

Carrageenan-induced granuloma and iron status in rats with dietary polyunsaturated fatty acid deficiency

BY T. CARBONELL, M. P. SAIZ AND M. T. MITJAVILA*

Departamento de Bioquímica y Fisiología, Facultad de Biología, Universidad de Barcelona, Avenida Diagonal 645, E-08071-Barcelona, Spain

AND P. PUIG-PARELLADA

Departamento de Farmacología, Facultad de Medicina, Universidad de Barcelona, E-08028-Barcelona, Spain

AND C. CAMBON-GROS, Y. FERNANDEZ AND S. MITJAVILA

Université Paul Sabatier, Institut de Physiologie, INSERM U-87, 31400-Toulouse, France

(Received 7 February 1990 – Accepted 6 September 1990)

Sprague–Dawley rats were fed for 4 months on a control diet or a polyunsaturated-fatty-acid (PUFA)-deficient diet. The combined effects of iron overload (Fe dextran) or Fe deficiency (desferrioxamine) on carrageenan-induced granuloma were studied. PUFA deficiency induced changes in Fe metabolism, but no alterations in lipid peroxidation variables were observed. Inflammation implied an increase in lipid peroxidation, Fe storage and caeruloplasmin concentration, together with symptoms of anaemia. PUFA deficiency in inflamed rats gave rise to a lower inflammatory response (granuloma weight and prostaglandin E₂ concentration) and ethane exhalation. Fe overload potentiated inflammatory and lipid peroxidation processes, whereas Fe deficiency decreased them.

Fatty acids: Iron status: Carrageenan-induced granuloma: Rat

Inflammatory processes are related to lipid peroxidation of the membranes mediated by low molecular weight iron (Gutteridge *et al.* 1981) and implicate an increase in prostaglandin E₂ synthesis (Yoshino *et al.* 1984). It has been suggested that Fe dextran induces an exacerbation of the inflammatory diseases (Winyard *et al.* 1987) while high levels of desferrioxamine (Fe-chelating drug) reduce inflammation but the effects are dependent on the administered dose (Blake *et al.* 1983).

The type of fatty acid in the diet strongly influences the fatty-acid composition of tissue and serum lipids (Morganroth *et al.* 1987), and eicosanoid metabolism is largely dependent on the amount and type of fatty-acid precursor in the diet (Codde *et al.* 1985; Terano *et al.* 1986). Thus, eicosapentaenoic acid (EPA; 20:5*n*-3), precursor of the *n*-3 eicosanoid family, has been relevant in the treatment of arthritis either in experimental models (McColl *et al.* 1987) or in clinical situations (Kremer *et al.* 1985, 1987; Moncada & Salmon 1986). Moreover, polyunsaturated-fatty-acid (PUFA)-deficient diet is associated with changes in physical properties of the membrane, which lead to altered bound-enzyme, transport proteins (Cambon-Gros *et al.* 1989) as well as lowering the production of free radicals, hydroperoxides and aldehydes (Christon *et al.* 1988). PUFA-deficient diet also induces the substitution of the physiological eicosanoid precursor by a non-suitable substrate for the cyclooxygenase enzyme system (EC 1.14.99.1; Ziboth *et al.* 1974).

* For reprints.

Direct proof for the combined role of Fe and PUFA in inflammation (carrageenan-induced granuloma in rats) has not been demonstrated. Accordingly we have studied the effect of Fe status (Fe overload or Fe deficiency) in inflamed rats submitted to a control or a PUFA-deficient diet. Indicators of inflammation, PUFA oxidation and Fe variables were determined.

MATERIALS AND METHODS

Animals and diets

The experiments were carried out on forty male Sprague–Dawley rats. After weaning they were separated in two equal groups. One group was fed with commercially available rat chow (UAR^R; Villemoison, France), containing 50 g fat/kg (25 g rapeseed oil and 25 g peanut oil/kg; control diet). The other group was offered a diet (UAR^R) containing 50 g tripalmitin/kg (PUFA-deficient diet). On analysis by flame atomic absorption spectrophotometry both diets contained 58 mg Fe/kg.

