Production of Lesions in Various Tissues by Amino Acids

This study is concerned with stress from an elevated concentration of a single amino acid directed on various tissues of organs including skin and internal organs. The amino acid selected for stress was from essential and non-essential amino acids. Amino acid stress was indicated by lesions, inflammation, cytolysis, and cytomorphosis.

Materials and methods. Daily application of 0.5 ml of 1 m*M* concentration of an individual amino acid of pH 7.2 in isotonic saline was applied on 2″ × 2″ gauze to dorsal skin quadrants of the guinea-pig, rabbit, and rhesus monkey using a modification of DRAIZE te al.[1,2]. Substances used for exposure to skin were L-aspartic acid (ASP), D-aspartic acid (D-ASP), carbamate[2], L-glutamic acid (GLU), D-glutamic acid (D-GLU), isotonic saline, or L-ornithine (ORN). The skin was evaluated[3] daily and new solutions were applied. Skin biopsies for cytopathological and histological evaluation were taken upon termination of a 21 day period of exposure.

Internal organs of Swiss White mice and Sprague Dawley rats were exposed to 0.5 ml containing 0.5 mmole of individual amino acid of pH 7.2 in isotonic saline. The volume and effective concentration of amino acid was determined from an earlier study[3]. Daily injections of B-alanine (B-ALA), L-citrulline (CIT), glycine (GLY), L-histidine (HIS), L-phenylalanine (PHE), L-tryptophan (TRP), or L-tyrosine (TYR) was delivered intraperitoneally for 10 days. An equal volume of isotonic saline was injected into control animals. Internal organs such as heart, lung, spleen, kidney, liver and intestine were biopsied for cytopathological and histological evaluation.

Results. Rabbit skin response to single amino acids are contained in Figure 1. Rabbit skin exposure to ASP produced erythema in 8 days, induration in 10 days, and vesiculation in 16 days. The skin of guinea-pig and rhesus monkey responded with eyrthema, induration, and vesiculation to ASP. GLU produced a definite erythema to the entire patch area in rabbit skin in 14 days. Carbamate produced bullous lesions in 3 days. ORN was an early irritant producing erythema in most patch areas and erythema and induration in some patch areas. Irritation caused by ORN was maximal at 10 days and subsided by 21 days.

Rabbit skin irritation was greater from exposure to L enantiomorphs of ASP and GLU than from D enantiomorphs as shown in the Table. Vesiculation was prominent in rabbit skin exposed to 1 m*M* ASP after 21 days. Erythema was prominent in rabbit skin exposed for the same period to 1 m*M* GLU. Less erythema was elicited in rabbit skin exposed to 1 m*M* D-ASP and the least amount of erythema was present in rabbit skin exposed to D-GLU.

Histological sections revealed heavy infiltrates of inflammatory cells in degenerated epithelium of rabbit skin exposed to carbamate for 21 days. The dermis had dense fibrosis with inflammatory infiltrates. Skin exposed to ASP (Figure 2a) manifested a thinned and occasional discontinuity of the epithelium. Fluid was accumulated in

