PHARMACOLOGICAL REVIEWS Vol. 20, No. 1
 Printed in U.S.A.

THE PROSTAGLANDINS: A FAMILY OF BIOLOGICALLY ACTIVE LIPIDS

SUNE BERGSTRÖM, LARS A. CARLSON AND JAMES R. WEEKS[1]

Department of Chemistry, Karolinska Institutet; Department of Internal Medicine and King Gustav V Research Institute, Karolinska Hospital, Stockholm, Sweden; and Pharmacology Research Laboratories, The Upjohn Company, Kalamazoo, Michigan

TABLE OF CONTENTS

An observation that the human uterus could react with either strong contractions or relaxation on instillation of fresh human semen was reported in 1930 by the two New York gynecologists Kurzrok and Lieb (225). A few years later M. W. Goldblatt (166, 167) in England and U. S. von Euler (153, 154) in Sweden independently observed and studied the strong smooth-muscle stimulating activity of human seminal plasma. Von Euler found similar effects in the seminal fluid of the monkey, sheep, and goat and in extracts of the vesicular glands of male sheep, but not in a number of other species. He prepared lipid extracts of these glands and found the activity to be associated with a fraction containing lipid-

[1] Dr. Weeks' address: The Upjohn Company, Kalamazoo, Michigan 49001.

soluble acids. The active factor was named "prostaglandin" and extensive studies of the pharmacological effects of these extracts were reported (155–158, 160, 167).

It is curious that these findings did not arouse more interest among scientists in the field, but the second World War and the new biomedical problems it brought into focus are the most probable explanations.

Starting in 1963 there was renewed interest in the biology of these compounds. Furthermore, several other tissue extracts were shown to contain prostaglandins as their active constituents, and this stimulated prostaglandin research in other areas. As a result, the prostaglandin literature is extremely varied, including much preliminary and sometimes contradictory information. Statements in this review must often be tentative and criticisms reserved pending future confirmatory and more detailed studies. The reviewers have intentionally included much preliminary data in order to stimulate interest and provide direction for further research. We hope this review will provide a key to all biological studies on prostaglandins currently available. In the interest of brevity, ancillary and historical literature citations have been held to a minimum.

I. CHEMISTRY

A. Isolation and structure

Early chemical exploratory work by Bergström (31) showed that the observed biological activity was due to a new type of highly active, lipid-soluble, unsaturated hydroxy acids. The isolation in pure crystalline form from sheep glands of the first two prostaglandins, now called PGE_1 and $PGF_{1\alpha}$, was reported in 1957 by Bergström *et al.* (54) and Bergström and Sjövall (61–63). Ultramicroanalysis and mass spectrometry of the first small amounts available proved that they had the empirical formulas $C_{20}H_{34}O_5$ and $C_{20}H_{36}O_5$ respectively (53). The complete structure of PGE_1 (fig. 1), (−) 11α, 15 (S)-dihydroxy-9-oxo-13-*trans*-prostenoic acid, was elucidated by various degradations, and again mass spectrometry in combination with gas chromatography played a decisive role (2, 55–58, 248). Several related prostaglandins have been isolated, mainly from human seminal plasma and sheep vesicular glands, and their structures determined (48, 49, 178, 180, 277–279, 281).

All the prostaglandins contain 20 carbon atoms and have the same basic carbon skeleton, "prostanoic acid." The complete stereochemical structure of all the "primary" prostaglandins, E_1, E_2 and E_3 and the corresponding F_α compounds, are shown in the first column of figure 1, together with those of a number of related naturally occurring prostaglandins.

All prostaglandins of the E type contain the characteristic 11α-hydroxy and 9-keto groups on a 5-membered ring. This structure is easily dehydrated by weak alkali to the 10:11 unsaturated ketone (PGA) that can rearrange to the doubly conjugated ketone (PGB). These compounds have UV absorption maxima at 217 and 278 mμ respectively, and were formerly designated PGE-217 and PGE-278 (181). The F prostaglandins are analogous to the E compounds but the 9-keto group is reduced to a hydroxyl. Chemical reduction of PGE yields two isomeric alcohols, F_α and F_β (53). Only the F_α isomer occurs naturally.

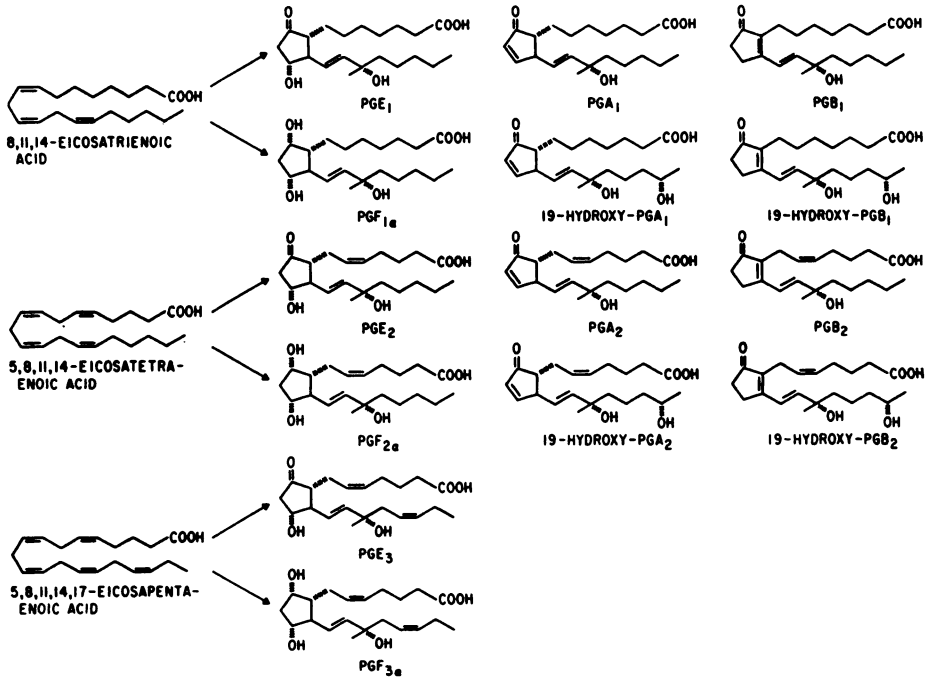

FIG. 1. Structural formulas of the naturally occurring prostaglandins and their precursors.

All the "primary" prostaglandins contain the 13:14-*trans* double bond. The E_1 and F_1 compounds contain only this one double bond whereas E_2 and F_2 have two bonds (5:6-*cis* additional) and E_3 and F_3 have three bonds (5:6-*cis* and 17:18-*cis* additional). The dehydration products, PGA and PGB corresponding to both PGE_1 and PGE_2, have all been isolated from human seminal plasma together with the corresponding 19-hydroxy-substituted compounds shown (178, 180). Of the prostaglandins shown in figure 1, all except $PGF_{3\alpha}$ occur in human seminal plasma.

For further information on the chemistry and biochemistry of the prostaglandins the reader is referred to other reviews (27, 32–34, 60, 283, and especially 267).

The actions of E, F and A prostaglandins are often dissimilar and sometimes are opposed. Thus, the PGEs are vasodepressor agents, decrease the motility of the human uterus at ovulation, of the fallopian tubes, and of the ureter, whereas the PGFs usually have the opposite effects. On the other hand, both PGEs and PGFs tend to increase cardiac output, probably as a result of increased return of blood to the heart, and to induce contraction of isolated gastrointestinal muscle and constriction of the iris. The PGAs are like the PGEs with respect to their vascular actions, but are virtually inactive as gastrointestinal contracting agents. The PGEs are the only prostaglandins so far tested which have significant antilipolytic activity.

B. Occurrence

Prostaglandins are ubiquitous among mammalian tissues. In most tissues investigated there are strong indications that prostaglandins are present, but as the concentrations are at most about 1 μg/g of wet tissue the analytical problems of separating and determining these relatively labile and closely related compounds have presented difficulties. Chromatographic procedures and isotope dilution analysis have been useful (49, 59, 97, 98, 172, 278). Thin layer chromatography combined with bioassays on smooth muscle has yielded very sensitive methods (section II A). There is, however, great need for even more sensitive and specific methods to reach the level necessary for many physiological studies.

With these methods the presence of primary prostaglandins has been demonstrated in a number of different tissues, such as lung (48, 281), thymus (59), brain and spinal cord (8, 124, 189, 200, 213, 280), kidney (130, 228, 229, 312), iris (8, 15, 332), umbilical cord (210) and human decidua (211), and there are strong indications of an occurrence in or release in low concentrations from many other tissues, such as fat (266, 296), adrenals (266, 268) ovaries (160), stomach (30, 123), intestines (8, 9, 318, 330) and nerves (269, 270). Prostaglandins also occur in menstrual and amniotic fluid (section IV C and D), darmstoff (318, 330), and SRS-C (slow reacting substance liberated by cobra or bee venoms) (330).

Human and sheep seminal plasma exceed other tissues in the number of different prostaglandins present and the total concentration of over 100 μg/ml (see section IV A).