Pretreatment of experimental animals

After 4 months of treatment each group was divided into four subgroups of five rats. All C and D groups received control and deficient diets respectively (see Table 1). The pretreatments for inflamed rats (I) were: on day 0, a carrageenan granuloma pouch (Fukuhara & Tsurufuji, 1969) was induced by a dorsal injection of 5 ml air, followed 24 h later by 4 ml carrageenan (20 g/l) (Viscarin 402, Marine Colloids, Springfield, NJ) (groups CI and DI). Fe overload (Imf) was induced by injection of Fe dextran (Imferon, Instituto Llorente, Barcelona, Spain) at 50 mg Fe/kg body-weight into the granuloma pouch on days 0, 3 and 5 (groups CIIImf and DIIImf). Fe deficiency (Dfx) was induced by a daily subcutaneous injection of desferrioxamine (Desferin, Ciba-Geigy, Barcelona, Spain) at 100 mg/kg body-weight, 2 d before the granuloma induction, and into the granuloma pouch on the following 5 d (groups CIDfx and DIDfx). Rats were killed 6 d after the granuloma induction. Desferrioxamine was dissolved in sterile saline (9 g sodium chloride/l) treated with Chelex-100 (Bio-Rad Laboratories, Richmond, CA). All groups received the corresponding volume of saline treated with Chelex-100 in order to obtain the same final liquid volume injected.

Analytical techniques

At the end of the pretreatment period, four rats of each group were placed together for 4 h in a specially-designed expired gases collection chamber which enabled the monitoring *in vivo* of the peroxidative degradation of polyunsaturated lipids. Ethane production is a good indicator of *n*-3 fatty acid peroxidation. Changes in the level of ethane production between groups may be due to a lower peroxidation of *n*-3 fatty acids or to a relatively higher content of other fatty acids, such as *n*-9 which gives octane, undetectable in expired gases. The ethane content of the gas phase was measured in samples of 10 ml injected into a gas-chromatograph equipped with a flame ionization detector. The column used was 2 m × 2 mm packed with Poropak Q (Waters Associates, Framingham, MA) 100–200 mesh. Gas flow was helium at 35 ml/min. The column temperature was programmed from 50° to 170°. Samples were taken within 5 min after the animals were sealed in the chambers (this value was subtracted from all subsequent ethane determinations) and at 1, 2, 3, and 4 h.

Then the animals were anaesthetized with diethyl ether and exsanguinated by cardiac puncture. The granuloma and the liver were removed and weighed. The non-haem-Fe (Torrance & Bothwell, 1968) and the ferritin-Fe (Drysdale & Munro, 1965) levels in the liver were measured. Packed cell volume, haemoglobin concentration (Drabkin & Austin,

Table 1. *Body-weight, liver-weight and liver iron content of rats offered different diets and pretreatments**

(Results are means with their standard errors for five rats)

Group	Body-wt (g)		Liver-wt (g)		Ferritin-Fe ($\mu\text{g/g}$)		Non-haem-Fe ($\mu\text{g/g}$)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
C	422 ^{ab}	13	10.3 ^{ab}	0.40	18 ^c	1.6	128 ^c	8
D	391 ^{bc}	11	10.7 ^a	0.52	60 ^{ab}	9.6	186 ^{cd}	22
CI	378 ^{bc}	22	9.8 ^{ab}	0.57	23 ^c	2.1	159 ^d	9
DI	396 ^{ab}	14	9.1 ^{bc}	0.35	48 ^b	4.2	253 ^{bc}	22
CIImf	458 ^a	23	9.7 ^{abc}	0.59	37 ^b	3.1	303 ^b	26
DIImf	395 ^{abc}	17	9.5 ^{abc}	0.38	70 ^a	5.0	519 ^a	37
CIDfx	437 ^a	12	9.8 ^{ab}	0.55	22 ^c	2.5	106 ^e	9
DIDfx	358 ^c	18	8.1 ^c	0.42	21 ^c	3.3	138 ^{de}	22

^{a, b, c, d, e} Values in the same column with different superscript letters were significantly different (Miller's multiple comparison test): $P < 0.05$.

