JOURNAL OF THE AMERICAN COLLEGE OF TOXICOLOGY
Volume 12, Number 5, 1993
Mary Ann Liebert, Inc., Publishers

Appendix to the Report on Arachidonic Acid

GENERAL

ARACHIDONIC ACID IS A POLYUNSATURATED fatty acid that in mammals can only be made from linoleic acid (Lehninger, 1982). However, linoleic acid cannot be synthesized by mammals and is obtained from plant sources; after linoleic acid is ingested, it can then be converted into Arachidonic Acid. Arachidonic Acid is metabolized to several biologically important molecules. Hence, the precursors of Arachidonic Acid, linoleic and linolenic acids, are required in the diet and are called essential fatty acids (EFAs).

The conversion of linoleic acid into Arachidonic Acid involves the desaturation of linoleic acid to γ-linolenic acid, which is then elongated to form eicosatrienoic acid. Eicosatrienoic acid is then desaturated to form Arachidonic Acid.

Arachidonic Acid is a normal constituent of cells and is mainly found in membrane-bound phospholipids (Samuelsson, 1987), particularly phostaphidylcholine. The concentration of Arachidonic Acid in most cells is normally less than 10^{-6} M (Morrison, 1986). Arachidonic Acid is released from membrane phospholipids primarily by the action of phospholipase A_2 and phospholipase C (Zoja et al., 1989).

METABOLIC PATHWAY

Arachidonic Acid is well absorbed from the gastrointestinal tract (Coots, 1965; Chow and Hollander, 1978; Ramesha et al., 1985) and the circulatory system (de Tomas and Mercuri, 1971, Puri et al., 1975; Zijlstra and Vincent, 1985); dermal absorption studies of pure Arachidonic Acid have not been reported. Arachidonic Acid distributes rapidly into the lipid compartment of the body (Zijlstra and Vincent, 1985) and is rapidly converted to phospholipid by the liver (de Tomas and Mercuri, 1971).

The rate-limiting step of Arachidonic Acid metabolite formation is the release of free Arachidonic Acid from the phospholipid (Malmsten, 1986). Direct release is catalyzed by phospholipase A_2. Phospholipase C first forms a diglyceride that yields free Arachidonic Acid by hydrolyzation. Cyclooxygenase and lipoxygenase facilitate hydrogen removal, double-bond rearrangement, and inclusion of oxygen to yield several unstable fatty acids (McGiff, 1987).

Arachidonic Acid can be metabolized by three different pathways (McGiff, 1987). If oxygenation occurs via the cyclooxygenase pathway, prostaglandins E_2, $F_{2\alpha}$, I_2, and D_2, (PGE_2, $PGF_{2\alpha}$, PGI_2, and PGD_2), and thromboxane A_2 (TxA_2) are formed (Zoja et al., 1989). If oxygenation occurs by the lipoxygenase pathway, hydroxy fatty acids and leukotrienes are formed.

The third pathway for the metabolism of Arachidonic Acid in animal tissues is by the cytochrome P450 system (Schwartzman et al., 1987), which is called the epoxygenase

pathway (Basu and Karmazyn, 1987). Cytochrome P450-dependent monoxygenase converts Arachidonic Acid to monohydroxyeicosatetraenoic acids, epoxyicosatetrienoic acid, and the ω,ω-1, and ω-2 hydroxylation products (Schwartzman et al., 1987). The formation of these metabolites is strictly dependent on molecular oxygen and NADPH (McGiff, 1987; Schwartzman et al., 1987).

Iron, or heme, is an essential cofactor for the cyclooxygenase, lipoxygenase, and peroxidase enzymes involved in the formation of platelet endoperoxides and thromboxanes (Rao et al., 1978). For the oxidation of Arachidonic Acid, iron must be in the ferrous form, and the reaction between Arachidonic Acid and iron is dependent on the presence of oxygen.

ABSORPTION, DISTRIBUTION, METABOLISM, EXCRETION

Absorption, distribution, metabolism, and excretion studies are summarized in Table 1.

Four healthy male subjects were used to determine the effect of the administration of ethyl Arachidonate on the Arachidonic Acid content in plasma and platelet lipids and on the changes of prostaglandin synthesis in humans (Seyberth et al., 1975). The study design consisted of three phases: (1) a control period consisting of 3 baseline days and 7 placebo days; (2) a period of oral administration of 6 g ethyl Arachidonate daily for 21 days to subjects 1 and 2 and for 19 and 14 days to subjects 3 and 4, respectively; and (3) a post-ethyl Arachidonate period of at least 10 days, during which placebo capsules were administered. The placebo that was used did not have any effect on PGE production. Diet was maintained isocaloric throughout the study.

Ethyl Arachidonate administration to subjects 3 and 4 was discontinued after 19 and 14 days, respectively, because of a decrease in the ADP threshold concentration; the threshold fell to 10–20% of the control. A decrease in the ADP threshold was also observed in the other two subjects, but it was not as great as it was for subjects 3 and 4. No symptoms or side effects were observed and the platelet count, blood pressure, sodium balance, renal creatinine clearance, and other parameters were unchanged in all the subjects.

Various physical and chemical parameters were measured on alternate days. Platelet aggregation studies were performed every 3–6 days; once changes in aggregation were observed, the studies were carried out every 1–2 days. Platelet lipids were determined at the end of each trial period. Blood samples were obtained for all tests from the antecubital vein in the morning following a 12 h period of fasting and no smoking.

Ethyl Arachidonate administration resulted in significant increases in the relative abundance of Arachidonate in triglycerides, phospholipids, and cholesteryl esters. Increased Arachidonic Acid concentration in the phospholipids and cholesteryl esters continued for 10 days after discontinuation of ethyl Arachidonate administration; elevated values still remained for subjects 3 and 4 sixteen days after dose discontinuation. A fall in plasma linoleic acid concentration accompanied these elevated values. No changes were observed in the absolute concentration of plasma lipids, including free plasma cholesterol. Arachidonic Acid concentration was also increased in platelet lipids after ethyl Arachidonate administration, with a corresponding decrease of linoleate. Although the accumulation of Arachidonic Acid in platelet phospholipids increased in three of the four subjects, the increase was not significant. The excretion of

Table 1. Arachidonic Acid Absorption, Distribution, Metabolism, Excretion Studies

Species and gender	*Methods*	*Results and comments*	*Reference*
Male humans	Four subjects were used in a study that consisted of the following three phases: (1) a control period consisting of 3 baseline days and 7 placebo days; (2) oral administration of 6 g ethyl Arachidonate daily for 21 days; (3) a post-Arachidonate period of at least 10 days, during which placebo capsules were given. Various parameters were measured on alternate days. Platelet aggregation studies were performed every 3–6 days; when aggregation changes were observed, the studies were performed every 1–2 days. Platelet lipids were determined at the end of each trial period. Blood samples were taken in the morning following a 12 h period of fasting and no smoking.	Dosing of subjects 3 and 4 was stopped after days 19 and 14, respectively, due to decreased ADP concentrations. Decreased ADP concentrations were observed for subjects 1 and 2, but the decrease was not as great. Significant increases of Arachidonic Acid concentration in the triglycerides, phospholipids, and cholesteryl esters were observed. Arachidonic Acid concentrations were elevated in the phospholipids and the cholesteryl esters 10 days after dosing; these values remained elevated 16 days in subjects 3 and 4. Plasma linoleic acid concentrations decreased; the absolute plasma lipid concentration did not change. Platelet lipid Arachidonic Acid concentration increased, with a decrease of linoleate. An increase in 7α-hydroxy-5, 11-diketotetranorprostane-1,16-dioic acid excretion was observed in 3 of 4 subjects; this value could not be determined for the other subject. The threshold ADP value returned to control value 2 wk after dosing; the primary response to ADP was unchanged in all 4 subjects throughout the study.	Frolich et al., 1975
Male Mol-Wistar rats	Weanling rats were fed a fat-free diet. After 12 wk, rats were placed in metabolism cages; 24 h urine samples were taken for 3 days. The rats were then dosed with 300 mg ethyl Arachidonate for 10 days, with 24 h urine samples taken the last 3 days. This procedure was repeated twice using 2 previously undosed rats.	No significant changes in urinary output were seen. A small but consecutive decrease in urinary AVP excretion was observed; this decrease was significant when day 10 of dosing was compared to the mean value for the 3 control days. Urinary PGE_2 was significantly increased. Renal analysis showed a peak area percentage of 29.1 $\pm$ 1.6 for Arachidonic Acid and 0.2 $\pm$ 0.1 for linoleic acid. A decrease in renal linoleic acid could be due to a displacement with Arachidonic Acid.	Hansen and Jensen, 1986

Table 1. Arachidonic Acid Absorption, Distribution, Metabolism, Excretion Studies (*Continued*)

Species and gender	*Methods*	*Results and comments*	*Reference*
Sprague-Dawley rats	Seven pregnant rats were fed a fat-free diet 1 wk prior to delivery and during lactation. After weaning, 8 males were fed a fat-free diet until weighing 180–200 g. The rats were given oral doses of Arachidonic Acid; various doses were given on various days for a total dose of approx. 386 mg. Urine and feces were collected every 24 h.	The average basal amount of radioactivity excreted on days 15–20 was equivalent to 6.0 mg Arachidonic Acid/day in the urine and 0.4 mg/day in the feces. The radioactivity in the aqueous fraction of the urine was equivalent to ≈0.4–0.8 mg Arachidonic Acid. On nondosing days, the urine radioactivity was equivalent to ≈0.2–0.8 mg Arachidonic Acid. As of day 8, the amount of aqueous radioactivity in the urine remained basically constant, regardless of dose. Arachidonic Acid dose termination resulted in a 30–40 fold decrease in the amount of radioactivity in the urine, which remained constant at the value of nondosing days. Approx. 40–100 μg equivalent of organic Arachidonic Acid metabolites was the basal amount of metabolite excretion in the urine. The kidneys contained the most Arachidonic Acid/g of tissue, followed by the heart, liver, lungs, and brain. Approx. 50% of the dosed Arachidonic Acid was accounted for as tissue ester, $^{3}H_2O$, or excreted metabolite. In the glycerophospholipids, Arachidonic Acid was mostly contained in PE; the plasma and adipose tissue contained large amounts of Arachidonic Acid in the form of TG.	Ramesha et al., 1985
Male albino Wistar rats	3 normal and 3 EFA-deficient rats were given an i.v. injection of approx. 20 μCi 1-^{14}C-Arachidonic Acid and were killed 5 min, 30 min, or 24 h after dosing.	For normal rats, the highest concentration of radioactivity, after 5 min, was present in the subcutaneous fat, perivertebral fat, liver, heart, muscle, kidneys, and adrenal glands; the same general results were obtained after 24 h. In EFA-deficient rats, the quantitative distribution was almost the same as in normal rats, with the exception that, in the EFA-deficient rats, the amount of radioactivity in the tissues was greater after 24 h.	Zijlstra and Vincent, 1985

Male albino Wistar rats	Normal and EFA-deficient rats, number unspecified, were given i.v. injections of 1 μCi 1-^{14}C-Arachidonic Acid and were killed 5 min, 30 min, 4 h, or 24 h after being dosed.	After 24 h, the amount of radioactivity was decreased in the heart, approx. the same in the liver and kidneys, and increased in the adrenal glands as compared to the earlier time periods. The amount of radioactivity was greater in the tissues of EFA-deficient rats than in normal rats.	Zijlstra and Vincent, 1985
Male albino Wistar rats	Normal and EFA-deficient rats, number unspecified, were given i.v. injections of 1.5 μCi ^{14}C-Arachidonic Acid and a group of normal control rats, number unspecified, were dosed with 2 μCi ^{3}H-Arachidonic Acid. All animals were placed in metabolism cages for 2–3 wk. Urine fractions were collected for 15 days; excreted radioactivity was determined daily. Urine was collected for 1 day after the control rats were dosed and metabolites were isolated.	The majority of the radioactivity excreted by rats dosed with 1.5 μCi ^{14}C-Arachidonic Acid occurred the first day. During the entire test period, the EFA-deficient rats excreted less radioactivity than did the normal group. The amount of excreted radioactivity by the EFA-deficient rats decreased more rapidly over the period when compared to normal rats; the amount excreted over 10 days was very small compared to the amount excreted after the first day. In the control rats, the majority of the radioactivity was present in the water soluble fraction of the urine.	Zijlstra and Vincent, 1985
Male albino Wistar rats	Normal and EFA-deficient rats were given i.v. injections of 1 μCi 1-^{14}C-Arachidonic Acid. $^{14}CO_2$ was collected in methanol/ethanolamine (4:1) in 15 min fractions for a 2 h period.	The radioactivity half-time in 8 normal rats was 28 ± 2.8 min; in 5 EFA-deficient rats it was 39 ± 2.9 min. The total amount of $^{14}CO_2$ respired represented 3.5 ± 1.1% and 3.7 ± 0.8%, respectively, of the injected radioactivity.	Zijlstra and Vincent, 1985
Male albino Wistar rats	The amount of radioactivity in the heart of a rat was determined 2 h after an i.v. injection of 1-^{14}C-Arachidonic Acid.	The distribution of radioactivity was as follows: 6.9 ± 0.6% in the PI; 44 ± 4.1% in the PC; 10.0 ± 1.0% in the PE; and 9.3 ± 1.6% in the neutral lipids.	Zijlstra and Vincent, 1985
Male albino Wistar rats	Over a 1 min period, an i.v. injection of 0.1 ml solution containing 10 μC of either 1-^{14}C-Arachidonic Acid, 1-^{14}C-linoleic acid, or 1-^{14}C-α-linolenic acid was given to 5, 6, or 7 rats, respectively.	There was no significant difference observed in the amount of each fatty acid incorporated into the rat hepatic lipids. A significantly greater amount of Arachidonic Acid was incorporated into the phospholipid fraction. Significantly less Arachidonic Acid was incorporated into the 1,2-diacylglycerol.	de Tomas and Mercuri, 1971

Table 1. Arachidonic Acid Absorption, Distribution, Metabolism, Excretion Studies (*Continued*)

Species and gender	*Methods*	*Results and comments*	*Reference*
Male Wistar rats	Fasted rats, 5/time period, were given i.v. injections of 1 μc methyl ^{14}C-Arachidonate in 0.5 ml rat serum. The rats were killed after 1, 4, or 24 h. Blood samples and the liver and testes were taken from each animal. The tissue lipids were separated.	The liver contained the greatest percentage of radioactivity at all 3 time periods; the most radioactivity was recovered after 1 h. The majority of the radioactivity was recovered in the phospholipid fraction of the liver and testes and in the glyceride and fatty acid fraction of the serum. After 4 and 24 h, the majority of the radioactivity remained in the phospholipid fraction of the liver and testes, while in the serum the majority was found in the cholesterol ester fraction, with the least being found in the glyceride and fatty acid fractions. After 24 h, 96% of the ^{14}C in the serum lipids was in the form of ^{14}C-Arachidonic Acid; in the liver, 93% was [^{14}C]-Arachidonic Acid; and in the testes, 75% was [^{14}C]-Arachidonic Acid. The remaining ^{14}C was generally long chain polyunsaturated fatty acids. The turnover time of liver and serum Arachidonic Acid was estimated to be 18–24 h.	Swell and Law, 1967
Male albino Holtzman rats	Four rats were fed a diet that included 7.5 μc/g soybean oil containing 1-^{14}C-Arachidonic Acid. Respiratory CO_2, urine, and feces were collected for 51 h, at which time the rats were killed.	The actual total recovery of ^{14}C was 96%. The majority of the radioactivity was recovered in the carcass, followed by in the respiratory CO_2, the gastrointestinal contents, the feces, and the urine.	Coots, 1985
Male albino Holtzman rats	Five rats were fed a liquid diet that included 7.5 μc/g soybean oil containing 1-^{14}C-Arachidonic Acid. For 42 h, lymph was collected and separated.	The total recovery of ^{14}C was 96.3%. Absorption peaked 12–16 h after feeding. The majority of the radioactivity was recovered in the glycerides, 90.0%; only 0.1% was recovered in the sterol esters. During the peak of absorption, the specific activity was 6.0 μc/g in the glycerides, 12.4 μc/g in the phospholipids, and 1.1 μc/g in the sterol esters.	Coots, 1965
Male albino Holtzman rat	A thoracic duct-cannulated rat was fed a liquid diet that included 7.5 μc/g soybean oil containing 1-^{14}C-Arachidonic Acid.	Of the recovered ^{14}C, amount not given, 5.4% was excreted as $^{14}CO_2$.	Coots, 1965

Male albino mice	Five mice/group, with previously transplanted neuroblastoma neoplasms, were given i.v. injections of 80 ci/mmol [^{3}H]-Arachidonic Acid dissolved in 0.1 ml ethanol. The mice were killed after 1, 6, or 24 h.	The highest ratios of activity, being expressed as tissue-to-neoplasm activity, were seen at 1 h. The distribution of [^{3}H]-Arachidonic Acid had the greatest activity in the kidneys, liver, and lungs.	Puri et al., 1975
Male Sprague-Dawley rats	Everted intestinal sacs were immersed into 50 ml of micellar incubation solution under various conditions. The solution was agitated for all tests, the pH was 7.4, and the incubation temp. was 37°C, unless otherwise noted. The conditions were as follows:		Chow and Hollander, 1978
	1. 4 rats were used and the incubation solution contained 2.1 mM Arachidonic Acid.	1. The relationship between Arachidonic Acid and absorption was linear; Arachidonic Acid absorption by the proximal jejunum and distal ileum were similar.	
	2. Arachidonic Acid concentration was varied from 0.21–8.36 mM; at least four rats were used at each concentration.	2. Absorption remained linear for all concentrations; Arachidonic Acid absorption increased with concentration increase. The absorption difference at different concentrations was significant; the difference in absorption rate between proximal and distal segments was not significant.	
	3. An Arachidonic Acid concentration range of 5–150 μM was used; at least four rats were used at each concentration.	3. The absorption vs. time relationship remained linear. Absorption process saturation was indicated at concentrations >100 μM.	
	4. 100 μM butyric, oleic, linoleic, and linolenic acids were added to micellular solution containing 100 μM Arachidonic Acid; at least four rats/acid were used.	4. The Arachidonic Acid and time relationship was linear. Arachidonic Acid absorption rate was unchanged by butyric acid; a significant and progressive decrease in absorption rate was seen with the addition of oleic, linoleic, and linolenic acids.	
	5. 0.1 mM 2,4-dinitrophenol, 1 mM potassium cyanide, or 1 mM sodium azide was added to micellar solution containing 100 μM Arachidonic Acid; at least four rats/group were used.	5. None of the added chemicals significantly altered the Arachidonic Acid absorption rate.	
	6. pH of an incubation fluid containing 100 μM Arachidonic Acid was varied: 7.4, 4 rats, 6.4, 5 rats, and 5.4, 5 rats.	6. Arachidonic Acid absorption increased with a decrease in pH.	

