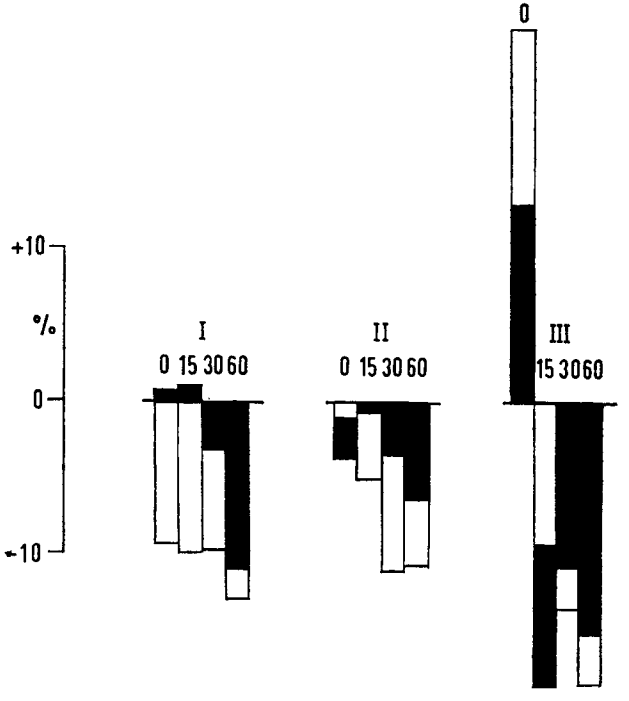

FIG. 1. The changes (%) in serum levels of cholesterol (I), phospholipid (II), and glyceride (III) in guinea-pigs immediately, 15, 30 and 60 min after anaphylactic shock (open columns) compared with those of control animals (solid columns). All columns originate from the base-line.

computed as percentage changes from the values determined for the control samples from each animal. The changes in serum lipid levels of animals exposed to aerosolised antigen are shown in Fig. 1, together with

the changes calculated from the two blood samples removed from the corresponding control groups of animals. Small but unequivocal decrements occurred in all three lipid fractions after anaphylaxis. These were similar but greater in magnitude than the changes occurring in control animals. Tests for statistical significance indicated that changes greater than 10% were statistically significant at $P = 0{\cdot}95$, except for the glyceride fraction where changes of around 20% were required for statistical significance at the same probability level.

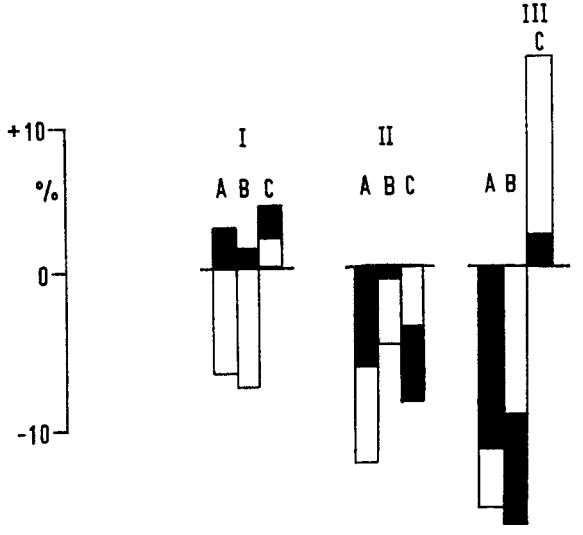

FIG. 2. The changes (%) in serum levels of cholesterol (I), phospholipid (II), and glyceride (III) in guinea-pigs after anaphylactic shock (B) compared with those in animals protected from anaphylaxis by pretreatment with mepyramine and ethanolamine (A) or mepyramine and hydrocortisone (C). The control responses in solid columns. All columns originate from the base-line.

The effect of pretreatment with ethanolamine plus mepyramine and hydrocortisone plus mepyramine upon the changes in serum lipid levels induced by anaphylactic shock can be noted from Fig. 2. The ethanolamine-treated animals still showed small decrements in cholesterol and glyceride similar to those in untreated animals with a somewhat greater loss of phospholipid. A loss of phospholipid still occurred after pretreatment with hydrocortisone but the cholesterol and glyceride fractions were slightly elevated.