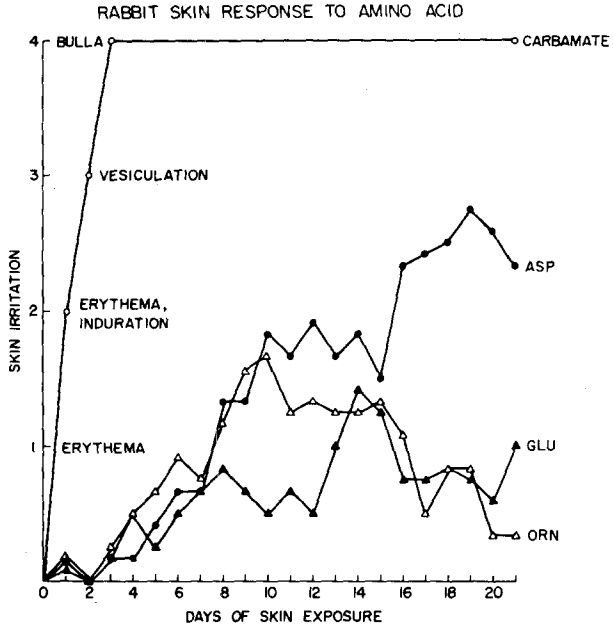

Fig. 1. Rabbit skin response to daily exposure to 0.5 ml of a 1 m*M* concentration of amino acid. Rabbit skin was exposed to carbamate (6)[a], ASP (12)[a], GLU (12)[a], and ORN (6)[a]. Scoring was according to modified DRAIZE[1]: 0, negative reading; 1, definite erythema of the entire patch area; 2, erythema and induration; 3, vesiculation; 4, bullous lesion. The mean values from the scoring of the skin was placed in the chart. [a] Number of animals used.

[1] J. H. DRAIZE, G. WOODARD and H. O. CALVERY, J. Pharmac. exp. Ther. *82*, 377 (1944).
[2] L. PHILLIPS, II, M. STEINBERG, H. I. MAIBACH and W. A. AKERS, Toxic. appl. Pharmac. *21*, 369 (1972).
[3] R. W. LONGTON, Ph. D. Thesis, Northwestern University, Evanston, Ill. (1971).

Rabbit skin response to L and to D amino acids

Days of exposure	ASP			GLU			D-ASP			D-GLU		
	$\bar{X}$	S.D.	S.E.	$\bar{X}$	S.D.	S.E.	$\bar{X}$	S.D.	S.E.	$\bar{X}$	S.D.	S.E.
1	0	0	0	0	0	0	0	0	0	0	0	0
5	0.3	0.5	0.2	0	0	0	0.7	0.8	0.3	0.3	0.5	0.2
10	1.2	1.0	0.4	0.2	0.4	0.3	1.0	0.6	0.3	0.5	0.8	0.3
15	1.0	0.9	0.4	1.0	1.3	0.5	1.2	1.0	0.4	0.7	1.2	0.5
20	2.8	1.2	0.5	1.5	1.4	0.6	1.0	1.3	0.5	0.5	0.6	0.3
21	3.2	1.0	0.4	1.0	1.6	0.6	0.8	1.0	0.4	0.5	0.8	0.3

$\bar{X}$, mean of 6 animal responses. 0, negative reading; 1, definitie erythema of the entire patch; 2, erythema and induration; 3, vesiculation; 4, bullous lesion.

