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The influence of corticosteroid pretreatment on anaphylactic bronchoconstriction in the guinea-pig

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Previous reports that cortisone and related compounds exerted a small degree of protection against antigen-aerosol-induced anaphylactic reactions, have been confirmed. Optimal effects were obtained 16 to 24 h after corticosteroid pretreatment. Marked effects were obtained from the more potent glucocorticoids, particularly water-soluble compounds. Similar pretreatment decreased the severity and modified the form of anaphylactic bronchoconstrictor responses induced by intravenous administration of antigen. In guinea-pigs pretreated also with mepyramine, the corticosteroids suppressed the residual and presumably non-histamine component of the anaphylactic bronchoconstrictions. Anti-anaphylactic potency was correlated with glucocorticoid and anti-inflammatory activity. A suggested mode of action involves a combination of weak antihistamine activity and inhibition of release of slow reacting substances of anaphylaxis.

An anti-anaphylactic property of corticosteroids in the guinea-pig may be consistently demonstrated (Goadby & Smith, 1964; Hicks, 1968) using microshock reactions, induced by inhalation of antigen aerosol and assessed by the preconvulsion time technique. In contrast, a review of the literature (Hicks, 1969) has revealed that previous attempts to demonstrate such an effect had failed to produce consistent results when anaphylactic reactions were induced by intravenous administration of antigen. The inadequacy of the latter experiments may have been due to the failure of the subjective scoring or mortality assessment techniques employed to reveal a not very pronounced effect. Alternatively, a qualitative difference in the nature of anaphylactic reactions, induced by either inhaled aerosol or intravenously administered antigen, may provide an explanation which would be of fundamental significance.

An attempt to resolve these possibilities and to investigate further the influence of corticosteroids on guinea-pig anaphylaxis has been made using both microshock reactions and direct evaluation of bronchoconstrictor responses *in vivo*. A range of corticosteroids has been investigated to see if a relation exists between anti-anaphylactic properties and glucocorticoid or anti-inflammatory potency.

EXPERIMENTAL

Materials and methods

Virgin female albino guinea-pigs (Dunkin Hartley Strain), 300–500 g body weight, were maintained on unrestricted supplies of water, and diet S.G.I. (Oxoid), supplemented by hay and green vegetables, or ascorbic acid (50 mg/day) in the drinking water. They were housed in well ventilated conditions at 65° F.

Sensitization. Active hypersensitivity was induced by single intraperitoneal injections of egg albumen (B.D.H. flake) in a dose of 50 mg/kg. Anaphylactic reactions were induced by further administration of the antigen 28 days later.

Corticosteroid preparations. The following suspensions were used: Cortisone acetate (25 mg/ml) (Boots), methylprednisolone acetate (40 mg/ml) (Upjohn), prednisolone acetate (25 mg/ml) (Pfizer), triamcinolone diacetate (25 mg/ml) (Lederle), hydrocortisone acetate (25 mg/ml) (Roussel). Fludrocortisone acetate (4 mg/ml) and paramethasone acetate (4 mg/ml) were both made up in suspension in Boots suspension vehicle.

The following were used in solution: Dexamethasone 21-phosphate (4 mg/ml) (Roussel), betamethasone disodium phosphate (4 mg/ml) (Glaxo), hydrocortisone hemisuccinate (50 mg/ml) in saline.

Antigen-aerosol-induced anaphylaxis. Antigen was administered to sensitized animals by an adaptation of the microshock method of Herxheimer (1952). Egg albumen aerosol (5% solution in water) was sprayed into a closed chamber from a *Wright* nebulizer using compressed air (10 lb inch^2). Guinea-pigs were placed singly in the chamber for sufficient time to inhale the antigen aerosol until the induced anaphylactic bronchoconstriction resulted in consistently visible symptoms of dyspnoea. The animal was then removed from the chamber, and the exposure time was recorded as the "preconvulsion time". The end-point used was the first spasm of the body wall in the upper abdominal-diaphragm area. Termination of exposure at this point averted the progression of the reaction to convulsions and death. All experiments were made using groups of 5 animals, and the results were taken as the mean values of individual preconvulsion times.

