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EFFECT OF IONIZING RADIATION ON PROSTAGLANDIN-LIKE ACTIVITY IN TISSUES

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1 One to 7 days after whole body exposure of mice to a single dose of 700 R of x-rays, little or no change was detected in prostaglandin-like activity in the brain, blood and seminal vesicles. Slight increases in intestinal and renal tissue were not significant. In the lung, mean activity rose from 62 ng/g to a transient peak of 145 ng/g wet weight on the fourth day ($P < 0.05$). In the spleen, mean levels rose steadily from 13.2 ng/g to 259 ng/g on the fourth day ($P < 0.01$), and were still 184.4 ng/g on the seventh day.

2 Prostaglandin-like activity was measured 4 days after single doses of 200–700 R. In the lung, a significant rise was produced by 600 and 700 R, and in the spleen by 200–700 R.

3 Thin layer chromatography showed that part of the prostaglandin-like activity in spleen extracts had an R_F similar to that of [^{3}H]-prostaglandin E_1, and part to that of [^{3}H]-prostaglandin $F_{2\alpha}$.

4 Splenic tissue from mice exposed to 700 R four days earlier, inactivated prostaglandin E_1 less potently than did tissue from non-irradiated mice.

Introduction

Prostaglandin levels in tissues and body fluids are affected by a large number of physical, chemical, hormonal, immunological, nervous and other endogenous and exogenous factors (Horton, 1972; Piper, 1973). The present paper suggests that ionizing radiation produces extensive changes in prostaglandin levels of several tissues of the mammalian body. The numerous biochemical changes induced in tissues by ionizing radiation have been extensively studied (Altman, Gerber & Okada, 1970; Berdjis, 1971). Most tissue constituents are affected, particularly in tissues whose radiosensitivity is high. In such tissues radiation acts on the enzyme systems responsible for DNA and RNA synthesis and degradation, protein and lipid turnover, oxidative phosphorylation in mitochondria and nuclei, glycolysis, and other cell functions; on water and electrolyte content; on histamine, 5-hydroxytryptamine, catecholamine, and acetylcholine levels. Changes in tissue constituents with potent pharmacological actions may obviously play a special role in the development of radiation sickness.

Methods

Male Balb/c strain or crossed strain C57 × Balb/c or NZB × Balb/c mice aged 3–7 months and weighing 25–40 g were exposed to 200–700 R of unfiltered x-radiation, at a dose rate of 140 R/min from a 230 kV and 15 mA source, and at a target distance of 60 centimetres. The mice received food and water *ad libitum* before and after irradiation. One, two, four or seven days after irradiation, the mice were anaesthetized with ether and exsanguinated through the axillary artery. Blood and organs were rapidly collected avoiding water loss by evaporation. The organs were placed in pre-weighed tubes with 5 ml of ice cold 0.9% w/v NaCl solution (saline) containing 5.6 μM indomethacin (2 μg/ml) to prevent prostaglandin formation during processing (Vane, 1971). They were then weighed and homogenized, and the prostaglandins extracted by a modification of the method of Unger, Stamford & Bennett (1971). The homogenates were mixed with 5 ml of absolute ethanol and stored overnight at 4°C (Greaves & McDonald-Gibson, 1972). They were then washed twice with 10 ml of petroleum ether. The water-ethanol phase was acidified with formic acid to a pH below 3, and extracted with 10 ml of chloroform. The chloroform extract was dried at 30°C under reduced pressure and excess formic acid removed by flushing with oxygen-free nitrogen. Recovery of prostaglandin E_1 was determined with internal standards, and found to be in the range of 80–98%. Prostaglandins were assayed on rat stomach strips (Vane, 1957) superfused with Krebs solution which contained the mixture of antagonists used by Gilmore, Vane & Wyllie (1968); 95% O_2 and 5% CO_2 was continuously bubbled through the solution.