Table 1. Arachidonic Acid Absorption, Distribution, Metabolism, Excretion Studies (*Continued*)

Species and gender	*Methods*	*Results and comments*	*Reference*
	7. 5, 10, 15, or 20 mM sodium taurocholate was added to solution containing 2.1 mM Arachidonic Acid. Four rats were used per group.	7. With 5–15 mM sodium taurocholate, the absorption rate was not significantly changed; the absorption rate was decreased with 20 mM sodium taurocholate.	
	8. 5 mM Pluronic F68, 6 rats, and 5 mM Tween 80, 7 rats, was added to solution containing 2.1 mM Arachidonic Acid. Four rats/group were used.	8. Arachidonic Acid absorption was greater with these chemicals than it was with the addition of 20 mM sodium taurocholate.	
	9. Oscillation speed was varied to 0, seven rats, 40, four rats, 80, four rats, and 120, seven rats, oscillations/min; the solution contained 2.1 mM Arachidonic Acid.	9. Arachidonic Acid absorption increased with speeds >40 oscillations/sec.	
	10. 2.1 mM dextrose, glutamic acid, lysine, or leucine was added to solution containing 2.1 mM Arachidonic Acid; four rats per group were used.	10. No significant difference in Arachidonic Acid absorption rate was observed.	
Mouse epidermis-derived cell line HEL/30	Cells were incubated with 10 μM ^{3}H-Arachidonic Acid 0.2 μCi/nmol, for 1 h in the presence of either 0.02% BSA or 10% FCS. The cells incubated with BSA were incubated in fresh medium for an additional 2 h. Cells were also incubated with TPA.	After incubation with BSA, 53.5 ± 0.9% of the radioactivity was incorporated into the cells. After an additional 2 h, 0.52 ± 0.03% of the radioactivity was released into the medium. Incubation with FCS produced a four-fold increase in radioactivity release by the cells. TPS addition significantly increase radioactivity release into the medium and PGE_2 formation.	Tragni et al., 1988
Murine macrophage-like neoplasm cell line J774.2	Exogenous Arachidonic Acid was added to the cells. Serum-treated zymosan and the ionophore A-23187 was added prior to Arachidonic Acid addition.	The cells metabolized Arachidonic Acid to PGE_2, 12-hydroxy-5,8, 10-heptadecantrienoic, TxB_2, PDG_2, and PGF_2. Over 80% of ≤10 μM Arachidonic Acid was metabolized within 3.5 min. Zymosan and A-23187 addition increased the proportion of exogenous Arachidonic Acid esterified into phospholipids and decreased the proportion metabolized by cyclooxygenase.	Stenson et al., 1981
Perfused rat lungs	Study examining the metabolism of exogenous Arachidonic Acid.	6-keto-PGF_2 and TxB_2 production was greater in male than in female lungs.	Maggi et al., 1980

7α-hydroxy-5,11-diketotetranorprostane-1,16-dioic acid increased significantly in three subjects; this could not be determined for the fourth subject because the value did not remain constant during and between the control and post-ethyl Arachidonate period. After 10–12 days of ethyl Arachidonate administration, the threshold concentration of ADP necessary to induce the secondary irreversible aggregation of platelet-rich plasma dropped significantly in all four subjects, as mentioned earlier. The threshold dose of ADP returned to control values 2 wk after dose discontinuation. The primary response to ADP was unchanged in all subjects throughout the study.

Ethyl Arachidonate was administered to EFA-deficient male Mol-Wistar rats to determine its effect on urine output and urinary excretion of arginine–vasopressin (AVP) and PGE_2 (Hansen and Jensen, 1986). Weanling rats were fed a fat-free diet; feed and water were available *ad libitum*. After 12 wk, two rats were placed in metabolic cages, where they still received feed and water, *ad libitum;* 24 h urine samples were collected for 3 days (period 1). After 3 days of being in the metabolic cages, the rats were given a daily oral supplement of 300 mg of ethyl Arachidonate for 10 days. Twenty-four h urine samples were collected during the last 3 days of dosing (period 2). This procedure was repeated twice, using two previously undosed rats each time.

No significant changes in urinary output due to ethyl Arachidonate were observed when period 1 was compared with period 2. A small but consecutive decrease in urinary AVP excretion was observed during period 2; the mean value of period 1 was compared to the value obtained on day 10 of dosing and a significant decrease was observed in urinary AVP excretion. Urinary PGE_2 was significantly increased by Arachidonate supplementation, with an approximately fivefold increase observed. Fatty acid analysis of total kidney lipids had a peak area percentage of 29.1 ± 1.6 for Arachidonic Acid and 0.2 ± 0.1 for linoleic acid. The feeding of Arachidonate to EFA-deficient rats resulted in the incorporation of linoleic acid into epidermal sphingolipids; this indicated that a decrease in renal linoleic acid could be due to a displacement with Arachidonic Acid. Arachidonate supplementation to EFA-deficient rats normalized transepidermal water loss.

Sprague-Dawley rats were used in a study to determine the amount of Arachidonic Acid absorbed and/or excreted when administered by oral intubation (Ramesha et al., 1985). Seven pregnant rats were fed a fat-free diet the week prior to delivery and during lactation. Following weaning, eight male pups were selected for study and fed a fat-free diet until reaching a body weight near the range of 180–200 g. The rats were then placed in metabolic cages where they received water and fat-free diet, *ad libitum*. Arachidonic Acid solution was prepared by mixing hexane solutions of $^2H_8$-Arachidonic Acid and $^3H_8$-Arachidonic Acid to give a specific activity of 1.14 μCi/mg. The solvent was evaporated from each aliquot to be used and then suspended in 10 mM phosphate buffer (pH 8.1) containing 75 mM NaCl. A 1 ml solution of the aqueous dispersion of the Arachidonate mixture was administered by oral intubation.

At study initiation, the rats received 48.7 mg of the Arachidonic Acid mixture. On days 3, 4, 5, 8, 9, and 13, the rats were given doses of 64.9, 75.7, 58.7, 67.5, 42.4, and 28.4 mg, respectively, of the same mixture for a total dose of approximately 386 mg. Urine and feces were collected every 24 h.

The average basal amount of radioactivity excreted by the animals after completion of dosing, days 15–20, was equivalent to about 0.6 mg and 0.4 mg of Arachidonic Acid per day in urine and feces, respectively. Approximately 20–30% of the radioactivity in the urine during the 24 h after the initial dose appeared to be ^3H_2O; the amount of radioactivity in the aqueous fraction was equivalent to about 0.4–0.8 mg of the

Arachidonic Acid. On the days when the animals were not dosed, the aqueous radioactivity in the urine was approximately 0.2–0.8 mg equivalent of the dosed Arachidonic Acid, but it represented 70–90% of the excreted radioactivity. As of day 8 of the study, the amount of aqueous radioactivity in the urine remained basically constant regardless of dose; this was probably attributable to the large amount of tritiated water that had accumulated in the tissues. About 70–80% of the urinary radioactivity measured on dosing days was organic material; this value was 10–30% on non-dosing days.

The discontinuation of Arachidonic Acid dosing resulted in a 30–40-fold decrease in the amount of radioactivity in the urine, which remained constant at the value of nondosing days. Therefore, approximately 40–100 μg equivalent of the organic Arachidonic Acid metabolites, the value of nondosing days, represented a basal amount of metabolite excretion in the urine. The fecal metabolites of Arachidonic Acid were fractionated. About 40–45% of the fecal radioactivity, which was equivalent to approximately 2 mg of Arachidonic Acid on a dosing day, did not bind to the resin or was washed from the column with water. The remaining activity, which was equivalent to 1.5–2.5 mg Arachidonic Acid on a dosing day and to 0.1–0.5 mg on a nondosing day, bound to the resin and was eluted with acetone. Lyophilization of the aqueous fraction revealed that 20–50% of the radioactivity was volatile, probably representing ^{3}H-water, while the rest was polar, water-soluble, nonvolatile material.

After 21 days, the radioactivity present in the $^{3}H_2O$ of the major tissues averaged 2.6×10^4 counts/min/g (cpm/g) of wet tissue. The organic soluble radioactivity in the tissues was primarily in the form of esterified Arachidonic Acid. In nearly all tissues, the Arachidonic Acid was mostly the deuterated material that was used in dosing. The kidneys contained the most Arachidonic Acid per gram of tissue (2.3 mg/g), followed by the heart (1.2 mg/g) and the liver, lungs, and brain, all of which contained approximately 0.5 mg/g. About 50% of the dosed Arachidonic Acid was accounted for as tissue ester, $^{3}H_2O$, or excreted metabolite. The distribution of Arachidonic Acid radioactivity was determined among the different glycerophospholipids of selected tissues at the end of dosing. The majority of the Arachidonic Acid was contained in phosphatidylethanolamine (PE), with the exception of plasma and adipose tissue, which contained large amounts of Arachidonic Acid in the form of triacylglycerol (TG).

Three normal and three EFA-deficient male albino Wistar rats were given an intravenous (i.v.) injection of approximately 20 μCi 1-[^{14}C]Arachidonic Acid (Zijlstra and Vincent, 1985). The animals were killed 5 min, 30 min, or 24 h after the injection. After 5 min, the greatest concentration of radioactivity in normal rats was present in the subcutaneous fat, perivertebral fat, liver, heart muscle, kidneys, and adrenal glands; the same general results were obtained after 24 h. In EFA-deficient rats, the quantitative distribution was approximately the same as in normal rats, with the exception that the amount of radioactivity in the tissues appeared to be greater after 24 h in the EFA-deficient rats than in the normal rats.

Normal and EFA-deficient male albino Wistar rats, number unspecified, were given i.v. injections of 1 μCi 1-[^{14}C]Arachidonic Acid and were killed 5 min, 30 min, 4 h, or 24 h after injection (Zijlstra and Vincent, 1985). The radioactivity in different tissues was measured at these time periods. After 24 h, the amount of radioactivity was decreased in the heart, approximately the same in the liver and kidneys, and increased in the adrenal glands compared to earlier time periods. The amount of radioactivity was higher in the tissues of EFA-deficient rats than of normal rats; in the liver, the

radioactivity per gram was, on the average, 2.38 times higher in the EFA-deficient rats than in the normal rats.

Normal and EFA-deficient male albino Wistar rats, number unspecified, were given i.v. injections of 1.5 μCi ^{14}C-Arachidonic Acid (Zijlstra and Vincent, 1985). A group of normal control rats, number unspecified, were injected with 2 μCi ^{3}H-Arachidonic Acid. All animals were placed in metabolism cages for 2–3 wks with feed and water, *ad libitum*. Urine fractions were collected for 15 days, and excreted radioactivity was determined daily. The largest amount of radioactivity excreted by the rats receiving 1.5 μCi ^{14}C-Arachidonic Acid occurred the first day. The amount of radioactivity excreted by the EFA-deficient group was less than that excreted by the normal group during the entire test period. Also, in the EFA-deficient group, the amount of radioactivity excreted decreased more rapidly over the period and the amount excreted after 10 days was very small compared to the amount excreted after the first day. Urine was collected for 1 day after normal rats were given injections of 2 μCi ^{3}H-Arachidonic Acid; metabolites were isolated and the majority of the radioactivity was present in the water-soluble fraction of the urine.

Normal and EFA-deficient male albino Wistar rats, varying in number, were given i.v. injections of 1 μCi 1-^{14}C-Arachidonic Acid (Zijlstra and Vincent, 1985). The $^{14}CO_2$ that formed was collected in methanol/ethanolamine (4:1), in 15 min fractions, using the method described by Lauterburg and Bircher (1976) for 2 h. The half-time for $^{14}CO_2$ in eight normal rats was 28 ± 2.8 min and in five EFA-deficient rats it was 39 ± 2.9 min. The total amount of $^{14}CO_2$ respired represented 3.5 ± 1.1 and 3.7 ± 0.8%, respectively, of the injected radioactivity.

The amount of radioactivity in the heart of a normal albino male Wistar rat was determined 2 h after an injection of 1-^{14}C-Arachidonic Acid (Zijlstra and Vincent, 1985). The radioactivity was expressed as a percentage of the total amount of radioactivity on the plate and was distributed over these compounds as follows: phosphatidylinositol (PI), 6.9 ± 0.6; phosphatidylcholine (PC), 44 ± 4.1; PE, 10.0 ± 1.0; and neutral lipids, 9.3 ± 1.6.

Male albino Wistar rats were used to study the molecular distribution of 1-^{14}C-Arachidonic Acid, 1-^{14}C-linoleic acid, and 1-^{14}C-α-linolenic acid (de Tomas and Mercuri, 1971). Each rat was given an i.v. injection of 0.1 ml of solution over a 1 min period; 0.1 ml of solution contained 10 μC of radioactive fatty acid. Five rats were given Arachidonic Acid, six rats were given linoleic acid, and seven rats were given α-linolenic acid, respectively. No significant difference was observed in the amount of each fatty acid incorporated into the rat hepatic lipids. However, a significantly greater amount of Arachidonic Acid than the other fatty acids was incorporated into the phospholipid fraction; significantly less Arachidonic Acid was incorporated into 1,2-diacylglycerol than was linoleic acid and α-linolenic acid.

Fasted male Wistar rats were used to study the amount of 1-^{14}C-Arachidonic Acid in the different lipid fractions of the liver, testes, and serum 1, 4, and 24 h after an i.v. injection of 1 μC methyl Arachidonate-1-^{14}C in 0.5 ml rat serum. The turnover of Arachidonic Acid in these fractions was also determined (Swell and Law, 1967). The rats were fasted following dosing and were killed after 1, 4, or 24 h; five rats were used per time period. Blood samples and the liver and testes were taken from each animal.

Of the examined tissues, the liver contained the greatest percentage of injected ^{14}C after all three time periods. The amount of ^{14}C-Arachidonic Acid recovered in the liver was 35.2 ± 4.5%, 24.1 ± 6.9%, and 9.1 ± 1.8% after 1 h, 4 h, and 24 h, respectively. After 1 h, 37% of the radioactive Arachidonic Acid was recovered in the examined

tissues, with 2.2 ± 1.0% being recovered in the serum and 0.2 ± 0.1% being recovered in the testes. Only a very small amount of the radioactivity was recovered as $^{14}CO_2$. After 24 h, less than 10% of the ^{14}C-Arachidonic Acid was recovered in the examined tissues, with 0.5 ± 0.2% being recovered in the serum and 0.2 ± 0.1% being recovered in the testes.

The tissue lipids were separated into cholesterol ester, glyceride and fatty acids, and phospholipid fractions. After 1 h, the majority of the recovered radioactivity was present in the phospholipid fraction of the liver and testes, 67.0% and 78.7%, respectively, and in the glyceride and fatty acid fraction of the serum, 83.9%. The glyceride and fatty acid fraction of the serum and liver was separated and 40–60% of the ^{14}C was in the free fatty acids after 1 h; after 4 and 24 h, this value was 5–10%. After 4 and 24 h, the majority of the radioactivity remained in the phospholipid fractions of the liver and the testes, with 89.8% and 95.0% being recovered in the liver phospholipids, respectively, and 84.4% and 83.1% being recovered in the testes phospholipids, respectively.

In the serum, the percentage of recovered ^{14}C after 4 h was 39.7% in the cholesterol ester fraction, 37.5% in the phospholipid fraction, and 22.8% in the glyceride and fatty acid fraction. In the serum after 24 h, 55.0% was recovered in the cholesterol ester fraction, 28.2% in the phospholipids, and 16.8% in the glyceride and fatty acid fraction. After 24 h, 96% of the ^{14}C in the serum lipids was in the form of ^{14}C-Arachidonic Acid. In the liver, 93% was ^{14}C-Arachidonic Acid and 7% was ^{14}C-long chain polyunsaturated fatty acids. In the testes, 75% was ^{14}C-Arachidonic Acid and 25% was [^{14}C]long chain polyunsaturated fatty acids. The turnover time of liver and serum Arachidonic Acid, as estimated from the disappearance of radioactivity, was 18–24 h.

Male albino Holtzman rats were used in a catabolism study and an absorption study to assess the metabolism of soybean oil containing 1-^{14}C-Arachidonic Acid (Coots, 1965). Four animals were fed the radioactive soybean oil, 7.5 μC/g, as part of a liquid diet in the catabolism study. Respiratory CO_2, urine, and feces were collected for 51 h and the amount of radioactivity was measured. After 51 h, the animals were killed and the disposition of ^{14}C was determined in the gastrointestinal contents and the carcass. The results were normalized to 100% recovery, meaning that they were expressed as a percentage of the total activity in all the fractions. The actual total recovery was 96%. The percentages of radioactivity recovered in the 51 h period were 40.2 ± 0.5 as respiratory CO_2, 0.8 ± 0.3 in the urine, 1.2 ± 0.4 in the feces, 1.9 ± 0.1 in the gastrointestinal contents, and 56.0 ± 0.9 in the carcass.

In the absorption study, five rats were fed the radioactive soybean oil, 7.5 μC/g, as part of a liquid diet. Lymph was collected from each animal for 42 h following dosing and isolated and separated into glyceride, phospholipid, and sterol ester fractions. A liquid scintillation counter was used to measure radioactivity and the data were reported as a percentage of the total activity recovered from the chromatographic column. Recovery of ^{14}C applied to the column was 96.3%. Absorption peaked 12–16 h after feeding. The percentages of recovered radioactivity were 90.0 in the glycerides, 9.9 in the phospholipids, and 0.1 in the sterol esters. During the peak of absorption, the specific activity of the fractions was 6.0 μC/g for the glycerides, 12.4 μC/g for the phospholipids, and 1.1 μC/g for the sterol esters.