C. Biosynthesis and metabolism

The elucidation of the structure showed that the primary prostaglandins differ only in their degree of unsaturation, the additional double bonds being located in relation to the carboxyl and methyl groups in the same way as in certain naturally occurring C-20 acids of the "essential" fatty acid group. This led to the independent discovery by van Dorp *et al.* (135, 136) and Bergström *et al.* (46, 47) that *dihomo-γ*-linolenic acid (all-*cis*-eicosa-8, 11, 14-trienoic acid), arachidonic acid (all-*cis*-eicosa-5,8,11,14-tetraenoic acid), and all-*cis*-eicosa-5,8,11,14,17-pentaenoic acid are transformed in high yields into PGE_1, PGE_2, and PGE_3, respectively, when incubated with whole homogenates of sheep vesicular glands. This biosynthetic reaction has also been demonstrated in bovine seminal vesicles (336), lung (18, 133, 246), iris (137), stomach (252), intestinal muscosa (133, 246, 331), brain (213, 252), and human endometrial curettings (133) and to a lesser extent in uterus, thymus, heart, liver, kidney, and human decidua (133, 246, 331).

The biosynthesis is effected by an enzyme system associated with the microsome fraction and a heat stable factor present in the supernatant (134, 285, 343). Whole homogenates of vesicular glands yield mainly E-compounds whereas lung homogenates yield comparable amounts of F_α- and E-compounds (or their metabolites). The yield and proportions can be varied by addition of gluthathione, tetrahydrofolate, hydroquinone *etc.* (246, 285). Under optimal conditions a yield of more than 60% of PGE_1 can be obtained. The enzyme system has recently been solubilized and partially purified (286).

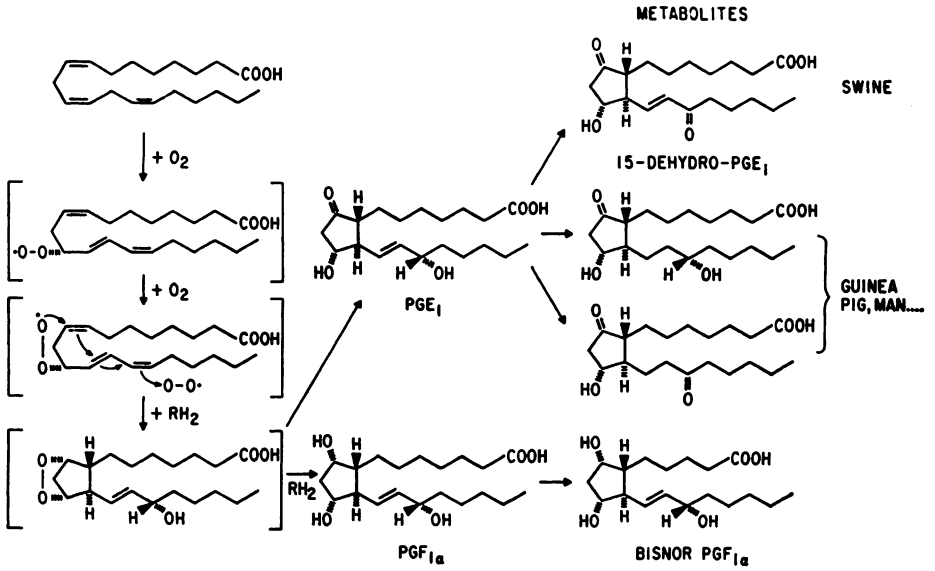

FIG. 2. Proposed mechanism of biosynthesis and the metabolism of prostaglandins E_1 and $F_{1\alpha}$.

Initial studies elucidating the mechanism of the biosynthesis were reported independently by Ryhage and Samuelsson (276) and by Nugteren and van Dorp (247). Extension of these studies, mainly by Samuelsson and associates, has led to the proposed mechanism summarized in figure 2 (183, 217, 246, 284, 286). The three oxygen atoms present at carbon atoms 9, 11 and 15 are derived from molecular oxygen and it is of special interest that the two oxygen atoms of C-9 and C-11 are derived from the same oxygen molecule. The stoichiometry of the biosynthesis of PGE_2 and $PGF_{2\alpha}$ is thus:

$$C_{20}H_{32}O_2 + 2O_2 \begin{cases} + RH_2 \rightarrow C_{20}H_{32}O_5\,(PGE_2) + R + H_2O \\ + 2RH_2 \rightarrow C_{20}H_{34}O_5\,(PGF_{2\alpha}) + 2R + H_2O \end{cases}$$

arachidonic acid

The proposed reaction mechanism is in accordance with very extensive studies with stereospecifically labeled precursors (179, 182, 217, 286).

The cyclic peroxide thus appears to be the direct precursor of both the E and F prostaglandins. From a physiological point of view it is of interest that the proportion of these two biologically quite distinct types of compound easily can be varied according to the amount and nature of the cofactor added (134). No direct interconversion of E and F compounds has yet been observed.

Studies of the substrate specificity has shown that the precursor acids can have various chain length (C-18 to C-24) as long as the methylene interrupted double bond system is located at ω-6 or in one case at ω-7 (46, 136, 314, 315).

Several isotopically labeled prostaglandins have been prepared from specifically labeled fatty acid precursors (136; for summary, see 182). For biological studies they have been prepared by partial reduction, with tritium gas, of other prostaglandins (46, 47, 135, 171, 282).

When biosynthesis is done in guinea pig lung homogenates, extensive further metabolism of the E-compounds formed is observed (14, 17, 18, 20). The two compounds shown in figure 2 are formed by dehydrogenation of the hydroxyl at C-15, reduction of the 13:14 double bond, or both. In pig lungs the 15-ketone is the only product. The dehydrogenase involved has been isolated. This enzyme has a high degree of specificity, and may be of value in a specific analytical method (19). These metabolites have some biological activity (12). Similar metabolism of E prostaglandins has been observed in several other tissues from several species including man (21). They have been identified in the blood of rats after administration of PGE_1 (282). A urinary metabolite of $PGF_{1\alpha}$ has been identified as the *dinor*-compound formed by β-oxidation (169), but many urinary metabolites of prostaglandins remain to be identified.

D. Distribution and excretion

After intravenous administration of minute amounts of tritium-labeled PGE_1 to rats, about 50% of the isotope is recovered in the urine and 10% in the feces within 20 hours. Fecal recovery is due to biliary excretion. The metabolites formed in lung homogenates as described above are detected in the plasma only, some unchanged PGE_1 with unidentified polar metabolites is found in the liver, and only polar metabolites are found in the urine (282). After intravenous administration in man, labeled material disappears very rapidly from the blood during the first 10 minutes; by 1 hour radioactivity in the blood reaches a low steady level. Excretion into the urine and feces is nearly complete in 10 hours. Total recovery of injected radioactivity is about 60%, 40% in the urine and 20% in the feces (170).

Studies of the distribution of PGE_1 in rats (282) and sheep (283) show high concentrations in the kidneys and liver, and a concentration slightly above blood level in the lungs and a number of endocrine organs. Significant concentrations of tritium were found in the uterus and fallopian tubes of the ewe and in the vesicular glands of the ram.

The distribution of small amounts of tritium-labeled PGE_1 in mice has been studied by means of autoradiography (184). The highest concentrations are observed in kidney, liver, and connective tissue with somewhat lower concentrations in lungs and myometrium. A similar distribution was found for $PGF_{2\alpha}$ (171).

Prostaglandins E_1 and E_2 are rapidly removed from the blood during one circulation through vascular beds (163). The prostaglandin was infused into the arterial supply and the venous effluent assayed by the blood-bathed organ technique of Vane (324). Disappearance was estimated by comparing the amount of prostaglandin required for an equivalent effect when infused directly into the venous effluent. Over 90% of infused prostaglandins disappeared in one circulation through the lungs of cats, rabbits and dogs, somewhat less through cat liver,

and 50 to 66 % through the cat hindquarters. Whether this disappearance represents uptake or metabolism cannot be answered at present. The observations are consistent with the rapid disappearance from the blood, metabolism observed in lung tissue *in vitro*, and concentration in liver tissue after intravenous injection discussed above. In anesthetized dogs, changing the route of infusion from intravenous to intra-aortic increased the vasodepressor and cardio-accelerator response (42, 114), but not to the degree which might have been anticipated for a 10- to 20-fold increase in effective blood level. It is possible that uptake mechanisms may have become saturated or that metabolites formed may have had activity.

II. ACTIONS ON SMOOTH MUSCLES *IN VITRO*

A. Bioassay of prostaglandins

Von Euler (158) defined a "prostaglandin unit" as the amount that would lower a rabbit's blood pressure about 30 %. This unit of activity was represented by 0.1 mg of the barium salt of prostaglandin. Eliasson (144) used a unit for a human seminal-fluid prostaglandin (HSF-PG) based upon similar biological activity, which on subsequent assay was equivalent to 1.5 Euler units. Hawkins and Labrum (186) used still another arbitrary unit. Somewhat later, a "PGE_1 intestinal unit," equivalent to 1 μg of pure PGE_1 assayed on rabbit duodenum, was used to measure activity during fractionation procedures (54, 279).