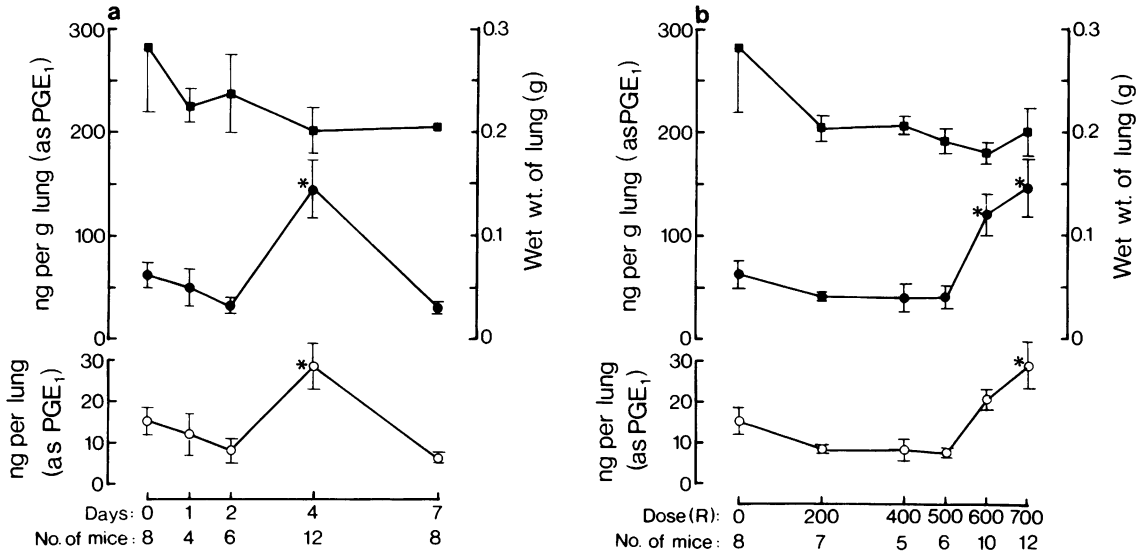

Figure 1 Effect of whole body exposure of mice to x-rays or prostaglandin-like activity in lung. (a) One to 7 days after exposure to 700 R; (b) 4 days after exposure to 200–700 R. Left-hand ordinate scales: prostaglandin (PG)-like activity (measured as PGE_1) per g wet wt. of lung (●, upper graph) and per lung (○, lower graph). Right-hand ordinate scale: fresh wet wt. of lungs (■, upper graph). Means ± s.e. mean are given; s.e. mean indicated by vertical lines, which are omitted when smaller than the symbol. Means significantly different from controls: * $P<0.05$; ** $P<0.01$.

Processing in the presence of indomethacin invariably reduced the prostaglandin level found in a tissue, by more than 50 per cent.

Prostaglandins E and F were differentiated by thin layer chromatography. After removal of chloroform and formic acid, the dry tissue extracts were redissolved in 0.2 ml of chloroform and applied to aluminum-backed silica thin-layer chromatography plates (Merck & Co.). The plates were developed by the AII system of Gréen & Samuelsson (1964). Water–ethanol solutions of [5, 6, 8, 11, 12, 14, 15(n)-^{3}H]-prostaglandin E_2 (0.1 μCi; 0.625 pmol) and of [5, 6, 8, 11, 12, 14, 15(n)-^{3}H]-prostaglandin $F_{2\alpha}$ (0.2 μCi; 1.3pmol) were run on the same plates. For bioassays, 1 cm strips of the gel were scraped, placed in 2 ml of Krebs solution, thoroughly mixed, and then centrifuged. For counting in a Packard Tri-carb Scintillation System, the scraped gels were placed in vials containing 5 ml of scintillation fluid.