The amount of ^{14}C in the respiratory CO_2 of a thoracic duct-cannulated male albino Holtzman rat fed a liquid diet containing the radioactive soybean oil, 7.5 μC/g, was measured (Coots, 1965). Of the recovered ^{14}C, amount not given, 5.4% was excreted as $^{14}CO_2$.

Male albino mice, five per group, with previously transplanted neuroblastoma neoplasms (C1300) were given i.v. injections of ^{3}H-Arachidonic Acid, 80 Ci/mmole, dissolved in 0.1 ml ethanol (Puri et al., 1975). The mice were killed after 1, 6, or 24 h, with tissues being weighed and neoplasm samples taken. The largest ratios of activity, with the results being expressed as ratios of tissue-to-neoplasm activity, were seen at 1 h. The organs with the greatest activity of ^{3}H-Arachidonic Acid were the kidneys, liver, and lungs when compared with the other tissues. The average ratios of activity for these tissues after 1 h were: 3.7 for kidney/neoplasm, 2.5 for liver/neoplasm, and 1.5 for lung/neoplasm. The ratios after 1 h for heart/neoplasm and spleen/neoplasm were 1.4 and 1.3, respectively. The average ratios of activity 24 h following injection were 1.6 for kidney/neoplasm, 1.4 for liver/neoplasm, 1.3 for lung/neoplasm, 1.1 for heart/neoplasm, and 1.5 for spleen/neoplasm. The percentage of the total injected [^{3}H]Arachidonic Acid in the major tissues 1 h following injection was 8% in the neoplasm, 2% in the kidney, 13% in the liver, and <1% in the lung, spleen, and heart.

The intestinal absorption of Arachidonic Acid was evaluated under various conditions using everted intestinal sacs from male Sprague-Dawley rats (Chow and Hollander, 1978). A micellar incubation solution containing Arachidonic Acid, concentration dependent upon experimental conditions, surfactant or bile salt, 22.82 mM sodium dihydrogen phosphate, 87.45 mM disodium hydrogen phosphate, and tracer amounts of ^{3}H-Arachidonic Acid and ^{14}C-inulin was used. The sac was immediately immersed into 50 ml of the micellar incubation solution and agitated at 80 oscillations/min. Preincubation samples, drawn in triplicate, were used for the calculation of the initial specific activity of Arachidonic Acid and inulin. Proximal and distal intestinal sacs were removed from the solution every 2 min, for a total of 8 min, and immediately rinsed for 15 sec in 200 ml of either 1 mM taurocholate, 0.1 mM Pluronic F 68, or 1 mM Tween 80 solution. All sacs were dried for 24 h and the sutured ends were removed. These methods were the same for all test conditions.

The first test condition used an incubation solution containing Arachidonic Acid at a concentration of 2.1 mM and a pH of 7.4; the incubation temperature was 37°C. Four rats were used. The incubation solution contained 10 mM of sodium taurocholate, an anionic surfactant. The relationship between Arachidonic Acid and absorption was linear for the 8 min of incubation. The absorption of Arachidonic Acid by the proximal jejunum and the distal ileum was similar.

The concentration of Arachidonic Acid in the solution was then varied from 0.21 to 8.36 mM; the remaining test conditions were the same as in the previous test. At least 4 rats were used at each concentration. Absorption remained linear for all concentrations, but the rate of Arachidonic Acid absorption by the segments increased with increases in concentration. The differences in absorption at each concentration were significant, but the difference between the rate of absorption by the proximal and distal segments was not. The relationship between the absorption rate of Arachidonic Acid and its concentration remained linear throughout the range of concentrations.

The absorption of Arachidonic Acid was examined at a concentration range of 5–150 μM Arachidonic Acid; the other conditions were the same as above. At least four rats were used at each concentration. The relationship of absorption versus time remained linear. However, the rate–concentration plot indicated saturation of the absorption process at concentrations above 100 μM.

Butyric, oleic, linoleic, and linolenic acids, each at a concentration of 100 μM, were added to micellar solution, pH 7.4, containing 10 mM sodium taurocholate and their effect on the absorption of 100 μM Arachidonic Acid was studied. At least four rats

were used with each fatty acid. The relationship between Arachidonic Acid and time was linear during the 8 min incubation period with the addition of all the fatty acids. The rate of Arachidonic Acid absorption was not changed by the addition of butyric acid, but a significant and progressive decrease in the Arachidonic Acid absorption rate by the jejunum and ileum was observed with the addition of oleic, linoleic, and linolenic acids.

The absorption of 100 μM and 2.1 mM Arachidonic Acid was measured after the addition of the following metabolic inhibitors and uncouplers to the incubation medium: 0.1 mM 2,4-dinitrophenol, 1 mM potassium cyanide, and 1 mM sodium azide. Four rats were used per group. The addition of these substances did not significantly alter the rate of Arachidonic Acid absorption compared to the rate at basal conditions for either concentration of Arachidonic Acid.

The effect of the incubation pH on Arachidonic Acid absorption was also examined. The incubation fluid contained 100 μM Arachidonic Acid and 10 mM sodium taurocholate. The pH of the solution was varied by changing the ratio of sodium dihydrogen phosphate and disodium hydrogen phosphate in the buffer solution. The pHs tested were 7.4 using four rats, 6.4 using five rats, and 5.4 also using five rats. The absorption of Arachidonic Acid increased with a decrease in pH of the incubation fluid.

The effect of 5, 10, 15, or 20 mM sodium taurocholate added to the standard phosphate buffer solution on the absorption rate of 2.1 mM Arachidonic Acid was tested. Four rats were used per group. The absorption rate was not significantly different at concentrations of 5–15 mM, but the rate was decreased with the addition of 20 mM sodium taurocholate compared to the baseline experiments using 10 mM sodium taurocholate.

The effect of 5 mM Pluronic F 68 and 5 mM Tween 80, both nonionic surfactants, on the rate of absorption of 2.1 mM Arachidonic Acid was examined. Six rats were used in testing the effect of Pluronic F 68 and seven rats were used to test the effect of Tween 80. The absorption rate of Arachidonic Acid in the presence of either nonionic surfactant was higher than the rate of Arachidonic Acid when solubilized in a 5 mM sodium taurocholate solution.

The oscillation speed of the incubation chamber was varied to determine the effect of the unstirred water-layer thickness on the absorption rate of 2.1 mM Arachidonic Acid. Speeds of 0, 40, 80, and 120 oscillations/min were tested and seven, four, four, and seven rats were used, respectively. The absorption rate at 0 oscillations was compared statistically to the absorption rates at 40, 80, and 120 oscillations/min. The absorption rate of Arachidonic Acid increased at speeds greater than 40 oscillations/min.

Finally, the influence of 2.1 mM dextrose, glutamic acid, lysine, or leucine on the absorption of 2.1 mM Arachidonic Acid was examined. Four rats were used per group. The addition of these nutrients caused no significant difference in the absorption rate of Arachidonic Acid.

The mouse epidermis-derived cell line HEL/30 was incubated with 10 μM ^{3}H-Arachidonic Acid, 0.2 μCi/nmol, for 1 h in the presence of either 0.02% bovine serum albumin (BSA) or 10% fetal calf serum (FCS) to study Arachidonic Acid metabolism (Tragni et al., 1988). When the cell line was incubated in the presence of BSA for 1 h, 53.5 ± 0.9% of the radioactivity was incorporated into the cells. After an additional 2 h of incubation in a fresh medium, 0.52 ± 0.03% of the radioactivity was released into the medium. The remaining radioactivity inside the cell was mainly associated with the cell membrane. When incubated in the presence of 10% FCS, a

fourfold increase in radioactivity released by the cells was observed; the authors stated that this was probably due to growth factors in the FCS. The addition of 1 μM 12-O-tetradecanoyl-phorbol-13-acetate (TPA) significantly increased the release of radioactivity into the medium and the formation of PGE_2. Thin-layer chromatographic (TLC) analysis of cell extracts revealed that the HEL/30 cell line metabolized Arachidonic Acid by both the cyclooxygenase and lipoxygenase pathways.

A study was conducted to define the metabolism of exogenous Arachidonic Acid in murine macrophage-like neoplasm cells and it was found that the cell line J774.2 metabolized Arachidonic Acid to PGE_2, 12-hydroxy-5,8,10-heptadecantrienoic acid, thromboxane B_2 (TxB_2), PGD_2, and $PGF_{2\alpha}$ (Stenson et al., 1981). Over 80% of 10 μM or less exogenous Arachidonic Acid was metabolized by J774.2 macrophages within 3.5 min of administration. The addition of serum-treated zymosan and the ionophore A-23187 prior to the addition of Arachidonic Acid increased the proportion of exogenous Arachidonic Acid esterified into phospholipids and decreased the proportion metabolized by cyclooxygenase.

Studies using perfused rat lungs have reported that metabolism of exogenous Arachidonic Acid, as measured by 6-keto-PGF_2 and TxB_2 production, was greater in male than female lungs (Maggi et al., 1980).

ARACHIDONIC ACID AND PROSTAGLANDIN PRODUCTION

The studies contained in this section and a number of similar studies concerning the effect of Arachidonic Acid on prostaglandin production are summarized in Table 2.

To identify and measure PGE_2 and $PGF_{2\alpha}$ in human urine, urine was collected from a varying number of females (Frolich et al., 1975). The subjects abstained from sexual intercourse 2 days prior to and during the study. During the acute study, a tampon was inserted. Twenty-four h urine specimens were collected from subjects who had not taken any drugs for at least 14 days prior to study initiation and urine was collected only outside the menstrual period. PGE_2 and $PGF_{2\alpha}$ were identified by mass spectrometry as the PGE_2 methyl ester converted to methoxime bis-acetate and $PGF_{2\alpha}$ methyl ester converted to its tris-trimethylsilylether.

Based on the amount of deuterated internal standard added to each aliquot of urine, 24 h urine samples of eight ambulatory females had a PGE_2 concentration of 374 ± 77 pg/ml. In 10 females, the concentration of $PGF_{2\alpha}$, measured by selected ion monitoring, was 386 ± 78 pg/ml.

Two to three weeks before initiation of a study examining prostaglandin production, a polyethylene matrix was implanted in the renal interstitium of the left kidney at the corticomedullary level of male Sprague-Dawley rats (Kinoshita et al., 1989). The open end of the tubing was sealed by tying it around itself, the abdominal cavity was rinsed with saline to remove any residual blood, and 15,000 U of penicillin G was injected intramuscularly. The rats were fasted 14–18 h before the start of the study. Six rats were used per group. The first group received i.v. injections of 3 mM sodium carbonate in isotonic saline at 0.375 ml/100 g body wt, followed by continuous i.v. infusion of the same solution at 0.375 ml/100 g body wt/h. Thirty minutes later, a 30 min control period was started during which mean arterial pressure and urinary excretion of PGE_2 and 6-keto-$PGF_{1\alpha}$ were measured. Following the control period, 100 μl of 10^{-4} M Arachidonic Acid in isotonic saline containing 0.06% ethanol was injected into the renal interstitium via the implanted matrix. A continuous infusion of

Table 2. Arachidonic Acid and Prostaglandin Production

Species and gender	*Methods*	*Results and comments*	*Reference*
Female humans	Urine $PGE_2$and $PGF_{2\alpha}$ concentrations were measured in females that abstained from intercourse for 2 days.	In 8 subjects, 24 h urine samples had a PGE_2 concentration of 374 ± 77 pg/ml. Ten subjects had a $PGF_{2\alpha}$ concentration of 386 ± 78 pg/ml.	Frolich et al., 1975
Male Sprague-Dawley rats	Two to 3 wk before study initiation, a polyethylene matrix was implanted in the renal interstitium of the left kidney of test rats; rats were fasted before study initiation. Six rats were given an i.v. injection, followed by continuous i.v. infusion, of 3 mM sodium carbonate at 0.375 ml/100 g body wt/h. 30 min later, there was a control period during which certain values were measured. Then, 100 μl 10^{-4} M Arachidonic Acid was injected into the renal interstitium via the matrix, followed by a continuous infusion of 10^{-4} M Arachidonic Acid at 10 μl/min. Another control period started 5 min later. The same procedure was followed in a second group, with the exception that a 3 mg/kg i.v. injection of indomethacin was given 30 min prior to the control period; a continuous infusion of 50 μg/kg/min indomethacin followed and continued through the study. The procedure was carried out for a third group, with the exception that vehicle was given instead of Arachidonic Acid; this group served as the controls.	Based on measurements from 4 animals, urinary PGE_2 and 6-keto-$PGF_{1\alpha}$ increased significantly with Arachidonic Acid infusion. Indomethacin inhibited the increase.	Kinoshita et al., 1989
Mongrel dogs	The inner medulla of the kidney was removed and slices were incubated in either 3.3×10^{-4}, 8.2×10^{-4}, or 1.6×10^{-3} M Arachidonic Acid.	PGE_2 production increased significantly with concentration increase.	Bolterman et al., 1989

Male and female mongrel dogs	Six dogs were used in studying the effects of Arachidonic Acid, 6-keto-PGF_1, and bradykinin infusion. There were six 30 min infusion periods. Urine was collected during the last 20 min of each period; blood samples were collected during urine collection mid-point. Periods 1, 3, and 5 were control and recovery periods. During period 2, Arachidonic Acid was infused into the renal artery at 15 μg/kg/min. 6-keto-$PGF_{1\alpha}$ and bradykinin were infused during periods 4 and 6, respectively.	The following parameters increased during Arachidonic Acid infusion: renal blood flow, urine flow, net renal venous 6-keto-PGF_1 outflow, urinary 6-keto-$PGF_{1\alpha}$ excretion, renal venous plasma 6-keto-$PGF_{1\alpha}$ concentration, arterial 6-keto-$PGF_{1\alpha}$ concentration, net renal venous outflow of PGE_2, urinary excretion of PGE_2, renal venous concentration of PGE_2, and the arterial PGE_2 plasma concentration.	Boyd et al., 1986
Male and female mongrel dogs	Dogs were fasted 18 h prior to study initiation. The study used a three-step procedure: blood and urine samples were collected after a period of equilibrium and stabilization; Arachidonic Acid was infused into the left renal artery; and recovery samples were obtained after 10–35 min. Conditions varied and were as follows: 1. Fifteen hydropenic dogs were given infusions of 160 μg/kg/min Arachidonic Acid. 2. Five hydropenic dogs were given infusions of 40 μg/kg/min Arachidonic Acid.	1. The arterial Arachidonic Acid concentration was 53.4 ± 4.1 μmol/L. Renal blood flow, renal venous PGE_2, urine PGE_2, urinary PGE_2 excretion, and total PGE_2 output increased significantly. Significant decreases were observed during recovery; total PGE_2 output remained slightly elevated. 2. The arterial Arachidonic Acid concentration was 26.1 ± 7.5 μmol/L. Renal blood flow, renal venous PGE_2 excretion, and total PGE_2 output increased significantly with Arachidonic Acid administration and decreased significantly during recovery. Urine PGE_2 decreased with Arachidonic Acid administration. Complete recovery was reached 30 min after dosing.	Sejersted et al., 1984

TABLE 2. ARACHIDONIC ACID AND PROSTAGLANDIN PRODUCTION *CONTINUED*

Species and gender	*Methods*	*Results and comments*	*Reference*
	3. After the hydropenic period, high urine flow was induced in 13 dogs by infusion of mannitol, saline, or saline and ECA. The three-step procedure was then followed.	3. Infusion with the three-substances did not produce any significant changes in PGE_2 output as compared to the recovery period; arterial PGE_2 concentration was not changed. Intrarenal Arachidonic Acid infusion produced a decrease in the urinary fraction of PGE_2. Stimulation of total PGE_2 output was not significantly different than during hydropenia. Arachidonic Acid might stimulate release into the renal vein.	Sejersted et al., 1984
Female CD-1 mice	Mice were given subcutaneous doses of 2 μg Arachidonic Acid during estrus. Uterine tissues were collected 7.5–8 h later.	Arachidonic Acid did not significantly affect uterine tissue PGF content; uterine concentration of PGF significantly increased. Uterine weight was significantly decreased by Arachidonic Acid administration.	Saksena et al., 1974
Male and female mongrel dogs	The effect of Arachidonic Acid infusion into the renal artery of hydropenic and volume-expanded dogs at a rate of 40 μg/kg/min was evaluated. Indomethacin was also given to some dogs.	Arachidonic Acid infusion significantly increased urinary and renal venous PGE_2 in nine hydropenic and five volume-expanded dogs. Indomethacin inhibited this effect.	Landberg et al., 1984
Male Sprague-Dawley rats	Hydropenic and saline-loaded rats were given intrarenal infusions of Arachidonic Acid at a dose of 45–65 μg/min/100 g body wt. A group of saline-loaded rats were given a priming dose and continuous infusion of indomethacin in addition to infusion of Arachidonic Acid. The number of animals used per group was not specified.	PGE_2 and $PGF_{2\alpha}$ excretion, urine flow, and osmolar excretion increased significantly in both hydropenic and saline-loaded rats. The $PGE_2/PGF_{2\alpha}$ ratio did not change significantly under either condition. Indomethacin and Arachidonic Acid increased PGE_2 and $PGF_{2\alpha}$ excretion significantly, but the increase was not as great as the increase caused by Arachidonic Acid alone.	Leyssac and Christensen, 1981
Mongrel dogs	Arachidonic Acid was infused into the left renal artery of six dogs at a rate of 30 μg/min. This procedure was repeated in another 6 dogs after a Blalock clamp was placed around the aorta above the renal arteries to maintain a renal perfusion pressure of approx. 80 mm Hg. Six dogs were given infusions of Arachidonic Acid at a rate of 3 mg/min 45 min after being given 1 mg/kg carprofen.	With and without the clamp, renal venous PGE concentration increased significantly. Renal venous PGE did not change significantly with the administration of carprofen; upon Arachidonic Acid infusion, renal venous PGE increased significantly.	Bay et al., 1979

Mongrel dogs	Six mongrel dogs were given infusions of sodium Arachidonate in 0.2 ml saline at a rate of 10 μg/min for 30 min. Tritiated Arachidonic Acid was added to nonradioactive Arachidonic Acid and infused into the renal artery of a dog at a rate of 10 μg/min for 30 min. With the beginning of infusion, a portion of the radioactive and nonradioactive Arachidonic Acid was added to the dog's urine which was collected during a control period.	Sodium Arachidonate infusion resulted in a significant increase in ipsilateral concentration and excretion of PGE. The urine of the dog given radioactive Arachidonic Acid contained significant amounts of PGE and PGF only after Arachidonic Acid infusion.	Frolich et al., 1975
Male and female mongrel dogs	Doses of 1.0, 3.0, 10.0, and 30.0 μg/kg sodium Arachidonate were administered to 12 dogs intrarenally.	A significant increase was observed in urinary prostaglandin-like material, PGE_2, and $PGF_{2\alpha}$ with the infusion of sodium Arachidonate at a rate of 10 μg/kg/min.	Tannenbaum et al., 1975
Rabbit ileal mucosa	In an *in vitro* study, rabbit ileum was exposed to Arachidonic Acid, $K_{1/2} \approx 10^{-6}$ M.	Electrolyte secretion, cAMP accumulation, and PGE_2 production were stimulated. Tachyphylaxis developed with the continued presence of Arachidonic Acid. Secretory and cAMP responses had a half-life of approx. 20 min. Subsequent additions of Arachidonic Acid produced little or no further response. PGE_2 production continued undiminished.	Field et al., 1981

10^{-4} M Arachidonic Acid at 10 μl/min was then administered. Five min after the start of this infusion, another 30 min control period was started, and the same factors were measured.