When pure prostaglandins became available, attempts were made to relate the other units to PGE_1. This could not be done with precision since the standards were mixtures of PGE and PGF prostaglandins (51, 88) and the amount needed for equivalent activity varied with the sensitivity of the assay tissue to the PGF component (51). Roughly, however, 1 Euler unit = 4.5 μg PGE_1 (51, 203), 1 Eliasson HSF-PG unit = 10 μg PGE_1 (90), and 1 Hawkins and Labrum unit = 20 μg PGE_1 (203).

Although chemical methods are available for assay of seminal prostaglandins (97), bioassays must still be used because of the minute amounts involved in fractionation procedures and in the identification and assay of prostaglandins in tissues and body fluids. Assays of prostaglandins can be performed on rabbit and guinea pig intestine (11, 13, 144, 194), rat stomach fundus (125) and uterus (144, 194), and hamster colon (6, 194). The effect on rabbit and rat blood pressure has been used to assay the vasodepressor action of prostaglandins, the latter species especially for renal vasodepressor lipids (section VI C) (188, 228, 312). In spite of the extensive use of bioassays, no careful evaluation of methods, with adequate dose-response curves and statistical analyses, has been reported. Dose-response curves have been published for PGE_1 (228) and $PGF_{2\alpha}$ (13) on rabbit intestine, PGE_1 and $PGF_{2\alpha}$ on guinea pig and rat uterus (316), PGE_1 on rat stomach (343), PGE_1 on dog blood pressure (245), and rabbit renal vasodepressor lipid on rat blood pressure (228, 312). Four- or 6-point assays have been used (11, 144), but generally unknowns and standards were compared by bracketing (125, 194, 203).

The choice of a bioassay system is determined by the substance to be assayed and the amount present. Since there are quantitative and qualitative differences among prostaglandins on various organs and systems (sections III and VI A), an

organ sensitive to the prostaglandin present must be used. Among sensitive tissues, there is no evidence for superiority of one tissue over another for assay of pure prostaglandins. Ambache and co-workers (7–9) stated than the ascending colon of the gerbil (jird) was the most sensitive preparation, yielded a steep dose-response curve, was insensitive to histamine, was free of rhythmic activity, and relaxed quickly between doses. For extremely small quantities, the 0.05 ml microbath of Gaddum and Szerb (165) has been used with goldfish intestine (265). However, for vasodepressor prostaglandins that do not affect these systems (*e.g.*, PGAs), a vascular system is indicated. Weanling and adult rats are equally sensitive to the hypotensive effect of PGE_1. Mice offer no advantage, requiring a 10-fold greater dose for equivalent effect (337). Vasodilator prostaglandin activity has been assayed by intra-arterial injection into a cat hind-limb perfused at a constant rate (344): as little as 10 ng of PGE_1 lowered perfusion pressure about 15 mm Hg. Rabbit intestine is preferable for mixtures of PGE and PGF, since it is sensitive to both (13, 51, 194, 195). For impure extracts, interference with other materials that affect smooth muscle becomes a problem. The effects of muscarinic substances, histamine, and serotonin may be blocked without interfering with the action of prostaglandin by adding atropine, an antihistaminic compound, or bromolysergic acid diethylamide, respectively, to the bath (7, 124, 125, 144, 265). Polypeptides may be destroyed by incubation with chymotrypsin (124, 265). Hydroperoxides derived by auto-oxidation from polyunsaturated fatty acids may be inactivated by triphenylphosphine or ascorbic acid without affecting prostaglandin activity (128, 188). Instead of antagonizing the interfering substances, one can use muscles insensitive to possible contaminants. Thus, rat and hamster colon are insensitive to histamine and hydroperoxides, and rat colon is either insensitive to or relaxed by bradykinin (7). However, it would seem preferable first to use extraction methods to eliminate interfering substances, second, to separate prostaglandins by thin layer chromatography using parallel reference spots of known prostaglandins, and finally to assay the extracts in small muscle baths (127). Parallel assays of unknown prostaglandins for their equivalent activity in terms of PGE_1 and $PGF_{1\alpha}$, with tissue having marked quantitative differences in sensitivity to E and F prostaglandins, has provided presumptive evidence of the type of prostaglandins (*e.g.*, 30, 123, 200, 210, 265).

The blood-bathed organ technique (324) has been adapted to assay prostaglandins in circulating blood (163). A combination of rat stomach strips, rat colon, and chicken rectum was used. All these preparations contract in the presence of 1 to 10 ng of prostaglandins per ml (E_1, E_2 and $F_{2\alpha}$). No other substance, in concentrations likely to be found in blood, contracts all three muscles. By infusing known amounts of prostaglandins into the perfusing blood, a rough quantitative estimate of the prostaglandin concentration may be obtained. This method was applied to the assay of PGE_2 in dog splenic venous blood and to studies on the disappearance of prostaglandins from the circulation (sections I C and V A).

The relative potency of prostaglandins in various assay preparations has been assembled in table 1. It must be emphasized that many of these comparisons were based upon only a few trials and the relationships were interpreted only as ap-

proximations. This table can serve as a guide to further details of prostaglandin actions on specific preparations.

B. Modification of prostaglandin actions on smooth muscles

In most instances prostaglandins cause contraction of smooth muscle, but there are some exceptions for specific tissues and prostaglandins. Responses may be modified by ionic composition of the medium, hormones, blocking agents, and interactions with other agonists. Full interpretation of these actions involves many aspects of the physiology and pharmacology of smooth muscle, a field too broad for this review.

Ionic composition of the medium. On the whole, the influence of changes in the concentration of Ca^{++}, Mg^{++} and K^{+} on the contractile responses to PGE_1 is like that of most other agonists (121, 125, 177, 234, 236, 254, 294, 343). The response is calcium-dependent and is still present in muscles depolarized by high potassium. Isolated human myometrial stripes are generally relaxed by PGEs but always stimulated by PGFs (section IV B). In this muscle, both low Ca^{++} and low K^{+} increased the inhibitory effect of PGE_1 (88). In contrast to PGE_1 in other muscle preparations, $PGF_{1\alpha}$ contractions were not affected by low Ca^{++} (88). No other studies of the effect of ions on PGFs have been reported. This lack of calcium dependence merits confirmation and further study.

Interactions with blocking agents and other agonists. The contractile or relaxing response to PGE_1 in various smooth muscles is not blocked by atropine or other antimuscarinic agent (51, 125, 153, 156, 254, 311, 333), antihistaminic compounds (144, 265, 269, 311, 322), antagonists to serotonin (125, 311), α-adrenergic blocking agents (145, 146, 254), or β-adrenergic blocking agents (145, 146, 311). Propranolol did prevent miosis induced by PGE_1 in rabbits (334). PGE_1 (20 μg/kg intra-arterially) did not reduce the response to preganglionic stimulation of the cat nictitating membrane, a result that implies a lack of effect on ganglionic transmission (190). The contractile response to $PGF_{2\alpha}$ was unaffected by atropine or lysergic acid diethylamide (13).

Superfusion of the isolated rat stomach fundus with procaine reduces by $\frac{1}{8}$ to $\frac{1}{10}$ the concentration of PGE_1 needed for a contraction. Bretylium, dichloroisoproterenol, Dibenamine, and hexamethonium also increase the sensitivity of the muscle to PGE_1 (125). These results suggest that blockade of intrinsic nerves, especially sympathetic fibers, contributed to the increased sensitivity. The observation that the paradoxical relaxation of rat duodenum by PGE_1 is secondary to catecholamine release is in line with this explanation (214). However, more may be involved than inhibition of catecholamines, since procaine superfusion of stomach muscle from reserpinized rats also increases sensitivity to PGE_1. Furthermore, even though such reserpinized muscles require higher concentrations of PGE_1 to elicit contractions, bretylium superfusion causes such a marked increase in sensitivity to PGE_1 that 0.01 ng/ml can cause contraction, compared to 0.2 to 0.5 ng/ml required for untreated muscles (125).

Epinephrine and norepinephrine do not affect the isolated rat stomach fundus, but, depending upon their concentration, increase the amount of PGE_1 needed

TABLE 1

Relative activity of prostaglandins (PGE_1 = 1.0)[a]

Species	Prostaglandin						References
	E_2	E_3	$F_{1\alpha}$	$F_{2\alpha}$	A_1	A_2	
Jejunum/duodenum (contraction)							
Rabbit	1.5 (±0.5)	0.99 (±0.9)	2.22 (±1.72)				194
	3.1 (CI 1.9–5.0)				0.010 (CI 0.0060–0.016)	0.084 (CI 0.040–0.14)	338
	5	0.5	2	8			279
				26 (R 5–100)			195
				R 3–30			13[b]
			R 1–3				51
	R 2.5–5	R 0.4–0.5					52
			R 1–2				91
			3.2				260
					0.01		129
						0.02[c]	228
					R 0.02–0.08	R 0.003–0.03	92
Rat	0.2						265
			R 0.05–0.2				51
				R 0.15–0.6			13[b]
Chicken			0.025				51
Ileum (contraction)							
Guinea pig	1.56 (±0.78)	0.23 (±0.19)	0.023 (±0.013)				194
	0.85 (CI 0.60–1.2)				0.0020 (CI 0.0012–0.0028)	0.0057 (CI 0.0040–0.0080)	338
	R 0.4–1.3	R 0.1–0.2					52
	R 0.1–0.2		0.5				265
				R 0.1–0.7			13[b]
			R 0.01–0.07				51
				0.55 (R 0.015–3)			195
					0.01		129