C, control diet; D, polyunsaturated fatty acid (PUFA)-deficient diet; CI, control diet and inflamed; DI, PUFA-deficient diet and inflamed; CIImf, control diet, inflamed and pretreated with imferon; DIImf, PUFA-deficient diet, inflamed and pretreated with imferon; CIDfx, control diet, inflamed and pretreated with desferrioxamine; DIDfx, PUFA-deficient diet, inflamed and pretreated with desferrioxamine.

* For details of diets and pretreatments, see p. 498.

1935), plasma Fe concentration and total Fe binding capacity (International Committee for Standardization in Haematology, 1971) were assayed immediately. The following variables were examined in plasma samples frozen in liquid nitrogen and stored at -80° for a few days: caeruloplasmin was measured as its *p*-phenylenediamine oxidase (*EC* 1.4.3.6) activity (Sunderman & Nomoto, 1970), malondialdehyde by the thiobarbituric acid reaction (Yagi, 1976) and prostaglandin E_2 by radioimmunoassay (Saragen Inc., Boston, Mass).

Statistical methods

Results are expressed as means for five rats with corresponding standard errors. Data were evaluated by the one-way analysis of variance with eight groups per variable. When the variable effect was statistically significant, the groups were compared pairwise by a multiple comparison method (Miller, 1966). The means are given several superscript letters thus allowing the comparison of the statistical significance between groups ($P < 0.05$). Groups from the same column having at least one common superscript letter are not statistically different.

RESULTS

The main object of this study was the effect of Fe status on inflammatory variables in normal and PUFA-deficient rats. The results (Table 1) show that no important alterations in body and liver weights were observed, except in group DIDfx. PUFA deficiency induced an increase in Fe storage in the liver. Inflammation had a significant increasing effect only in non-haem-Fe stores in the liver of control rats, while Fe-overloading groups gave the highest content. A clear decrease in liver Fe content was observed when comparing the corresponding inflamed groups (CI and DI) with their respective desferrioxamine pretreated groups (CIDfx and DIDfx).

The more relevant results in blood variables related directly or indirectly to Fe (Table 2)

Table 2. *Packed cell volume (PCV), blood haemoglobin (Hb), plasma iron concentration, total Fe-binding capacity (TIBC), transferrin saturation and caeruloplasmin (Cp) concentration in rats offered different diets and pretreatments**

(Results are means with their standard errors for five rats)

Group	PCV		Hb (g/l)		Plasma Fe (μ g/l)		TIBC (μ g/l)		Transferrin (saturation %)	Cp (mg/l)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	Mean	SE
C	0.46 ^{ab}	0.013	144 ^a	4.0	1310 ^c	160	4910 ^b	290	27	270 ^d	25
D	0.46 ^{ab}	0.013	139 ^a	2.7	1730 ^{bc}	330	5020 ^b	320	34	320 ^d	34
CI	0.42 ^b	0.019	111 ^c	7.8	1120 ^c	50	3590 ^c	120	31	490 ^c	15
DI	0.46 ^{ab}	0.006	136 ^{ab}	3.4	1910 ^b	110	5140 ^b	260	37	710 ^{ab}	10
CIImf	0.47 ^{ab}	0.002	121 ^c	1.6	4870 ^a	120	7300 ^a	500	67	530 ^{bc}	34
DIImf	0.48 ^a	0.012	129 ^{bc}	3.2	7660 ^a	1550	10260 ^a	1590	75	710 ^a	36
CIDfx	0.47 ^{ab}	0.007	116 ^c	4.7	1570 ^{bc}	310	4810 ^b	140	33	540 ^{bc}	43
DIDfx	0.46 ^{ab}	0.002	126 ^{bc}	3.9	1540 ^{bc}	490	4610 ^b	290	33	670 ^{ab}	56

^{a, b, c, d} Values in the same column with different superscript letters were significantly different (Miller's multiple comparison test): $P < 0.05$.

C, control diet; D, polyunsaturated fatty acid (PUFA)-deficient diet; CI, control diet and inflamed; DI, PUFA-deficient diet and inflamed; CIImf, control diet, inflamed and pretreated with imferon; DIImf, PUFA-deficient diet, inflamed and pretreated with imferon; CIDfx, control diet, inflamed and pretreated with desferrioxamine; DIDfx, PUFA-deficient diet, inflamed and pretreated with desferrioxamine.