In a second group, this procedure was repeated with the exception that 3 mg/kg indomethacin was given as an i.v. injection 30 min prior to the control period. After this injection, indomethacin was then infused at a rate of 50 μg/kg/min and infusion continued throughout the study.

In the control group, the initial procedure was carried out, with the exception that 0.6% ethanol in isotonic saline was infused instead of the Arachidonic Acid solution.

The results were that urinary PGE_2 and 6-keto-$PGF_{1\alpha}$ increased significantly with Arachidonic Acid infusion. (These results were based on measurements from four animals.) Indomethacin blocked these increases.

The kidneys of mongrel dogs were removed and used in determining the effect of Arachidonic Acid on PGE_2 production (Bolterman et al., 1989). The inner medulla was removed from the kidneys and slices were incubated with 3.3×10^{-4}, 8.2×10^{-4}, or 1.6×10^{-3} M Arachidonic Acid. PGE_2 production was significantly increased from a basal value of 314 ± 81 pg/mg to 956 ± 249 pg/mg, 1315 ± 202 pg/mg, and 2688 ± 569 pg/mg as the doses increased. The increase of PGE_2 production due to 1.6×10^{-3} M Arachidonic Acid was approximately 11-fold when compared to the basal rate.

Six male and female dogs, strain and number/gender unspecified, were used to study the effects of Arachidonic Acid, 6-keto-PGF_1, and bradykinin infusion on renal venous and urinary outflows of 6-keto-PGF_1 and PGE_2 (Boyd et al., 1986). After equilibration, there were six 30 min infusion periods; urine samples were collected during the last 20 min of each period, and arterial and renal venous blood samples were collected at the midpoint of urine collection. Periods 1, 3, and 5 were control and recovery periods, with 0.9% NaCl being infused into the renal artery. Arachidonic Acid was infused into the renal artery at 15 μg/kg/min. During periods 4 and 6, respectively, 6-keto-$PGF_{1\alpha}$ and bradykinin were infused.

During Arachidonic Acid infusion, renal blood flow increased from 171 ± 27 ml/min to 205 ± 15 ml/min. The urine flow rate increased to 180% of the control. Glomerular filtration rate and blood pressure were not altered.

Net renal venous 6-keto-$PGF_{1\alpha}$ outflow increased approximately 14-fold, from 8 ng/min to >100 ng/min. Urinary 6-keto-$PGF_{1\alpha}$ excretion increased less than threefold, from 3.2 ± 0.6 ng/min to 8.7 ± 1.3 ng/min. The renal venous plasma concentration of 6-keto-$PGF_{1\alpha}$ was 648 ± 165 pg/ml during period 1; it increased to 2121 ± 451 pg/ml with the infusion of Arachidonic Acid. The arterial plasma concentration of 6-keto-$PGF_{1\alpha}$ was 645 ± 150 pg/ml during period 1, which increased to 1183 ± 465 pg/ml with the infusion of Arachidonic Acid.

The net renal venous outflow and the urinary excretion of PGE_2 increased approximately twofold. The renal venous plasma concentration of PGE_2 was 344 ± 122 pg/ml during period 1, and 614 ± 180 pg/ml with the infusion of Arachidonic Acid. The arterial PGE_2 plasma concentration was 122 ± 25 pg/ml during period 1, and it was 146 ± 43 pg/ml with the infusion of Arachidonic Acid.

Male and female mongrel dogs, which were fasted 18 h prior to study initiation, were used in examining the relationship between renal venous PGE_2 output and urinary PGE_2 excretion during various experimental diuretic conditions (Sejersted et al., 1984). A standard three-step procedure was used for the different diuretic states tested: (1)

blood and urine samples were collected after a period of equilibration and stabilization; (2) Arachidonic Acid, 160 μg/kg/min, was infused into the renal artery; and (3) recovery samples were obtained after 10–35 min. The Arachidonic Acid was dissolved in 1 ml of 70% ethanol and diluted with buffer to the desired concentration; the pH was adjusted to 7.4. Infusion of the buffer and ethanol without Arachidonic Acid did not affect renal function or PGE_2 output.

The procedure was first performed using 15 dogs in a hydropenic state. The infusion of 160 μg/kg/min produced an arterial Arachidonic Acid concentration of 53.4 ± 4.1 μmol/L. Renal blood flow, renal venous PGE_2, urine PGE_2, urinary PGE_2 excretion, and total PGE_2 output increased significantly with intrarenal Arachidonic Acid infusion; significant decreases were observed during recovery. Arterial PGE_2 did not change significantly with Arachidonic Acid infusion. The total PGE_2 output increased by 3.06 ± 0.51 pmol/g/min with the infusion of 160 μg/kg/min Arachidonic Acid and it remained slightly elevated. Renal blood flow increased 63 ± 6% and urine flow increased from 0.2 ± 0.1 to 1.9 ± 0.2 ml/min.

After the recovery period, the procedure was repeated using five hydropenic dogs; infusions of 40 μg/kg/min instead of 160 μg/kg/min Arachidonic Acid were administered. The concentration of arterial Arachidonic Acid at this dosage was 26.1 ± 7.5 μmol/L. Renal blood flow, renal venous PGE_2, and total PGE_2 output increased significantly during Arachidonic Acid infusion; these values decreased significantly during recovery. Arterial PGE_2 did not change significantly. Urine PGE_2 decreased with the administration of 40 μg/kg/min Arachidonic Acid; a significant decrease was seen when the recovery value was compared to the value during the administration of Arachidonic Acid. The total PGE_2 output increased by 0.90 ± 0.30 pmol/g/min with the infusion of 40 μg/kg/min Arachidonic Acid. Renal blood flow increased 43 ± 20% while urine flow increased from 0.9 ± 0.8 to 1.9 ± 0.7 ml/min. Complete recovery was reached 30 min after the termination of Arachidonic Acid infusion.

The arterial and venous plasma concentrations of PGE_2 averaged 0.072 ± 0.017 and 0.067 ± 0.013 nmol/L during the control period prior to any infusion; this value was determined using 15 dogs. During the infusion of Arachidonic Acid at either dosage, renal venous PGE_2 concentrations were always elevated while arterial PGE_2 concentrations did not change significantly. Regardless of the dose, very little of the Arachidonic Acid (on the average, a fraction of 2×10^6) was converted to PGE_2 and appeared in venous plasma and urine. There was a linear relationship with a high correlation coefficient between the different doses and the total PGE_2 output during renal infusion. Arachidonic Acid infusion was carried out in three dogs before and after i.v. administration of 10 mg/kg indomethacin to determine whether this response was due to intrarenal PGE_2 synthesis; indomethacin reduced venous PGE_2 output by 83.2 ± 3.8% during the infusion of 40 μg/kg/min Arachidonic Acid. Urinary PGE_2 excretion was linearly related to total PGE_2 output. During PGE_2 recovery, the fraction of PGE_2 in the urine averaged 17%.

After the hydropenic period, high urine flow was induced in 13 dogs by infusion of mannitol, saline, or saline and ethacrynic acid (ECA); the three-step procedure was then followed. Infusion with these substances did not produce any significant change in total renal PGE_2 output as compared to the recovery period; arterial PGE_2 concentrations were not changed. During intrarenal infusion of 160 μg/kg/min Arachidonic Acid, the urinary fraction of PGE_2, on the average, decreased. Stimulation of total renal PGE_2

output during high urine flow was not significantly different compared to the value during hydropenia. Therefore, at high urine flow, Arachidonic Acid might stimulate release of PGE_2 into the renal vein.

Five CD-1 female mice were given subcutaneous doses of 2 μg Arachidonic Acid at 3:00 a.m. of estrus to determine the effect of Arachidonic Acid on the production of PGF by uterine tissue (Saksena et al., 1974). Uterine tissues were collected between 10:30 and 11:00 a.m. on the day of estrus. Arachidonic Acid did not significantly affect uterine tissue content of PGF; however, uterine concentration of PGF significantly increased. Uterine weight was significantly decreased by Arachidonic Acid administration.

ARACHIDONIC ACID AND RENAL FUNCTION

Arachidonic Acid and renal function studies are summarized in Table 3.

Arachidonic Acid metabolites modulate hemodynamics and excretory function in the kidneys by influencing renal blood flow, glomerular filtration, the release of renin, and the urinary excretion of electrolytes and water by direct action and by modulating other hormones (Zoja et al., 1989). It has been suggested that a complex interaction exists between angiotensin II and Arachidonic Acid metabolites of glomerular origin in the regulation of glomerular hemodynamics. Normal human and rat glomerular tissue synthesizes both vasodilatory and vasoconstrictor Arachidonic Acid derivatives; it has receptors for angiotensin II that are linked to a specific activation of the Arachidonic Acid metabolic pathway.

The enzymes for Arachidonic Acid metabolism in the kidneys are not evenly distributed; the cyclooxygenase, lipoxygenase, and cytochrome P-450 enzymes are used in directing the renal metabolic pathways (Morrison, 1986).

In the study performed by Kinoshita et al. (1989) that was described earlier in the section Arachidonic Acid and prostaglandin production, the effect of Arachidonic Acid on renal function was also measured. Recollection micropuncture at the superficial late proximal tubules of six male Sprague-Dawley rats showed that Arachidonic Acid, 10^{-4} M infused at a rate of 10 μl/min, significantly increased the tubular flow rate and the fractional delivery of sodium at late proximal tubules; it significantly decreased the tubular fluid-to-plasma inulin concentration ratio. Infusion of indomethacin, at a rate of 50 μg/kg/min throughout the study, along with Arachidonic Acid infusion did not produce any significant changes in these values nor any other values when measured using six rats.

Male and female mongrel dogs were used to study the effect of Arachidonic Acid on kidney function during hydropenic and volume-expanded conditions (Landberg et al., 1984). Nine hydropenic dogs were given Arachidonic Acid infusions into the renal artery at a rate of 40 μg/kg/min; five of the dogs were subsequently given a dose of 10 mg/kg indomethacin. The volume-expanded group, which consisted of five dogs, was given i.v. infusions of a modified Ringer solution at a volume corresponding to approximately 10% of body weight, and they received a priming dose of 3 mg/kg ethacrynic acid followed by continuous infusion of ethacrynic acid at a rate of 1.5 mg/kg/h. The infusion of the modified Ringer solution continued throughout the experiment at a rate that balanced urine loss. Arachidonic Acid in saline was infused at a rate of 40 μg/kg/min and measurements were taken. A dose of 10 mg/kg indomethacin was then administered. A Blalock clamp placed above the origin of the renal arteries was used to control renal arterial perfusion pressure. In the hydropenic dogs, renal

Table 3. Arachidonic Acid and Kidney Function

Species and gender	*Methods*	*Results and comments*	*Reference*
Male Sprague-Dawley rats	For six rats, recollection micropuncture samples were taken at the superficial late proximal tubules after infusion of 10^{-4} M Arachidonic Acid at 10 μl/min. In six rats, indomethacin was infused throughout the study at 50 μg/kg/min.	Arachidonic Acid significantly increased tubular flow rate and fractional sodium delivery at the late proximal tubules. The tubular fluid-to-plasma inulin concentration ratio significantly decreased. Infusion with indomethacin did not produce any significant changes in these or other values.	Kinoshita et al., 1989
Male and female mongrel dogs	Nine hydropenic dogs were given infusions of 40 μg/kg/min Arachidonic Acid into the renal artery; five of these dogs were subsequently dosed with 10 mg/kg indomethacin. Five volume-expanded dogs were dosed with 40 μg/kg/min Arachidonic Acid; a dose of 10 mg/kg indomethacin was then given. Renal arterial perfusion pressure was controlled with Blalock clamp.	In the hydropenic dogs, renal blood flow, glomerular filtration rate, urine flow, sodium excretion, sodium reabsorption, chloride excretion, and chloride reabsorption increased significantly with Arachidonic Acid infusion. Indomethacin reduced renal blood flow. Renal blood flow and glomerular filtration rate could not be restored by increased renal arterial perfusion pressure. In the volume-expanded dogs, Arachidonic Acid significantly increased renal blood flow; indomethacin reduced this value to less than the control value. Arachidonic Acid infusion following indomethacin infusion had no effect on renal blood flow.	Landberg et al., 1984
Mongrel dogs	Seven dogs were given infusions of 20 μg/kg/min into the left renal artery during pericardial tamponade and after pericardiocentesis. There were four experimental periods for group 1. Nine dogs were given infusions of 20 and 80 μg/kg/min Arachidonic Acid; group 2 had five experimental periods.	In group 1, Arachidonic Acid infusion increased sodium excretion during pericardial tamponade and after pericardiocentesis. Urine flow rate was increased during each period. Between tamponade and pericardiocentesis, renal blood flow increased significantly. Peripheral blood PGE_2 concentration was increased by Arachidonic Acid during tamponade and remained elevated after pericardiocentesis; Arachidonic Acid increased PGE_2 concentration insignificantly during pericardiocentesis. After pericardiocentesis, mean arterial pressure increased and venous pressure, hematocrit, and plasma renin activity decreased; however, Arachidonic Acid did not affect these values. In group 2, sodium excretion increased in a steplike fashion with concentration. One hour after dosing, sodium excretion was near control values. Pericardiocentesis increased sodium excretion. Arachidonic Acid did not affect renal blood flow or plasma protein concentration. In both groups, Arachidonic Acid did not affect glomerular filtration rate, mean arterial pressure, venous	Boudreau and Mandin, 1981

TABLE 3. ARACHIDONIC ACID AND KIDNEY FUNCTION (CONTINUED)

Species and gender	*Methods*	*Results and comments*	*Reference*
		pressure, or absolute blood flow to any cortical zone. Following pericardiocentesis, blood flow to cortical zone Z_1 increased in both groups.	Boudreau and Mandin, 1981
Mongrel dogs	Six dogs were given infusions of 300 μg/min Sodium Arachidonate into the left renal artery. Another six dogs were given the same dose, with the exception that a Blalock clamp was used to maintain a perfusion pressure of ≈ 80 mm Hg. A third group was first given 1 mg/kg carprofen and, 45 min later, 3 mg/min Arachidonic Acid.	In group 1, renal blood flow, urinary volume, urinary sodium excretion increased significantly; filtration fraction decreased significantly. In the controlled-pressure group, no significant changes were observed for these values. Carprofen administration resulted in a significant decrease in renal blood flow, urinary volume, and urinary sodium excretion. Subsequent Arachidonic Acid infusion returned renal blood flow to a near-control value and urinary volume to a value significantly greater than control value. Urinary sodium excretion was increased to a value that was slightly less than the control value.	Bay et al., 1979
Male and female mongrel dogs	Twelve dogs were given infusions of Sodium Arachidonate into the renal artery at doses of 1.0, 3.0, 10.0, or 30.0 μg/kg/min. The procedure was repeated in seven dogs with the simultaneous infusion of 0.3 μg/kg/min 5,8,11,14-eicosatetraynoic acid. Test article was infused for two 5 min periods at each dose.	The maximum effects of Arachidonic Acid were observed at 30 μg/kg/min. Arachidonic Acid infusion produced a significant increase in urine flow, sodium excretion, potassium excretion, and the tubular rejection fraction of sodium. 30 μg/kg/min Sodium Arachidonate produced a significant increase in renal blood flow. Free water clearance, glomerular filtration rate, and filtration fraction did not significantly change with Arachidonic Acid infusion. 5,8,11,14-eicosatetraynoic acid inhibited Sodium Arachidonate's effect on urine flow, urinary sodium excretion, potassium excretion, tubular rejection fraction of sodium, and renal blood flow.	Tannenbaum et al., 1975
Sprague-Dawley rats	Normally hydrated rats were given Arachidonic Acid infusions into the aorta over a 25 min period. Mildly volume-expanded rats were also given Arachidonic Acid infusions. A group of rats was used as volume-expanded controls.	Immediately after Arachidonic Acid infusion, a significant increase in plasma renin activity occurred in the normal group; this returned to a control value in 90–120 min. Urine volume and sodium excretion did not change significantly. In the volume-expanded rats, plasma renin activity increased. Urine volume and urinary sodium excretion remained almost unchanged when compared to volume expanded controls. Arachidonic Acid administration with saline prevented the increase in renal blood flow and glomerular filtration rate normally seen with volume-expansion due to saline.	Weber et al., 1975

blood flow, glomerular filtration rate, urine flow, sodium excretion, sodium reabsorption, chloride excretion, and chloride reabsorption increased significantly with the administration of Arachidonic Acid.

Subsequent infusion of indomethacin in five of the nine hydropenic dogs reduced renal blood flow by 19 ± 3%; renal blood flow and glomerular filtration rate could not be restored by raising renal arterial perfusion pressure. In volume-expanded dogs, renal blood flow significantly increased with Arachidonic Acid infusion. Subsequent infusion of indomethacin reduced renal blood flow to a value lower than that of the controls; Arachidonic Acid infusion following indomethacin administration had no effect on renal blood flow.