Colon/rectal caecum (contraction)							
Guinea pig	5		0.01	0.05			208
Hamster	2.75 (±1.5)	0.19 (±0.14)	0.26 (±0.16)				194
	0.02			0.2			265
				3.5 (R 1–8)			195
Gerbil	0.02		0.2	0.2			265
Chicken				R 0.7–6.0			13[b]
			R 0.7–3.0				51
Stomach (contraction)							
Rat			0.9				343
	3.3 (±0.5)		0.028 (±0.004)	0.19 (±0.03)			123
Uterus (rat contraction, human relaxation)							
Rat (anestrus)	0.25		0.25	0.25			265
				5			316
				R 2–10			13[b]
			R 0.9–1.55				3
Rat (estrogen-treated)	1.09 (±0.37)	0.31 (±0.03)	0.94 (±0.75)				194
				8 (R 5–15)			195
				50			13[d]
Rat (pregnant)			2				51
Man	R 0.7–0.9	R 0.65–0.85			R 0.03–0.3	R 0.01–0.1	89, 92
	0.83	0.67	>0.2	>0.2			91
Fallopian tube (relaxation)							
Rabbit	0.93 (±0.08)	0.43 (±0.16)					194
Trachea (relaxation)							
Cat				0.03 (R 0.0025–0.05)			195
	1.0	0.2	0.002				231
Iris (contraction)							
Cow			<0.03				51

TABLE 1—*Continued*

Species	Prostaglandin						References
	E_2	E_3	$F_{1\alpha}$	$F_{2\alpha}$	A_1	A_2	
Intravenous injection (sedation)							
Chick	0.67	0.25					192
Antilipolytic (epinephrine stimulated)							
Rat epididymal fat	2.9 (CI 1.7–5.2)				0.0037 (CI 0.0022–0.0060)	0.029 (CI 0.018–0.049)	338
Blood platelets (inhibition of aggregation)							
Rabbit	0.005				0.0006	<0.0005	338
Blood flow (increase)							
Cat (muscle)	0.76 (±0.31)	0.53 (±0.46)	0.22				194
				<0.05			195
Cat (skin)	0.91	0.23	0.22				194
Blood pressure (fall)							
Rabbit	1.0 (±0.0)	0.32 (±0.03)	0.075 (±0.04)				194
	R 0.45–0.6	R 0.25–0.4					52
				0.11 (R 0.08–0.2)			195
			<0.05				51
Rat	0.13 (CI 0.04–0.27)				0.30 (CI 0.12–0.53)	0.33 (CI 0.14–0.58)	338
Dog	0.71 (CI 0.46–1.13)				2.7 (CI 1.5–4.1)	2.5 (CI 1.4–3.9)	338

[a] Values in some cases recalculated in terms of PGE_1 taken as unity. The symbols are: ±, standard error; R, range of values; and CI, the 95% confidence interval evaluated from parallel line assay.

[b] Values in terms of PGE_1 calculated from $PGF_{2\alpha}/PGF_{1\alpha}$ values and $PGF_{1\alpha}$ activity in Bergström *et al.* (51).

[c] Tested as medullin.

[d] Value calculated as footnote *b* above from Horton and Main (194).

for a given contraction from 2- to 37-fold (125). If one assumes that the catecholamines showed no smooth-muscle relaxing action because the isolated stomach muscle is without intrinsic tone, the explanation need not be blockade of prostaglandin, but merely simple antagonism of a contracting and relaxing agent.

Both E and F prostaglandins, applied to a variety of smooth muscles in subthreshold amounts, also diminish the effects of catecholamines (epinephrine, norepinephrine, phenylephrine, isoproterenol) (119, 120). For example, norepinephrine blocks the contraction of isolated guinea pig uterus induced by vasopressin. In this tissue, $PGF_{2\alpha}$ causes contraction at 10 ng/ml, but 0.001 ng/ml diminishes the norepinephrine's inhibition of vasopressin's effect. Diminution of the catecholamine effect was independent of the direction of the prostaglandin or catecholamine activity. Thus, norepinephrine action on guinea pig uterus (relaxation) or on rat seminal vesicle (contraction) were both decreased by $PGF_{2\alpha}$. In some tissues, the antagonism was not evident until repeated challenging doses of catecholamine were given for 90 minutes or more. Where catecholamine caused contraction, the first responses were often potentiated (see discussion following). This early potentiation was especially prominent in the guinea pig seminal vesicle and can account for the apparently contradictory report by Eliasson and Risely (149, 150) that PGE_1 potentiated catecholamines in that tissue.

Intriguing as these observations are, a few words of caution are in order. Evaluation of potentiation or inhibition by changes in threshold or comparison of responses before and after an experimental procedure may lead to serious errors in interpretation. The best method requires assessment of horizontal shifts of dose-response curves. These problems have been concisely reviewed in relation to sympathomimetic amines by Trendelenburg (320) and the principles apply generally. Furthermore, in some of the studies described above (119, 120), only illustrative cases were presented without statistical evaluation. Finally, before this antagonism can be fully accepted, control experiments are needed to show that the responses to catecholamines remain constant during the test period. This point is especially important since in some cases the inhibition did not appear until after repeated challenges with catecholamine.

Potentiation. In addition to causing contraction of smooth muscles, PGE_1 and PGE_2 can induce a persistent nonspecific increase in sensitivity to other agonists (121, 177, 263). Thus, in isolated guinea pig uterus, when the dose-response relation was determined for vasopressin and PGE_1 was then added and washed out, as little as $\frac{1}{8}$ to $\frac{1}{10}$ as much vasopressin is subsequently required for the same effect. Contractions do not return to control levels for an average of 45 minutes. In the guinea pig uterus, vasopressin was most carefully studied; but responses to oxytocin, histamine, electrical field stimulation, and even $PGF_{2\alpha}$ are enhanced. This action was apparently limited to PGE_1 and PGE_2, not being seen after $PGF_{1\alpha}$, and $PGF_{2\alpha}$, ricinoleic acid, sodium phosphatidate, hydroperoxide-containing linolenic acid, or oxytocin (121, 316). The degree of potentiation was roughly parallel to the PGE_1 concentration in the range of 0.0075 to 1.2 ng/ml; higher concentrations gave no greater potentiation. Although high Mg^{++} or low Ca^{++} decrease contractile responses to PGE_1, they did not affect potentiation of vaso-

pressin or electrically induced contractions by PGE_1 (121, 177). In guinea pig uteri depolarized by high K^+, potentiation of contractions produced by electrical field stimulation was not seen, although potentiation of vasopressin's effect did occur as long as Ca^{++} was low (persistent contracture precluded testing vasopressin in K^+-depolarized preparations with normal Ca^{++}) (121).

Similar potentiation has been reported in other tissues. PGE_1 (but not $PGF_{1\alpha}$) enhances responses to epinephrine, norepinephrine, tyramine, acetylcholine, serotonin, and hypogastric nerve stimulation in rat and guinea pig seminal vesicles (149, 150, 234), and PGE_1 enhances the response to acetylcholine in the rat stomach fundus (125) and rat and guinea pig bladder (205). Subthreshold concentrations of PGE_1 also potentiate angiotensin and serotonin (but not norepinephrine) contractions in the rabbit aortic strip, and acetylcholine-induced contractions of the frog rectus abdominus muscle preparation, and they both decreased the threshold voltage and increased the magnitude of the contraction in electrically stimulated cat carotid arteries (214). Bradykinin, normally inactive on the rabbit aortic strip, caused it to contract after subthreshold concentrations of PGE_1 (214). On the other hand, potentiation was not observed after PGE_1 in electrically stimulated rat uterus (316) or in rat or cat seminal vesicle (234). In the rabbit seminal vesicle, PGE_1 reduced the responses to hypogastric nerve stimulation (234).

PGE_1 (0.33 ng/ml) has only a slight positive inotropic effect on isolated, electrically driven rabbit auricles. A subthreshold concentration of ouabain became equivalent to a therapeutic concentration in the presence of the PGE_1, and a therapeutic concentration became toxic. Tissue electrolyte studies showed that both PGE_1 and ouabain causes loss of intracellular K^+ but that PGE_1, unlike ouabain, also inhibits Na^+ uptake, resulting in a net decrease in intracellular cation concentration (321). These studies do not explain the mechanism of the potentiation, but do indicate that PGE_1 may affect transport systems for other ions than Ca^{++}.

Over 30 years ago, in his original study on prostaglandin, Goldblatt observed that a threshold dose of epinephrine on the isolated guinea pig seminal vesicle would potentiate the effect of prostaglandin added some time later (167). This reverse situation, potentiation of prostaglandin by other agonists, has not been studied further.

