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Inhibition of Anaphylaxis in Mice by Pre-treatment with Cysteine, Lysine Ethyl Ester, and Tyrosine Ethyl Ester[1]

Immune hemolysis, according to the studies of BECKER[2], proceeds by activation of the first component of complement (C^1 1) to form an esterase, with activity directed primarily toward *p*-toluene sulfonyl arginine methyl ester. This compound inhibits immune hemolysis, apparently by its action as a competitive substrate. CUSHMAN *et al.*[3] also have demonstrated the inhibitory action of certain other compounds, among which were cysteine, lysine ethyl ester, and tyrosine ethyl ester. In view of increasing evidence for the participation of complement (C^1) as a primary factor in hypersensitivity reactions, this exploratory study on the effect of these materials on systemic anaphylaxis in the mouse was undertaken.

Methods. Swiss and CFW mice (6–8 weeks) obtained from Thomas Euers, Austin (Texas), were employed. Where mice of both sexes were used, they were distributed as uniformly as possible. Each experiment was performed with a separate lot of mice and was completed within one day. Antiserum was prepared in 3 adult New Zealand white rabbits by 3 weekly subcutaneous injections of 100 mg crystalline egg albumin (CEA) Armour) in complete Freund's adjuvant (Difco), with exsanguination at the fourth week. The pooled antiserum contained 390 μg antibody N/ml by the usual quantitative precipitin techniques. The following compounds were prepared at 0·1 M in buffered saline (0·0054 M Na_2HPO_4, 0·0013 M KH_2PO_4, 0·135 M NaCl) and, except where otherwise stated, were administered by intraperitoneal injection at a dosage of 1 ml/20 g body weight (0·005 M/kg): L-cysteine HCl, DL-tyrosine ethyl ester HCl (TEE), L-lysine ethyl ester diHCl (LEE), and *p*-toluene sulfonyl-L-arginine methyl ester HCl (TAME). All shocking injections were given intravenously into a tail vein. Rectal temperatures were recorded, just prior to the shocking injection and 30 min afterwards, using a direct reading thermistor device (Tele-Thermometer, Yellow Springs Inst. Co.). The rectal probe was introduced to 2 cm and the meter allowed to stabilize. This method has been found to be a reliable measure of the anaphylactic response in this laboratory and by others[4]. In this study anaphylaxis has been arbitrarily defined as reactions resulting in a 1°C drop 30 min after the shocking injection, or death within 30 min. This designation has the advantage of complete objectivity. Since most animals which show a 5°C drop within 30 min have lethal shocks, this value was assigned to animals dying within 30 min in calculating mean values. Additional information concerning methods will be given below.

Results. Preliminary toxicity studies with the 4 compounds showed TAME to be too toxic for further evaluation. Levels higher than 0·0015 M/kg uniformly resulted in deaths, while dosages less than this had no discernible effect on mouse anaphylaxis.

Reactions in actively sensitized mice (*Table I*). In Experiment 1 CFW mice were injected with 0·75 mg CEA in

[1] Aided by Research Grant (E 1563) from National Institute of Allergy and Infectious Diseases, USPHS.

[2] E. L. BECKER, J. Immunol. *77*, 469 (1956).

[3] W. F. CUSHMAN, E. L. BECKER, and G. WIRTZ, J. Immunol. *79*, 80 (1957).

[4] P. MORGAN, N. P. SHERWOOD, and A. A. WERDER, J. Immunol. *79*, 46 (1957).