* For details of diets and pretreatments, see p. 498.

Table 3. *Granuloma-weight and blood prostaglandin E₂ (PGE₂) and malondialdehyde (MDA) concentrations in rats offered different diets and pretreatments**

(Results are means with their standard errors for five rats)

Group	Granuloma wt (g)		PGE ₂ (μ g/l)		MDA (μ mol/l)	
	Mean	SE	Mean	SE	Mean	SE
C	—	—	1.68 ^{bc}	0.33	2.00 ^c	0.11
D	—	—	1.06 ^d	0.08	2.13 ^c	0.13
CI	24.7 ^{ab}	2.67	3.86 ^a	0.71	2.88 ^b	0.19
DI	14.1 ^c	1.15	0.88 ^d	0.10	2.38 ^{bc}	0.33
CIImf	27.6 ^a	2.46	2.30 ^{abc}	0.53	3.45 ^a	0.09
DIImf	18.2 ^{bc}	2.36	1.19 ^{cd}	0.49	1.55 ^d	0.09
CIDfx	20.9 ^{ab}	1.00	2.50 ^{ab}	0.23	1.13 ^d	0.15
DIDfx	16.1 ^c	1.69	1.28 ^{cd}	0.30	1.29 ^d	0.14

^{a, b, c, d} Values in the same column with different superscript letters were significantly different (Miller's multiple comparison test): $P < 0.05$.

C, control diet; D, polyunsaturated fatty acid (PUFA)-deficient diet; CI, control diet and inflamed; DI, PUFA-deficient diet and inflamed; CIImf, control diet, inflamed and pretreated with imferon; DIImf, PUFA-deficient diet, inflamed and pretreated with imferon; CIDfx, control diet, inflamed and pretreated with desferrioxamine; DIDfx, PUFA-deficient diet, inflamed and pretreated with desferrioxamine.

* For details of diets and pretreatments, see p. 498.

were the alterations induced by inflammation itself or by the PUFA deficiency in haemoglobin, plasma Fe, total Fe-binding capacity, transferrin saturation and caeruloplasmin with regard to their respective controls. In all groups, inflammation induced a significant increase in caeruloplasmin levels, which was more important in PUFA-

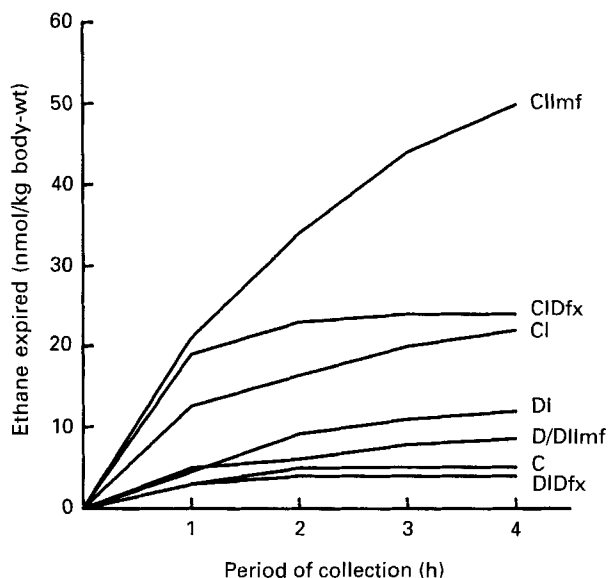


Fig. 1. Accumulated values of ethane expired by different groups of four rats placed together in a collection chamber and offered different diets and pretreatments. For details of diets and pretreatments see p. 498. C, control diet; D, polyunsaturated fatty acid (PUFA)-deficient diet; CI, control diet and inflamed; DI, PUFA-deficient diet and inflamed; CIIImf, control diet, inflamed and pretreated with imferon; DIIImf, PUFA-deficient diet, inflamed and pretreated with imferon; CIDfx, control diet, inflamed and pretreated with desferrioxamine; DIDfx, PUFA-deficient diet, inflamed and pretreated with desferrioxamine.

deficient rats. This seemed to be independent of Fe because both overloading and desferrioxamine treatments were without effect.