Degradation of prostaglandins by spleen homogenates was measured by a modification of the method of Bedwani & Marley (1975). Spleens from freshly killed mice were homogenized in Krebs solution modified by the addition of 3.6 mM $MgCl_2$ and 27.6 mM nicotinamide. Large particles were removed by centrifuging for 10 min at 4000 *g*; 0.5 ml aliquots of the supernatant were added to 4.5 ml of the modified Krebs solution containing also 2 mM NAD plus 0.5 μg of prostaglandin E_1. The reaction mixture was incubated at 30°C. Aliquots (0.1 ml) were taken at intervals and quickly frozen. They were melted and diluted 10 times with Krebs solution immediately before bioassay.

Inactivating potency was also measured in a crude preparation of soluble cytoplasmic enzymes. Spleen homogenates were centrifuged at 10,000 *g* for 10 minutes. The separated supernatant was centrifuged at 95,000 *g* for 70 minutes. The prostaglandin-destroying activity in the final supernatant was assayed as described, except that the supernatant constituted a larger proportion (25%) of the reaction mixture.

Results

The effect of a single exposure to 700 R on prostaglandin-like activity in blood, brain, seminal vesicles, small intestine, kidney, lung and spleen was examined. Little or no change was detected in brain, seminal vesicles and blood.

There was considerable scatter in the prostaglandin levels of the small intestine (from the pylorus to the caecum). A fall in the prostaglandin-like activity per g wet wt of intestine from a control level of 217.75 ± 50 ng (s.e. mean) to 132.42 ± 16.1 ng at day 2 after irradiation, and a subsequent rise to 422 ± 150 ng at day 7, were not significant ($P>0.05$).

Prostaglandin-like activity per g of kidney fell from

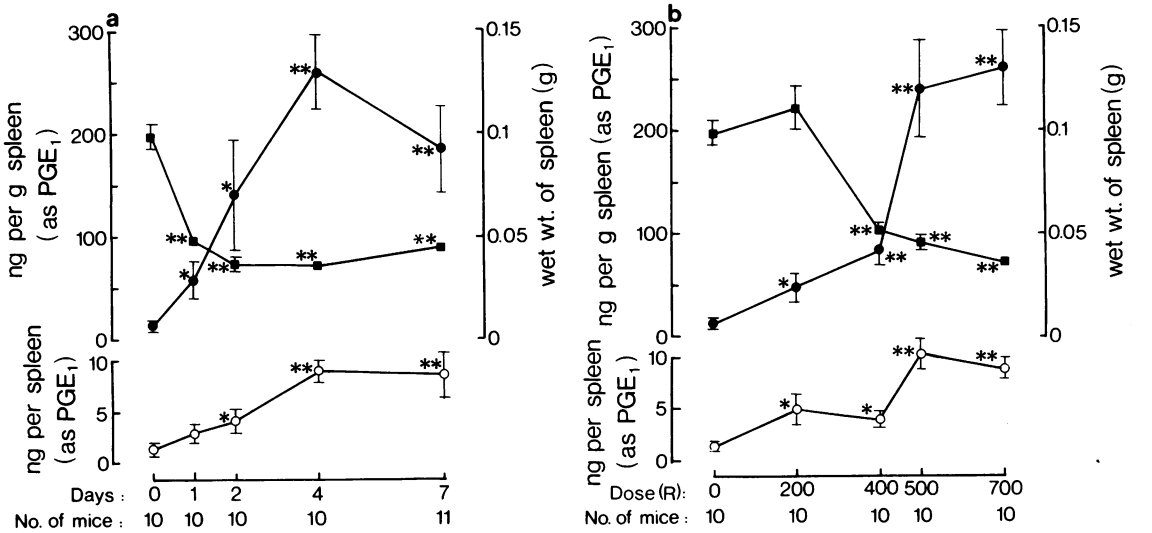

Figure 2 Effect of whole body exposure of mice to x-rays on prostaglandin-like activity in spleen. (a) One to 7 days after exposure to 700 R; (b) 4 days after exposure to 200–700 R. Left-hand ordinate scales: prostaglandin (PG)-like activity (measured as PGE_1) per g spleen (●, upper graph) and per spleen (○, lower graph). Right-hand ordinate scale: wet wt. of spleen (■, upper graph). For means, s.e. means, and P values see legend to Figure 1.