LIPIDS IN EXTRACELLULAR FLUID OF THE LUNGS

In Table 1 the lipid present in extracellular lung fluid from animals exposed to aerosolised antigen and the corresponding control animals is recorded. The lipid content and volume of fluid collected were maximal 15 min after anaphylaxis, and fell to their lowest value 1 hr after shock. The lipid contents of the samples from the control groups were fairly

constant except for an increase in the glyceride content in the 15 min sample. From Table 2 it can be seen that protection from anaphylaxis using ethanolamine or hydrocortisone in combination with mepyramine reduced the volume of extracellular fluid and also its lipid content.

TABLE 1. THE VOLUME AND CHOLESTEROL, PHOSPHOLIPID, AND GLYCERIDE CONTENTS OF THE EXTRACELLULAR FLUID IN THE LUNGS OF GUINEA-PIGS AT VARIOUS TIMES AFTER ANAPHYLACTIC SHOCK COMPARED WITH THOSE OF CONTROL ANIMALS

Time min	Vol ml	Cholesterol		Phospholipid		Glyceride	
		mg/ml	Total	mg/ml	Total	mg/ml	Total
		Shock group					
0	1·5	0·28	0·42	3·02	4·53	0·47	0·70
15	2·2	0·29	0·62	3·23	6·95	0·32	0·69
30	1·3	0·38	0·59	3·54	4·61	0·42	0·55
60	1·0	0·15	0·15	2·63	2·63	0·20	0·20
		Control group					
0	0·5	0·24	0·12	4·50	2·25	0·24	0·12
15	0·9	0·24	0·22	2·65	2·39	0·50	0·45
30	0·7	0·26	0·18	3·61	2·53	0·34	0·24
60	0·6	0·39	0·23	6·50	3·90	0·53	0·32

TABLE 2. THE CHOLESTEROL, PHOSPHOLIPID, AND GLYCERIDE CONTENTS OF THE EXTRACELLULAR FLUID IN THE LUNGS OF ANIMALS AFTER ANAPHYLACTIC SHOCK COMPARED WITH THOSE OF ANIMALS PROTECTED FROM ANAPHYLAXIS BY TREATMENT BEFOREHAND WITH MEPYRAMINE AND ETHANOLAMINE OR WITH MEPYRAMINE AND HYDROCORTISONE

Group	Vol ml	Cholesterol		Phospholipid		Glyceride	
		mg/ml	Total	mg/ml	Total	mg/ml	Total
Mepyramine and ethanolamine							
Shock ..	1·45	0·19	0·28	2·78	4·00	0·18	0·25
Treated ..	0·95	0·23	0·22	3·60	3·42	0·23	0·22
Mepyramine and hydrocortisone							
Shock ..	1·70	0·24	0·40	3·64	6·19	0·26	0·44
Treated ..	0·70	0·20	0·14	5·11	3·57	0·27	0·19

TABLE 3. THE ALBUMIN AND GLOBULIN CONTENTS IN PERCENTAGES OF TOTAL PROTEIN OF THE EXTRACELLULAR FLUID IN GUINEA-PIG LUNGS AT VARIOUS TIMES AFTER ANAPHYLACTIC SHOCK

Time after shock in min	Shock group		Control group	
	Albumin %	Globulin %	Albumin %	Globulin %
0	35·5	64·5	33·7	66·3
15	34·1	65·9	34·3	65·7
30	37·6	62·4	32·2	67·8
60	32·4	67·5	31·3	68·7

PROTEIN IN EXTRACELLULAR FLUID AND SERUM

Table 3 shows that the relative proportions of albumin and globulin in extracellular fluid from the lungs of shocked animals and corresponding

controls showed no significant differences. The distribution pattern was observed to be diffuse and the bands on cellulose acetate were not easily distinguishable. Albumin appeared as a distinct band, often associated with a pre-albumin fraction, but all the globulin fractions tended to fuse into each other and form one diffuse band. A small rise in the proportion of albumin (statistically significant at $P = 0{\cdot}90$) can be observed 30 min after anaphylaxis. There were no noticeable changes in the protein composition of serum samples examined in these experiments. Since anaphylaxis had no pronounced effect on the protein content of extracellular fluid or serum, the effects of drug pretreatment were not investigated.

Discussion

These results show that anaphylaxis in guinea-pigs induces a fall in serum lipids; that observed for glyceride was preceded by a rise. These effects were also noted in control animals, indicating that the procedure used for collecting the blood samples (cardiac puncture) itself affected the serum lipid levels. A similar difficulty was experienced by Page, Pasternak & Burt (1931) whilst investigating the action of adrenaline on plasma lipids in the rabbit. Even so, the present results indicate that there is certainly no rise in serum lipids following anaphylaxis.