Table 3 shows results concerning the inflammatory process. In all groups offered a deficient diet (groups D, DI, DIIImf and DIDfx) in relation to their respective controls (C, CI, CIIImf and CIDfx) there was a significant decrease in granuloma weight (with the exception of groups C and D, which were not inflamed), and in plasma prostaglandin E_2 concentration. In group CI, prostaglandin E_2 and malondialdehyde levels were significantly increased in comparison with group C. The Fe pretreatment in inflamed rats (CIIImf) increased the malondialdehyde levels whereas desferrioxamine (CIDfx) had an opposite effect with regard to group CI. The differences in the malondialdehyde level were only statistically significant in DIIImf rats. Variations in ethane exhalation were also observed (Fig. 1): group CIIImf showed the greatest values while PUFA deficiency induced a decrease in the ethane exhaled by inflamed rats pretreated with Imferon or with desferrioxamine (DIIImf and DIDfx) when compared with their controls (CIIImf and CIDfx).

DISCUSSION

In relation to the control group, PUFA deficiency gives rise to a less inflammatory activity which is expressed as a lower granuloma weight together with a lower prostaglandin E_2 concentration in the plasma, suggesting that the inflammatory response is directly related to the lipid constituents of the diet. In this sense, it has been observed that when rats are maintained on a PUFA-deficient diet, arachidonic acid (20:4n-6) and homo γ -linolenic acid (20:3n-6) (prostaglandin precursors) are mainly substituted by eicosatrienoic acid (20:3n-9) (Morganroth *et al.* 1987) which is not a proper substrate for the cyclooxygenase enzyme system activity (Ziboth *et al.* 1974).

Inflammation induces an increase in the synthesis of some acute-phase proteins such as ferritin (Konijn *et al.* 1981) and caeruloplasmin (Rice, 1961). Surprisingly enough, in

inflamed PUFA-deficient rats, which consequently show a low inflammatory response, the caeruloplasmin concentration is more elevated and related to increased Fe variables in blood and liver. Osaki & Johnson (1969) postulated that caeruloplasmin is required for the oxidation of Fe at the cell surface before this Fe can be bound to transferrin. In addition, caeruloplasmin acts as a superoxide radical scavenger (Goldstein *et al.* 1979) and according to Samokyszyn *et al.* (1989) it also inhibits superoxide and ferritin-dependent lipid peroxidation, largely via its ability to reincorporate reductively-mobilized-Fe back into ferritin by feroxidase activity. These facts could explain the increase in caeruloplasmin levels in control inflamed rats and in PUFA-deficient rats as a defence mechanism in the presence of increased iron stores.

As the diet is implicated in the inflammatory response, we became interested in the effect of dietary lipids on the peroxidation capacity of the cell. In this sense, we must take into account the fact that the oxidative degradation of 20:3n-9 leads to the formation of other biologically-active metabolites such as 4-hydroxyundecenal and other compounds (Benedetti *et al.* 1980). This activity is not very well known, but may explain the maintenance of lipid peroxidation levels in rats offered the PUFA-deficient diet (group D). However, the PUFA-deficient diet in the Fe-overload group gives rise, in relation to its control group, to a decrease in the production of malondialdehyde levels together with an increase in plasma Fe. We believe that these alterations are independent: the decrease in lipid peroxidation is related, according to Lefkowitz (1988), to the depressed levels of macrophages in the exudate as a result of the diet; the changes observed in Fe metabolism as a consequence of the PUFA-deficient diet cannot easily be explained and need further research. However, findings have been published on the interrelationship of dietary factors and Fe absorption (Davis & Deller, 1966; Van Campen, 1974; Monsen & Cook, 1979), and according to Van Dokkum *et al.* (1983) an increase of linoleic acid (18:2n-6) intake causes a reduction in the Fe balance.

In our experimental work some efforts have been directed to the role of Fe deficiency in inflammation. Desferrioxamine pretreatment induces alterations in lipid peroxidation, possibly related to its activity as an Fe-chelating agent. The anti-inflammatory action of this drug supports the suggestion that Fe has an important role in the initiation and maintenance of inflammation (Blake *et al.* 1983), but high doses of desferrioxamine must be administered as it is excreted very rapidly, making it difficult to achieve concentrations high enough to chelate Fe effectively (Peters *et al.* 1966).

