of the plasma membrane (Figure 2). These alterations are characteristically exhibited by sick or dying cells. The changes do not appear to be a consequence of defective tissue fixation and processing since they were not seen in fibroblasts or other cells of the NCT – nor in the non-fibroblastic cells of the ICT fraction.

The changes observed in the fibroblasts of the ICT appear to be associated with the activity of lymphoid cells. There is a strong positive correlation ($r = +0.55$) between the increasing fibroblast size and increasing numbers of medium-size lymphocytes and immunoblasts (Figure 1). Furthermore, lymphocytes were observed frequently in intimate contact with the altered fibroblasts in the ICT-fraction (Figure 2) while these associations were not observed in the NCT-fraction.

Recently it was shown that peripheral blood lymphocytes obtained from patients with inflammatory gingival disease are sensitized to antigenic substances present in human dental microbial plaque[6]. These cells undergo blast transformation when cultured in vitro in the presence of plaque antigens, and fluids from these cultures exert a marked cytotoxic effect on gingival fibroblasts[7]. The morphologic and morphometric data presented here support the idea that a phenomenon similar to that observed in vitro may be occurring in the gingival tissue in early gingival disease.

The observations provide a plausible basis for the early lesion in gingivitis. In susceptible individuals, lymphoid cells become sensitized to microbiologic substances present in dental plaque. Upon encountering these substances in the region of the dento-gingival area, the cells undergo blast transformation and interact directly or through mediators with resident fibroblasts to induce cytopathic alterations. The affected fibroblasts are no longer able to produce and maintain the connective tissue substance of the gingiva and this leads to the observed loss of connective tissue substance and loss of normal function.

Zusammenfassung. Eine morphometrische Analyse menschlicher, entzündeter Gingiva ergab im Vergleich zu normalem Bindegewebe 70% weniger Kollagenfasern, dreifach vergrösserte und pathologisch veränderte Fibroblasten und eine kleine Population charakteristischer Immunoblasten mit einer Zellansammlung, die zu 76% aus Zellen der Lymphozytenserie bestand. Die Grösse der Fibroblasten war positiv mit der steigenden Zahl der Lymphozyten korreliert. Diese Befunde weisen auf eine Immunreaktion mit zytotoxischen Auswirkungen auf Fibroblasten des gingivalen Bindegewebes hin.

H. E. SCHROEDER and R. PAGE

Department of Oral Structural Biology, Dental Institute, University of Zürich, CH-8028 Zürich (Switzerland), and Center of Research in Oral Biology and Department of Pathology, Medical School, University of Washington, Seattle (Washington, 98195 USA), 4 May 1972.

[6] L. IVANYI and T. LEHNER, Archs oral Biol. *15*, 1089 (1970). L. IVANYI, J. M. A. WILTON and T. LEHNER, Archs oral Biol. *16*, 1117 (1971). J. E. HORTON, J. J. OPPENHEIM and S. E. MERGENHAGEN, Int. Ass. dent. Res. *50*, G. M. Abstr. No. 369 (1972).

[7] J. E. HORTON, J. J. OPPENHEIM and S. E. MERGENHAGEN, Int. Ass. dent. Res. *50*., G. M. Abstr. No. 369 (1972).

Antiphlogistic Activity of L-Phenylalanine and L-Tryptophane within the Mouse Peritoneal Cavity

Since the administration of irritants into the abdominal cavity produces a predictable inflammatory response characterized by an increased % polymorphonuclear leukocytes (PMN) I have attempted to inhibit the infiltration of PMNs into an inflamed peritoneal cavity induced by an intraperitoneal injection of gelatin solution by injecting L-phenylalanine and L-tryptophane[1]. MCGOWAN and I (1968) have found that in women with inflammation of the peritoneal cavity the PMN counts were elevated 2 or 3 times above normal[2]. The possibility that some amino acids have anti-inflammatory activity was suggested to us by the observation that certain amino acids maintain life and increase liver glycogen in adrenalectomized rats[3–5]. Both biological activities characterize adrenal steroids which have anti-inflammatory activity. Furthermore, we have shown that L-phenylalanine and L-tryptophane inhibited PMN infiltration into an area of local gelatin-induced inflammation under the skin of adrenalectomized rats[6] – a reliable procedure for testing the local antiphlogistic activity of glucocorticosteroids[7].

Adult female CF-1 mice (25–30 g) were given a single i.p. injection of aqueous 1% gelatin solution and we injected control animals with water. Aqueous L-phenylalanine and L-tryptophane were each administered i.p. at 25 mg/kg simultaneously but separately with gelatin. The 1% gelatin solution was injected on a 100 mg/kg per 10 ml basis whereas the 0.25% amino acid solutions were administered on the basis of 25 mg/kg/10 ml. The 10 ml volume per kg of body weight was employed for control animals. When the gelatin solution was injected simultaneously with the amino acid the total volume was maintained equal to the gelatin alone so that we would not obtain a response due to a gelatin dilution factor. In 3 h peritoneal fluid (sometimes only one drop) was aspirated and spread on an albumin-coated slide. Each coded slide was fixed in 95% alcohol and stained with buffered Wright's stain. 200 cells were consecutively and randomly counted so that we could determine the % distribution of lymphocytes, polymorphonuclear leukocytes, monocytes, eosinophils, other mononuclear cells and mast cells present in each cytologic specimen. This procedure of randomly counting a fixed number of cells rather than counting the number of cells in an absolute fluid volume eliminates the extreme variation resulting from counting cells per unit volumes because of the small amount of fluid aspirated. The average values reported represent mean % distribution of cells obtained

[1] L. MCGOWAN and R. H. DAVIS, J. Path. *100*, 210 (1970).