Anaphylactic bronchoconstriction induced by intravenous antigen. Sensitized animals were anaesthetized with pentobarbitone (60 mg/kg, i.p.). Cannulae were inserted into an external jugular vein for saline drug infusions and into the trachea for artificial ventilation. Each animal received heparin 1000 units/kg intravenously, following the cannulation. Artificial ventilation was maintained from a miniature Starling Ideal pump, with a stroke volume of 1 cc of air per 100 g body weight, plus an arbitrary amount depending upon the dead space of the system. Ventilation was at a rate of 36 strokes/min. Bronchiolar tone, as indicated by resistance to positive pressure inflation, was recorded by the method of Dixon & Brodie (1903) as employed by Hicks & Leach (1963). Challenging doses of antigen were administered intravenously and were infused with 0·5 ml of heparinized saline.

RESULTS

Effects of corticosteroids on the severity of antigen-aerosol induced-anaphylaxis: determination of optimal time interval

Single doses of either cortisone (25 mg/kg, i.m.), prednisolone (5 mg/kg, i.m.) or dexamethasone (4 mg/kg, s.c.) were administered to groups of sensitized guinea-pigs. Separate groups from batches receiving each type of treatment were exposed to the antigen aerosol after intervals of 4, 8, 12, 16, 18, 20 or 24 h. Preconvulsion times were measured. Control groups pretreated with equivalent volumes of the vehicle used in the corticosteroid preparation, were similarly exposed to the aerosol and anaphylactic preconvulsion times were measured. Control and test group responses were compared statistically using Student's *t*-test. Results are in Table 1.

Table 1. *Anaphylactic preconvulsion times in guinea-pigs pretreated with corticosteroids. Reactions induced at various times after treatment.* Results expressed as mean preconvulsion times (s) ± standard errors. Control groups treated with appropriate vehicle

Interval (h)	Treatment	Cortisone (25 mg/kg)	Prednisolone (5 mg/kg)	Dexamethasone (4 mg/kg)
4	Control	52·0 ± 5·5	47·0 ± 4·4	49·8 ± 2·9
	Test	58·0 ± 5·6	47·0 ± 4·7	55·8 ± 3·7
8	Control	52·0 ± 5·5	47·0 ± 4·4	49·8 ± 2·9
	Test	65·0 ± 11·4	49·7 ± 6·8	51·0 ± 3·5
12	Control	29·0 ± 7·0	47·0 ± 4·4	35·6 ± 4·2
	Test	37·8 ± 3·2	43·5 ± 2·2	37·6 ± 4·0
16	Control	48·8 ± 3·4	43·5 ± 3·7	35·6 ± 4·2
	Test	67·7 ± 6·6*	50·2 ± 4·3	58·8 ± 2·2
18	Control	52·0 ± 3·0	28·4 ± 4·6	51·6 ± 4·0*
	Test	77·2 ± 8·3*	38·0 ± 2·3	87·8 ± 3·2
20	Control	52·0 ± 5·5	43·5 ± 3·7	35·6 ± 4·2*
	Test	58·0 ± 9·8	54·2 ± 2·6	58·3 ± 5·0
24	Control	52·0 ± 5·5	43·5 ± 3·7	39·8 ± 2·9
	Test	55·0 ± 3·8	47·6 ± 5·0	51·6 ± 4·0

* denotes significant difference $P<0·05$.

Cortisone pretreatment caused significant prolongation of anaphylactic preconvulsion times when reactions were induced 16 and 18 h after its administration. Dexamethasone similarly resulted in significant prolongation of preconvulsion times after 18 and 20 h. No significant effects were observed in animals pretreated with prednisolone.

Influence of corticosteroid pretreatment on antigen aerosol-induced reactions 18 h later

Groups of sensitized guinea-pigs were pretreated with single doses of various corticosteroids, and, after 18 h, were exposed to the antigen aerosol. Cortisone, hydrocortisone (acetate and hemisuccinate), prednisolone, triamcinolone, fludrocortisone, dexamethasone, betamethasone, paramethasone, or methylprednisolone, were investigated, and a range of doses of each was used in different groups of animals. Preconvulsion times of treated animals were compared statistically with those of animals in appropriate control groups (vehicle treated). Effects were calculated as percentage prolongations of preconvulsion times. Optical doses and the maximal prolongation effects observed with each compound were as shown in Table 2.

No significant effects were observed with any of the doses of methylprednisolone or hydrocortisone acetate, administered. At 18 h after pretreatment with each of the other corticosteroids significant prolongation of preconvulsion times were observed with at least one of the dose levels administered. In general, the more potent substances (indicated by lower optimal dosage) caused a greater prolongation of effects.

