THE EFFECTS OF HYDROCORTISONE ON THE CHANGES IN LIPID METABOLISM INDUCED IN GUINEA-PIG LUNG TISSUE BY ANAPHYLAXIS *IN VIVO*

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Exposure of sensitised guinea-pigs to aerosolised antigen causes alterations in the lipid metabolism of their lung tissue. The responses of control animals exposed to aerosolised distilled water has suggested that these metabolic disturbances are probably a manifestation of a "stress reaction". Pretreatment of sensitised animals with anti-anaphylactic dosage of sodium hydrocortisone hemisuccinate abolished this reaction.

CERTAIN forms of stress influence the anaphylactic reaction in man; thus localised infections, traumatic shock, surgical shock and pregnancy all temporarily relieve asthmatic symptoms. But attempts by Samter and Kofoed (1952) and Feinberg, Malkiel and McIntire (1953) to demonstrate a similar protection in sensitised guinea-pigs using sterile abscesses and treatment with piromen were unsuccessful.

The hypothesis that the beneficial effects of stress were mediated by the adrenal-hypophyseal axis received some confirmation when good clinical results were obtained in asthmatic patients treated with cortisone or ACTH (Bordley, Carey, Harvey, Howard, Kattus, Newman and Winkwerder, 1949; Carryer, Koelsche, Prickman and Maytum, 1950; M.R.C., 1956). In addition, Eriksson-Lihr (1951), Rose, Fyles and Venning (1955) and Siegel, Ely, Birnberg and Kelley (1956) found diminished 17-ketosteroid excretion in asthmatics and this seemed to be related to the severity of the disease. Furthermore, Kenipow (1922) found that partially adrenalectomised guinea-pigs showed increased sensitivity to anaphylactic reactions.

But it is usually impossible in a sensitised guinea-pig to prevent death from anaphylactic shock with cortisone or ACTH (Freidlander and Friedlander, 1950; Leger, Leith and Rose, 1948; Dworetsky, Code and Higgins, 1950; Malkiel, 1951). Herxheimer and Rosa (1952) also showed that a single injection of cortisone given a short time before exposure to aerosolised antigen did not influence the time for production of dyspnoea and cough in actively sensitised guinea-pigs. In 1953, Feinberg, Malkiel and McIntire reported cortisone to be capable of prolonging the "pre-convulsion time" in passively sensitised animals, provided that the drug was administered 18 hr. before exposure to aerosolised antigen.

Recent investigations by Smith (1962) using an isolated, sensitised guinea-pig lung have demonstrated marked changes in lipid content during anaphylactic shock. Alterations in the lipid metabolism of several organs as a result of cortisone administration have been reported

in man (Adlersberg, Schaefer and Dritch, 1950), in rabbits (Adlersberg, Schaefer and Wang, 1951) and in rats and guinea-pigs (Hausberger, 1958).

The purpose of this investigation was to examine any changes occurring in the lung lipid content of intact sensitised guinea-pigs when exposed to aerosolised antigen and to determine possible modifications induced by corticosteroid therapy.

Methods

Pharmacological

Guinea-pigs, 250 to 350 g., fed on Diet 18 (Oxo) and receiving 50 mg. of ascorbic acid every morning in solution in drinking water contained in amber glass bottles were sensitised to egg albumin (BDH) by the intraperitoneal injection of 100 mg. (5 per cent) in water.

Three weeks after sensitisation, eight animals were killed by a blow on the head, their lungs were excised and perfused for 10 min. at 1 ml./min. through the pulmonary artery with Tyrode's solution at 37° to remove blood, and then chopped into small pieces and freeze dried. This was the control group.

After sensitisation a second group of eight animals was injected with 50 mg./animal (about 100 mg./kg.) of sodium hydrocortisone hemisuccinate intramuscularly, killed, and their excised lungs treated similarly. This was the "treatment control group".