[2] L. MCGOWAN and R. H. DAVIS, Obstet. Gnyec. *31*, 589 (1968).

[3] R. H. DAVIS, Thesis, Rutgers University, New Brunswick, N.J., USA (1958).

[4] J. H. LEATHEM, Recent Progr. Hormone Res. *14*, 141 (1958).

[5] Y. KOTAKE and K. INOUYE, J. Biochem., Tokyo *42*, 513 (1955).

[6] R. H. DAVIS, J. S. FISHER and L. MCGOWAN, J. Endocr. *41*, 603 (1968).

[7] D. E. HOLTKAMP, D. E. BATES, A. E. HEMING, C. E. POETSCH, C. J. LAWRENCE and H. E. BUELL, Acta endocr., Copenh. *21*, 268 (1956).

Table I. Inhibition of polymorphonuclear leukocyte infiltration into gelatin-induced inflamed peritoneal cavities of female CF-1 mice by injected L-phenylalanine

	Control (injected water	1% Gelatin	
		Alone	Plus L-phenylalanine
No. of mice	13	13	18
Intraperitoneal dose (mg/kg)	—	100	25
	% distribution of cells (mean ± S.E.)		
Lymphocytes	61.3 ± 3.9	39.6 ± 1.6	52.0 ± 1.6
Polymorphonuclear leukocytes	27.2 ± 3.0	48.0 ± 3.1	37.1 ± 2.6[a] (22.7%)
Monocytes	0.9 ± 0.5	0.5 ± 0.2	0.4 ± 0.1
Eosinophils	0.4 ± 0.2	0.3 ± 0.1	0.6 ± 0.1
Mononuclear cells	10.0 ± 1.6	9.6 ± 1.7	9.6 ± 1.7
Mast cells	0.3 ± —	0.9 ± 0.5	0.1 ± —

[a] Significant difference compared with polymorphonuclear leukocytes of the gelatin controls is $P < 0.02$. The % inhibition below the gelatin control value is shown in parenthesis. ± S.E. = standard error.

Table II. Inhibition of polymorphonuclear leukocyte infiltration into gelatin-induced inflamed peritoneal cavities of female CF-1 mice by injected L-tryptophane

	Control (injected water)	1% Gelatin	
		Alone	L-tryptophane
No. of mice	15	16	13
Intraperitoneal dose (mg/kg)	—	100	25
	% distribution of cells (mean ± S.E.)		
Lymphocytes	58.6 ± 3.7	23.4 ± 2.7	23.4 ± 4.2
Polymorphonuclear leukocytes	20.5 ± 2.3	49.2 ± 3.9	34.2 ± 4.4[a] (30.5%)
Monocytes	3.3 ± 0.6	2.0 ± 0.5	1.8 ± 0.5
Eosinophils	1.2 ± 0.6	1.4 ± 0.2	1.1 ± 0.3
Mononuclear cells	14.2 ± 7.1	21.0 ± 3.3	36.0 ± 6.0
Mast cells	2.4 ± 1.2	3.2 ± 1.4	3.6 ± 0.8

[a] Significant difference compared with polymorphonuclear leukocytes of the gelatin controls is $P < 0.02$. The % inhibiton below the gelatin control value is shown in parenthesis. ± S.E. = standard error.

from 13–18 mice. Probability values were obtained by using Student's *t*-test.

Administration of L-phenylalanine and L-tryptophane each inhibit gelatin-induced inflammation of the mouse abdominal cavity (Tables I and II). Gelatin injections alone produced a significant increase in the proportion of PMNs infiltrating into the peritoneal cavity as compared to controls. A 22.7% inhibition of PMN infiltration was obtained with 25 mg/kg L-phenylalanine ($P < 0.02$) as compared to 30.5% for 25 mg/kg L-tryptophane ($P < 0.02$). The distribution of lymphoctyes was lower in animals receiving L-tryptophane plus the irritant than those receiving the L-phenylalanine combination. The proportions of monocytes, eosinophils and mast cells were similar in all three test groups for each amino acid but the % distribution of mononuclear cells (other than lymphocytes and monocytes) was higher in specimens obtained from gelatin plus tryptophane treated animals than mice receiving 1% gelatin alone or controls. The amphoteric buffering properties of amino acids cannot account for the anti-inflammatory activity because certain amino acids such as proline and hydroxyproline are actually inflammatory. We therefore feel the response is specific for certain amino acids which mimic anti-inflammatory hormones and should not be explained merely on a physiochemical basis. Other amino acids will be investigated.

Résumé. L'inflammation de la cavité abdominale provoquée chez la souris par injection de gélatine a été inhibé par l'administration intrapéritonéale de 25 mg/kg de L-phénylalanine et de L-tryptophane. Ce fait a été constaté aussi en mesurant la distribution des cellules dans le liquide péritonéale aspiré.

R. H. Davis

Department of Obstetrics and Gynecology, Department of Physiology and Biophysics, Hahnemann Medical College and Hospital, Philadelphia (Pennsylvania 19102, USA),
3 May 1972