Table 2. *Prolongation of anaphylactic preconvulsion times* 18 *h after single dose corticosteroid administration. Relation of optimal dose to maximal effects*

Drug	Range of doses investigated (mg/kg)	Optimal dose (mg/kg)	Mean prolongation of preconvulsion times (%) at optimal dosage	*P*
Cortisone	0·1–50	10	29·3	<0·05
Hydrocortisone (acet.)	1·0–50	50	22·9	>0·05*
Hydrocortisone (hemisuccinate) ..	2·0–50	25	49·9	<0·05
Prednisolone	0·5–20	10	43·5	<0·05
Fludrocortisone	0·5–10	10	65·0	<0·001
Triamcinolone	0·5–20	10	38·0	<0·05
Methylprednisolone	0·5–20	10	22·0	>0·05*
Paramethasone	0·2– 8	4	54·1	<0·01
Betamethasone	0·1– 4	2	42·5	<0·01
Dexamethasone	0·1– 8	4	64·0	<0·001

* Denotes no significant difference from control groups.

Effects of corticosteroids in mepyramine-treated animals

Groups of 6 sensitized guinea-pigs were treated with single doses of cortisone (10 mg/kg, i.m.), dexamethasone (4 mg/kg, i.m.) or fludrocortisone (4 mg/kg, i.m.), followed 17 h later with, in each case, a single subcutaneous dose of mepyramine (1 mg/kg). One h after the final treatment, each animal was exposed to the aerosol and anaphylactic preconvulsion times were recorded. Similar investigations were made on animals pretreated solely with mepyramine, and provided control values. Results were as shown in Table 3.

Table 3. *Effects of corticosteroids on anaphylactic preconvulsion times in mepyramine treated guinea-pigs.* Results recorded as mean preconvulsion times (s) ± standard errors

Corticosteroid	Preconvulsion times: Mepyramine alone (1 mg/kg, s.c.)	Mepyramine + corticosteroid	% Prolongation	*t*	*P*
Cortisone .. .. (10 mg/kg, i.m.)	158 ± 18	231 ± 34	46	3·28	<0·01
Dexamethasone .. (4 mg/kg, i.m.)	177 ± 14	409 ± 49	132	6·35	<0·001
Fludrocortisone .. (4 mg/kg, i.m.)	177 ± 14	340 ± 34	92	4·29	<0·01

Mean preconvulsion times in guinea-pigs treated with mepyramine plus cortisone, fludrocortisone, or dexamethasone, were very significantly longer than those in animals pretreated only with mepyramine. End points were difficult to observe particularly in those most prolonged exposures.

Effects of corticosteroids on anaphylactic bronchoconstrictor responses

Corticosteroids shown to exert significant influence on aerosol-induced anaphylaxis, were investigated for their ability to produce comparable effects against directly recorded anaphylactic bronchoconstrictor responses in hypersensitive guinea-pigs *in vivo*.

Single doses of either cortisone (10 mg/kg, i.m.), dexamethasone (4 mg/kg, i.m.) or fludrocortisone (4 mg/kg, i.m.) were administered to sensitized guinea-pigs. 17 h after treatment the animals were anaesthetized and prepared for artificial ventilation and intravenous infusion. At 18 h after treatment a dose of egg albumen antigen (200 or 500 μg/kg) was injected intravenously, and the consequent anaphylactic bronchoconstrictor response was recorded. The antigen doses chosen were those which previously were found to induce either small, or large but still submaximal, anaphylactic bronchoconstrictor responses in guinea-pigs under similar conditions of sensitization. The severity of bronchoconstrictor responses in corticosteroid-treated animals was compared with those of similar reactions induced in control animals which received pretreatment only with solvent or suspending fluid. It was impracticable to perform all the experiments on the same day, but, to standardize conditions, similar numbers of treated and control animals were investigated in each session. Records of bronchoconstrictor responses were evaluated according to the method of Hicks & Leach (1963). Results are in Table 4.