119 ± 43 ng (s.e. mean) in controls to 69.3 ± 8.9 ng at 2 days after irradiation with 700 R. It attained 210 ± 81.2 ng on day 4, and had decreased to 156 ± 22.5 ng on day 7. The difference between the levels on days 2 and 4 was significant ($P < 0.05$), but none of the irradiated groups differed significantly from control kidneys.

The most pronounced changes were found in lung and spleen, and these tissues were examined in greater detail. In the lung (Figure 1a) prostaglandin-like activity fell slightly ($P > 0.1$) for the first two days, and then rose to a sharp transient peak on day 4 (145 ± 28.0 ng, s.e. mean). On day 7, the activity was slightly lower than in non-irradiated lungs. The fall in wet weight 1–7 days after irradiation was not significant ($P > 0.05$).

Since highest levels were found 4 days after irradiation, mice were exposed to doses ranging from 200 to 700 R, and prostaglandin-like activity measured 4 days later (Figure 1b). Up to 500 R produced no significant change, whereas 600 R was nearly as effective as 700 R.

The spleen reacted very promptly to irradiation with 700 R (Figure 2a). After 24 h, most of the extensive weight loss had already occurred, and the rise in prostaglandin-like activity per g spleen was already significant. Highest concentrations (259 ± 36.6 ng s.e. mean) were found on day 4; this was a nearly 20-fold increase from control levels (13.2 ± 4.9 ng). The rate of increase on the 4 days following exposure appeared to be fairly constant at about 60 ng per g spleen per day. Seven days after exposure, the concentration of prostaglandins in the spleens was still some 15 times higher than in controls. In spite of the great reduction in size and weight of the spleens, the prostaglandin content per spleen was increased about six-fold.

Exposure of mice to doses of 200 to 700 R (Figure 2b) showed that 4 days later the group which had received 200 R had slightly enlarged spleens, whilst the greatest reduction in weight occurred in the dose-range between 200 and 400 R. In contrast, the greatest increase in prostaglandin-like activity was found between the groups given 400 and 500 R.

Identification of prostaglandin-like activity

Extracts of spleens from control mice and from mice irradiated with 700 R 4 days earlier, were examined by thin layer chromatography (Figure 3) using the AII system of Gréen & Samuelsson (1964). The distribution of prostaglandin-like activity was compared with that of [^{3}H]-prostaglandin E_2 and [^{3}H]-prostaglandin $F_{2\alpha}$.

Assays on the rat stomach strip showed that about two-thirds of the total activity in the spleen extracts resembled prostaglandin E_2 in its mobility in the AII

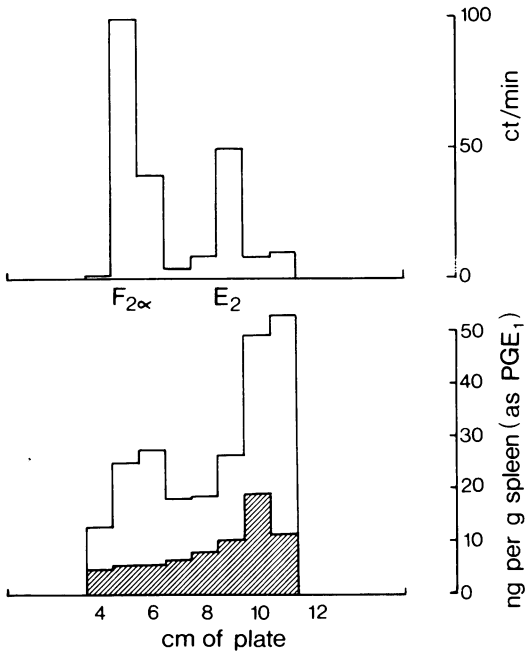

Figure 3 Thin layer chromatography of [^{3}H]-prostaglandins E_2 and $F_{2\alpha}$ (upper graph) and of extracts of spleens (lower graph) from mice irradiated with 700 R (clear area) and control mice (hatched area). Origin on left, solvent front on right.