Two groups of mongrel dogs, gender unspecified, were used to study the effect of Arachidonic Acid on renal function during pericardial tamponade (Boudreau and Mandin, 1981). Arachidonic Acid was infused into the left renal artery of seven dogs at a rate of 20 μg/kg/min during pericardial tamponade and after pericardiocentesis. A set of two clearance collections was taken during four periods: during pericardial tamponade; during Arachidonic Acid administration; dosing was stopped, the pericardial tamponade was drained, and a third set of collections was taken 1 h following dose completion; and the last set was taken during a second Arachidonic Acid infusion.

A second group of nine dogs was given infusions of 20 and 80 μg/kg/min of Arachidonic Acid. For this group there were five experimental periods during which collections were made: during pericardial tamponade; during tamponade plus the administration of 20 μg/kg/min Arachidonic Acid; during tamponade 1 h after dose completion; during tamponade plus administration of 80 μg/kg/min Arachidonic Acid; and during pericardiocentesis 1 h following dose administration.

In the first group, Arachidonic Acid infusion increased sodium excretion during pericardial tamponade and following pericardiocentesis. Urine flow rates were increased in each experimental period by Arachidonic Acid infusion. Between the tamponade and pericardiocentesis plus Arachidonic Acid periods, renal blood flow increased significantly. PGE_2 concentrations in peripheral blood were increased by Arachidonic Acid administration during tamponade and remained elevated following pericardiocentesis; Arachidonic Acid increased PGE_2 concentrations insignificantly following pericardiocentesis. Pericardiocentesis increased urine flow and sodium excretion. Following pericardiocentesis, mean arterial pressure increased and venous pressure, hematocrit, and plasma renin activity decreased; however, Arachidonic Acid infusion did not affect hematocrit or plasma renin activity.

In the second group, sodium excretion increased in a steplike fashion with increased Arachidonic Acid concentration. Sixty min following Arachidonic Acid infusion, sodium excretion decreased to near control values. Pericardiocentesis produced an increase in sodium excretion. Arachidonic Acid affected neither renal blood flow nor plasma protein concentration at either dose.

In both groups, Arachidonic Acid infusion did not affect glomerular filtration rate, mean arterial pressure, venous pressure, or absolute blood flow to any cortical zone; following pericardiocentesis, blood flow to cortical zone Z_1 increased in both groups.

Mongrel dogs, gender not specified, were used to examine the effect of Arachidonic Acid infusion into the left renal artery on renal function (Bay et al., 1979). Six dogs were given infusions of sodium Arachidonate at a rate of 300 μg/min. The same procedure was repeated using another six dogs, with the exception that a Blalock clamp was placed around the aorta, above the renal arteries, to maintain a perfusion pressure of

approximately 80 mmHg. A third group of six dogs was given 1 mg/kg carprofen and, after 45 min, an infusion of Arachidonate at a rate of 3 mg/min.

In the group that received Arachidonic Acid without controlled perfusion pressure, renal blood flow, urinary volume, and urinary sodium excretion increased significantly; filtration fraction decreased significantly. With a constant perfusion pressure, no significant changes were observed in any of these values. Administration of carprofen resulted in significant decreases in renal blood flow, urinary volume, and urinary sodium excretion. Subsequent infusion of Arachidonic Acid returned renal blood flow to a value similar to the control value and urinary volume to a value significantly greater than the control value. Urinary sodium excretion was increased to a value slightly lower than the control value.

Twelve male and female mongrel dogs were given infusions of sodium Arachidonate through the renal artery at a dose of 1.0, 3.0, 10.0, or 30.0 μg/kg/min and the effect on renal function was evaluated (Tannenbaum et al., 1975). In seven dogs, this procedure was repeated with the simultaneous infusion of 0.3 μg/kg/min of 5,8,11,14-eicosatetraynoic acid. The test article was infused into the renal artery for two 5 min periods at each of the doses.

The maximum effects of Arachidonic Acid infusion were observed at 30 μg/kg/min. Arachidonic Acid infusion produced significant increases in urine flow, sodium excretion, potassium excretion, and the tubular rejection fraction of sodium. A significant increase in renal blood flow was seen with the infusion of 30.0 μg/kg/min sodium Arachidonate. Free water clearance, glomerular filtration rate, and filtration fraction did not change significantly with Arachidonic Acid infusion. The effect of sodium Arachidonate on urine flow, urinary sodium excretion, potassium excretion, tubular rejection fraction of sodium, and renal blood flow was inhibited by 5,8,11,14-eicosatetraynoic acid.

The effect of Arachidonic Acid on plasma renin activity, urinary sodium excretion, and water excretion was studied using normally hydrated and mildly volume-expanded Sprague-Dawley rats, gender not specified (Weber et al., 1975). Six rats were infused with saline at a rate of 0.5 ml/hr/100 g body wt for 180 min to replace physiological fluid loss. After a control period of 60 min, Arachidonic Acid was infused into the aorta, just above the renal artery, over a 25 min period. Two group of rats, five rats per group, were given an infusion of saline at a rate of 0.5 ml/hr/100 g body wt for 1 h. The saline infusion rate was then increased to 1.5 ml/hr/100 g body wt for an additional 120 min in order to produce mild volume expansion. Arachidonic Acid was administered to one group during the first 25 min of the higher saline infusion rate. The other group served as a control and received solvent only.

In the normally hydrated group, a significant increase in plasma renin activity was observed immediately after completion of Arachidonic Acid infusion; 90–120 min later, plasma renin activity returned to a control value. Urine volume and sodium excretion did not change significantly. In the volume-expanded treated rats, plasma renin activity increased significantly. Urine volume and urinary sodium excretion remained almost unchanged when compared to the significant increases observed in the volume-expanded controls. Inulin and para-amino-hippuric acid clearances increased in the volume-expanded control group but remained almost unchanged in the volume-expanded test group. Also, administration of Arachidonic Acid with saline prevented the increase in glomerular filtration rate and renal blood flow that is normally observed with volume-expansion with saline.

In studies of renal metabolism of Arachidonic Acid, a significant increase in

glomerular synthesis of thromboxane A_2 (TxA_2), measured as immunoreactive thromboxane B_2 (TxB_2) and urinary excretion of TxB_2 occurred during adriamycin-induced nephrosis; adriamycin is a commonly used chemotherapeutic agent (Zoja et al., 1989). No significant changes were observed in the glomerular synthesis and urinary excretion of the Arachidonic Acid metabolite 6-keto-$PGF_{1\alpha}$ during adriamycin-induced nephrosis.

It has been reported that Arachidonic Acid metabolism in peripheral blood monocytes and in smooth muscle cells in culture has been altered by cyclosporin A, a potent immunosuppressive drug which can cause nephrotoxicity (Zoja et al., 1989).

ARACHIDONIC ACID AND CARDIAC TISSUE

Arachidonic Acid is a major unsaturated fatty acid in cardiac tissue that can produce direct toxic effects on the heart (Basu and Karmazyn, 1987). Arachidonic Acid release has been associated with cardiac injury associated with ATP depletion. The results of a study examining the role of Arachidonic Acid in cardiac tissue injury suggested that accumulation of intracellular unesterified Arachidonic Acid, which may have resulted from peroxidation of membrane lipids, increased tissue injury caused by exogenous free radicals.

A study was conducted to examine the influence of Arachidonic Acid on normal isolated rat hearts and hearts subjected to ischemia and reperfusion (Karmazyn and Moffat, 1985). Arachidonic Acid, 10 μg/ml, was initially a positive inotropic agent when added to three normally perfused rat hearts. After 10 min of Arachidonic Acid perfusion, contractility (dF/dt) approximately doubled; this effect started to decrease after approximately 13 min, and within 90 min, recovery of contractility (+dF/dt) was 15–20% below normal value. Pretreatment with vitamin E, a free radical scavenger, significantly reduced the degree of myocardial depression due to prolonged Arachidonic Acid perfusion, but it did not prevent the inotropic effect of Arachidonic Acid.

Arachidonic Acid, 10 μg/ml, significantly increased creatine–phosphokinase efflux from rat hearts prior to ischemia initiation. During the ischemic perfusion phase, Arachidonic Acid had little effect on the enzyme release profile or the depression of contractility. Under control conditions, there was approximately a 50% recovery of myocardial contractility following reperfusion of ischemic myocardium; 10 μg/ml Arachidonic Acid significantly reduced the recovery of contractility. Coronary pressure was significantly increased by 10 μg/ml Arachidonic Acid perfusion. Arachidonic Acid reduced the activity of sarcolemmal Na^+/K^+-ATPase. The damaging effect of Arachidonic Acid is probably attributable either to its peroxidation, or oxidation, to free radicals. Arachidonic Acid may provide a significant contribution towards cardiac damage via free radical generation, particularly under stressful generation, because only when ischemia and subsequent reperfusion were initiated did large deleterious effects of Arachidonic Acid result.

Arachidonic Acid was bound to albumin (6:1) and added to primary cultures of rat heart muscle and endothelioid cells at concentrations of 5×10^{-5} M and 5×10^{-4} M (Wenzel and Hale, 1978). Arachidonic Acid, 5×10^{-5} M, was toxic for both muscle and endothelioid cell cultures after 8 h. During viability tests with ^{51}Cr, more than twice the amount of ^{51}Cr was released from muscle cells than from endothelioid cells. Arachidonic Acid, 5×10^{-4} M, was toxic to both cell types after 2 h. Activation of lysosomal acid phosphatase by 5×10^{-5} M Arachidonic Acid occurred after 2 h.

Arachidonic Acid also increased mitochondrial permeability in both cell types after 4 h; muscle mitochondria were injured as early as 2 h.

ARACHIDONIC ACID AND INFLAMMATION

Arachidonic Acid metabolites are involved in the inflammatory process (Zoja et al., 1989). Metabolism of Arachidonic Acid via the cyclooxygenase pathway produces the metabolites PGI_2 and PGE_2, which can be vasodilators (Zoja et al., 1989), and the prostaglandin endoperoxidases G_2 and H_2 and the thromboxane A_2, which induce rapid, irreversible aggregation of human platelets and are inhibitors of smooth muscle contraction (Malmsten, 1986). TxA_2 is a potent vasoconstrictor (Zoja et al., 1989).

Metabolism of Arachidonic Acid occurs by the lipoxygenase pathway in cells involved in host inflammatory responses; these cells include human neutrophil polymorphonuclear leukocytes, eosinophils, monocytes, alveolar macrophages, and mast cells (Lee et al., 1984). Hydroxyeicosatetraenoic acids (HETEs) and leukotriene B_4 are products of the lipoxygenase pathway of Arachidonic Acid metabolism that have chemotactic and chemokinetic activity. Leukotriene B_4 is the most active compound derived from Arachidonic Acid to exert chemotactic effects on polymorphonuclear leukocytes (Malmsten, 1986). The inflammatory response appears to begin with, *in vivo,* adherence of leukocytes to the endothelium of microvessels near inflammatory areas. *In vitro,* this seems to correspond with leukocyte aggregation and adherence. Leukotrienes C_4 and D_4, also products formed by the lipoxygenase pathway, induce a contractile response to decrease the surface area of isolated glomeruli and cause changes in the shape of cultured mesangial cells (Zoja et al., 1989). Leukotrienes C_4 and D_4, and also leukotriene E_4, do not induce chemotaxis, enzyme release, or leukocyte aggregation, but do have a potent effect on smooth muscle in the peripheral airway and the ability to increase macromolecular permeability in venules (Malmsten, 1986).

In a subchronic skin inflammatory reaction induced by repeated application of Arachidonic Acid to the pinnae of OF1 mice, an acute vascular phase was first observed; this was replaced by a cellular phase characterized by leukocyte infiltration (Bouclier et al., 1989). Initially, the cell infiltrates observed were mostly polymorphonuclear infiltrates in the dermis or epidermis. Later, mononuclear cell infiltrations appeared in the dermis.

Arachidonic Acid metabolism by cultured rat peritoneal macrophages, important cellular components of inflammation, was examined using peritoneal macrophages collected and pooled from several rats (Marshall, 1988). In a 4 h period, 62.8 ± 2.8% of 3 μCi ^{3}H-Arachidonic Acid was incorporated by the macrophages. The radioactive macrophages were treated with either pure serum-free medium or medium containing either indomethacin or nordihydroguaiaretic acid (NDGA) for 30 min prior to a 1 h exposure to pure medium (unstimulated) or TPA. The unstimulated cultures released 3.1 ± 1.2% of the radioactivity into the medium in a 1 h period. Indomethacin and NDGA exposure did not affect this value. When [^{3}H]Arachidonic Acid macrophages were stimulated with TPA, 10.5 ± 0.5% of the radioactivity was released. Treatment with at least 0.003 μM indomethacin inhibited radioactivity release. This release was not altered by NDGA until 10 μM was added to the medium. Ninety percent of the incorporated ^{3}H-Arachidonic Acid localized in the phospholipids, 8.3% in the free fatty acid, and 1.5% in neutral lipid fractions. Macrophage stimulation with TPA for 1 h

resulted in a marked deacylation of radioactive fatty acid from phosphatidylcholine, phosphatidylserine, and phosphatidylinositol and an increase in radioactivity found as unesterified fatty acid, neutral lipid, or released into the medium. During stimulation, liberated ^{3}H-Arachidonic Acid was converted into eicosanoids, which were released into the medium. Excess free ^{3}H-Arachidonic Acid was evidenced by the increase in cellular free Arachidonic Acid and its reacylation into neutral lipid pools.

Tumor necrosis factor (TNF), a cytokine released by macrophages, stimulated Arachidonic Acid metabolism and caused mitogenesis in several cell types, particularly fibroblasts (Burch and Tiffany, 1989). The ability of TNF to increase Arachidonic Acid metabolism in response to other agonists may be important in the dysregulation that occurs in a variety of inflammatory responses.

ARACHIDONIC ACID AND DIABETES

A significant decrease in the Arachidonic Acid content of erythrocytes and platelets has been observed in patients with poorly controlled diabetes; this could be due to an increase in free radical activity (Taylor et al., 1987).

Normal and streptozotocin-induced diabetic male albino Wistar rats were used to determine the incorporation ratio of ^{3}H-Arachidonic Acid and ^{14}C-linoleic acid into the lipid components of liver cells (Mercuri and de Tomas, 1971). The injection solution contained ^{3}H-Arachidonic Acid and ^{14}C-linoleic acid; 0.1 ml of solution contained 5 μC of ^{3}H-Arachidonic Acid and 10 μC of ^{14}C-linoleic acid. Five rats were given i.v. injections of 0.1 ml of solution over 1 min; five diabetic rats were given 0.1 ml over 1 min; and four diabetic rats were given i.v. injections of 0.1 ml of injection solution and 1 U of insulin over a 1 min period.

Total radioactivity incorporation per gram of liver was approximately five times greater in normal rats than in diabetic rats, with or without insulin. The ^{3}H/^{14}C ratio for the total radioactivity incorporated into the hepatic lipids was constant in all three groups, but the distribution varied among the groups. The ^{3}H/^{14}C ratio for 1,2-diacylglycerol in the neutral fraction and in the 3-sn-glycerophosphorylcholine fraction was significantly higher in diabetic rats than in normal rats. The incorporation of Arachidonic Acid and linoleic acid into hepatic lipids was reduced in rats with severe diabetes and insulin was unable to correct this reduction.

The effect of Arachidonic Acid on alloxan-induced diabetic rats was investigated using male Wistar rats (Brenner et al., 1968). Alloxan diabetes significantly decreases the fatty acid desaturating activity of hepatic microsomes. A group of 11 alloxan-diabetic rats was fed a relatively fat-free diet rich in linoleic acid and a group of eight alloxan-diabetic rats was fed the same diet along with daily oral doses of 100 mg ethyl Arachidonate. The animals were killed after 83 days and certain organs were removed for macroscopic and microscopic examination.

No significant decrease was observed between the two diabetic groups in fatty acid desaturating activity; both of these groups had decreased activity compared to the controls. A significant decrease in the percentage of palmitoleic acid and the ratio of Arachidonic/linoleic acid was observed, and a significant increase in linoleic acid was seen when both diabetic groups were compared to controls.

Atrophy of the seminal vesicles was observed in the diabetic rats not given ethyl Arachidonate supplementation; this atrophy was not observed in rats given ethyl Arachidonate. The seminal vesicles of the diabetic rats had greater amounts of total PGE

per gram of tissue than the diabetic rats given Arachidonic Acid; the total amount per rat was comparable between the two groups. Testes weight was decreased in diabetic rats when compared to diabetic rats given ethyl Arachidonate and normal rats. Spermatogenesis was usually inhibited after the spermatogonia stage in most of the diabetic rats; however, most of the diabetic rats given Arachidonic Acid had normal spermatogenesis.

The renal tubules of both diabetic groups had severe cellular damage and tubular dilation, and deposits of periodic acid-Schiff (PAS)-positive material were found in the cells and tubular lumens; these lesions were more prominent in the distal than the proximal tubules. These tubules and interstitial lesions were probably due primarily to alloxan. Ethyl Arachidonate administration did not prevent the development of cataracts in the diabetic rats.

ARACHIDONIC ACID AND MAJOR DISEASES

A chronic cellular imbalance between Arachidonic Acid, γ-linolenic acid, and eicosapentaenoic acid, and of their respective eicosanoid derivatives, may have major health implications (Booyens and van der Merwe, 1985). It appears that such an imbalance may be involved in diseases such as atherosclerotic heart disease, arterial hypertension, hypercholesterolemia, chronic inflammatory and autoimmune disorders, allergic eczema, and other atopic disorders. It has been proposed that, in part, these diseases arise from a chronic excess of Arachidonic Acid with a relative deficiency of γ-linolenic and eicosapentaenoic acid in the membrane phospholipids of many cells.

ARACHIDONIC ACID AND ALLERGIC RESPONSE

Native slow reacting substances (SRSs) are mixtures of different thioether leukotrienes (Malmsten, 1986). The ratio of leukotrienes C_4, D_4, and E_4 and the 11-*trans*-isomers varies in different SRSs depending on the source, stimulus, time, or other various cofactors. Leukotrienes C_4, D_4, and E_4 are potent constrictors of guinea pig ileum preparations and are efficient bronchoconstrictors; all three are more constrictive than histamine. Leukotrienes C_4 and D_4 cause rapid arteriolar constriction and they actively promote plasma leakage in postcapillary venules. The thioether leukotrienes C_4, D_4, and E_4 and the 11-*trans*-isomers slow the rate of mucous clearance from the airways of asthmatic subjects after antigen inhalation; they may also increase the amount of mucous glycoprotein synthesized by human airways.