Three weeks after sensitisation six groups of eight animals were exposed to an aerosol of 1 per cent egg albumin. Thereafter they were exposed to antigen at weekly intervals (Herxheimer, 1951). After exposures to determine their "normal collapse time" (Smith, 1961), each group was divided into two subgroups of four animals of approximately equal sensitivity to antigen. One week later one subgroup was exposed to antigen as before and the other subgroup received aerosolised distilled water for a period of time equivalent for each animal to its normal collapse time.

The animals in Groups I, II, III received no premedication but the animals in Groups IV, V, VI received 50 mg. per animal of sodium hydrocortisone hemisuccinate intramuscularly 18 hr. before exposure to aerosol. Group I and IV were killed 15 min. after exposure to aerosol. Groups II and V were killed 30 min. and Groups III and VI 1 hr. after exposure to aerosol. All the lungs were excised, chopped and freeze dried.

Biochemical

Each freeze dried lung was powdered and then extracted for 24 hr. with 200 times its weight of chloroform : methanol (12 : 1). After filtration, the extract was dried *in vacuo*, redissolved in the original volume of chloroform and stirred for 5 min. with 5 g. of silicic acid (Malinkrodt). The filtrate was examined for cholesterol by the method of Hanel and Dam (1955) and glyceride by the method of Van Handel and Zilversmit (1957). The silicic acid was allowed to dry and transferred to a volume of methanol equivalent to the original volume of extract. After 5 min.

the methanol was filtered and examined for lipid phosphorus (Bartlett, 1959). The fatty acids of the lipids present in the chloroform and methanol were methylated. An aliquot (5 ml.) of the methanol (phospholipid) solution was dried *in vacuo*, dissolved in 1 ml. of 1·7N methanolic hydrochloric acid and kept overnight at 37°. Water (2 ml.) was added and the solution extracted with 4 ml. of light petroleum (60–80°). The separated light petroleum was dehydrated with anhydrous sodium sulphate and then dried. The separated fatty acid methyl esters were dissolved in 0·2 ml. of light petroleum (80–100°). A larger aliquot (20 ml.) of the chloroform (neutral lipid) solution was first dried, and dissolved in 0·5 ml. of 0·1N ethanolic potassium hydroxide. After 30 min. at 37°, 0·5 ml. of 0·4N sulphuric acid was added, and the solution extracted with 4 ml. of light petroleum (60–80°). After separating and dehydrating the light petroleum layer, it was dried *in vacuo*. The fatty acids were then methylated with 1·7N methanolic hydrochloric acid.

The methyl esters of both neutral and phospholipids were examined by gas-liquid chromatography (Pye Argon). The stationary phases were Apiezon L grease at 190° and polyethylene glycol adipate at 175°. Identification of individual fatty acids was carried out by measuring the log relative retention times on these two columns (James, 1959).

Reagents

Hydrogen peroxide (100 vol.) (phosphorus free) was kindly supplied by Laporte Chemicals, Luton. A range of pure fatty acid standards for gas chromatography was kindly supplied by Prices (Bromborough) Ltd., Bromborough Pool, Near Birkenhead.

Other reagents and solvents were Analar grade, except zinc chloride, which was reagent grade.

TABLE I

LIPID CONTENT OF LUNGS FROM SENSITISED GUINEA-PIGS EXPOSED TO AN AEROSOL OF DISTILLED WATER OR AEROSOLISED ANTIGEN (EGG ALBUMIN). RESULTS AS MEAN ± STANDARD ERROR

Lipid fraction	Controls	Time after aerosol (hr.)		
		0·25	0·5	1·0
		Distilled water		
Cholesterol	19·45 ± 0·78	29·06 ± 2·17	19·14 ± 1·02	22·75 ± 2·84
Glyceride	19·10 ± 1·25	21·83 ± 2·28	81·53 ± 5·73	21·64 ± 3·76
Phospholipid	121·00 ± 4·50	130·97 ± 9·24	100·79 ± 9·59	77·04 ± 4·89
		Egg albumin		
Cholesterol	19·45 ± 0·78	21·98 ± 3·90	17·34 ± 0·39	17·39 ± 1·43
Glyceride	19·10 ± 1·25	30·08 ± 2·85	66·31 ± 14·58	21·16 ± 5·47
Phospholipid	121·00 ± 4·50	117·16 ± 15·25	101·81 ± 4·08	85·93 ± 4·88