Table I
Anaphylactic Reactions in Actively Sensitized Mice

Pre-Shock Treatment (IP Injection)	Pre-Shock Temperature (°C) Mean S. E.	Temperature 30 min after shock Mean* S. E.	Difference of Means	Deaths	Shock/ total	(% Shock)	P**
Exp. 1 ♀ CFW							
None	36·3 ± 0·3	30·9 ± 1·0	−5·4	6	10/10	(100)	—
Cysteine 60 min	35·0 ± 0·5	33·2 ± 1·1	−1·8	0	4/9	(44)	0·05
Lee 60 mm.	36·9 ± 0·2	34·8 ± 1·2	−2·1	0	7/9	(78)	0·50
Saline 60 min.	37·8 ± 0·1	34·6 ± 0·8	−3·2	0	4/5	(80)	0·75
Exp. 2 ♂ and ♀ Swiss							
None	36·4 ± 0·2	32·1 ± 0·3	−4·3	4	6/6	(100)	—
Cysteine 60 min	35·3 ± 0·3	33·8 ± 0·6	−1·5	0	3/6	(50)	0·20
Saline 60 min	36·7 ± 0·4	32·0 ± 0·5	−4·7	3	5/5	(100)	—
Exp. 3 ♂ Swiss							
None	37·6 ± 0·3	35·2 ± 0·6	−2·4	0	5/5	(100)	—
Cysteine 60 min	34·6 ± 0·3	33·6 ± 0·9	−1·0	1	1/5	(20)	0·10
Cystine 60 min	36·6 + 0·3	34·8 ± 1·0	−1·8	2	2/5	(40)	0·25
Lee 60 min	35·4 ± 0·4	34·8 ± 0·5	−0·6	0	2/5	(40)	0·25
Lysine 60 min	36·2 ± 0·1	32·6 ± 1·0	−3·6	4	5/5	(100)	—
Tee 60 min	34·4 ± 0·3	35·0 ± 0·1	+0·6	0	0/5	(0)	0·025
Tyrosine 60 min	35·0 ± 0·3	33·2 ± 1·4	−1·8	2	2/5	(40)	0·25

* Deaths considered as − 5·0°C Temperature drop.

** Determined from Chi-square values using Yates correction.

Freund's adjuvant and on the 24th day given a shocking injection of 0·2 mg CEA in 0·2 ml. Experiments 2 and 3 were similar except for the use of Swiss mice. Cysteine given 1 h before shocking reduced the intensity and incidence of the anaphylactic shock. LEE appeared to be less effective, while TEE showed a significant inhibitory effect. In most instances these compounds alone had a moderate hypothermic effect; however, the degree of hypothermia would not seem to be of sufficient magnitude to prevent an evaluation of the anaphylactic reactions by temperature depression. The absence of fatal shock in the saline-treated animals in Experiment 1 should be noted. Statistical analysis of the combined Experiments 1, 2, and 3 showed cysteine treatment to be inhibitory at the 98% level.

All surviving animals were injected again with the shocking dose of CEA 24 h after the initial injection. Both pretreated and the untreated animals were equally refractory to anaphylaxis. Animals which had been treated but not shocked 24 h previously were not protected from shock.

Reactions in mice shocked by soluble antigen-antibody complexes (*Table II*). In Experiment 4–8 a soluble antigen-antibody complex prepared at 10 × optimal antigen concentration (500 μg CEA N/ml antiserum) was employed. Antigen and Antiserum were allowed to react in buffered saline at 37°C for 30 min, then at 2°C for 48 h before centrifugation at 2°C. At this antigen level 40 μg ppt N was formed. A total volume of 0·3 ml of the supernate obtained was injected. These experiments demonstrated the variability of response in different groups of mice (e.g. compare cysteine at 60 min in Experiment 4 and 6). However, cysteine, LEE, and TEE were protective in most instances when administered 60 min prior to shocking. LEE produced the least hypothermia of the 3 compounds, while TEE gave the greatest temperature reductions. TEE was the most potent compound in modifying the anaphylactic response. In these, as in the previous experiments, surviving animals challenged 24 h after the initial injection were refractory to anaphylaxis. This could not be related to persistence of the drug effect.

Reactions in passively sensitized mice (*Table III*). All animals in Experiment 9 were shocked passively by the intravenous injection of 0·15 mg CEA in 0·2 ml within 1 min after the intravenous injection of 0·5 ml of a 1:5 dilution of antiserum (39 μg antibody N). Typical specific anaphylactic reactions are regularly produced in mice by this procedure[5]. Although statistical significance cannot be assigned to the findings in this small sample, results with cysteine and LEE at 60 min suggest a protective effect.