Numerous studies using guinea pig lungs have examined the relationship between Arachidonic Acid and SRS during allergic response. Watanabe-Kohno and Parker (1980) demonstrated that Arachidonic Acid is a biosynthetic precursor of SRS-A in anaphylactically stimulated guinea pig lung fractions. Arachidonic Acid, slow-reacting substance of anaphylaxis (SRS-A), slow-reacting substance from egg yolk (SRS-C), and bradykinin infusions into the pulmonary artery, as well as anaphylaxis, caused the release of prostaglandins and rabbit aorta-contracting substance (RCS) from isolated guinea pig lung preparations (Palmer et al., 1973). Arachidonic Acid caused a prolonged release of RCS and prostaglandins as opposed to bradykinin. Vargaftig and Dao (1971) reported that SRS-C and Arachidonic Acid released from guinea pig lungs a

material that contracts the spiral strip of rabbit aorta. They stated that this material was similar to RCS released from the lungs by anaphylaxis, bradykinin, and SRS-A.

Nonsteroidal anti-inflammatory agents antagonize the ability of Arachidonic Acid, SRS-A, and SRS-C to increase the resistance of inflation by guinea pig lungs (Vargraftig and Dao, 1970).

It has been determined that products of Arachidonic Acid metabolism are involved in modulation of immediate-type immunologic reactions in the lungs of humans (Fish et al., 1981). However, the participation of Arachidonic Acid metabolites differs in asthmatic and nonasthmatic subjects, as was evidenced in a study using a group of subjects with asthma and a nonasthmatic group with allergic rhinitis. An indomethacin-related increase in antigen sensitivity was observed in nonasthmatic patients; this may possibly have been due to an increase in lipoxygenase metabolism with enhanced production of the leukotriene constituents of SRS-A and other lipoxygenase products capable of augmenting preformed mediator release. In asthmatic subjects, indomethacin had no significant effect on antigen sensitivity. Also, there is evidence to the fact that people with asthma are more sensitive to the bronchoconstrictor effects of some prostaglandins, such as $PGF_{2\alpha}$.

To examine the role of platelets in atopic disease and asthma, and the importance of Arachidonic Acid metabolites in the pathogenesis of these diseases, a study was conducted using atopic subjects having seasonal allergic rhinitis and/or asthma and nonatopic control subjects (Audera et al., 1988). A significant increase was observed in the mean platelet volume of atopic subjects as compared to the controls. No significant changes were observed in platelet count or serum TxB_2.

Male albino guinea pigs were used to examine changes in airway insufflation pressure due to i.v. injection of Arachidonic Acid (Mathe et al., 1977). Injection of 50–250 μg/kg Arachidonic Acid into the jugular vein increased the airway pressure. Indomethacin inhibited this response.

ANTIMICROBIAL ACTIVITY

Fatty acids can inhibit the growth of fungi, protozoa, viruses, and many types of bacteria (Knapp and Melly, 1986). Polyunsaturated fatty acids generally affect only certain types of bacteria and are toxic at relatively low concentrations in a pH-independent manner.

In an *in vitro* killing assay, all the gram-positive species of bacteria tested were susceptible to 10^{-5} M Arachidonic Acid (Knapp and Melly, 1986). *Lactobacillus acidophilus* was the most susceptible of the species tested and only rarely survived 1 h of exposure. *Bacillus megaterium* was the least susceptible, with >98.8% of the inoculum being killed within 1 h of exposure. The gram-negative species *Neisseria, Haemophilus,* and *Branhamella* were as readily killed by Arachidonic Acid as were the gram-positive bacteria.

Escherichia coli, Pseudomonas aeruginosa, and Klebsiella pneumoniae were not susceptible to killing by Arachidonic Acid, even when being incubated with 3×10^{-4} M Arachidonic Acid for 6 h.

Within 2 h, an entire 10^6 inoculum of *Staphylococcus aureus* was killed by 10^{-5} M Arachidonic Acid. Arachidonic Acid, 5×10^{-6} M, slowly killed >99.9% of the *S. aureus;* 10^{-6} M Arachidonic Acid had a more modest effect over several hours.

Some morphologic changes were observed in the bacteria that were caused by Arachidonic Acid; these changes were not observed in *E. coli*. Arachidonic Acid

peroxidation products exhibited low antimicrobial activity. Transition metal chelators and some thiols were highly protective of the bacteria. *S. aureus* grown in iron-supplemented broth had increased iron content and Arachidonic Acid susceptibility.

The minimum inhibitory concentration (MIC) of Arachidonic Acid against two gram-positive bacteria, one gram-negative bacteria, and their wild counterparts was investigated (Raychowdhury et al., 1985). The MICs of Arachidonic Acid were: penicillin G-resistant, 749/C, *Bacillus licheniformis,* 6 μg/ml; penicillin-sensitive B. licheniformis, 8 μg/ml; methicillin-resistant, metR 18, *S. aureus,* 4 μg/ml; methicillin-sensitive *S. aureus,* 6 μg/ml; cloxacillin-resistant *E. coli,* 50 μg/ml; and cloxacillin-sensitive *E. coli,* 70 μg/ml.

Arachidonic Acid, 1.0 mg/ml at 3–6.0 mM, has no inhibitory effect for pneumococci, *Streptococcus* group A, *Streptococcus* beta-hemolytic non-A, *Candida albicans,* or *S. aureus* (Kabara, 1984). The MIC of Arachidonic Acid for *Clostridium welchii* was 6.09 mg/100 ml at pHs of 6.5 and 7.5.

Dive et al. (1978) reported that Arachidonic Acid was greatly toxic for the protozoan *Colpidium campylum.*

CYTOTOXICITY

The studies contained in this section and a number of similar studies concerning Arachidonic Acid and cytotoxicity are summarized in Table 4.

Monolayer cultures of MCF-7 human breast cancer cell lines were treated with 10^{-5}–10^{-11} M Arachidonic Acid in order to determine Arachidonic Acid's cytotoxic potential (Najid et al., 1989). A significant decrease in cell number was observed at various times when MCF-7 cells cultured in 5% fetal bovine serum (FBS) were treated with Arachidonic Acid; the relative potencies were not significantly different between small and great concentrations. A concentration of approximately 10^{-6} M Arachidonic Acid inhibited 50% cell growth.

The cytotoxic effects of Arachidonic Acid against oral origin mucoepidermoid carcinoma, A-253, and squamous cell carcinoma, SCC-15, were examined (Takeda et al., 1989). Arachidonic Acid had cytotoxic effects against A-253, but not against SCC-15. The addition of ferrous iron to the medium enhanced the cytotoxic effects, while vitamin E inhibited these effects.

In a cytotoxicity study using human breast carcinoma cell line ZR-75-1, the cytotoxic potential of a fatty acid varied with the acid's ability to stimulate the production of superoxide radicals (Begin et al., 1988). The effectiveness of a fatty acid in killing cancer cells corresponded to the intracellular thiobarbituric acid-reactive material (TBARM) content; γ-linoleate and Arachidonic Acid produced the most TBARM and were the most cytotoxic fatty acids evaluated. The authors concluded that the effectiveness of a fatty acid in killing cancer cells correlated with the extent of lipid peroxidation of the added fatty acid in the cells.

The cytotoxicities of autoxidized polyunsaturated fatty acids, including autoxidized Arachidonic Acid, toward human umbilical vein endothelial cells were examined (Kaneko et al., 1988). Autoxidized linoleic acid was more cytotoxic than autoxidized linolenic and Arachidonic Acids. Autoxidized linoleic acid was more toxic than linoleic acid, autoxidized linolenic acid, and autoxidized Arachidonic Acid. Autoxidized Arachidonic Acid was as toxic as linoleic acid or Arachidonic Acid. The toxic component of Arachidonic Acid was determined to be (E)-4-hydroxy-2-nonenal.

Table 4. Cytotoxicity of Arachidonic Acid

Species and gender	*Methods*	*Results and comments*	*Reference*
Human breast cancer cell line, MCF-7	Monolayer cultures in 5% FBS were treated with 10^{-5}–10^{-11} M Arachidonic Acid.	A significant decrease in cell number was observed at various times. The relative potencies were not significantly different between high and low concentrations. Approx. 10^{-6} M Arachidonic Acid inhibited 50% cell growth.	Najid et al., 1989
Mucoepidermoid carcinoma, A-253 and squamous cell carcinoma, SCC-15	The cytotoxic effects of Arachidonic Acid on these cell lines were examined. Ferrous iron and vitamin E were added to the medium.	Arachidonic Acid had cytotoxic effects on A-253 but not SCC-15. Addition of ferrous iron enhanced cytotoxic effects; vitamin E inhibited the effects of Arachidonic Acid.	Takeda et al., 1989
Human breast carcinoma cell line, ZR-75-1		A fatty acid's cytotoxic potential varies with the acid's ability to stimulate the production of superoxide radicals. The fatty acid's effectiveness in killing cancer cells corresponded to the intracellular thiobarbarituric acid-reactive material (TBARM) content. Of the acids evaluated, γ-linoleate and Arachidonic Acid produced the most TBARM and were the most cytotoxic. The fatty acid's effectiveness in killing cancer cells correlated with the extent of lipid peroxidation.	Begin et al., 1988
Human umbilical vein endothelial cells		Autoxidized linoleic acid was more cytotoxic than autoxidized linolenic acid and Arachidonic Acid. Autoxidized linoleic acid was more toxic than linoleic acid, autoxidized linolenic acid, and autoxidized Arachidonic Acid. Autoxidized Arachidonic Acid was as toxic as linoleic acid or Arachidonic Acid. The toxic component of Arachidonic Acid was (E)-4-hydroxy-2-nonenal.	Kaneko et al., 1988

Table 4. Cytotoxicity of Arachidonic Acid (*Continued*)

Species and gender	*Methods*	*Results and comments*	*Reference*
Normal human fibroblasts and neoplasm cells, ZR-75-1	Fibroblasts and neoplasmic cells were treated with Arachidonic Acid and polyunsaturated fatty acids *in vitro*.	Polyunsaturated fatty acid addition to normal and neoplasmic cells produced selective cytotoxicity and killed neoplasmic, but not normal, cells. Arachidonic Acid, γ-linoleate, and eicosapentaenoate caused a significant enhancement of NBT-reduction by ZR-75-1 cells; they had a much lesser effect in normal cells. Arachidonic Acid, linoleate, and eicosapentaenoate uptake was at least twice as great in fibroblasts than in neoplasmic cells. After 72 h incubation, Arachidonic Acid incorporation in the neoplasmic cells was less for Arachidonic Acid than the other two acids; in normal cells, the rate was approx. equivalent for all three acids. Normal cells incorporated more fatty acids than neoplasmic cells. Arachidonic Acid and eicosapentaenoate-treated neoplasmic cells had a higher capacity to reduce NBT than did normal cells. Some polyunsaturated fatty acids may have selective neoplasmicidal actions.	Das et al., 1987
Mat 1376b ascites neoplasm cells	A volume of 0.025 ml of 8% neoplasmic cells was added to Arachidonic Acid colloids. Two control mixtures containing cells with either salt solution alone or salt solution containing alcohol at a concentration of 100 mg/100 ml were also tested.	Morphological transformation of the neoplasmic cells was observed after exposure to Arachidonic Acid; no detectable morphological changes were observed in controls. The minimal toxic concentration that caused >90% of the neoplasmic cells to become permeable to trypan blue after 60–90 min incubation at 37°C was 1.25 mg/ml, or 4×10^{-5} M, Arachidonic Acid.	Siegal et al., 1987

Human lung carcinoma, A-549; human breast carcinoma, ZR-75-1; human prostatic adenocarcinoma PC-3; normal simian cells, CV-1 and BSC-1; canine kidney cells, MDCK; normal human fibroblasts, CCD-41SK	All cell lines were grown in 0.5 ml of experimental growth medium, with and without Arachidonic Acid and other fatty acids. One day after seeding, one set of cultures was supplemented with 20 μg/ml of various polyunsaturated fatty acids; another set of unsupplemented cultures was treated with ethanol. Cells were harvested 9–14 days after supplementation. Serum concentration and seeding density were varied in the presence of 5 or 20 μg/ml polyunsaturated fatty acid. Cocultures were made with normal human fibroblasts and one of the human neoplasm cell lines. The first coculture had a neoplasmic cell-to-normal cell ratio of 1:80; the second coculture had a ratio of 5:1.	Extensive cytopathic effects were observed for neoplasmic cells treated with 20 μg/ml polyunsaturated fatty acid; lipid droplet-filled cytoplasms were observed in normal cells. The cytopathic effects appeared on day 4, increased rapidly, and reached a maximum at >7 days supplementation. Arachidonic Acid effectively killed neoplasmic cells. While it did not kill normal cells, Arachidonic Acid lowered their rate of division. Arachidonic Acid selectively eliminated neoplasmic cells in a mixed culture. Arachidonic Acid was one of the most effective polyunsaturated fatty acids tested in eliminating neoplasmic cells clones. The extent of cytostatic and cytotoxic effects depended on the cell density, fatty acid concentration, cell type, and the fatty acid itself. The LD_{50} value of Arachidonic Acid was 8 μg for ZR-75-1 cells and 7 μg for PC-3 cells.	Begin et al., 1986
Human breast cancer cell line, ZR-75-11; human lung cancer cell line, A-549; human prostate cancer cell line, PC-3; normal human fibroblasts, CCD-41SK; monkey kidney cell lines, CV-1 and BSC-1; dog kidney cell line, MDCK	Cells were seeded and grown in medium with and without fatty acid supplementation. One day after seeding, various fatty acid esters and control solutions were added. Cells were harvested 10–15 days after the start of culture. The effects of cell density and polyunsaturated fatty acid concentration were examined. Cocultures of a cancer cell line and normal fibroblasts were incubated with and without polyunsaturated fatty acids. Various cyclooxygenase and lipoxygenase inhibitors were added to some cocultures. The effects of three antioxidants added to the cultures were also examined.	Arachidonic Acid was effective in killing malignant cells; Arachidonic Acid had little effect on normal cells. Arachidonic Acid was as effective as α-linolenic acid and dihomo-α-linolenic acids; Arachidonic Acid was not as selective. Polyunsaturated fatty acids were seeding density- and fatty acid concentration-dependent. In cocultures, Arachidonic Acid consistently completely eliminated neoplasmic cells, leaving a clean fibroblast monolayer. Cyclooxygenase and lipoxygenase inhibitors did not affect polyunsaturated fatty acid cytotoxic action; indomethacin may have enhanced polyunsaturated fatty acid cytotoxic effects in neoplasmic cells. The antioxidants blocked the polyunsaturated fatty acids' actions.	Begin et al., 1985

TABLE 4. CYTOTOXICITY OF ARACHIDONIC ACID (*CONTINUED*)

Species and gender	*Methods*	*Results and comments*	*Reference*
Human osteogenic sarcoma cells, MG63	Cultures seeded with 0.14×10^6 cells were incubated for 2 days in standard growth media. The media were then replaced with media containing 5, 10, 20, 40, 60, 80, or 100 μg/ml Arachidonic Acid or other polyunsaturated fatty acids. Cells were supplemented on days 3, 5, and 7. Duplicate cultures treated with 10 μl/ml of ethanol on the same days test cultures were treated served as controls.	Arachidonic Acid showed a more marked suppression of cell proliferation than α-linolenic acid. In a concentration range of 60–100 μg/ml Arachidonic Acid, no cells were found.	Booyens et al., 1984
Intestinal epithelium; colonic neoplasms	Male Sprague-Dawley rats were dosed with 1,2-dimethylhydrazine dihydrochloride to induce colonic neoplasms. Rats were given i.p. injections of 10 or 100 mg/kg Arachidonic Acid in peanut oil; control rats were dosed with peanut oil alone. In some rats, a cyclooxygenase inhibitor, two cyclooxygenase and lipoxygenase inhibitors, or a TxA_2 synthesis inhibitor was injected 15 min prior to Arachidonic Acid administration.	Arachidonic Acid, 100 mg/kg, significantly retarded the metaphase rate in jejunal crypts; 10 mg/kg did not affect the rate. Both 10 and 100 mg/kg Arachidonic Acid significantly retarded cell proliferation in colonic crypts and colonic carcinomas. Peanut oil did not have a significant effect on any of these 3 areas. Administration of the inhibitors 15 min prior to Arachidonic Acid injection had the following effects: Cyclooxygenase inhibitor: prevented the inhibition of cell proliferation in jejunal crypts and colonic carcinomas. Cell proliferation in colonic crypts remained retarded. Cyclooxygenase and lipoxygenase inhibitors: one prevented the inhibition of cell proliferation in colonic crypts, but not in the jejunal crypts or colonic carcinomas; the other prevented the inhibition of cell proliferation rates in colonic crypts and colonic carcinomas, but not in jejunal crypts. TxA_2 synthesis inhibitor: inhibited the effect of Arachidonic Acid in colonic neoplasms; did not affect Arachidonic Acid's effect on colonic and jejunal crypts.	Petry et al., 1984
Human glioma cell clone; fetal brain cells	Proliferating glioma and fetal neural cells were incubated with Arachidonic Acid.	Arachidonic Acid inhibited cell proliferation in both types of cell cultures. Cell proliferation inhibition increased with increased Arachidonic Acid concentration.	Liepkalns et al., 1982
Ehrlich ascites carcinoma; TA_3 mammary carcinoma	Neoplasmic cells and Arachidonic Acid were mixed and 10 nonspecific Connaught mice were inoculated.	Susceptibility of each of the neoplasmic cell lines to Arachidonic Acid varied.	Tolnai, 1966

The effect of Arachidonic Acid and other polyunsaturated fatty acid supplementation on nitroblue tetrazolium reduction in normal and neoplastic cells, *in vitro,* was examined in order to determine whether polyunsaturated fatty acid-induced cytotoxicity to neoplastic cells was mediated by enhanced production of free radicals (Das et al., 1987). Addition of polyunsaturated fatty acids to normal and neoplastic cells can elicit selective cytotoxicity with neoplastic cells, but not normal cells, being killed.