The lungs of guinea-pigs release large amounts of histamine in a vasoactive form as a result of anaphylaxis, and become oedematous in consequence. The isolation and biochemical estimation of the oedema fluid so formed presents technical difficulties. The procedure we used for recovering extracellular fluid from the lungs was devised in the belief that it represented oedema fluid diluted with Tyrode solution present in the pulmonary circulation. This belief is supported by the recovery of larger amounts of fluid from shocked compared with control lungs and also a small rise in its albumin content 30 min after anaphylaxis. The latter would be expected to follow an increase in lung capillary permeability since the molecular size of albumin is known to be smaller than that of the globulins, so that enrichment of tissue fluid with plasma protein would be expected to lead to an increase in albumin relative to globulin. The small extent of the albumin enrichment of extracellular fluid probably accounts for our failure to detect loss of albumin from serum. Anaphylaxis caused an increase in the lipid content of extracellular fluid followed by a return towards the control values. These changes suggest that lipid lost from lungs during anaphylaxis accumulates in oedema fluid in the lung and is then removed, presumably by lymphatic drainage. The reductions in volume of extracellular fluid and its lipid content observed in animals protected from fatal anaphylaxis by drug pretreatment are compatible with this interpretation.

The relationship between oedema fluid and phospholipid loss from lung tissue observed in these experiments is of interest in view of the previous findings of Kohler & Barbe (1954a,b). These workers reported the loss of total lipid from the lungs of rabbits following oedema induced by adrenaline and hypnotics. During anaphylaxis, therefore,

lipid loss from lung tissue may well be caused by the development of oedema in that organ.

The significance of the fall in serum lipids induced by anaphylaxis is unknown as yet. The fact that cardiac puncture itself induces changes essentially similar to those observed after anaphylaxis suggests that some "stress reaction" involving perhaps adrenaline- or ACTH-release might be operating (Page & others, 1931; Conn, Vogel, Louis & Fajans, 1950; Selye, 1950; Adlersberg, Shaefer & Drachman, 1951). The organ removing lipids from the serum under these experimental conditions is unknown. It might well be the liver. These tentative hypotheses invite early confirmation, but only in experiments in which adequate blood samples can be removed from guinea-pigs by a technique shown to be lacking in stimuli to the pituitary-adrenal axis. Otherwise, as in the present experiments, there remains the possibility that the technique of blood sampling may induce responses capable of modifying the subsequent anaphylaxis.

References

Adlersberg, D., Shaefer, L. E. & Drachman, S. R. (1951). *J. clin. Endocrinol.*, **11**, 67–83.

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

Brocklehurst, W. E. (1960). *J. Physiol.*, **151**, 416–435.

Conn, J. W., Vogel, W. C., Louis, L. H. & Fajans, S. S. (1950). *J. lab. clin. Med.*, **35**, 504–517.

Dawson, R. M. C. (1960). *Biochem. J.*, **75**, 45–53.

DiLuzio, N. R., Shore, M. L. & Zilversmit, D. B. (1954). *Metabolism*, **3**, 424–432.

Goadby, P. & Smith, W. G. (1962). *J. Pharm. Pharmacol.*, **14**, 739–745.

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

Herxheimer, H. (1951). *J. Physiol.*, **117**, 251–255.

Kohler, D. & Barbe, M. (1954a). *C.R. Soc. biol., Paris*, **148**, 299–302.

Kohler, D. & Barbe, M. (1954b). *Ibid.*, **148**, 494–497.

Kohn, J. (1960). *Chromatographic and Electrophoretic Techniques*, Vol. II, pp. 56–90. Editor: Smith, I. London: Heinemann.

Marquis, V. O. & Smith, W. G. (1963). *J. Pharm. Pharmacol.*, **15**, 652–659.

Mendelsohn, D. & Antonis, A. (1961). *J. Lipid Res.*, **2**, 45–50.

Page, I. H., Pasternak, L. & Burt, L. M. (1931). *Biochem. Z.*, **232**, 295–309.

Selye, H. (1950). *Brit. med. J.*, **1**, 1383–1392.

Smith, W. G. (1961). *J. Pharm. Pharmacol.*, **13**, 1–11.

Smith, W. G. (1962). *Biochem. Pharmacol.*, **11**, 183–186.

Smith, W. G. (1964). *Allergy and Tissue Metabolism*, pp. 81–90. London: Heinemann.

Van Handel, E. & Zilversmit, B. B. (1957). *J. Lab. clin. Med.*, **50**, 152–157.