Discussion. A small measure of uncertainty in evaluating anaphylaxis by body temperature measurements has been introduced into these studies by a hypothermic response seen in some groups of animals after pretreatment with the compounds under study and before the shocking dose was given. No significant correlation was found to

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Table II
Anaphylactic Reactions in Mice Using Soluble Antigen-Antibody Complex

Pre-Shock Treatment (IP Injection)	Pre-Shock temperature (°C) Mean S. E.	Temperature 30 min after shock Mean* S. E.	Difference of Means	Deaths	Shock/ total	(% Shock)	P**
Exp. 4 ♂ and ♀ Swiss							
None	37·2 ± 0·3	33·5 ± 0·5	−3·7	2	10/11	(91)	—
Cysteine 60 min	34·8 ± 0·3	34·6 ± 0·6	−0·2	1	6/14	(43)	0·10
Lee 60 min	36·4 ± 0·3	36·4 ± 0·4	0	0	3/10	(30)	0·025
Tee 60 min	35·7 ± 0·3	35·8 ± 0·4	+0·1	0	3/14	(21)	0·005
Saline 60 min	36·5 ± 0·4	33·2 ± 0·7	−3·3	1	12/15	(80)	0·50
Exp. 5 ♂ and ♀ Swiss							
None	37·0 ± 0·2	33·6 ± 0·5	−3·4	0	6/6	(100)	—
Cysteine 30 min	36·4 ± 0·3	35·0 ± 0·3	−1·4	0	3/4	(75)	0·70
Cysteine 40 min***	36·8 ± 0·3	35·5 ± 0·8	−1·3	0	3/6	(50)	0·20
Cysteine 60 min	34·4 ± 0·2	35·4 ± 0·5	+1·0	0	0/5	(0)	0·01
Lee 60 min	37·6 ± 0·5	36·8 ± 0·3	−0·8	0	3/5	(60)	0·80
Lee (0·05 *M*) 60 min	38·2 ± 0·2	36·2 ± 0·5	−2·0	0	5/5	(100)	—
Saline 60 min	37·4 ± 0·6	33·2 ± 1·5	−4·2	0	4/4	(100)	—
Exp. 6 ♂ Swiss							
None	37·4 ± 0·2	35·0 ± 0·7	−2·4	1	8/10	(80)	—
Cysteine 15 min	36·7 ± 0·1	34·3 ± 0·3	−2·4	1	13/15	(87)	0·50
Cysteine 30 min	35·7 ± 0·5	32·7 ± 0·6	−3·0	3	5/7	(71)	0·98
Cysteine 60 min	36·4 ± 0·2	34·7 ± 0·5	−1·7	2	9/16	(56)	0·50
Cysteine 150 min	37·0 ± 0·2	35·6 ± 0·5	−1·4	0	7/14	(50)	0·30
Cysteine 180 min	37·0 ± 0·3	35·7 ± 0·4	−1·3	0	5/7	(71)	0·98
Exp. 7 ♂ and ♀ Swiss							
None	36·5 ± 0·3	32·9 ± 0·4	−3·6	3	10/10	(100)	—
Tee 30 min	33·1 ± 0·3	33·1 ± 0·4	0	1	8/21	(38)	0·005
Tee 60 min	35·0 ± 0·3	34·2 ± 0·7	−0·8	0	4/9	(44)	0·05
Exp. 8 ♂ Swiss							
None	37·2 ± 0·2	33·6 ± 0·5	−3·6	2	18/19	(95)	—
Tee (0·1 *M*) 60 min	35·6 ± 0·4	33·7 ± 0·6	−1·9	2	9/14	(64)	0·10
Tee (0·2 *M*) 60 min	33·8 ± 0·2	33·2 ± 0·6	−0·6	3	4/19	(21)	0·001
Saline 60 min	37·8 ± 0·2	34·8 ± 0·9	−3·0	4	8/10	(80)	0·70

* Deaths considered as − 5·0°C temperature drop.
** Determined from Chi-square values using Yates correction.
*** Cysteine injected intravenously.