Table 4. *Anaphylactic bronchoconstrictor responses in guinea-pigs, 18 h after pretreatment with corticosteroids.* Severity of responses expressed as % reduction in tidal volume

Treatment	% reduction in tidal volume (mean ± s.e.)	Number of animals	t	P
Antigen (200 μg/kg)				
Cortisone (10 mg/kg, i.m.) ..	17·3 ± 5·4	6	1·76	>0·05
Control	37·7 ± 8·9	7		
Dexamethasone (4 mg/kg, i.m.)	14·4 ± 4·3	6	2·35	<0·05
Control	37·7 ± 8·9	7		*
Antigen (500 μg/kg)				
Cortisone (10 mg/kg, i.m.) ..	67·9 ± 11·7	8	1·41	>0·05
Control	86·9 ± 6·8	8		
Dexamethasone (4 mg/kg, i.m.)	59·5 ± 8·2	8	2·58	<0·05
Control	86·9 ± 6·8	8		*
Fludrocortisone (4 mg/kg, i.m.)	62·6 ± 8·8	9	2·19	<0·05
Control	86·9 ± 6·8	8		*

* Denotes significant difference.

Anaphylactic bronchoconstrictor responses induced by either 200 or 500 μg/kg doses of antigen, were significantly less severe in animals treated with dexamethasone or fludrocortisone, 18 h previously. No significant decrease resulted from the cortisone treatment.

Other sensitized animals received similar pretreatment with corticosteroids, and 17 h later a single injection of mepyramine (1 mg/kg, s.c.) was administered. The animals were anaesthetized, and 18 h after administration of corticosteroids anaphylactic bronchoconstriction was induced and recorded. The antigen dose was 500 μg/kg. Results are in Table 5.

In control animals treated with mepyramine alone before administration of the challenging dose of antigen, the anaphylactic bronchoconstrictor responses were markedly reduced. In such animals, pretreated also with any one of the corticosteroids, the bronchoconstrictor response severity was reduced even further, being significantly smaller than in animals treated with mepyramine alone.

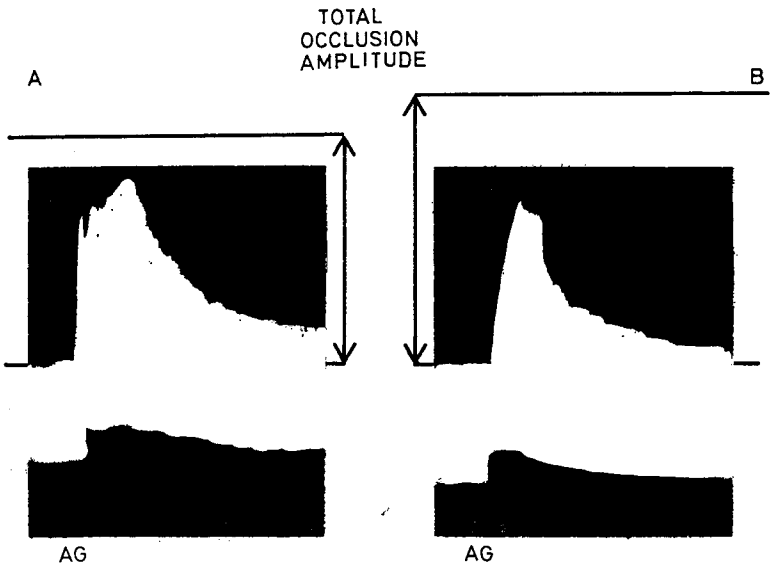

FIG. 1. Anaphylactic bronchoconstrictor responses induced by egg albumen antigen AG (500 μg/kg, i.v.) in sensitized, anaesthetized guinea-pigs. Recorded using method of Dixon & Brodie (1903). Record A is from a saline treated control animal. Record B is from an animal pretreated with dexamethasone (4 mg/kg, i.m.) 18 h previously.