C. Mechanism of action

The contractile action of PGE_1 seems coupled with oxidative metabolism. In the rat uterus, aeration with nitrogen reduces the response to PGE_1 without affecting the contraction induced by acetylcholine (254). In the rat stomach fundus, nitrogen aeration, cyanide, and carbon monoxide aeration (all of which should block oxidative metabolism) also inhibit PGE_1 and not acetylcholine responses (125). Conversely, ascorbic acid and reduced (but not oxidized) glutathione increase the response to PGE_1. Furthermore, PGE_1 seems to be bound to the muscle in an inactive form under anoxic conditions. Application of PGE_1 during nitrogen aeration causes a very small contraction, which is not concentra-

tion-related. Washing with oxygenated solution results in a typical concentration-related contraction. However, when the muscle is washed with nitrogen aerated solution after adding PGE_1, contraction still occurs on subsequent oxygenation. Similarly, when PGE_1 is added in the presence of azide or cyanide, contraction follows washing out of the inhibitor.

A unifying mechanism of action which accounts not only for qualitative difference in tissue responses but also for the potentiation of other agonists has not yet been proposed. Action in the presence of a wide variety of pharmacological blocking agents and in depolarized muscles implies a non-neural action and the interaction of prostaglandin with a specific discrete receptor in the cell membrane (254). In support of this concept are the observations that botulinum toxin, which inactivates neural mechanisms (10), did not affect PGE activity in guinea pig taeni coli (236) and that PGE did not affect the glycerinated intestinal smooth muscle-ATP system or its relaxing factor (294). The relationships of PGE_1 to Ca^{++} in the membrane and medium has been discussed at length by Paton and Daniel (254). Vogt (329) suggested that the hydroxy fatty acids that cause smooth muscle contraction might provide chelating sites and carry Ca^{++} through cell membranes. Courtald molecular models showed this to be feasible for PGE and PGF_α (263).

Membrane depolarization and release of bound calcium seems reasonable to explain stimulation (125, 254, 311). Clegg *et al.* (121) suggested that direct effects of the prostaglandins involved the cell membrane, while potentiation was intracellular by facilitation of excitation-contraction coupling. Some evidence in favor of these hypotheses is the increased rate of exchange of $^{45}Ca^{++}$ in isolated hearts stimulated by PGE_1 (22, 216). Strong and Bohr (311) have proposed that very low PGE_1 and PGA_1 concentrations (1–100 ng/ml) may render intracellular ionic Ca^{++} inactive and thereby bring about relaxation of vascular smooth muscle through decrease in activity of the actomyosin ATPase system. Potentiation of other agonists is explained by hypopolarization of the cell membrane, possibly by decreasing Ca^{++} at sites which normally fix Ca^{++} (214, 311). The observation that prostaglandins lower the threshold and increase the response to electrical stimulation supports this concept (121, 214). Khairallah *et al.* (214) have discussed discrepancies between this hypothesis and that of Clegg *et al.* (121).

Most mechanism studies have used PGE_1. Although the actions of PGE_1 and $PGF_{1\alpha}$ are qualitatively similar on many smooth muscles (except with respect to potentiation), different receptors may be involved. Thus, after tachyphylaxis was induced in isolated rat uterus to either PGE_1 or $PGF_{1\alpha}$ there was still a normal response to the other prostaglandin (3).

III. SMOOTH MUSCLE ORGANS

The discussion in the preceding section was primarily concerned with general actions of the prostaglandins. Here, prostaglandin actions on specific organs, both *in vivo* and *in vitro*, will be considered.

Uterus and fallopian tube. See section IV B.

Stomach. Prostaglandins E_1, E_2 and A_1 are powerful inhibitors of gastric secre-

tion in dogs (273). Prostaglandins were given by intravenous infusion to trained unanesthetized dogs with either innervated (Pavlov) or denervated (Heidenhain) pouches after stimulation of secretion by either food or infusion of histamine. Almost total inhibition of secretion, judged by volume, acid, or pepsin, is obtained after infusion of 1 μg/kg/min of PGE_1 or PGE_2 for 30 to 45 minutes. The ED50 (the dose inhibiting gastric secretion by 50 %) is about 0.5 μg/kg/min. PGA_1 does not affect histamine-induced secretion, but strongly inhibits food-induced secretion (ED50 0.08–0.1 μg/kg/min). On cessation of prostaglandin infusions, gastric secretion resumes gradually. $PGF_{2\alpha}$ (tested only against histamine-induced secretion) was inactive at 1 μg/kg/min. The demonstration of prostaglandin liberation by rat stomach (section V A) suggests that prostaglandins may have a role in the control of gastric secretion (30, 123).

Intestine and colon. There are few studies of prostaglandin action on intestinal function as such. These tissues have been used for bioassay and for studies on factors influencing prostaglandin on smooth muscle. *In vitro*, certain intestinal and colon preparations contract in concentrations of 1 ng/ml or less, but much variation has been reported. PGFs are generally more potent than their corresponding PGEs on rabbit intestine, but they are manyfold less potent on guinea pig ileum and colon (51, 194, 195, 210). PGE_1 (5 ng/ml) relaxes rat duodenum (214). This relaxation becomes contraction either in the presence of combined α- and β-adrenergic blockade (phentolamine plus propranolol) or in muscles from reserpinized rats. Such contraction can in turn be blocked by bromolysergic acid. Presumably, PGE_1 liberates both catecholamines and serotonin from the rat duodenum. Biogenic amine liberation should be considered in the action of prostaglandins on intestinal muscle from other species. *In vivo*, an intestinal stimulant action of crude prostaglandin was observed through a window in a rabbit abdomen (159), and inferred from semifluid feces after subcutaneous injection in mice (156) and from abdominal cramping in man during intravenous infusion of PGE_1 (103, 106). The PGAs, while lowering the blood pressure, are virtually devoid of intestinal stimulant activity (129, 228, 264, 338). With the demonstration of PGE_1 in darmstoff (318) and in plexus-containing longitudinal muscle of rabbit and guinea pig ileum (8, 9), attention should be given to possible function of related lipids in intestinal physiology (see review, ref. 27).

Ureter. Ureteral muscle resembles that of the fallopian tube in its response to prostaglandins. Ureteral motility was recorded in trained unanesthetized dogs with exteriorized bladders. In these animals, intravenous PGE_1 decreases or stops ureteral peristalsis (81) while $PGF_{2\alpha}$ increases it (226). The effects of higher doses lasted up to 30 minutes, much longer than the cardiovascular effects. *In vitro*, PGE_1 inhibits and $PGF_{1\alpha}$ stimulates dog, monkey, and baboon ureters (81, 311, 317).

Lung and trachea. The effect of prostaglandins has been studied on tracheal chains and on the resistance to inflation of the lungs by the method of Konzett and Rössler (219). PGE_1, PGE_2, PGE_3, $PGF_{1\alpha}$ and $PGF_{2\alpha}$ all relax tracheal muscle, the PGFs being much less potent than the corresponding PGEs (195, 214, 231). Main (231) studied PGE_1 on the tracheal muscle of 7 species. Threshold

activities range from 1 ng/ml in the cat to 3 μg/ml in the sheep, with the guinea pig, ferret, monkey, rabbit and pig intermediate. In the Konzett and Rössler preparation, PGE_1 alone did not affect resistance to inflation in the guinea pig (191) but antagonized the increase in resistance induced by vagal stimulation in the rabbit and guinea pig (231). In cats both PGE_1 and $PGF_{2\alpha}$ increase the resistance of the lungs (13, 231). $PGF_{2\alpha}$ also increases the resistance in the guinea pig, an action not blocked by calcium acetylsalicylate given in a dose that blocks the actions of bradykinin and SRS-A (slow reacting substance liberated in anaphylaxis) (64).

Eye. Stimulation of the trigeminal nerve in rabbits leads to a long-lasting, atropine-resistant miosis. This was observed long ago by both Magendie and Bernard, then subsequently by many authors (6, 332). Ambache (5, 6) characterized a lipid extract of rabbit iris, which he named irin, as an unsaturated hydroxy fatty acid and reported chemical and biological characteristics of this extract which were compatible with those of prostaglandins. Later, $PGF_{2\alpha}$ was identified in sheep iris (15), and mixed prostaglandins in rabbit iris (332, 335). Arachidonic acid and enzymes for biosynthesis of prostaglandin are present in pig iris (137). Injection of PGE_1 into the anterior chamber of rabbit eyes (threshold dose 10 ng) causes a persistent miosis and a rise in intraocular pressure. PGE_2 is equally effective but $PGF_{1\alpha}$ relatively inactive (333). Intravenous PGE_1, in depressor doses, also elevates intraocular pressure, whereas a lowering of intraocular pressure is usually associated with vasodilation. Miosis without change in intraocular pressure is seen after intraocular PGE_1 in cats and monkeys (333, 334). The mechanism of the increased pressure in rabbits is unknown, but it is apparently not due to interference with aqueous drainage. PGE_1 reverses atropine mydriasis in cats (333); but, in rabbits, propranolol blocked the miosis produced by prostaglandin (334). However, neither phenoxybenzamine nor propranolol affected the rise in intraocular pressure in rabbits These studies implied that the effects on intraocular pressure and miosis involved different mechanisms.