Arachidonic Acid, γ-linolenate, and eicosapentaenoate caused a significant enhancement in nitroblue tetrazolium ion (NBT) reduction by ZR-75-1 cells; these fatty acids had a much lesser effect on normal human fibroblasts. The ZR-75-1 cells treated with these acids are almost twice as effective as linoleate-treated cells in inducing NBT reduction. Uptake of Arachidonic Acid, linoleate, and eicosapentaenoate was at least twice as great in the fibroblasts compared to the neoplastic cells.

After 72 h of incubation, the incorporation of Arachidonic Acid into the neoplastic cells was at least 1.5 times less than the incorporation of linoleate and eicosapentaenoate; the rate was approximately equivalent for all three in the normal cells. Normal cells incorporated more fatty acid than neoplastic cells when incubated under the same conditions.

Although neoplastic cells incorporated less Arachidonic Acid, linoleate, and eicosapentaenoate than normal cells, Arachidonic Acid and eicosapentaenoate-treated neoplastic cells had a greater capacity to reduce NBT, probably due to enhanced superoxide production. Also, some polyunsaturated fatty acids may have selective neoplasmicidal actions because superoxide radicals and other free radicals can initiate lipid peroxidation and are toxic to neoplastic cells.

FATTY ACID DEFICIENCY

Deprivation of EFAs, including Arachidonic Acid, results in scaliness of the skin and increased water loss in humans (Wilkinson and Moore, 1982). Topical application of sunflower seed oil can reverse these conditions.

In rats, deprivation of certain long-chain polyunsaturated fatty acids can result in scaliness of skin and hair loss. Also in rats, EFA deficiency creates an atrophy of the testes that deteriorates the germinal epithelium (Brenner et al., 1968).

Essential fatty acid-deficient rats that do not have the substrates for the synthesis of prostaglandins and TxA_2 have been found to be resistant to bacterial endotoxin (Cook et al., 1979).

GENERAL IMMUNOLOGIC AND PHARMACOLOGIC EFFECTS

Male DBA/2 mice were used in a study to determine the potential role of Arachidonic Acid and its metabolites in ultraviolet (UV) light-induced cutaneous immune suppression (Rheins et al., 1987). Several groups of mice were fed 0.15 mg/kg indomethacin in normal feed on days 1–5. These and other groups of mice, which were fed normal diet, were given dermal applications of 100 μl of Arachidonic Acid on their shaved backs on days 2–5. On day 6, all rats were fed control feed and sensitized with applications of 25 μl of 0.5% 2,4-dinitro-1-fluorobenzene (DNFB) to their backs. On day 13, baseline measurements of pinnal thickness were taken and the mice were then challenged with 20 μl of 0.2% DNFB. Pinnal thickness was measured 24 h later to

determine the reaction to DNFB. Negative controls were challenged with DNFB but were not previously sensitized.

Mice treated with only Arachidonic Acid did not respond significantly differently to DNFB than did the negative controls. The mice treated with Arachidonic Acid and indomethacin had a significantly increased degree of pinnal swelling compared to mice given Arachidonic Acid only. The response to the DNFB challenge for the mice treated with Arachidonic Acid and indomethacin was not significantly different from mice treated with diluent, dimethyl sulfoxide (DMSO)/water, or indomethacin only. Arachidonic Acid caused an increase in the number of epidermal pigment cells, melanocyte hyperplasia, and induced melanogenesis; indomethacin, 0.15 mg/kg, abrogated Arachidonic Acid's immunosuppressive effects. The authors proposed that Arachidonic Acid altered the cutaneous immune response, possibly by prostaglandin production.

Arachidonic Acid was applied to mouse skin to determine its effect on epidermal Langerhans cells and cutaneous immune reactivity (Rheins and Nordlund, 1986). Arachidonic Acid, 0.05 or 2%, was applied either to the back or to the pinnae on days 1–7; controls were treated with DMSO/water diluent.

Upon microscopic examination on day 8, it appeared that Arachidonic Acid had a biphasic effect on Langerhans cells. A dose of 0.05% Arachidonic Acid increased identifiable ATPase and immune-associated (Ia)-positive Langerhans cells; 2% Arachidonic Acid decreased the identifiable cells. A distal effect on Langerhans cell populations was not observed at either dose.

Mice that were dosed on the back (both dose groups) were sensitized with 0.5% DNFB on days 8 and 10; on day 15, the untreated pinnae were challenged with 0.2% DNFB. A 62% increase in pinnal edema was observed in the group dosed with 0.05% Arachidonic Acid; a 24% increase in edema was observed for DMSO-treated controls. Mice that demonstrated a reduction in identifiable ATPase and Ia-positive Langerhans cells after being dosed with 2% Arachidonic Acid had only a 21% increase in pinnal edema; DMSO-treated controls had a 34% increase in edema. When the mice dosed with 2% Arachidonic Acid on the pinnae were sensitized and challenged as above, the Arachidonic Acid-treated pinnae with fewer ATPase/Ia-positive cells had a 20% increase in edema after 24 h; the DMSO-treated controls had a 37% increase in edema.

A study was conducted to examine whether sodium Arachidonate could trigger the reaction between normal human lymphocytes and rat isolated atria, thereby replacing the initial triggering step provided by lectin stimulation of the lymphocyte's membrane (Borda et al., 1984). It was found that normal human lymphocytes incubated with sodium Arachidonate *in vitro* had positive inotropic and chronotropic effects. Stimulation of normal lymphocytes with phytohemagglutinin resulted in a prompt release of Arachidonic Acid to the medium.

Exogenous Arachidonic Acid was added to phagocytic cells to determine the effects of Arachidonic Acid on various functions of human polymorphonuclear and mononuclear leukocytes (Henricks et al., 1984). The effects of Arachidonic Acid on the phagocytic cell function were specific for neutrophils. Incubation with Arachidonic Acid produced greater amounts of oxygen consumption, superoxide production, and chemiluminescence generation by neutrophils; these results were not observed for monocytes. Neutrophils aggregated to a much larger degree than monocytes upon Arachidonic Acid incubation. Neutrophil chemotaxis towards an attractant was lost and phagocytosis of bacteria decreased when incubated with Arachidonic Acid; at 0°C, Arachidonic Acid incubation did not affect neutrophil uptake of staphylococci. Mononuclear phagocytes were not affected at any temperature.

The oxidative degradation of unsaturated fatty acids was followed by the determination of the amount of malonyldialdehyde, a metabolite formed during lipid peroxidation by oxygen metabolites. A dose-dependent response was observed when polymorphonuclear leukocytes were incubated with Arachidonic Acid: a greater amount of malonyldialdehyde was formed with a greater dose of Arachidonic Acid. Incubation of neutrophils with indomethacin prior to Arachidonic Acid incubation reduced the effects of Arachidonic Acid on the stimulation of neutrophil metabolism. Indomethacin partly abolished the decreasing effect of Arachidonic Acid on phagocytosis and it inhibited the chemiluminescence response; indomethacin did not inhibit oxygen consumption and glucose metabolism after Arachidonic Acid stimulation. Scavengers of oxygen species interfered with the effect of Arachidonic Acid on phagocytosis.

A study examining the effects of chronic intracutaneous administration of Arachidonic Acid into the posterior aspect of both ears of female albino guinea pigs found only minor dermal changes due to Arachidonic Acid administration (Ruzicka and Burg, 1987). Positive results were obtained by injection of some metabolites of Arachidonic Acid. Based on these results, the authors suggested that, apart from hyperproliferative and inflammatory dermatoses, lipoxygenase-derived Arachidonic Acid metabolites may be involved in the pathophysiology of vasculitis and dermatoses characterized by tissue eosinophilia.

Arachidonic Acid metabolism in the skin of normal female Hartley guinea pigs, guinea pigs with contact dermatitis induced by 1-chloro-2,4-dinitrobenzene (DNCB), and guinea pigs with irritant dermatitis induced by a nicotinic acid derivative was compared (Ruzicka and Printz, 1982). The principal metabolite of exogenous Arachidonic Acid in normal skin was HETE; in the presence of cofactor, reduced glutathione and increased cyclooxygenase activity was observed and PGD_2 was the principal product. A marked inhibition of the lipoxygenase pathway and the production of PGD_2 from Arachidonic Acid were found with contact dermatitis. Dermal application of DNCB in unsensitized animals resulted in increased PGE_2 production. With irritant dermatitis, inhibition of the cyclooxygenase pathway occurred in the epidermis; however, stimulation of this pathway occurred in the dermis, and PGE_2 was the principal metabolite. PGH_2 metabolism was unchanged; the lipoxygenase pathway was slightly stimulated.

A study was conducted to determine the effect of Arachidonic Acid on isolated human umbilical veins, particularly to determine whether endothelial injury occurs (Mehta et al., 1985). At concentrations of 0.01–1.0 mM, Arachidonic Acid disruption of the endothelial surface, exposure of subendothelial cells, and adherence of blood cells to the vascular surface were not observed. No separation occurred between cells and the underlying basal lamina and no exposure of the muscular layer was produced. Prostacyclin production from human umbilical veins was stimulated with low concentrations of Arachidonic Acid. A significant increase in the amount of 6-keto-$PGF_{1\alpha}$ released was observed with 0.01–0.1 mM Arachidonic Acid; this significant increase was not observed using 0.1–1.0 mM Arachidonic Acid.

Arachidonic Acid injection into the carotid artery of rabbits caused ipsilateral cerebrovascular injuries (Fujimoto et al., 1988). Immediately after injection, deendothelialization was observed; this may be due to the detergent effect of Arachidonic Acid, which injures endothelial cells directly.

A study was performed to examine the role of Arachidonic Acid in human chorionic gonadotropin (hCG)-induced steroidogenesis in rat Leydig cells (Didolkar and

Sundaram, 1987). Elevation of intracellular amounts or exogenous addition of Arachidonic Acid resulted in an increase in testosterone secretion. Small doses of Arachidonic Acid enhanced hCG-induced testosterone secretion; larger doses of Arachidonic Acid, which were stimulatory in the absence of hCG, inhibited hCG-induced secretion of testosterone. These results indicated that intracellular concentrations of Arachidonic Acid that result during hCG stimulation are critical in determining the steroidogenic activity of Leydig cells. Arachidonic Acid had a biphasic effect on hCG-stimulated cyclic AMP (cAMP) secretion by Leydig cells; Arachidonic Acid itself did not have an effect on cAMP secretion.

The deciduogenic action of intraluminal instillation of Arachidonic Acid into the uterus of immature female Sprague-Dawley rats sensitized with progesterone was examined (Sananes et al., 1981). After a dose of 3 mmol/L Arachidonic Acid, the mean weight of the decidualized uterine horns was almost five times greater than the mean weight of the controls.

Arachidonic Acid's effect on the resting tone of guinea pig tracheal smooth muscle was examined (Mansour and Daniel, 1986). The resting basal tone in guinea pig tracheal smooth muscle appeared to be dependent on Arachidonic Acid metabolites and not on intrinsic innervation; it was found that cyclooxygenase products were the main mediators of the resting tone. Responses to Arachidonic Acid were dependent on concentration and on the resting tone of the tissue. Tissues with slight resting tone in both saline and ovalbumin saline samples responded to exogenous Arachidonic Acid by an increase in tension; tissues with a greater resting tension responded with either a reduction in tone or no change. The results of the study suggested that the metabolism of endogenous Arachidonic Acid responsible for tone maintenance of guinea pig tracheal smooth muscle may differ from the metabolism of exogenous Arachidonic Acid leading to active tension. Endogenous Arachidonic Acid was metabolized primarily to excitatory cyclooxygenase products, while exogenous Arachidonic Acid was metabolized to either excitatory or inhibitory cyclooxygenase products, depending on resting tone. $PGF_{2\alpha}$ was presumed to be the excitatory product while PGE_2 was presumed to be inhibitory, although low concentrations of PGE_2 may be excitatory.

To study Arachidonic Acid-induced bronchoconstriction, guinea pigs were administered 0.5 mg succinylcholine and 3 mg/kg propranolol, intraperitoneally, and 1 mg/kg, intravenously, and were then stabilized for 20 min (de Clerck et al., 1985). The animals were then challenged at 15 min intervals with increasing i.v. doses of 200, 400, 600, and 800 μg/kg Arachidonic Acid. Intravenous injection of Arachidonic Acid produced a dose-related reduction of tidal volume.

The effect of Arachidonic Acid on polyphosphoinositide turnover and protein kinase C activity in colonic epithelium was studied (Craven and DeRubertis, 1988). Intracolonic instillation of Arachidonic Acid induced the translocation of protein kinase C from the soluble fraction to the particulate fraction of mucosa homogenates. A concentration of 5 mM, or 15.6 mg/kg, Arachidonic Acid resulted in a 30-fold increase in colonic mucosal ornithine decarboxylase; no evidence of either superficial cell lysis or inflammation was observed. It was indicated by the results of this study that Arachidonic Acid may activate protein kinase C through a direct interaction with the enzyme or indirectly through membrane action that stimulates breakdown of inositol phospholipids. The addition of Arachidonic Acid to enzyme reaction mixtures directly increased partially purified soluble protein kinase C activity from colonic epithelium and Arachidonic Acid stimulated inositol phosphate accumulation in isolated colonic crypt epithelium, implying enhanced polyphosphoinositide breakdown.

In a study conducted to examine the effects of exogenous Arachidonic Acid on volume regulation by the ion-transporting systems in Ehrlich ascites neoplasm cells, it was found that Arachidonic Acid inhibited cell shrinkage during regulatory volume decrease and after addition of the calcium (Ca) ionophore A23187 plus Ca (Lambert, 1987). Addition of Arachidonic Acid to Ehrlich cells in isotonic solution led to a net loss of potassium (K). In sodium (Na)-containing medium, Arachidonic Acid increased cellular Na uptake under isotonic and hypotonic conditions. In cardiac sarcolemmal vesicles, Arachidonic Acid had no significant effect on Na, K-ATPase activity; however, a 50% inhibition of beef brain Na, K-ATPase activity was caused by 21 μM Arachidonic Acid. Ehrlich cells, which synthesize and release prostaglandins and leukotrienes, increased the production and release of PGE_2 and $PGF_{2\alpha}$ after the addition of Arachidonic Acid. The net loss of potassium chloride (KCl) observed in Ehrlich ascites neoplasm cells following hypotonic swelling has been found to involve activation of separate, Ca/calmodulin-activated conductive K and Cl pathways. The authors concluded that the inhibitory effect of Arachidonic Acid on volume regulation in Ehrlich cells suspended in Na-free media was due to inhibition of the volume-induced K and Cl pathways; this was caused by a nonspecific detergent effect.

Incorporation of Arachidonic Acid into the phospholipids of animal cells induced a change in the fluidity of the cells' membranes (Kohn et al., 1980). Addition of Arachidonic Acid at concentrations not harmful to animal cells inactivated Sindbis virus proportionally to the concentration administered. Arachidonic Acid, up to 25 μg/10^6 cells/ml, did not have any effect on baby hamster kidney (BHK) cells. Influenza and Sendai viruses, titrated in embryonated eggs, lost their infectivity when incubated with 10 μg Arachidonic Acid for 15–18 min. The virus envelopes disintegrated with the addition of Arachidonic Acid and this accounted for their loss of infectivity.

Splenic lymphoid cells from BDF_1 mice were sensitized with sheep erythrocytes and exposed to 0.1–100.0 μg/ml Arachidonic Acid, *in vitro,* for 90 min and were then examined in a hemolytic plaque assay (Masaki et al., 1978). All concentrations of Arachidonic Acid used caused a stimulation of the number of plaque-forming cells; significant stimulation was observed using 10.0 μg/ml Arachidonic Acid.

A dose of 100 μg/ml Arachidonic Acid produced a marked transient increase in the concentration of cAMP in primary epithelial cell cultures obtained from C3H mouse mammary neoplasms (Burstein et al., 1977). A two- to threefold increase was seen at 2 min; the concentration of cAMP returned to basal value within 30 min. Substantial increases in the amount of cAMP were observed at a dose of 25 μg Arachidonic Acid, but the peak occurred at approximately 100 μg Arachidonic Acid; levels >100 μg produced lower concentrations of cAMP. A dose of 20 μg/ml naproxen inhibited the effect of Arachidonic Acid.

The effect of the addition of Arachidonic Acid to a reaction mixture containing rat hepatic and pulmonary microsomes or cytosols on the activation of benzo[a]pyrene was studied (Nemoto and Takayama, 1984). Addition of Arachidonic Acid to the reaction mixture resulted in the binding of benzo[a]pyrene metabolites to the proteins. Arachidonic Acid was the most efficient cofactor with cytosolic proteins and Arachidonic Acid was less efficient than linoleic acid with microsomal proteins.

EFA-deficient rats were resistant to the lethal effects of *Salmonella enteritidis* endotoxin; a less severe thrombocytopenic response to the endotoxin and a reduction in serum fibrin/fibrinogen degradation products were observed (Cook et al., 1981). EFA-deficient Long-Evans rats were given an intraperitoneal (i.p.) injection of 100 mg ethyl Arachidonate 2 days prior to an i.v. injection of the endotoxin. Plasma TxB_2 was

significantly increased when compared to untreated control EFA-deficient rats. The response was even greater in rats that were given four 100 mg doses of ethyl Arachidonate on alternate days and were then injected with the endotoxin on the second day after the last Arachidonate injection.

EFA deficient rats had a 48 h mortality rate of 24% following endotoxin administration versus a 92% 48 h mortality rate in normal rats. EFA deficient rats were either placed on a normal diet for 2 wks, given one injection of ethyl Arachidonate, or given four injections of ethyl Arachidonate. A 71% mortality rate, 100% mortality rate in 48 h, and a 100% mortality rate within 6 h, respectively, were observed. The increase in mortality was attributed to elevated TxB_2 concentrations.

In order to verify that the increased mortality induced by Arachidonic Acid was not nonspecific to fatty acids, the effect of docosahexaenoic acid, a C_{22} fatty acid, was studied. Docosahexaenoic acid administration did not sensitize EFA-deficient rats or raise plasma thromboxane concentrations to the extent that ethyl Arachidonate did. After 15 min and 240 min following endotoxin administration, the severity of thrombocytopenia was significantly less in EFA-deficient rats than normal rats; ethyl Arachidonate administration resulted in a significant increase in severity compared to the EFA-deficient rats. Serum fibrin degradation product concentrations were comparable between Arachidonic Acid-treated rats and normal rats following endotoxin administration; these values were significantly greater than the values in the EFA-deficient group.