system, whilst one-third resembled prostaglandin $F_{2\alpha}$. In these bioassays, all prostaglandin levels were measured and expressed in terms of the prostaglandin E_1 standards used. Since the rat stomach strip is five to ten times more sensitive to prostaglandins E than to prostaglandin $F_{2\alpha}$ (Vane, 1969), the assays would underestimate the amount of any prostaglandin $F_{2\alpha}$ present, and it is probable that the spleen extracts contained a higher proportion of prostaglandin $F_{2\alpha}$ than is suggested by Figure 3.

Irradiation slightly increased the proportion of activity due to prostaglandin $F_{2\alpha}$.

Effect of radiation on prostaglandin degradation

The possibility that radiation raised tissue levels of prostaglandins by depressing their enzymatic degradation was tested by measuring this process in spleens from control mice and from mice exposed to 700 R 4 days earlier. Figure 4a shows that prostaglandin E_1 was inactivated much faster by whole spleen homogenates (protein concentration 4.4 mg/ml) obtained from control mice, than by homogenates (protein concentration 6.4 mg/ml) from irradiated mice. In the soluble cytoplasmic fraction, inactivating potency per mg protein measured over 3 h at 30°C and pH 8.0 was also reduced (Figure 4b). To exclude the possibility that these results were distorted by

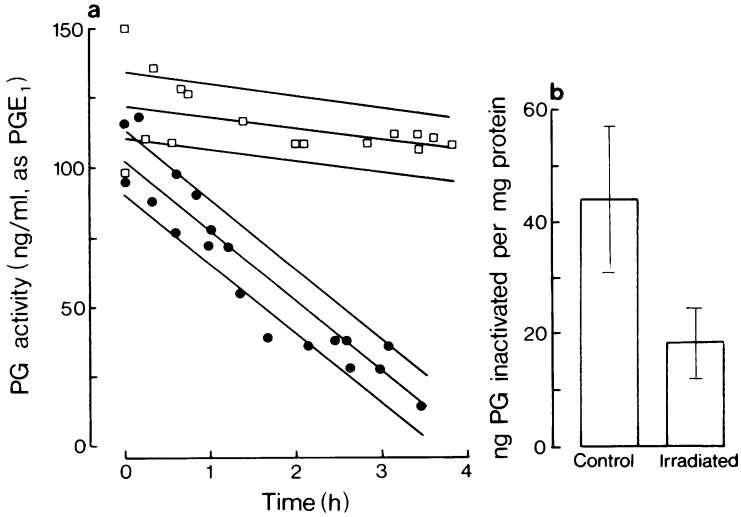

Figure 4 Effect of whole body exposure to 700 R on inactivation of prostaglandin (PG) by spleen tissue. Prostaglandin E_1, 100 ng/ml was added at 0 hours. (a) Time course of inactivation by homogenates (4.4 mg/ml) of spleens from control mice (●) and by homogenates (6.4 mg/ml) from mice irradiated 4 days earlier (□). Regression lines (calculated by method of least squares) ± s.d. of observed values are shown. Note that some initial values were raised above the total added prostaglandin E_1 by prostaglandin present in the homogenates. (b) Inactivation of prostaglandin E_1 in 3 h at 30°C and pH 8.0, by 1 mg protein of soluble cytoplasmic fraction of spleen ($P < 0.1$).

concurrent prostaglandin synthesis, 7 μM indomethacin was added in some assays to the reaction mixture. This reduced the apparent rate of prostaglandin degradation suggesting that in the conditions of the assay no prostaglandin was formed; it also suggested that indomethacin depressed one or more steps of prostaglandin catabolism.

Discussion

The results strongly suggest that ionizing radiation disturbs the turnover of prostaglandins, particularly in the highly radiosensitive spleen. The effects were seen with doses of 500–700 R which are in the range of the LD_{50} for mice when estimated by survival 30 days after exposure. Whilst comparable exposure would not be applied clinically to the whole human body, individual sites and organs are often treated with very high doses.