Table III
Anaphylaxis in Passively Sensitized Mice

Pre-Shock Treatment (IP Injection)	Pre-Shock Temperature (°C) Mean S. E.	Temperature 30 min after shock Mean* S. E.	Difference of Means	Deaths	Shock/ total	(% Shock)	P**
Exp. 9 ♂ and ♀ Mice							
None	36·4 ± 0·2	33·8 ± 0·8	−2·6	0	4/5	(80)	—
Cysteine 30 min.	35·0 ± 0·2	33·2 ± 0·6	−1·8	1	3/4	(75)	0·50
Cysteine 60 min.	35·0 ± 0·6	33·6 ± 1·2	−1·4	0	1/5	(20)	0·25
Cysteine 120 min.	35·0 + 0·5	34·2 ± 0·7	−0·8	0	2/5	(40)	0·70
Tee 30 min.	34·0 ± 0·6	32·0 ± 0·7	−2·0	1	4/5	(80)	—
Tee 60 min.	36·4 ± 0·5	35·4 ± 0·8	−1·0	0	3/5	(60)	—
Tee 120 min.	37·2 ± 0·2	34.0 ± 0·3	−3·2	0	5/5	(100)	—
Lee 30 min.	36·0 ± 0·3	33·6 ± 0·8	−2·4	0	3/5	(60)	—
Lee 60 min.	36·2 ± 0·5	36·5 ± 0·4	+0·3	0	0/5	(0)	0·10
Lee 120 min.	36·0 ± 0·3	33·8 ± 0·7	−2·2	1	4/5	(80)	—
Saline 30 min.	36·5 ± 0·4	34·9 ± 0·7	−1·6	0	4/5	(80)	—
Saline 60 min.	36·7 ± 0·2	32·4 ± 0·6	−4·3	3	5/5	(100)	—
Saline 120 min.	37·0 ± 0·3	34·7 ± 0·9	−2·3	1	4/5	(80)	—

* Deaths considered as — 5·0 °C temperature drop.

** Determined from Chi-square values using Yates correction.

exist, however, between preshock temperature and susceptibility to anaphylaxis. It was noted, furthermore, that the usual clinical indications of anaphylaxis in mice (e.g. lethargy and paralysis) were absent except in those animals showing a depression of body temperature following injection of the shocking material.

A definitive explanation of the modifying effect of these drugs cannot now be offered for lack of direct proof. It is possible that their action may be concerned with their capacity to inhibit $C^1$1, which is activated by antigen-antibody complexes[2]. It is tempting to speculate that the shocked animals may have been refractory to further attempts to produce an anaphylactic response within 24 h in part because of depletion of C^1 1.

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Résumé

L'injection intrapéritonéale chez la souris de cystéine, tyrosine-éthylester ou lysine-éthyl-ester, 1 h avant la production du choc anaphylactique diminue d'une façon significative la sensibilité des animaux à la réaction. Il est possible que cela soit dû à une modification de l'effet du premier composé actif du complément.

[6] SUMMER FELLOW. Allergy Foundation of America, 1957–1958.

Intracellular Peptides of Escherichia coli

One of the ways which could contribute to the elucidation of the mechanism of protein synthesis consists in finding intermediate products of peptide nature. Although free peptides have been detected in various organisms, their relationship to protein synthesis has not been demonstrated conclusively[1–5]. Gram-negative bacteria contain much less intracellular free ninhydrin-positive substances than Gram-positive ones, and glutathione is the only substance of peptidic character which has definitely been detected so far[6]. Further, non-identified ninhydrin-positive substances were described from *E. coli* cells[6] and from the nutrient medium remaining after the growth of these bacteria[7]. Recently, two pure dipeptides were found and identified which accumulate in *E. coli* cells particularly during growth in the presence of chloramphenicol[8].

In further work, other ninhydrin-positive substances from *E. coli B* extract prepared in cold with 5% trichloracetic acid were investigated. This extract of bacteria from 20 l of synthetic medium was treated with ethyl ether to remove trichloroacetic acid, freeze-dried and oxidized with performic acid[9], so that glutathione and possibly other cysteine-containing peptides were present in a single oxidized form. After de-salting on Dowex-50 in the hydrogen form, amino acids and peptides were eluted with ammonia, volume reduced by evaporation and chromatography carried out in a mixture of butanol-acetic acid. In this way free amino acids were separated from other ninhydrin-positive substances which remained at the start of the chromatogram.

After elution, substances from the chromatogram start were separated by high-voltage paper electrophoresis in a pyridine-acetate buffer at pH 5·6 (2·5 h, 29 V/cm)[10].

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