RESULTS

The changes in the lung lipids seen in animals exposed to aerosolised distilled water are shown in Table I, together with corresponding changes in animals exposed to aerosolised antigen. The most prominent changes are a fall in phospholipid content, and an increase in the glyceride content which is pronounced 30 min. after exposure to aerosol. One hr. after

exposure, the glyceride content had returned to normal but the phospholipid level appeared to be still falling.

In Table II the lipid fractions of the "control group" and the "treatment control group" are compared. It can be observed that hydrocortisone treatment did not appreciably alter the lipid content of the lungs.

TABLE II

LIPID CONTENT OF LUNGS FROM GUINEA-PIGS IN THE CONTROL GROUP COMPARED WITH LUNGS FROM GUINEA-PIGS PRETREATED WITH HYDROCORTISONE. RESULTS AS MEAN ± STANDARD ERROR

Lipid fraction	Treatment Controls	Hydrocortisone treated
Cholesterol.. ..	19·45 ± 0·78	20·09 ± 0·55
Glyceride	19·10 ± 1·25	17·15 ± 1·68
Phospholipid ..	121·00 ± 4·50	125·29 ± 2·91

The results obtained in animals pretreated with hydrocortisone are given in Table III. In animals exposed to either antigen or distilled water there are only small changes of the lipid fractions.

TABLE III

LIPID CONTENT OF LUNGS FROM SENSITISED GUINEA-PIGS EXPOSED TO AN AEROSOL OF DISTILLED WATER OR TO AEROSOLISED ANTIGEN, 18 HR. AFTER PRETREATMENT WITH HYDROCORTISONE. RESULTS AS MEAN ± STANDARD ERROR

Lipid fraction	Treatment Controls	Time after aerosol (hr.)		
		0·25	0·5	1·0
		Distilled water		
Cholesterol.. ..	20·09 ± 0·55	19·78 ± 0·87	19·63 ± 0·88	19·45 ± 0·01
Glyceride	17·15 ± 1·68	22·28 ± 1·23	25·10 ± 2·57	16·95 ± 1·43
Phospholipid ..	125·29 ± 2·91	140·10 ± 3·43	126·60 ± 0·86	119·75 ± 9·91
		Antigen		
Cholesterol ..	20·09 ± 0·55	18·77 ± 0·44	17·55 ± 0·44	19·45 ± 0·02
Glyceride	17·15 ± 1·68	23·02 ± 3·05	25·15 ± 1·57	23·20 ± 4·31
Phospholipid ..	125·29 ± 2·91	131·25 ± 6·65	126·50 ± 3·02	116·95 ± 6·23

The results of the gas chromatographic analysis of the neutral and phospholipid fractions of some groups of animals are given in Table IV. These examinations were confined to the controls and lungs obtained 30 min. after exposure to aerosol.

From Table IV it can be concluded that the constituent fatty acids of both the neutral lipid and phospholipid fractions were substantially the same in both the "controls" and "treatment controls". By comparing the data in Table IV (A and B) the effects of exposing animals to either distilled water or antigen can be deduced. The constituent fatty acids of the neutral lipids of animals exposed to distilled water have higher proportions of 16:1 and 18:1 acids and less 18:0 acid than the controls. The neutral lipids of animals exposed to antigen are also different in proportional fatty acid content from their controls. They have less 18:0 and more 18:1 and 18:3 acids. The phospholipid fractions also show differences. Animals exposed to distilled water have less 14:0 and more 16:1 and 18:1 acids than their controls. Animals exposed to