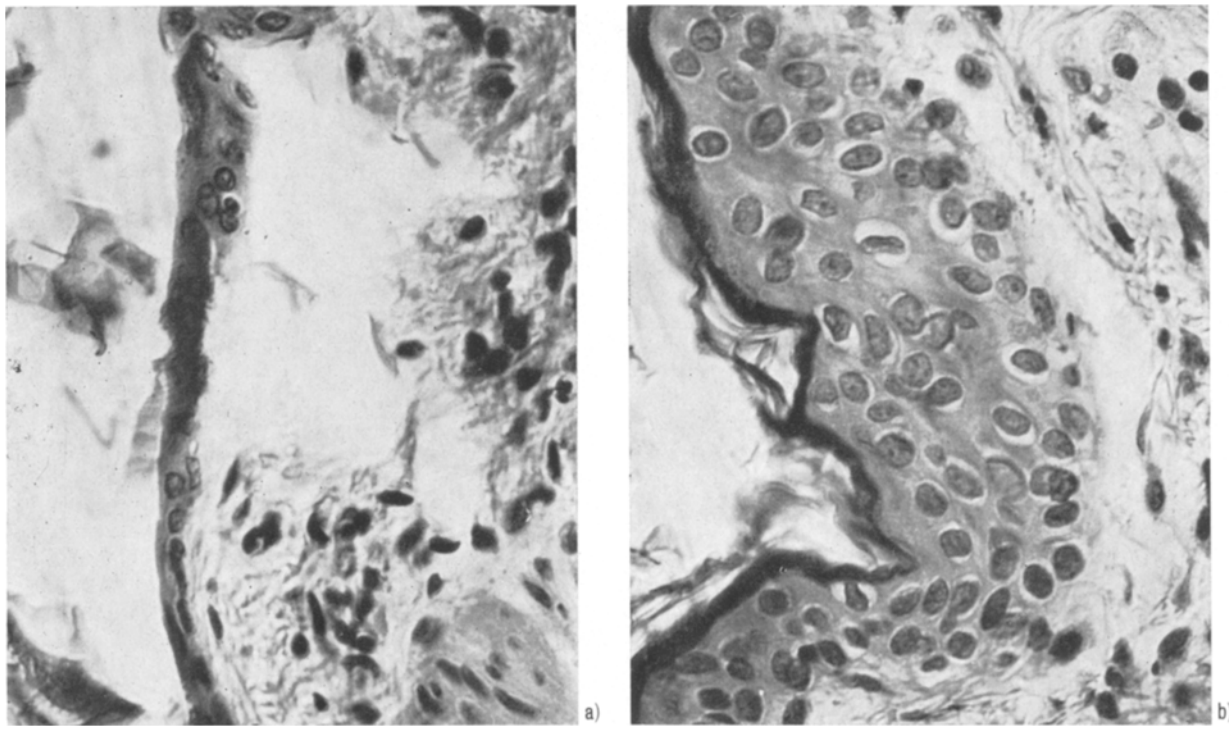

Fig. 2. Photographs of histological sections of rabbit skin after 21 days of exposure to 0.5 ml of 1 m*M* of amino acid. Thinned epidermis covered fluid areas which were surrounded by loose connective tissue of the dermis which contained few inflammatory cells was observed in skin exposed to ASP (a). Thickened epidermis with a moderate infiltrate of inflammatory cells in the dermis was observed in the skin exposed to GLU (b). ×500.