Vascular muscle. Prostaglandins E_1, E_2, A_1 and $F_{1\alpha}$ were studied in isolated vascular strips (311). Smooth muscle from dog peripheral resistance vessels (0.2–1 mm o.d.) shows a biphasic dose-response curve, relaxation in concentrations from 1 to 100 ng/ml, then contraction up to 10 μg/ml. PGA_1 is the most potent relaxant, followed in order by PGE_2, PGE_1 and $PGF_{1\alpha}$. Since the high concentrations would not ordinarily be achieved *in vivo*, these results are related to the peripheral vasodilating action of prostaglandins, even to the point of relative potency (see table 1). Rabbit aortic and dog coronary arterial strips show only contractile responses to these prostaglandins, even at threshold concentrations of 0.1 ng/ml (214, 311).

Isolated superior mesenteric veins have spontaneous rhythmic activity. PGE_1 enhances contractions in rabbit and rat veins but $PGF_{1\alpha}$ is inactive. Conversely, in dog veins PGE_1 depresses and $PGF_{1\alpha}$ enhances the activity (206, 311).

Bladder. PGE_1 increased the tone and motility of the rat and guinea pig bladder *in vivo*, and increased the response to hypogastric nerve stimulation. However,

it did not cause responses of the bladder *in vitro*, but nevertheless potentiated the action of acetylcholine (see section II B) (205).

IV. REPRODUCTIVE SYSTEM

Prostaglandins occur not only in human semen but also in association with female reproductive organs from menstruation through pregnancy. Natural occurrence of prostaglandins in a tissue does not necessarily mean that the compounds must serve a physiological function. Several physiological functions have been proposed, and they may be considered attractive hypotheses, which remain to be proved.

A. Seminal prostaglandins

Human seminal plasma contains the highest concentration of prostaglandins found so far: an average of about 50 μg/ml of PGE compounds, 8 μg/ml of PGF compounds, 50 μg/ml of PGA and PGB compounds combined, and 200 μg/ml of 19-hydroxy prostaglandins (89, 93, 98, 180). Ram seminal plasma differs in that it contains neither the dehydrated PGA and PGB prostaglandins nor their 19-hydroxylated derivatives (94, 181). Monkey (35), goat (144), and rabbit (203) semen also contain appreciable amounts of prostaglandins. No prostaglandin was detected in stallion, bull, or boar semen or male accessory glands of the bull, boar, dog, cat, rabbit, guinea pig, hamster, rat, mouse, ferret, or elk (144, 153, 160, 203).

Seminal prostaglandin levels have been studied in relation to suspected infertility (23, 98, 186). The levels bear no relation to sperm count, motility, or morphology. One study (98) reported that two (out of a total of 10) subfertile patients had abnormally low seminal prostaglandins. A larger series of both subfertile and normal subjects must be studied before any conclusions may be drawn.

Vaginal absorption of seminal prostaglandin was shown by depositing seminal plasma containing a tracer amount of ^{3}H-PGE$_1$. After 24 hours, 10 to 20 % of the radioactivity was recovered in the urine (293). Whether such absorption plays a significant role under physiological conditions in relation to coitus and fertilization is not yet clear.

B. Action on uterus and tubes

In vitro. The action of the various prostaglandins on the uterus and tubes both *in vitro* and *in vivo* has been studied extensively. Isolated uteri of rats and guinea pigs are caused to contract by both E and F$_\alpha$ prostaglandins (13, 51, 144, 194, 195, 265, 316). In the guinea pig uterus, as in the intestine, smaller doses of PGE are required than of the corresponding PGF$_\alpha$ (51, 316), but their relative potencies in the rat are not clear (51, 265, 316). In most cases human semen inhibits the motility of isolated human myometrium (126, 143, 225).

The three PGE compounds all decrease the tonus, frequency, and amplitude of the spontaneous contractions of human uterine strips (88, 90, 261, 289, 291). The related PGA, PGB, and 19-hydroxylated compounds present in human seminal plasma have similar effects but require higher concentrations. In mix-

tures the effect of the compounds are additive, and both the dehydrated and 19-hydroxylated prostaglandins make an appreciable contribution to the total effect of the human seminal plasma (92). $PGF_{1\alpha}$ and $PGF_{2\alpha}$ differ from the PGEs in that they both always cause a contraction of the myometrium (88, 261, 292).

The hormonal state clearly influences the sensitivity of isolated human myometrial strips. They are 3 to 5 times more sensitive to the relaxant action of PGE_1 at about ovulation time (88, 90, 289). Conversely, just before menstruation such strips have been reported to be more sensitive to the "menstrual stimulant" $PGF_{2\alpha}$ (142, 256, 262), and they are also more sensitive to PGF compounds during pregnancy (88, 93).

Progesterone added *in vitro* depresses the sensitivity of both guinea pig and rat uterus to PGE_1 and $PGF_{2\alpha}$ (316). Treatment of the animal with estrogen or combined estrogen and progesterone has been reported to have little effect (144, 316) or to increase (13) or decrease (72, 263, 265) the sensitivity of the isolated uterus.

When the human fallopian tube is divided into four parts of about equal length, and each mounted in an isolated smooth muscle bath, PGE_1 as well as PGE_2 induces contraction in the segment nearest the uterus and relaxation in the other three (288–291). There is no apparent change in sensitivity with the menstrual cycle (290). $PGF_{1\alpha}$ and $PGF_{2\alpha}$ caused contraction of all segments of the fallopian tube (292). The motility of the fallopian tube of the rabbit was reduced by PGE_1 *in vitro* and inconsistent results were obtained in sheep oviducts (202).

In vivo. Cat and rat uteri do not react to vasodepressor doses of PGE_1 (66, 144). Guinea pig uterus contracts after 1 μg/kg injected intravenously (66). Various results have been reported for rabbit uterus (24, 66, 144, 202).

Either stimulation or stimulation followed by inhibition of the nonpregnant human uterus is obtained after depositing semen or human seminal fluid extract (HSF-PG) in the vagina (147, 212). Release of posterior pituitary hormones during coitus has been reported (for references, see 147). The uterine response to HSF-PG during intravenous infusion of posterior pituitary hormones was consistently inhibition. It was proposed that such inhibition would facilitate sperm transport (147). With the Rubin technique, tubal resistance increases markedly in some patients after vaginal application of HSF-PG (148). It was also proposed that seminal prostaglandins might function to retain the ovum in the fallopian tube to allow fertilization (290). Motility of rabbit fallopian tubes is reduced by all PGEs *in vivo* (194), and intravenous PGE_1 causes relaxation of the isthmic circular muscles and thereby reduces the opening pressure (24, 82). PGF_αs stimulate the rabbit fallopian tube *in vivo* (194, 195). In sheep, crude prostaglandin (intravenous or intra-aortic) stimulates the oviduct of some animals but relaxes others (202). The doses needed are considerably in excess of the prostaglandin content of one ram ejaculate and no correlation with the hormonal status of the animal was observed. These results were taken to weigh against a physiological role of seminal prostaglandins on the fallopian tube of sheep.

Recent studies on the effect of various pure prostaglandins on the pregnant human uterus, by the technique of Caldeyro-Barcia and co-workers (99, 100) have yielded interesting results. The studies were made on midpregnant volun-

teers (14 to 22 weeks) admitted to the hospital for therapeutic abortion, and on pregnant patients at or near term (34 to 40 weeks). PGE_1 and PGE_2 infused intravenously increased the uterine motility. The effect was similar to that obtained *in vitro* with a low dose of PGE_1: an increase of tonus followed by increased irregular contractile activity. In some cases a more regular activity was induced. The threshold dose was 0.6 to 4 μg/min for both midpregnancy and term patients. The lower dose corresponded to about 10 ng/kg/min. In no cases was an inhibition of the motility found. Similar but less pronounced effects were obtained with $PGF_{2\alpha}$. No effect on the motility was recorded after intravenous infusion of $PGF_{1\alpha}$, $PGF_{1\beta}$ and PGA_1 although much larger doses than of PGE_1 were given (95, 96). Of special interest is that the doses that had a clear effect on uterine motility were below the doses that cause a demonstrable fall in blood pressure.

In another study (342), single intravenous or intramuscular injection of 100 μg of PGE_1 or 500 μg of $PGF_{2\alpha}$ considerably increased the tonus of the uterus in midpregnant patients. The effect was like that of 0.2 mg of ergot alkaloids (Methergine) given by intravenous injection. This result indicates the possibility of using the prostaglandins in medical practice for preventing atonic uterine bleeding after abortion or delivery.

C. Menstrual prostaglandins

Clitheroe and Pickles (122) separated from menstrual blood two components that stimulate smooth muscle. Component A was primarily $PGF_{2\alpha}$ with a lesser amount of PGE_2 (142). The menstrual prostaglandins are probably formed in the endometrium during menstruation, since the amount of prostaglandins discharged considerably exceeded the amount estimated to be present in the endometrium (142), and since synthesis of prostaglandins *in vitro* by endometrial curettings has been demonstrated (133). In a study on one subject during puberty, her prostaglandin loss in anovular cycles was about ⅕ that in ovular cycles (258, 259). This suggests that menstrual prostaglandin formation may be under hormonal control.

Pickles suggested that dysmenorrhea might be associated with either overproduction or an impediment to outflow of menstrual lipids (26, 257). In support of this view it may be noted that $PGF_{2\alpha}$ (0.5 μg) instilled into the uterus after dilatation and curettage stimulated contractile activity (151). On the other hand, one study on menstrual blood and endometrial curettings from dysmenorrheic patients showed such great individual variations that no conclusions could be drawn (262).