In summary, our findings show that a PUFA-deficient diet is able to reduce the inflammatory response and alter the peroxidation capacity of the cells. Fe plays a different role in lipid peroxidation according to the presence or absence of PUFA in the diet.

This work was supported by the Comisión Asesora Científica y Técnica (CAICYT) PB85-0234 and by the Acción Integrada Hispano-Francesa 1987 (16/130) y 1988(9/101). The authors also thank Miss J. Valentin for her technical assistance.

REFERENCES

- Benedetti, A., Comporti, M. & Esterbauer, H. (1980). Identification of 4-hydroxynonenal as a cytotoxic product originating from the peroxidation of liver microsomal lipids. *Biochimica et Biophysica Acta* **620**, 281-296.
- Blake, D. R., Hall, N. D., Bacon, P. A., Dieppe, P. A., Halliwell, B. & Gutteridge, J. M. C. (1983). Effect of a specific iron chelating agent on animal models of inflammation. *Annals of Rheumatic Diseases* **42**, 89-93.
- Cambon-Gros, C., Fernandez, Y., Mitjavila, M. T., Carbonell, T., Puig-Parellada, P. & Mitjavila, S. (1990). Combined effect of a PUFA deficient diet and iron levels on lipid peroxidation induced by CCl₄. *Food Additives and Contaminants* **7**, 5108-5110.
- Christon, R., Fernandez, Y., Cambon-Gros, C., Periquet, A., Deltour, P., Leger, C. & Mitjavila, S. (1988). The effect of dietary essential fatty acid deficiency on the composition and properties of the liver microsomal membrane of rats. *Journal of Nutrition* **112**, 1311-1318.