Further work is required to determine which steps in the synthesis and/or inactivation of prostaglandins are affected. It is likely that several, if not all enzymes involved in these processes are altered after irradiation, possibly to various extents and by different dose-ranges. This implies that the resulting changes in prostaglandin levels will not show a clear-cut dose-response relationship. The finding that irradiated spleen inactivated prostaglandin E_1 much more slowly, may be mainly due to reduced activity of 15-hydroxy-prostaglandin dehydrogenase (PGDH). In the kidney and lung, PGDH is short-lived and its activity falls rapidly when cellular protein synthesis is inhibited by cycloheximide or puromycin (Blackwell, Flower & Vane, 1975). Since x-rays depress synthesis of many proteins in lymphoid tissues (Altman *et al.*, 1970), similar falls in splenic PGDH levels may be expected. Since the assay system contained primarily the co-factors required by PGDH, it is probable that indomethacin inhibited prostaglandin degradation by acting on PGDH. Evidence that indomethacin and other analgesics inhibit prostaglandin catabolism is listed by Flower (1974).

The observed effects on prostaglandin levels were not closely related to the radiosensitivity of the organs. For example, prostaglandin levels rose considerably in the fairly radioresistant lung, but changed only little in the highly sensitive intestine. It should be remembered however, that most organs contain elements of diverse radiosensitivity. In the lung, alveolar cells of type II have a three times higher rate of turnover than type I cells, and are therefore more sensitive to radiation (Bertalanffy & Leblond, 1953). Alveolar macrophages are prone to damage by many noxae, amongst them immunosuppressive agents (Harris, Swenson & Johnson, 1970). Capillary endothelial cells resemble type I cells in their low turnover rate.

The fall in size and weight of the spleen occurs within hours of exposure to high doses of radiation, and is mainly due to pyknotic changes and death of the highly radiosensitive lymphoid cells of the white pulp. Myeloid cells which constitute a considerable part of the mouse spleen, are less sensitive. The damaged lymphoid cells are promptly ingested by macrophages and reticulum cells (Jordan, 1967). Phagocytosis is maximal 3 h after exposure, and almost complete at 24 h; there is little sign of it after 3 days. This process could contribute to accumulation of prostaglandins in the spleen, since Higgs, McCall & Youlten (1975) found that white blood cells engaged in phagocytosis release prostaglandins which attract further phagocytes. However, highest prostaglandin levels were found 4 days after irradiation; at this time, phagocytic activities have subsided and regenerative mitoses are only just beginning (Jordan, 1967).

The role of higher prostaglandin levels in the reactions to ionizing radiation is not yet known. In view of the pharmacological properties of prostaglandins, it is possible that they contribute to some of the signs of radiation sickness, such as diarrhoea, nausea and pain. Mennie, Dalley, Dinneen & Collier (1975) have therefore examined the effect of inhibition of prostaglandin synthesis by acetylsalicylate in patients with gastrointestinal distress caused by radiotherapy. Abdominal pain, flatulence and bowel motions were significantly reduced.

On the other hand, a possible radioprotective role of prostaglandins cannot be excluded. Prasad (1972) found that prostaglandin E_1, and other agents which raise the cellular level of cyclic adenosine 3′,5′-monophosphate (cyclic AMP) reduce *in vitro* radiation damage. This may be achieved by the depression of cell growth by cyclic AMP. Prostaglandins E could also protect from the effects of radiation by their ability to stimulate haemopoiesis (Fehér & Gidáli, 1974), and by acting as calcium ionophores (Kirtland & Baum, 1972) which increase the calcium content in cells thereby rendering them more resistant to radiation.

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References

ALTMAN, K.I., GERBER, G.B. & OKADA, S. (1970). *Radiation Biochemistry*, Vol. II: *Tissues and body fluids*. New York and London: Academic Press.