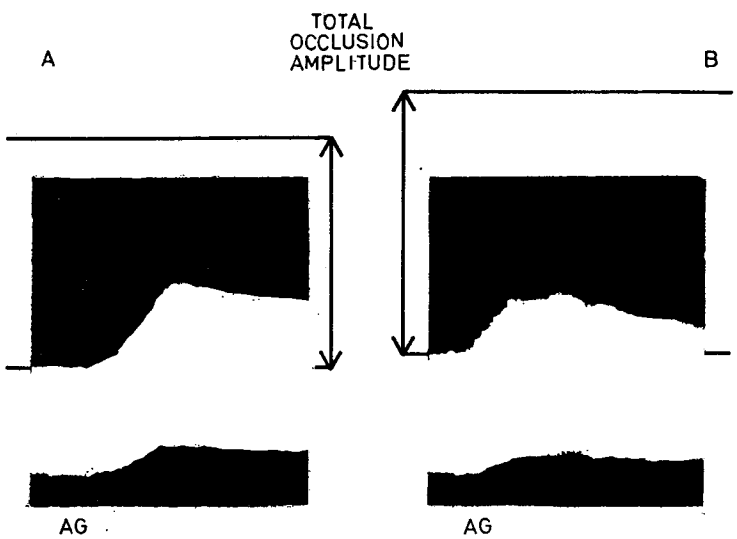

FIG. 2. Anaphylactic bronchoconstrictor responses induced by egg albumen antigen AG (500 mg/kg, i.v.) in sensitized, anaesthetized guinea-pigs. Recorded using method of Dixon & Brodie (1903). Animals pretreated with mepyramine (1 mg/kg, s.c.) 1 h before administration of antigen. Record A is from a saline treated control animal. Record B is from an animal pretreated with dexamethasone (4 mg/kg, i.m.) 18 h previously.

Table 5. *Anaphylactic bronchoconstrictor responses in guinea-pigs pretreated with mepyramine and corticosteroid.* Antigen administration (500 μg/kg) 18 h after corticosteroid given intramuscularly and 1 h after mepyramine given subcutaneously. Severity of responses expressed as % reduction in tidal volume

Treatment	% reduction in tidal volume (mean ± s.e.)	Number of animals	t	P
Mepyramine (1 mg/kg) alone ..	39·1 ± 4·7	8	5·79	<0·001
Control	86·9 ± 6·8	8		*
Cortisone (10 mg/kg) + mepyramine (1 mg/kg)	20·4 ± 4·5	7	2·86	<0·05
Mepyramine (1 mg/kg)	39·1 ± 4·7	8		*
Dexamethasone (4 mg/kg) + mepyramine (1 mg/kg)	12·8 ± 4·1	6	4·24	<0·01
Mepyramine (1 mg/kg)	39·1 ± 4·7	8		*
Fludrocortisone (4 mg/kg) + mepyramine (1 mg/kg)	20·7 ± 7·2	8	2·30	<0·05
Mepyramine (1 mg/kg)	39·1 ± 4·7	8		*

* Denotes significant difference.

Corticosteroid pretreatment qualitatively modified the character of the anaphylactic bronchoconstriction records, in addition to the suppression of amplitude. As illustrated in Fig. 1 the onset of the response and the position of the peak amplitude was similar in both control and pretreated animals. However, the duration of the response was noticeably less in the corticosteroid-treated animals. In mepyramine-treated animals (Fig. 2), the responses were much modified both in amplitude and character. Presumably as a result of antagonism to the histamine-like component of the reaction, the residual responses had a much slower onset and attained a less pronounced peak at a later time after antigen administration. Additional corticosteroid pretreatment markedly modified the amplitude of these residual responses, but the smooth protracted nature was unchanged.

DISCUSSION

These experiments confirm and extend previous reports that cortisone and related compounds exerted small, but significant, protection against aerosol-induced anaphylaxis in the guinea-pig (Herxheimer & Rosa, 1952; Feinberg, Malkiel & McIntire, 1953; Goadby & Smith, 1964). It has been shown that significant prolongation of preconvulsion times resulted from single dose pretreatment, optimally effective after an interval of 18 h. Only marginal effects were observed using insoluble corticosteroids in suspension. In contrast, marked anti-anaphylactic effects were exerted by the water soluble hydrocortisone hemisuccinate, while an even greater degree of protection was conferred by those compounds which were both water soluble and more potent glucocorticoids. Early investigations were necessarily limited by the availability of only the less potent and relatively insoluble steroids such as cortisone acetate. This may have been a feature contributing to the inconclusive nature of such experiments in a species which is relatively insensitive to corticosteroids (Hicks, 1969). Another factor contributing to early failures to

demonstrate this effect was the absence of experiments featuring the optimal time interval of 18 h.

It has been demonstrated that administration of fludrocortisone or dexamethasone in single doses, similar to those shown to prolong preconvulsion times and given 18 h before the intravenous doses of antigen, significantly decreased the severity of anaphylactic bronchoconstrictor responses *in vivo*. The bronchoconstrictor effects were evaluated directly, and compared with submaximal responses in control animals. In 1962, Bush stated that the mechanism whereby cortisone exerted its small protective effect was by the increased tolerance of guinea-pigs to anoxia. An alternative suggestion was that corticosteroids might modify the permeability of lung tissue to the absorption of antigen administered in the form of inhaled aerosol. These possibilities are largely precluded by the fact that the protective influence, at least of the most potent corticosteroids is exerted mainly on the primary response to the anaphylactic reaction rather than any secondary consequence of that response. In addition the effect is independent of the route of administration of the antigen inducing the reaction.