D. Amniotic fluid prostaglandins

A wide variety of smooth muscle stimulants has been reported present in amniotic fluid (see review 227), including some lipids from a neutral ether extract which probably are not prostaglandins (1). Karim (208, 210) and Karim and Devlin (211) identified prostaglandins E_1, E_2, $F_{1\alpha}$ and $F_{2\alpha}$ in human amniotic fluid and umbilical cord vessels, but not in placental tissue. Before term, half of the patients studied had no detectable prostaglandins in their amniotic fluid and the others had only a trace of PGE_1 (average about 1 ng/ml). At term, $PGF_{2\alpha}$

became the predominating prostaglandin (average 22 ng/ml) in all patients, with lesser amounts of both $PGF_{1\alpha}$ and PGF_2. In contrast, term patients not in labor had no detectable F_α prostaglandins. In one patient, who had a spontaneous abortion at 16 weeks, the amniotic fluid was similar with respect to prostaglandins to that during labor at term. These prostaglandins were thought to originate in the decidua, since the prostaglandin concentrations there were 10 to 30 times greater than in the amniotic fluid and since human decidua *in vitro* are able to synthesize prostaglandin (246). As with the amniotic fluid, decidua early in gestation contained only a relatively small amount of PGE_1, while at term in labor there were large amounts of $PGF_{1\alpha}$ and $PGF_{2\alpha}$ (600 and 800 ng/g respectively). The uterine secretory endometrium, which forms the menstrual prostaglandins, gives rise to the decidua. These factual observations do not indicate whether amniotic prostaglandins initiate labor or *vice-versa*. Karim (209) proposed that they might constrict umbilical vessels at birth.

V. NERVOUS SYSTEM

A. Prostaglandin release on nerve stimulation

Prostaglandins and prostaglandin-like substances occur in the brain (6, 124, 198, 200, 280) and in superfusates of cat cerebral and cerebellar cortex (124, 265). Spontaneous release from the cat cerebral cortex was 0.05 to 0.5 ng of PGE_1 equivalent/cm^2/min (266). The amount released from the somatosensory cortex can be greatly increased by direct electrical stimulation and is simultaneously evoked from the contralateral cortex. Section of the corpus callosum limits release to the stimulated side only. Furthermore, stimulation of the radial nerve evokes release only from the contralateral cortex. The fact that this release is frequency-dependent, being maximal at 0.25 to 1/sec and minimal at 30 to 100/sec, suggests the involvement of a synaptic pathway. The central stimulants picrotoxin, pentylenetetrazol (leptazol), and strychnine also evoke cerebral release of prostaglandins. From the behavior of the superfusates in bioassay and thin layer chromatography, it was concluded that the major component of the spontaneously released material was $PGF_{2\alpha}$, while the evoked material was a mixture of PGE and PGF_α (265). Spontaneously released prostaglandin from the cat cerebellum also shows PGF_α activity (124). On subfractionation of rat cerebral cortex homogenates, most of the E prostaglandins is in the nerve ending fractions while F prostaglandins are more evenly distributed. Only the lighter microsomes can synthesize prostaglandins (213).

The perfused spinal cord of the frog liberates at least 6 smooth-muscle stimulants, among them substances tentatively identified as PGE_1 and $PGF_{1\alpha}$ (269). PGE_1 is released spontaneously, while $PGF_{1\alpha}$ release is evoked by stimulation of the hind limbs, and by serotonin, dopa, or tranylcypramine but not by epinephrine. After treatment of frogs with tranylcypramine, both spontaneous and evoked release are abolished, a result that suggests that prostaglandin stores might have been depleted.

Prostaglandin release has been associated with events related to nerve activity in several unrelated peripheral tissues. In the cat adrenal, perfused *in situ* with

Locke's solution, $PGF_{1\alpha}$ release is evoked by acetylcholine (268). In perfused rat adrenals, both ACTH and acetylcholine evoke release of prostaglandin-like material (297). Acetylcholine-evoked release in cat adrenals does not occur in the absence of calcium (297). Prostaglandin release is independent of catecholamine release in that both nicotine and the re-introduction of calcium after calcium-free perfusion stimulate only catecholamine release (297). Perfusion of epinephrine or norepinephrine does not evoke prostaglandin release from the cat adrenal (268), nor does intra-arterial injection of PGE_1 stimulate catecholamine release (191). The prostaglandin released from the cat adrenal was probably synthesized or liberated from a bound form, since the prostaglandin content of the gland is only about $\frac{1}{10}$ the amount recovered in the venous effluent and the content of glands stimulated by acetylcholine is about the same as that of unstimulated glands (268). Furthermore, both acetylcholine and ACTH can induce prostaglandin formation in rat adrenal homogenates (297).

In the isolated rat diaphragm-phrenic-nerve preparation, either nerve stimulation or addition of epinephrine or norepinephrine evoke a release prostaglandin-like material and free fatty acids. The major component seems to be PGE_1. By inference, release is associated with activity at the nerve endings, rather than with the response of the diaphragm, since it is neither increased nor decreased by addition of physostigmine, acetylcholine, or tubocurarine (266, 270).

Prostaglandins are released from the isolated rat stomach after vagal or transmural stimulation. This observation was made independently by two laboratories. Bennett *et al.* (30) found little prostaglandin release from unstimulated stomach and mostly PGE_1 released on stimulation. Coceani *et al.* (123) found $PGF_{1\alpha}$ released most abundantly from unstimulated stomach and PGE_2 and $PGF_{2\alpha}$ released on stimulation. A strain difference in rats is the only apparent explanation for this discrepancy at present. Prostaglandin release was blocked by hyoscine (123) and reduced by hexamethonium or tetrodotoxin (30). The released prostaglandins probably came from muscle cells, since stomachs cold-stored for 3 days to allow degeneration of nerve fibers still release prostaglandin (123). A hypothesis was proposed that these prostaglandins originate by biosynthesis from arachidonic acid combined as phospholipid in the cell membranes. This phospholipid predominated in the stomach polyunsaturated lipids, but there was very little free arachidonic acid (123, 252).

Stimulation of the splenic nerve in the dog leads to the appearance of up to 200 ng/ml of PGE_2 in the venous effluent (30, 131). Intravenous or splenic arterial injection of epinephrine or norepinephrine also leads to prostaglandin release. Pretreatment with phentolamine or phenoxybenzamine abolishes both the splenic contraction and the prostaglandin output in response to either stimulation or catecholamine injection.

In isolated rat epididymal fat pads (see section VII), there is a small spontaneous release of prostaglandin-like material and this is elevated severalfold either by nerve stimulation or by addition of catecholamines. Release by nerve stimulation requires glucose in the medium, but release by catecholamines does not (266, 296). The amount of prostaglandin formed may have some physiological signifi-

cance, since as little as 1 ng/ml of PGE_1, which has no effect on spontaneous lipolysis, completely blocks the release of fatty acids during nerve stimulation (71). In this connection, one should note an intriguing preliminary report by Rosell (274) that α-adrenergic blockade potentiates the release of fatty acids *in vivo* during sympathetic nerve stimulation of dog adipose tissue. If one assumes that α-adrenergic blockade prevents evoked prostaglandin release in adipose tissue as in the spleen (30, 131), the increased release could be due to selective removal of an inhibition of fatty acid release by prostaglandins.

Beck *et al.* (see 29) have demonstrated the existence of a sustained dilatation in the hind limb of the dog after sympathetic nerve stimulation in the presence of certain adrenergic nerve-blocking agents (*e.g.*, xylocholine, reserpine, or guanethidine). This sustained dilatation is not mediated by acetylcholine, histamine, adenine nucleotides, or bradykinin and is mimicked by intra-arterial PGE_1. Attempts to identify prostaglandins in the effluent blood were unsuccessful. This sustained dilatation is reduced by intra-arterial infusion of graded doses of PGE_1 to a much greater degree than either the dilatation caused by intra-arterial acetylcholine or the transient, cholinergically mediated dilatation that precedes the sustained dilatation (28). These observations are consistent with the hypothesis that the sustained dilator mediator and PGE_1 occupy the same receptors.

The association of prostaglandin release with nerve activity under so many diverse conditions suggests that it may have some fundamental role in nerve or cell membrane physiology.

B. Actions on the central nervous system

In addition to being present in and released from the central nervous system, prostaglandins themselves have central actions, and there has been speculation about their physiological functions in the central nervous system (200, 201, 265, 295).

In unanesthetized cats, intraventricular injection of PGE_1 in doses ranging from 7 to 20 μg/kg leads to a decrease in spontaneous activity, stupor, and catatonia, complete recovery requiring as long as 48 hours (192). When given intravenously, however, 20 μg/kg of PGE_1 only slightly reduces spontaneous activity. In young chicks, before the development of the blood-brain barrier, intravenous PGE_1, PGE_2 and PGE_3 cause a profound decrease in spontaneous activity. Intraventricular injection of $PGF_{2\alpha}$ in similar doses in cats has no observable effect, but in young chicks intravenous $PGF_{2\alpha}$ causes an immediate tonic extension of the limbs.