BEDWANI, J.R. & MARLEY, P.B. (1975). Enhanced inactivation of prostaglandin E_2 by the rabbit lung during pregnancy or progesterone treatment. *Br. J. Pharmac.*, **53**, 547–554.

BERDJIS, C.C. (1971). *Pathology of irradiation*. Baltimore: Williams & Wilkins.

BERTALANFFY, F.D. & LEBLOND, C.P. (1953). The continuous renewal of the two types of alveolar cells in the lungs of the rat. *Anat. Rec.*, **115**, 515–525.

BLACKWELL, G.J., FLOWER, R.J. & VANE, J.R. (1975). Rapid reduction of prostaglandin 15-hydroxydehydrogenase activity in rat tissue after treatment with protein synthesis inhibitors. *Br. J. Pharmac.*, **55**, 233–238.

FEHÉR, I. & GIDÁLI, J. (1974). Prostaglandin E_2 as stimulator of haemopoietic stem cell proliferation. *Nature*, 247, 550–551.

FLOWER, R.J. (1974). Drugs which inhibit prostaglandin biosynthesis. *Pharmac. Rev.*, **26**, 33–67.

GILMORE, N., VANE, J.R. & WYLLIE, J.H. (1968). Prostaglandins released by the spleen. *Nature, Lond.*, **218**, 1135–1140.

GREAVES, M.W. & McDONALD-GIBSON, W. (1972). Extraction of prostaglandin-like activity from whole human blood. *Life Sciences*, **II**, part 1, 73–81.

GRÉEN, K. & SAMUELSSON, B. (1964). Prostaglandins and related factors: XIX. Thin-layer chromatography of prostaglandins. *J. Lipid. Res.*, **5**, 117–120.

HARRIS, J.D., SWENSON, E.W. & JOHNSON, J.E. (1970). Human alveolar macrophages: comparison of phagocytic ability, glucose utilization, and ultrastructure in smokers and non-smokers. *J. clin. Invest.*, **49**, 2086–2096.

HIGGS, G.A., McCALL, E. & YOULTEN, L.J.F. (1975). A chemotactic role for prostaglandins released from polymorphonuclear leucocytes during phagocytosis. *Br. J. Pharmac.*, **53**, 539–546.

HORTON, E.W. (1972). *Prostaglandins*. Berlin: Springer-Verlag.

JORDAN, S.W. (1967). Ultrastructural studies of spleen after whole body irradiation of mice. *Experimental and Molecular Pathology*, **6**, 156–171.

KIRTLAND, S.J. & BAUM, H. (1972). Prostaglandin E_1 may act as a 'calcium ionophore'. *Nature, New Biol.*, **236**, 47–49.

MENNIE, A.T., DALLEY, V.M., DINNEEN, L.C. & COLLIER, H.O.J. (1975). Treatment of radiation-induced gastrointestinal distress with acetylcalicylate. *Lancet*, **ii**, 942–943.

PIPER, P.J. (1973). Distribution and metabolism. In *The Prostaglandins, Pharmacological and Therapeutic Advances*, ed. Cuthbert, M.F., pp. 125–150. J.B. Lippincott Co: Philadelphia.

PRASAD, K.N. (1972). Radioprotective effect of prostaglandin and an inhibitor of cyclic nucleotide phosphodiesterase on mammalian cells in culture. *Int. J. Radiat. Biol.*, **22**, 187–189.

UNGER, W.G., STAMFORD, I.F. & BENNETT, A. (1971). Extraction of prostaglandins from human blood. *New Biol., Nature*, **233**, 336–337.

VANE, J.R. (1957). A sensitive method for assay of 5-hydroxytryptamine. *Br. J. Pharmac. Chemother.*, **12**, 344–349.

VANE, J.R. (1969). The release and fate of vaso-active hormones in the circulation. *Br. J. Pharmac.*, **35**, 209–242.

VANE, J.R. (1971). Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. *Nature, New Biol.*, **231**, 232–235.

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