Mepyramine treatment effected a marked prolongation of anaphylactic preconvulsion times, indicating the participation of a large histamine-like component in the reaction. This is consistent with the observation that release of histamine in anaphylaxis is rapid (Brocklehurst, 1960), as are its stimulant effects on bronchiolar smooth muscle. As preconvulsion time evaluation measures the onset of the early symptoms of anaphylaxis, it may, therefore, be considered that the microshock reactions are, if unmodified, largely due to histamine. An explanation of the mode of action of pretreatment with corticosteroids alone may, therefore, be sought in the possibility of antihistamine effects. Such effects have been reported for high doses of corticosteroids, by Lefcoe (1956), Huidobro (1960), Goadby & Smith (1964), Dawson & West (1965) and particularly for water-soluble corticosteroids by Zicha, Scheiffarth & others (1960, a, b, c). The latter authors showed significant antagonism to the effects of histamine aerosols in guinea-pigs, to be exerted by a wide range of glucocorticoid drugs. Water-soluble esters produced almost immediate effects lasting for a few hours, whereas those of insoluble esters did not appear for several days. It is suggested that the inactivity of cortisone is explained by its lack of antihistamine activity, possibly as a result of its insolubility. The significant anti-anaphylactic effect of dexamethasone is associated with significant antihistamine action.

With mepyramine treated animals, it may be assumed that the contribution of histamine to the total anaphylactic reaction is suppressed (Goadby & Smith, 1964; Collier & James, 1967). Any effect of corticosteroid pretreatment observable in such animals may, therefore, be considered to arise from an influence on the residual response induced by slow reacting substance of anaphylaxis (SRS-A), bradykinin or possibly other mediators. The combined effects of mepyramine with either fludrocortisone or dexamethasone resulted in prolongation of preconvulsion times, and are greater than those expected from a summation of their individual effects. Such a potentiation is similar to that described by Goadby & Smith (1964). The observed effects are thus consistent with a corticosteroid influence upon those components of the anaphylactic reaction whose bronchoconstrictor effects are delayed in onset, but sustained in action, and whose formation or release may possibly come later than that of histamine. In the case of microshock reactions such an influence of cortico-

steroids would be masked by the predominance of the histamine-like component, unless this is removed by simultaneous antihistamine administration.

Support for this interpretation is also derived from the changes in character of bronchoconstriction records. Treatment with fludrocortisone or dexamethasone accentuated the initial peaks of the anaphylactic responses, giving recoveries that were rapid in comparison to untreated controls. This is consistent with suppression of the later non-histamine response. In mepyramine-treated animals the initial rapid peak response was markedly suppressed and the corticosteroid influence on the delayed and sustained components was revealed.

The suppression of the residual component of guinea-pig anaphylaxis after corticosteroid treatment could be explained by a reduction in the production of smooth muscle stimulant mediators. This suggestion is supported by observations of significant reduction in the quantity of SRS-A in perfusates of anaphylactic lungs from guinea-pigs treated with corticosteroid 18 h previously (Goadby & Smith, 1964; Hicks, 1966).

The relative potencies of the steroids examined closely resemble their relative glucocorticoid potencies. It is of interest to contrast this finding with a previous observation that some mineralocorticoid hormones potentiated the severity of anaphylactic reactions (Hicks, 1968). With the potent mineralocorticoid, fludrocortisone, the anaphylactic properties appear to be more closely associated with the equally potent glucocorticoid properties which it also possesses. The main systemic metabolic effects resulting from administration of high doses of glucocorticoids would be hyperglycaemia (Kovacs & Suffiad, 1968) due to glyconeogenetic and diabetogenic mechanisms. It may be of significance to note reports that insulin hypoglycaemia aggravated anaphylaxis in the rat (Sanyal, Spencer & West, 1959; Dhar & Sanyal, 1963; Adamkiewicz, Sacra & Ventura, 1964) whereas in animals rendered hyperglycaemic with glucose or alloxan the severity of anaphylaxis was reduced.