Female goats were used to study the effect of nonfebrile intracerebroventricular infusions of Arachidonic Acid on fluid balance (Leksell, 1978). Hydrated and nonhydrated goats were given 30 min infusions into the lateral cerebral ventricle of 150 or 300 ng/kg/min Arachidonic Acid in 0.15 M NaCl. No or little reduction of water diuresis was observed during infusion of either dose in the hydrated goat. A temporary postinfusion decrease in the renal free water clearance was observed; this decrease was greater after administration of 300 ng/kg/min. No effect on renal sodium excretion or body temperature was seen.

ANIMAL TOXICOLOGY

Acute Toxicity

Intravenous

Acute toxicity data are summarized in Table 5.

Myers et al. (1988) stated that the approximate LD_{80} of i.v. injections of Arachidonic Acid for male CD-1 mice was 75 mg/kg. Sudden death was due to thrombotic/ischemic mechanisms.

The LD_{50} value of a fast (<2 sec) i.v. injection of Arachidonic Acid for male Swiss mice was 39.2 mg/kg within 1 h; i.v. injection of Arachidonic Acid had a dose-related toxic effect (de Clerck et al., 1985). Arachidonic Acid , ⩾50 mg/kg, resulted in a highly significant mortality rate of 75%. A proportional reduction of survival time was observed.

Intravenous administration of 45–50 mg/kg Arachidonic Acid to 339 mice produced a mortality rate of 90%; median survival time was 95 sec, with 75% of the animals alive 60 sec following injection and 25% surviving 160 sec after injection. Some comparatively large doses of fatty acid cyclooxygenase inhibitors, comparatively

Table 5. Acute Toxicity of Arachidonic Acid (I.V. Administration)

Species and gender	*Methods*	*Lethal Dose$_x$*	*Results and comments*	*Reference*
Male CD-1 mice		LD_{80} = 75 mg/kg	Arachidonic Acid, by i.v. injection, caused a thrombotic/ischemic death.	Myers et al., 1988
Male Swiss mice	A fast, <2 sec, injection of Arachidonic Acid was given.	LD_{50} = 39.2 mg/kg (in 1 h)	Arachidonic Acid, i.v. injection, had a dose-related toxic effect.	de Clerck et al., 1985
Male Swiss mice	Mice were given ⩾50 mg/kg Arachidonic Acid.		The mortality rate, 75%, was highly significant. A proportional reduction in survival time was observed.	de Clerck et al., 1985
Male Swiss mice	Arachidonic Acid, 45–50 mg/kg, was given to 339 mice.		The mortality rate was 90%. Medial survival time was 95 sec.	de Clerck et al., 1985
Male Swiss mice	A slow, 10 sec, dose of 50 mg/kg Arachidonic Acid was given.		No mortality was observed. Short-lasting respiratory distress was observed.	de Clerck et al., 1985
Male Swiss mice	Arachidonic Acid was aged for 24 h at 4°C in ethanolic suspension.		Toxic effects due to Arachidonic Acid was decreased.	de Clerck et al., 1985
Male Swiss mice	The effects of sodium Arachidonate were compared to Arachidonic Acid.		75 mg/kg: Arachidonic Acid killed six of six mice; sodium Arachidonate killed three of five mice 50 mg/kg: Arachidonic Acid killed four of six mice; sodium Arachidonate did not kill any mice	de Clerck et al., 1985
Male and female CD-1 mice	Mice were dosed with Arachidonate.	LD_{50} = 33 mg/kg (males) LD_{50} = 46 mg/kg (females)	Male mice are more susceptible to Arachidonate-induced sudden death than female mice.	Myers et al., 1983
Male and female CD-1 mice	Mice were dosed with 100 mg/kg Arachidonate.		The mortality rate was 100% for males and females. Sudden death was probably due mainly to pulmonary thrombosis and ensuing hypoxia. Massive platelet aggregation occurred in the pulmonary vessels.	Myers et al., 1983

TABLE 5. ACUTE TOXICITY OF ARACHIDONIC ACID (I.V. ADMINISTRATION) *CONTINUED*

Species and gender	*Methods*	*Lethal Dose$_x$*	*Results and comments*	*Reference*
Male and female CD-1 mice	Various doses of Arachidonate were administered to 50-day old mice over a 10 sec period.		Mortality rates: 12.5 mg/kg: 10% in males and 2% in females 25 mg/kg: 38% in males and 16% in females 50 mg/kg: 67% in males and 44% in females 100 mg/kg: 100% in males and females At 25 and 50 mg/kg, the mortality rate was significantly higher in males than in females	Myers et al., 1982
Male and female CD-1 mice	Immature mice of different ages were dosed with 50 mg/kg Arachidonate over 10 sec.		Mortality rates: 23 day old: 56% in males and 47% in females 29 day old: 62% in males and 48% in females 35 day old: 80% in males and 57% in females At 35 days of age and maturity, the mortality rate with 50 mg/kg Arachidonic Acid was significantly greater in males than in females.	Myers et al., 1982
Male and female CD-1 mice	Mice were gonadectomized at 23 days of age and dosed with Arachidonate over 10 sec at 50 days of age.		There was no significant difference in the mortality rate between males and females or in within-sex comparisons of intact and gonadectomized mice.	Myers et al., 1982
Male and female CD-1 mice	Mice were pretreated with estradiol and dosed with sodium Arachidonate over 10 sec. Males and females were gonadectomized.		The mortality rate in immature intact males and immature intact and gonadectomized females pretreated with estradiol was lower than the mortality rate in the intact control males and gonadectomized females.	Myers et al., 1982

Male and female CD-1 mice	Mice were pretreated with testosterone and dosed with sodium Arachidonate over 10 sec. Some mice of each sex were gonadectomized.	Testosterone had no effect on mortality in intact or gonadectomized mice.	Myers et al., 1982
Male CD-1 mice	A dose of 50 or 75 mg/kg sodium Arachidonate was given over 10 sec.	50 mg/kg: mortality rate of 71% 75 mg/kg: mortality rate of 80% Platelet aggregates were observed in four mice at microscopic examination of the lungs.	Rabbani et al., 1981
Male and female CF1 mice	A dose of 50 mg/kg sodium Arachidonate was given over 10 sec. Some mice were pretreated with cortisone acetate for 4 days or indomethacin 2 h before dosing.	The mortality rate was 56.7% for males and 36.7% for females. Cortisone and indomethacin pretreatment significantly reduced mortality.	Penhos et al., 1979
Male and female CF1 mice	Adrenalectomized mice were dosed with 50 mg/kg Arachidonate. Some mice were pretreated with cortisone.	A 100% mortality rate was observed for both sexes. Cortisone pretreatment significantly reduced the lethal effects of Arachidonate and eliminated sex-dependent survival.	Penhos et al., 1979
Male and female CF1 mice	Intact and gonadectomized mice were dosed with 26 mg/kg Arachidonate.	A significant difference in mortality rate was observed between male and female mice. Gonadectomy did not significantly protect males or females from Arachidonate-induced death.	Penhos et al., 1979
Male and female CF1 mice	Adrenalectomized mice were dosed with 26 mg/kg Arachidonate. Some mice were also gonadectomized.	The mortality rate was 60% and 45% for adrenalectomized males and females, respectively. Castration did not significantly affect Arachidonate-induced mortality.	Penhos et al., 1979

Table 5. Acute Toxicity of Arachidonic Acid (I.V. Administration) *Continued*

Species and gender	*Methods*	*Lethal Dose$_x$*	*Results and comments*	*Reference*
Male Fullinsdorf albino, SPF, mice	Ten mice were dosed with 90 mg/kg sodium Arachidonate.		90–100% mortality in 1–2 min.	Strub and Muller, 1979
Male CF1 mice	Mice were dosed with 50 or 100 mg/kg sodium Arachidonate.		50 mg/kg: mortality rate of 0–10%; respiratory distress, which lasted for an average of 7.5 min 100 mg/kg: mortality rate of 100% in 1–3 min. Platelet aggregates were observed in the lungs.	Kohler et al., 1976
Sprague-Dawley (CD) rats (sex not specified)	Rats were dosed with 10, 20, 50, or 100 mg/kg sodium Arachidonate.		10 mg/kg: 0 deaths; 4.4 min respiratory distress 20 mg/kg: 0 deaths; 6.7 min respiratory distress 50 mg/kg: 0 deaths; 11 min respiratory distress 100 mg/kg: 100% mortality rate in 1–3 min	Kohler et al., 1976
Male or female rabbits (strain not specified)	45 rabbits were dosed with 1.5 mg/kg sodium Arachidonate over 3 sec. Some rabbits were given i.v. infusions of PGE_2, PGD_2, and PGI_2 for 8 min. Arachidonate was injected 3 min after the start of infusion. Some rabbits were dosed with indomethacin 20 h prior to Arachidonate administration.		The mortality rate was 75.6%; 35/45 rabbits died in 1–4 min. Of the prostaglandins infused, only PGI_2 reduced mortality in a dose-related manner. Mortality was attributed to occlusive thrombi formation in the pulmonary microvascular bed. Indomethacin abolished the toxic effects of Arachidonate, with 0% mortality observed.	Bayer et al., 1979

New Zealand White rabbits (sex not specified)	Rabbits were dosed with 0.5–0.75, 1.0, or 10.0 mg/kg sodium Arachidonate.		0.5–0.75 mg/kg: 0 deaths; no obvious respiratory effects 1.0 mg/kg: 100% mortality in 1–3 min 10.0 mg/kg: 100% mortality in 1–2 min	Kohler et al., 1976
New Zealand White rabbits (gender not specified)	Rabbits were dosed with 0.5, 0.7, 1.0, 1.4, or 6.0 mg/kg sodium Arachidonate. Five rabbits that survived a dose of <1.4 mg/kg were later challenged with 1.4 mg/kg Arachidonate.	$LD_{50} \approx 1.0$ mg/kg	0.5 mg/kg: 1/5 died in 2 min; no gross toxic effects were observed in 4 surviving rabbits 0.7 mg/kg: rapid respiration observed in 3 rabbits 1.0 mg/kg: 2/4 rabbits died rapidly; rapid respiration and gasping observed in 2 surviving rabbits 45 sec after dosing 1.4 mg/kg: 15/15 rabbits died in 15 sec–3 min 6.0 mg/kg: 1 rabbit died in 2 min 5 challenged: 5/5 died in 2 min All rabbits that died had platelet aggregates in the pulmonary microcirculation.	Silver et al., 1974

small doses of specific inhibitors of thromboxane synthetase, and some drugs provided protection against mortality due to Arachidonic Acid injection.

A slow (10 sec) i.v. injection of 50 mg/kg Arachidonic Acid caused short-lasting respiratory distress but did not cause mortality. Toxic effects of Arachidonic Acid were decreased by aging the ethanolic suspension of Arachidonic Acid for 24 h at 4°C before use.

The mortality produced by i.v. injection of sodium Arachidonate versus Arachidonic Acid was compared: at 75 mg/kg, Arachidonic Acid killed six of six mice while sodium Arachidonate killed three of five; at 50 mg/kg, Arachidonic Acid killed four of six mice while sodium Arachidonate did not kill any mice.

Male CD-1 mice were more susceptible to Arachidonic Acid-induced sudden death than were female CD-1 mice (Myers et al., 1983). The i.v. LD_{50} was 33 mg/kg for male mice and 46 mg/kg for female mice. A dose of 100 mg/kg Arachidonic Acid produced 100% mortality in both male and female mice. The authors concluded that the sudden death induced by Arachidonic Acid was probably due mainly to pulmonary thrombosis and ensuing hypoxia because massive platelet aggregation occurred in the pulmonary vessels; aggregation did not occur in the coronary vasculature.

Ten sec of i.v. infusion of sodium Arachidonate at various doses using male and female 50 day old CD-1 mice produced the following results: 12.5 mg/kg Arachidonate produced 10% and 2% mortality in males and females, respectively; 25 mg/kg Arachidonate produced 38% and 16% mortality in males and females, respectively; 50 mg/kg Arachidonate produced 67% and 44% mortality in males and females, respectively; and 100 mg/kg Arachidonate produced 100% mortality in both genders (Myers et al., 1982). At 25 and 50 mg/kg Arachidonate infusion, the mortality rate for males was significantly greater for females.

Intravenous administration of 50 mg/kg Arachidonate in immature mice produced 56% and 47% mortality in male and female mice, respectively, at 23 days of age; 62% and 48% mortality for males and females, respectively, at 29 days of age; and 80% and 57% mortality for males and females, respectively, at 35 days of age. The mortality rate of male mice due to i.v. infusion of 50 mg/kg Arachidonate was significantly greater at 35 days of age and maturity than it was for females at these ages.

Male and female mice were gonadectomized at 23 days of age and infused with Arachidonate at 50 days of age. There was no significant difference in mortality between the two groups; the mortality rate was 78% for males and 60% for females. Within gender comparisons between intact and gonadectomized 50-day old mice were not significant.

Arachidonate-induced mortality in immature, intact males and intact and gonadectomized females pretreated with estradiol was lower than the mortality in the intact control males and intact and gonadectomized control females. Testosterone had no affect on mortality in intact or gonadectomized male or female mice.

In male CD-1 mice, an i.v. injection of 50 mg/kg sodium Arachidonate over 10 sec resulted in 71% mortality and 75 mg/kg Arachidonate resulted in 80% mortality (Rabbani et al., 1981). At microscopic examination, the lungs of four mice after Arachidonate-induced death had platelet aggregates.

A dose of 50 mg/kg sodium Arachidonate infused i.v. for 10 sec resulted in 56.7% mortality in male CF1 mice and 36.7% in female CF1 mice (Penhos et al., 1979). Mortality was significantly reduced by indomethacin administration 2 h before Arachidonate infusion and by 4 days pretreatment with cortisone acetate, 10 mg/kg by subcutaneous injection.

A mortality rate of 100% was observed for male and female mice in which the

adrenal glands were removed 4 days prior to i.v. infusion of 50 mg/kg Arachidonate. Cortisone pretreatment significantly reduced the lethal effects of Arachidonate; it also eliminated the gender-dependent difference in survival rate.

A significant difference in mortality was observed between male and female mice after administration of 26 mg/kg Arachidonate; 10 of 20 males and 5 of 21 females died.

Gonadectomized males and females were also dosed with 26 mg/kg Arachidonate; 7 of 21 males and 5 of 20 females died. Therefore, castration did not significantly protect males or females from mortality due to Arachidonate administration.

Adrenalectomized mice were dosed with 26 mg/kg Arachidonate; the mortality rate was 60% for males and 45% for females. Castration did not significantly affect the mortality rate due to Arachidonate administration in adrenalectomized mice.

Ten male Fullinsdorf albino, SPF, mice were given an i.v. injection of 90 mg/kg sodium Arachidonate (Strub and Muller, 1979). This dose resulted in 90–100% mortality in 1–2 min.

In male CF1 mice, an i.v. dose of 50 mg/kg sodium Arachidonate produced 0–10% mortality (Kohler et al., 1976). Respiratory distress, which lasted for an average of 7.5 min and was characterized by cyanosis, interrupted breathing rate, and gasping, was observed. A dose of 100 mg/kg Arachidonate resulted in 100% mortality in 1–3 min. Platelet aggregates were seen in the lungs, but not in the heart or brain.

An i.v. dose of 100 mg/kg sodium Arachidonate given to Sprague-Dawley (CD) rats, sex unspecified, resulted in 100% mortality in 1–3 min (Kohler et al., 1976). Doses of 10, 20, and 50 mg/kg Arachidonate resulted in no mortality and average periods of respiratory distress of 4.4, 6.7, and 11.0 min, respectively.

Male or female rabbits, strain not specified, were given i.v. injections of 1.5 mg/kg sodium Arachidonate over a 3 sec period (Bayer et al., 1979). Following the injection, severe respiratory distress, decreased blood pressure, and severe arrhythmias were observed. Within 1–4 min, 34 of 45 rabbits died, a 75.6% mortality rate. Mortality was attributed to the formation of occlusive thrombi in the microvascular bed of the lungs.

Intravenous doses of PGE_2, PGD_2, and PGI_2 were infused into the femoral vein at a rate of 0.2 ml/min for 8 min; 3 min after the start of infusion, Arachidonic Acid was given. Only PGI_2 reduced mortality in a dose-related manner. Seven rabbits were given i.p. injections of 10 mg/kg indomethacin 20 h prior to Arachidonate administration; indomethacin abolished the toxic effects of Arachidonic Acid, with no deaths occurring.

Sodium Arachidonate given by i.v. injection to New Zealand White rabbits, gender unspecified, at a dose of 1 mg/kg resulted in 100% mortality in 1–3 min and 10 mg/kg Arachidonate resulted in 100% mortality in 1–2 min (Kohler et al., 1976). A dose of 0.5–0.75 mg/kg Arachidonate produced no obvious respiratory effects and did not produce any deaths.

Various doses of sodium Arachidonate were administered intravenously to New Zealand rabbits, gender unspecified, with the following results: one rabbit died 2 min after an injection of 6.0 mg/kg Arachidonic Acid; 15 animals dosed with 1.4 mg/kg Arachidonic Acid died within 15 sec to 3 min after administration, with most dying within 2 min; two of four rabbits injected with 1.0 mg/kg Arachidonate died rapidly, the other two had rapid respiration and gasping 45 sec after dosing; three rabbits injected with 0.7 mg/kg Arachidonate had rapid respiration; and one of five rabbits injected with 0.5 mg/kg Arachidonate died within 2 min after dosing, while the other four had no gross toxic effects (Silver et al., 1974).

Five of the rabbits that survived a dose of <1.4 mg/kg Arachidonate were later challenged with 1.4 mg/kg Arachidonate; all five rabbits died within 2 min. An

approximate LD_{50} was 1.0 mg/kg. All rabbits killed by Arachidonic Acid had platelet aggregates in the microcirculation vessels of the lungs; platelet thrombi were not observed in the microvasculature of other organs.

ACKNOWLEDGMENT

Monice M. Zondlo, Scientific Analyst and Writer, prepared this Appendix to the Report on Arachidonic Acid.

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