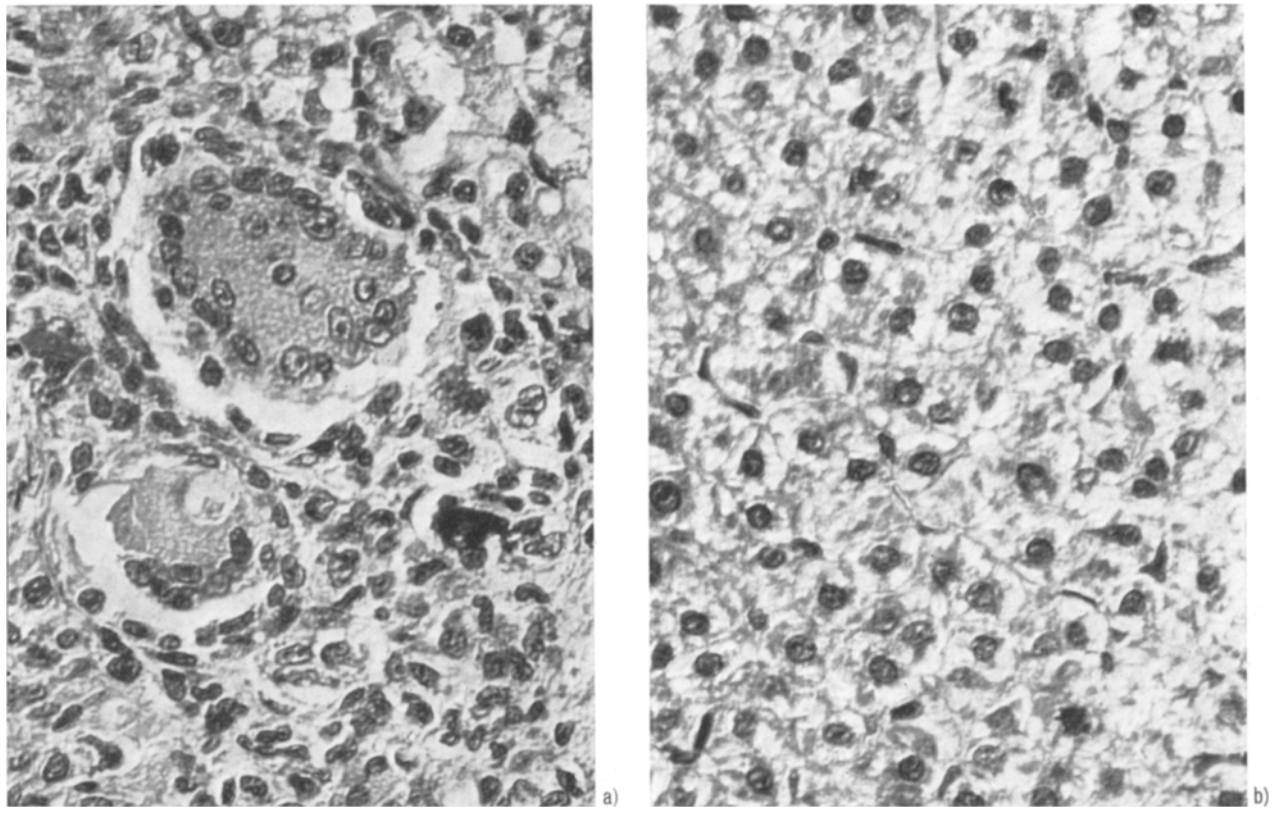

Fig. 3. Photographs of histological sections of liver from Sprague-Dawley rats after 10 days of exposure to daily i.p. injection of 0.5 ml containing 0.5 mmole HIS (a), and 0.5 ml of isotonic saline (b). Giant cells were observed in the parenchyma of the liver after the injection series with HIS (a). Normal liver was observed after the injection series with isotonic saline (b). ×500.

dermis beneath the basal layer of epidermis. Thickened epithelium (Figure 2b) and inflammatory infiltrates were present in skin exposed to GLU.

Pathological changes of internal organs were fibrosis of liver and intestines after i.p. exposure to TYR for 10 days. Perivascular aggregation of eosinophiles were observed in organs which contained high fibrogenic activity after exposure to TYR. Hemorrhage and engorgement with red blood cells in lungs were observed after exposure of B-ALA. Subcapsular hemorrhage of kidneys occurred after i.p. exposure of mice and rats to 0.5 mmole of B-ALA. Morphological changes such as giant cell formation were present in liver (Figure 3a) after exposure to HIS. The liver was normal (Figure 3b) after similar injections with isotonic saline. The bones of these animals injected with HIS were soft and friable and were less calcified than isotonic saline injected controls.

Discussion. Pathological changes which resulted after the series of i.p. injections appeared to be specific for the amino acid in excess. Different pathological changes were seen for each of the amino acids in excess.

The mechanism(s) of action of these essential and non-essential amino acids in producing lesions is unknown. Acidosis per se[4] is not a controlling mechanism since ASP caused the greatest rabbit skin irritation, GLU less, and D-ASP and D-GLU caused the least irritation. Structure and steric configuration of amino acid appears important. Amino acids may become decarboxylated producing very active biogenic amines. Activation of proteolytic enzymes[5] may occur due to amino acids or their products. It is possible that amino acids produced by bacteria[6] or amino acid metabolites activate mycoplasma and viruses present in tissues to produce cytopathology. No precedent appears to exist in literature for viral activation by an amino acid. Viral activation remains a possibility.

Zusammenfassung. Es wird der Einfluss einer Aminosäure auf verschiedene Gewebsarten und auch auf die Haut kontrolliert. Unterschiedlich starke, z.T. schwere Degenerationen, Entzündungen und auch Zellauflösungen waren festzustellen. Teilweise kam es zu Riesenzellbildungen oder zu Fibrosen in der Leber. Knochennekrosen und Blutungen wurden festgestellt.

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Naval Medical Research Institute, National Naval Medical Center, Bethesda (Maryland, USA), 15 February 1973.

[4] W. C. HEIRD, R. B. DELL, J. M. DRISCOLL, B. GREBIN and R. W. WINTERS, New Engl. J. Med. *287*, 943 (1972).
[5] K. C. KHURANA, V. K. PASRICHA, V. K. KURANA and S. K. DUGGAL, Dermatologica *141*, 428 (1970).
[6] R. W. LONGTON, J. S. COLE, III, H. D. TOW and V. J. BERZINSKAS, Influence of substrate on small metabolite production by selected streptococci, 50th Gen. Session, International Association for Dental Research, Program and Abstracts, 133 (1972).
[7] Thanks to K. T. GIVENS for carbamate, Hercules Incorporated, Wilmington, Delaware.
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[11] The assertions and opinions contained herein are those of the author and are not to be construed as reflecting the views of the Navy Department or the naval service at large.