Glucocorticoid properties are closely correlated with anti-inflammatory effects. Recent reports have implicated lysosomes of polymorphonuclear leucocytes in anaphylactic reactions in the mouse (Treadwell, 1965), rat (Orange, Valentine & Austen, 1967) and in heterologous passive cutaneous anaphylactic reactions in the guinea-pig (Movat, di Lorenzo & others, 1967), as well as a variety of inflammatory conditions. No direct evidence, however, has yet been produced suggesting that polymorphonuclear lysosomal enzymes play any part in active systemic anaphylaxis in the guinea-pig, although this possibility has been implied (Brocklehurst, 1967). Weissmann & Thomas (1966) reported that anti-inflammatory steroids inhibited the release of lysosomal enzymes. Furthermore, it has been suggested that these effects were associated with the marked ability of lysosomes to concentrate within themselves compounds including cortisone and dexamethasone (Allison & Young, 1964). It is also well known that glucocorticoid drugs exert a significant leucopenic action. The association of these facts prompts the speculation that the formation of non-histamine mediators of systemic anaphylaxis in the guinea-pig may be the result of the release of lysosomal enzymes, possibly of polymorphonuclear neutrophil origin. The anti-anaphylactic suppression of the non-histamine-like component of anaphylaxis by corticosteroids, could thus be explained by an interaction with this hypothetical lysosome involvement.

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REFERENCES

ADAMKIEWICZ, V. W., SACRA, P. J. & VENTURA, J. (1964). *J. Immunol.*, **92**, 3–7.

ALLISON, A. C. & YOUNG, M. R. (1964). *Life Sci.*, **3**, 1404–1414.

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

BROCKLEHURST, W. E. (1967). In *Modern Trends in Immunology*, 2. Editors: Cruikshank, R. & Weir, D. M., pp. 235–249, London: Butterworth.

BUSH, I. (1962). *Pharmac. Rev.*, **14**, 318–445.

COLLIER, H. O. J. & JAMES, G. W. L. (1967). *Br. J. Pharmac. Chemother.*, **30**, 283–301.

DAWSON, W. & WEST, G. B. (1965). *Ibid.*, **24**, 725–734.

DHAR, H. L. & SANYAL, R. K. (1963). *J. Pharm. Pharmac.*, **15**, 628.

DIXON, W. E. & BRODIE, T. G. (1903). *J. Physiol., Lond.*, **29**, 97–173.

FEINBERG, S. M., MALKIEL, S. & MCINTIRE (1953). *J. Allergy*, **24**, 302–308.

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

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

HERXHEIMER, H. & ROSA, L. (1952). *Ibid.*, **118**, 7P.

HICKS, R. & LEACH, G. D. H. (1963). *Br. J. Pharmac. Chemother.*, **21**, 441–449

HICKS, R. (1966). *Ph.D. Thesis*, University of London.

HICKS, R. (1968). *J. Pharm. Pharmac.*, **20**, 497–504.

HICKS, R. (1969). *Ibid.*, **21**, 202–203.

HUIDOBRO, H. (1960). *J. Physiol., Paris*, **52**, 121–122.

KOVACS, E. M. & SUFFIAD, K. (1968). *Br. J. Pharmac.*, **32**, 262–272.

LEFCOE, N. M. (1956). *J. Allergy*, **26**, 352–358.

MOVAT, H. Z., DI LORENZO, N. L., TAICHMAN, N. S., BERGER, S. & STEIN, H. (1967). *J. Immunol.*, **98**, 230–235.

ORANGE, R. P., VALENTINE, M. D. & AUSTEN, K. F. (1967). *Science, N.Y.*, **157**, 318–319.

SANYAL, R. K., SPENCER, P. S. J. & WEST, G. B. (1959). *Nature, Lond.*, **184**, 2020.

TREADWELL, P. E. (1965). *J. Immunol.*, **94**, 692–695.

WEISSMANN, G. & THOMAS, L. (1964). *Recent Progr. Horm. Res.*, **20**, 215–245.

ZICHA, L., SCHEIFFARTH, F., SCHMID, E., KOSCHERA, H. & GRAF, N. (1960a). *Arzneimittel-Forsch.*, **10**, 728–731.

ZICHA, L., SCHEIFFARTH, F., SCHMID, E. & WESCHTA, W. (1960b). *Ibid.*, **10**, 890–893.

ZICHA, L., SCHMID, E., SCHEIFFARTH, F., GRAF, N. & KOSCHERA, H. (1960). *Ibid.*, **10**, 831–834.