- Codde, J. P., Beilin, L. J., Croft, K. D. & Vandongen, R. (1985). Study of diet and drug interactions on prostanoid metabolism. *Prostaglandins* **29**, 895–910.
- Davis, P. S. & Deller, D. J. (1966). Prediction and demonstration of iron chelating ability of sugars. *Nature* **212**, 404–405.
- Drabkin, D. L. & Austin, J. H. (1935). Spectrophotometric studies II. Preparation from washed blood cells: nitric oxide hemoglobin and sulphohemoglobin. *Journal of Biological Chemistry* **112**, 51–64.
- Drysdale, J. W. & Munro, H. N. (1965). The separation of ferritin and hemosiderin for studies in the metabolism of iron. *Biochemical Journal* **95**, 851–858.
- Fukuhara, M. & Tsurufuji, S. (1969). The effect of locally injected anti-inflammatory drugs on the carrageenan granuloma in rats. *Biochemical Pharmacology* **18**, 475–484.
- Goldstein, I. M., Kaplan, H. B., Edelson, H. S. & Weissmann, G. (1979). Caeruloplasmin: a scavenger of superoxide anion radicals. *Journal of Biological Chemistry* **252**, 4040–4045.
- Gutteridge, J. M. C., Rowley, D. A. & Halliwell, B. (1981). Superoxide-dependent formation of hydroxyl radicals in the presence of iron salts. *Biochemical Journal* **199**, 263–265.
- International Committee for Standardization in Haematology (1971). Proposed recommendations of serum iron in human blood. *British Journal of Haematology* **20**, 451–453.
- Konijn, A. M., Carmel, N., Levy, R. & Hershko, C. (1981). Ferritin synthesis in inflammation. II. Mechanism of increased ferritin synthesis. *British Journal of Haematology* **49**, 361–370.
- Kremer, J. M., Bigaouette, J., Michalek, A. V., Timchalk, M. A., Lininger, L., Rynes, R. I., Huyck, C., Zieminski, J. & Bartholomew, L. E. (1985). Effects of manipulation of dietary fatty acids on clinical manifestations of rheumatoid arthritis. *Lancet* **i**, 184–187.
- Kremer, J. M., Jubiz, W., Michalek, A., Rynes, R. I., Bartholomew, L. E., Bigaouette, J., Timchalk, M., Beeler, D. & Lininger, L. (1987). Fish-oil fatty acid supplementation in active rheumatoid arthritis. A double-blind, controlled, crossover study. *Annals of Internal Medicine* **106**, 497–503.
- Lefkowitz, J. B. (1988). Essential fatty acid deficiency inhibits the in vivo generation of leukotriene B₄ and suppresses levels of resident and elicited leukocytes in acute inflammation. *Journal of Immunology* **140**, 228–233.
- McCull, M. R., Cleland, L. G., Whitehouse, M. W. & Vernon-Roberts, B. (1987). Effect of dietary polyunsaturated fatty acid (PUFA) supplementation on adjuvant induced polyarthritis in rats. *Journal of Rheumatology* **14**, 197–201.
- Miller, R. G. Jr (1966). *Simultaneous Statistical Inference*. New York, McGraw-Hill.
- Moncada, S. & Salmon, J. A. (1986). Leukocytes and tissue injury: The use of eicosapentaenoic acid in the control of white cell activation. *Wiener Klinische Wochenschrift* **98**, 104–106.
- Monsen, E. R. & Cook, J. D. (1979). Food iron absorption in human subjects. V. Effects of the major dietary constituents of a semisynthetic meal. *American Journal of Clinical Nutrition* **32**, 804–808.
- Morganroth, M. L., Pickett, W. C., Worthen, S., Mathias, M., Reeves, J. T. & Voelkel, N. F. (1987). Decreased pulmonary vascular responsiveness in rats raised on an essential fatty acid deficient diet. *Prostaglandins* **33**, 181–197.
- Osaki, S. & Johnson, D. A. (1969). Mobilization of liver iron by ferroxidase (ceruloplasmin). *Journal of Biological Chemistry* **244**, 5757–5758.
- Peters, G., Keberle, H., Schmid, K. & Brunner, H. (1966). Distribution and renal excretion of desferrioxamine and ferrioxamine in the dog and in the rat. *Biochemical Pharmacology* **15**, 93–109.
- Rice, E. W. (1961). Evaluation of the role of caeruloplasmin as an acute-phase reactant. *Clinica Chimica Acta* **6**, 652–655.
- Samokyszyn, V. M., Miller, D. M., Reif, D. W. & Aust, S. D. (1989). Inhibition of superoxide and ferritin-dependent lipid peroxidation by ceruloplasmin. *Journal of Biological Chemistry* **264**, 21–26.
- Sunderman, F. W. Jr & Nomoto, S. (1970). Measurement of human serum ceruloplasmin by its *p*-phenylenediamine oxidase activity. *Clinical Chemistry* **16**, 903–910.
- Terano, T., Salmon, J. A., Higgs, G. A. & Moncada, S. (1986). Eicosapentaenoic acid as a modulator of inflammation. Effect on prostaglandin and leukotriene synthesis. *Biochemical Pharmacology* **35**, 779–785.
- Torrance, J. D. & Bothwell, T. M. (1968). A simple technique for measuring storage iron concentrations in formalinized liver samples. *South African Journal of Medicine* **33**, 9–11.
- Van Campen, D. (1974). Regulation of iron absorption. *Federation Proceedings* **33**, 100–105.
- Van Dokkum, W., Cloughley, F. A., Hulshof, K. F. A. M. & Oosterven, L. A. M. (1983). Effect of variations in fat and linoleic acid intake on the calcium, magnesium and iron balance of young men. *Annals of Nutrition and Metabolism* **27**, 361–369.
- Winyard, P. G., Blake, D. R., Chirico, S., Gutteridge, J. M. C. & Lunec, J. (1987). Mechanism of exacerbation of rheumatoid synovitis by total-dose iron-dextran infusion: in vivo demonstration of iron-promoted oxidant stress. *Lancet* **i**, 69–72.
- Yagi, K. (1976). A simple fluorimetric assay for lipoperoxide in blood plasma. *Biochemical Medicine* **15**, 212–216.
- Yoshino, S., Blake, D. R. & Bacon, P. A. (1984). The effect of desferrioxamine on antigen-induced inflammation in the rat air pouch. *Journal of Pharmacy and Pharmacology* **36**, 543–545.
- Ziboth, V. A., Vanderhoek, J. T. & Lands, W. M. (1974). Inhibition of sheep vesicular gland oxygenase by unsaturated fatty acids from skin of essential fatty acids deficient rats. *Prostaglandins* **5**, 233–240.