Structural Coupling Between Pancreatic Islet Cells

There is circumstantial evidence that ionic[1-3] and metabolic[4] coupling observed between cells in a variety of tissues may be due to intercellular specializations of the membranes known as 'gap junctions'[5], which seem to represent low resistance pathways through which ions and low molecular weight substances can diffuse from one cell to the other[4, 6-13]. Thus far, no such junctions have been identified between pancreatic islet cells. The recent introduction of freeze-etching technique[14, 15] has greatly facilitated the identification of cell junctions[16-19]. This technique has now allowed us clearly to demonstrate for the first time in islet cells the presence of junctional complexes, in the form of small gap junctions and focal tight junctions[20].

Isolated islets were obtained by collagenase digestion[21] from pancreases of albino rats. Rats were chosen because of the characteristic topological relationship between the two main types of islet cells: indeed, glucagon-producing α-cells are disposed peripherally as a mantle around centrally located insulin-producing beta cells[22]. Pelleted islets were briefly fixed with 2% glutaraldehyde in phosphate buffer and soaked in 20% phosphate-buffered glycerol solution before freezing. Freeze-cleaving was performed according to the method of MOOR and MÜHLETHALER[23] in a Balzers Freeze-Etch Unit. Fracturing and etching temperature were –100 °C. Etching time was 1 min. The cleaned replicas were observed in a Philips EM 300

[1] W. R. LÖWENSTEIN, Ann. N.Y. Acad. Sci. *137*, 441 (1966).
[2] M. V. L. BENNETT, Ann. N.Y. Acad. Sci. *137*, 509 (1966).
[3] E. J. FURSHPAN and D. D. POTTER, Curr. Topics devel. Biol. *3*, 95 (1968).
[4] N. B. GILULA, O. R. REEVES and A. STEINBACK, Nature, Lond. *235*, 262 (1972).
[5] J. P. REVEL and M. J. KARNOVSKY, J. Cell Biol. *33*, C7 (1967).
[6] M. M. DEWEY and L. BARR, Science *137*, 670 (1962).
[7] J. J. DREIFUSS, L. GIRARDIER and W. G. FORSSMANN, Pflügers Arch. ges. Physiol. *292*, 13 (1966).
[8] L. BARR, W. BERGER and M. M. DEWEY, J. gen. Physiol. *51*, 347 (1968).
[9] M. W. BRIGHTMAN and T. S. REESE, J. Cell Biol. *40*, 648 (1969).
[10] B. W. PAYTON, M. V. L. BENNETT and G. D. PAPPAS, Science *166*, 1641 (1969).
[11] R. G. JOHNSON and J. SHERIDAN, Science *174*, 717 (1971).
[12] G. D. PAPPAS, Y. ASADA and M. V. L. BENNETT, J. Cell Biol. *49*, 173 (1971).
[13] J. P. REVEL, A. G. YEE and A. J. HUDSPETH, Proc. natn. Acad. Sci. USA *68*, 2924 (1971).
[14] R. L. STEERE, J. biophys. biochem. Cytol. *3*, 45 (1957).
[15] H. MOOR, K. MÜHLETHALER, H. WALDNER and A. FREY-WYSSLING, J. biophys. biochem. Cytol. *10*, 1 (1961).
[16] L. A. STAEHELIN, T. M. MUKHERJEE and A. M. WILLIAMS, Protoplasma *67*, 165 (1969).
[17] D. A. GOODENOUGH and J. P. REVEL, J. Cell Biol. *45*, 272 (1970).
[18] J. P. CHALCROFT and S. BULLIVANT, J. Cell Biol. *47*, 49 (1970).
[19] N. B. GILULA, D. BRANTON and P. SATIR, Proc. natn. Acad. Sci. USA *67*, 213 (1970).
[20] M. G. FARQUHAR and G. E. PALADE, J. Cell Biol. *17*, 375 (1963).
[21] P. E. LACY and M. KOSTIANOVSKY, Diabetes *16*, 35 (1967).
[22] H. FERNER, *Das Inselsystem des Pankreas* (Thieme, Stuttgart 1962), p. 119.
[23] H. MOOR and K. MÜHLETHALER, J. Cell Biol. *17*, 609 (1963).