TABLE IV

THE PROPORTIONAL FATTY ACID ANALYSIS OF THE NEUTRAL LIPID AND PHOSPHOLIPID FRACTIONS OF SENSITISED GUINEA-PIG LUNGS (A) FROM CONTROL GROUPS, (B) OBTAINED 30 MIN. AFTER EXPOSURE TO AEROSOLS OF DISTILLED WATER OR ANTIGEN, (C) OBTAINED 30 MIN. AFTER EXPOSURE TO AEROSOLISED DISTILLED WATER OR ANTIGEN 18 HR. AFTER PRETREATMENT WITH HYDROCORTISONE. THE RESULTS ARE EXPRESSED AS THE MEAN PER CENT AND STANDARD ERROR

	A				B				C			
	Neutral lipids		Phospholipids		Neutral lipids		Phospholipids		Neutral lipids		Phospholipids	
Fatty* acid	Controls	Treatment controls	Controls	Treatment controls	Distilled water	Antigen	Distilled water	Antigen	Distilled water	Antigen	Distilled water	Antigen
C14·0	2·77 S.E. 0·33	2·63 S.E. 0·12	3·30 S.E. 0·24	3·13 S.E. 0·20	2·46 S.E. 0·17	2·11 S.E. 0·20	2·47 S.E. 0·18	2·30 S.E. 0·37	3·37 S.E. 0·34	3·34 S.E. 0·38	3·51 S.E. 0·35	3·78 S.E. 0·27
U_1	Nil	0·12 S.E. 0·03	0·10 S.E. 0·03	0·14 S.E. 0·09	0·22 S.E. 0·04	0·19 S.E. 0·04	0·39 S.E. 0·08	0·46 S.E. 0·12	0·50 S.E. 0·17	0·44 S.E. 0·70	0·32 S.E. 0·19	0·63 S.E. 0·22
U_2	0·54 S.E. 0·09	0·43 S.E. 0·06	0·66 S.E. 0·07	0·67 S.E. 0·10	Nil	Nil	Nil	Nil	Nil	Nil	0·79 S.E. 0·30	0·25 S.E. 0·15
U_3	0·38 S.E. 0·38	0·15 S.E. 0·04	2·87 S.E. 0·09	2·31 S.E. 0·15	Nil	Nil	1·88 S.E. 0·43	1·67 S.E. 0·11	Nil	Nil	1·84 S.E. 0·68	1·93 S.E. 0·21
C16·0	43·37 S.E. 1·13	44·83 S.E. 1·02	51·37 S.E. 1·17	51·23 S.E. 1·07	49·63 S.E. 5·24	39·54 S.E. 5·13	47·21 S.E. 4·94	49·62 S.E. 1·19	42·47 S.E. 1·48	49·98 S.E. 1·53	46·29 S.E. 3·49	50·85 S.E. 1·54
C16·1	2·74 S.E. 0·28	3·92 S.E. 0·35	3·97 S.E. 0·37	4·65 S.E.0·26	4·00 S.E. 0·12	3·61 S.E. 1·24	7·80 S.E. 1·53	6·46 S.E. 0·74	4·59 S.E. 0·89	3·49 S.E. 0·25	3·43 S.E. 0·68	3·61 S.E. 0·65
C18·0	9·25 S.E. 1·30	9·52 S.E. 0·34	7·89 S.E. 0·05	8·00 S.E. 0·28	4·40 S.E. 0·60	4·47 S.E. 0·14	7·04 S.E. 0·79	6·37 S.E. 0·30	7·58 S.E. 1·25	7·20 S.E. 1·04	9·05 S.E. 0·66	7·53 S.E. 0·30
C18·1	23·36 S.E. 0·63	21·16 S.E. 1·19	14·04 S.E. 0·26	13·64 S.E. 0·39	26·18 S.E. 1·00	31·11 S.E. 1·53	16·36 S.E. 0·41	15·54 S.E. 0·56	24·80 S.E. 0·65	22·09 S.E. 1·57	17·10 S.E. 0·96	15·60 S.E. 0·93
C18·2	15·43 S.E. 1·95	12·77 S.E. 0·76	11·89 S.E. 0·64	10·53 S.E. 0·48	11·58 S.E. 1·10	15·89 S.E. 1·95	12·67 S.E. 2·31	12·76 S.E. 0·80	15·13 S.E. 0·78	12·18 S.E. 0·86	13·64 S.E. 0·77	10·92 S.E. 1·06
C18·3	2·15 S.E. 0·19	1·79 S.E. 0·19	0·14 S.E. 0·14	0·55 S.E. 0·17	2·26 S.E. 0·25	4·82 S.E. 0·33	0·23 S.E. 0·23	0·50 S.E. 0·69	1·50 S.E. 0·13	1·26 S.E. 0·27	0·43 S.E. 0·16	0·37 S.E. 0·21
C20·4	Nil	Nil	3·79 S.E. 0·22	5·14 S.E. 0·30	Nil	Nil	3·17 S.E. 1·10	4·33 S.E. 0·51	Nil	Nil	3·16 S.E. 0·21	4·53 S.E. 0·30

* Figures before decimal point = number of carbon atoms; those after the decimal point, the number of double bonds.

antigen have less 18:0 acids and more 16:1 and 18:1 acids than their controls. One general change detected throughout is an increase in unsaturated acids at the expense of saturated ones.

A parallel comparison of the constituent fatty acids of the neutral and phospholipid fractions of animals pretreated with hydrocortisone can be made by comparing the data in Table IV (A and C). The neutral lipids of the animals exposed to aerosolised distilled water showed an increase in 16·1 fatty acids and a decrease in 18·0 acids when compared with the hydrocortisone treated controls. The animals exposed to antigen showed an increase in 16·0 acids. The phospholipid fraction of the lungs of the animals exposed to distilled water showed an increase in 18·0 and 18·1 acids whilst the 20·4 acids showed a decrease. The phospholipid fraction of the lungs of animals exposed to antigen showed no changes.

Discussion

There is evidence that exposing guinea-pigs to aerosolised antigen altered the lipid content of their lungs. Lung phospholipid fell after exposure to aerosol and was still continuing to fall 1 hr. afterwards. During the same period of time, there was a substantial rise in glyceride content 30 min. after exposure, but this had returned to normal at 1 hr. Changes in the component fatty acids of both neutral and phospholipid fractions indicated that these fractions were in a state of metabolic turnover.

These changes are of sufficient magnitude to influence, at least transiently, the whole of the intermediary metabolism of lung tissue. Whilst the experiments were designed with the expectation that this might be so in animals exposed to antigen, it is surprising to observe changes which were almost identical in animals exposed to distilled water. There are two possible explanations.

The changes were determined in animals receiving antigen in the form of an aerosol for the fourth time. Earlier exposures were made to measure their sensitivity and express it in the form of a "collapse time". This was done to enable the subgroups to be matched in terms of antigen sensitivity. It is thus possible that the changes in animals exposed to distilled water were a conditioned response in the sense that the animals were expecting to be exposed to antigen with its resultant severe symptoms. Alternatively, it can be reasoned that the actual procedure of aerosol exposure (involving transfer to a noisy and uncomfortable aerosol chamber) constituted a form of stress followed by a "stress reaction" on the part of the lung metabolism.

Irrespective of the reasons for the metabolic changes which followed exposure to distilled water, it is significant that animals pretreated with hydrocortisone did not show a response to aerosols of distilled water or of antigen. The "stress reaction" or "conditioned response" did not occur.

This particular experimental study is thus of interest since it offers evidence from which it may be concluded that pretreatment of animals with hydrocortisone at a dose which imparts anti-anaphylactic activity

(Feinberg and others, 1953; Goadby and Smith, unpublished) in some way protects the lung tissue of these animals from disturbances of their lipid metabolism. It implies that the anti-anaphylactic activity of hydrocortisone may have a metabolic basis.

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