By contrast, in spinal or decerebrate cats and chicks, intravenous injection of either PGE_1 or $PGF_{2\alpha}$ leads to an increase in tension of the gastrocnemius muscle which is reversed by sciatic nerve section but not by dorsal root section (196, 199, 201). Doses of prostaglandins below those causing increased muscle tension enhance crossed extensor reflexes in spinal animals. There is no effect upon the patellar reflex. These results imply an action on the spinal cord. The action is presumably stimulation of excitatory pathways, rather than an inhibition of inhibitory pathways, since PGE_1 did not affect inhibition of the crossed extensor

reflex brought about by stretching of the ipsilateral hamstring muscle or by electrical stimulation of the peroneal nerve.

These experiments point to a primary effect on the spinal cord, but the presence of an intact brain prevents muscular effects in cats and allows $PGF_{2\alpha}$ effects to be manifest in chicks. In contrast to the negative results previously reported in anesthetized cats (221), Avanzino *et al.* (25) showed that prostaglandins may have specific actions on higher centers in unanesthetized, decerebrate cats. They applied PGE_1, PGE_2, or $PGF_{2\alpha}$ in the region of individual neurones in the brain stem and evaluated the rate of firing. About ¼ of the neurones tested were affected, and excitation predominated over inhibition. After repeated application, tachyphylaxis occurred in the case of the prostaglandins, but not in the case of acetylcholine or norepinephrine. There was no cross tachyphylaxis among the three prostaglandins, but their effects on the same neurone, if any, were always in the same direction. Acetylcholine also produced either excitation or inhibition, but the direction of its effect was not related to that of the prostaglandins.

VI. CARDIOVASCULAR SYSTEM

A. Acute cardiovascular actions

Blood pressure and cardiac output. PGE_1 uniformly lowers arterial pressure in the dog (42, 65, 114), cat (65, 190), rabbit (52, 194), guinea pig (65), rat (140, 190, 341), mouse (337), and chick (197, 199). For most species, the minimally effective single intravenous doses range between 1 and 10 μg/kg. The depressor action persists after atropine (51, 153), antihistaminic compounds (51), ganglion-blocking agents (103, 114, 140, 228), pretreatment with reserpine (103, 114, 140), and β-adrenergic blocking agents (65, 114, 245).

There appeared to be an antagonism between PGE_1 and catecholamines on blood pressure (42, 307) (*cf.* section II B), but since in dogs receiving continuous infusions of PGE_1 sufficient to lower blood pressure, infusions of norepinephrine or epinephrine were still pressor (114), the antagonism was probably merely summation of opposite effects rather than adrenergic blockade. This conclusion is supported by the fact that infusion of the ganglion stimulating agent dimethylphenylpiperazinium bromide (DMPP) or electric stimulation of the central ends of the cut vagus nerves raises the blood pressure, presumably by increasing sympathetic nerve discharge, in dogs receiving continuous infusion of PGE_1 (114). Furthermore, PGE_1 diminishes pressor responses not only of catecholamines but also of vasopressin and angiotensin (190, 341). In the dog, the heart rate increases with the depressor response, but this is most likely a compensatory reaction through the sympathetic nervous system to lowered blood pressure, since the increase is prevented by β-adrenergic blockade (114, 245).

PGE_1 increases the cardiac output in dogs, either anesthetized (228, 245) or unanesthetized with chronically implanted electromagnetic flowmeters (338), and in unanesthetized rats (341). Since blood pressure falls while cardiac output rises, the primary mechanism of the blood pressure fall is decreased peripheral resistance. In intact dogs, the increase in cardiac output after intravenous PGE_1 is associated with an increase of myocardial contractile force (245). Injection into

a coronary artery increases the contractile force without affecting blood pressure or heart rate. Coronary flow does increase, but an increase in flow alone does not affect the force of myocardial contraction (244).

PGE_2 and PGE_3 are like PGE_1 in their effect on blood pressure but less potent (42, 194). The relative depressor activity of PGE_1, PGE_2, PGA_1 and PGA_2 was determined in anesthetized, vagotomized rats treated with pentolinium, in similarly prepared dogs, and in unanesthetized dogs under basal conditions. These same prostaglandins were also compared for effect on calculated peripheral resistance of unanesthetized dogs with chronically implanted electromagnetic flow-probes (338). Depressor activity of PGAs was greater than that of the corresponding PGEs in dogs but not in rats. Relative activities are given in table 1. Likewise, intravenous infusion of PGA_1 in anesthetized dog produces a greater fall in blood pressure than the same dose of PGE_1 (45).

There were marked differences in sensitivity of individual dogs to prostaglandins. In dogs anesthetized with pentobarbital, the infusion rate of PGE_1 needed to obtain a moderate depressor effect ranged from 0.1 to 1.2 μg/kg/min (114). Likewise, in unanesthetized dogs, the single intravenous dose of PGE_1 required for a 10 to 12 mm Hg fall in blood pressure ranged from 0.56 to 3.2 μg/kg. These differences were consistent for the four prostaglandins compared over the several days required for the test (338). In unanesthetized dogs a blood pressure fall of more than about 25 mm Hg seemed to disturb the dogs and the resulting movement and apprehension counteracted depressor action. This reaction may explain the brief increase in systolic pressure noted by Steinberg and Pittman (304) after large intravenous doses (20 μg/kg) of PGE_1 in unanesthetized dogs.

From these and other experiments it is evident that in dogs the combination of pentobarbital anesthesia, vagotomy, and ganglion blockade by pentolinium increases by at least 20-fold the sensitivity to the depressor action of PGs (338). However, in comparative experiments on the same rats, anesthesia and pentolinium do not change the depressor response to PGE_1 (140), although the lower initial pressure that resulted may have masked increased sensitivity.

The route of administration is of importance for the cardiovascular effects of PGE_1. Intra-aortic infusion produced more pronounced changes than intravenous (42), and infusion into the thoracic aorta was more depressor than infusion into the lower abdominal aorta (114). Uptake and metabolism of PGE_1 in the lungs (16, 163, 184) as well as rapid diffusion from the blood there may explain these findings. Infusion of PGE_1 into a common carotid artery raised blood pressure slightly; and the fact that this effect was reversed after ganglionic blockade suggests reflex sympathetic vasoconstriction (114). However, PGE_1 may also have a central vasomotor effect, since it was pressor when injected intra-arterially into the vascularly isolated, neurally intact head perfused by a donor dog when the carotid sinus and carotid body areas of the recipient had been denervated (207). The failure of Nakano and McCurdy (245) to see such systemic effect after intracarotid injection may have been due to smaller doses (0.1 μg/kg as a single injection *vs.* 5 μg/kg or 0.4 μg/kg/min infusion).

Lee *et al.* (228) studied a rabbit renal medullary lipid "medullin," which sub-

sequently was identified as PGA_2 (section VI C) (229). Its action on arterial pressure and cardiac output in the dog was qualitatively and quantitatively like that of PGE_1.

The cardiovascular actions of $PGF_{2\alpha}$ are complicated by qualitative species variations. It is depressor in the cat and rabbit (13, 195), and pressor in the rat and dog (140), and in the spinal chick (197). In the anesthetized, intact chick, the effect may be pressor, depressor, or biphasic pressor-depressor (199). In the dog and rat (138), and in the rabbit (194, 195) $PGF_{2\alpha}$ is about five times more potent than $PGF_{1\alpha}$. The pressor activity of $PGF_{2\alpha}$ in spinal chicks persists after hexamethonium, phenoxybenzamine, or pronethalol (197, 199), and, in rats, it is augmented by ganglion blockade with pentolinium or by pretreatment with reserpine (140). Thus it is probably not mediated *via* the autonomic nervous system. In unanesthetized dogs, the pressor action of $PGF_{2\alpha}$ is accompanied by an increase in cardiac output and right atrial pressure, but calculated peripheral resistance is essentially unchanged (139, 140).

Peripheral and pulmonary circulation. The effect of a substance on a specific vascular bed is frequently evaluated through changes in blood flow or perfusion pressure after intra-arterial injection. In such experiments, PGE_1 decreases the calculated resistance in the hind limbs of dogs (228, 245, 300), cats (190), rabbits (29) and frogs (156). Carotid, renal and cutaneous blood flow are increased (194, 245). Although less potent than bradykinin and eledoisin on dog femoral flow, it is (on a molar basis) more potent than glyceryl trinitrate, acetylcholine, isoproterenol, or histamine (245, 300). The vasodilator effect is not influenced by atropine, the antihistamine tripelennamine, or the β-adrenergic blocking agent propranolol (245, 300). PGE_2 is about as potent as PGE_1, but PGE_3 is less potent (194).

The activity of $PGF_{2\alpha}$ on the peripheral circulation, as on blood pressure, is also complicated by species variations. In the cat, in which $PGF_{2\alpha}$ lowers blood pressure, it dilates muscle vessels but does not affect the renal vessels (13, 195). In the dog, in which it is pressor, $PGF_{2\alpha}$ has a very different action. Analysis of segmental vascular resistances by the method of Haddy *et al.* (173) revealed little effect on perfusion pressure in the limb of the dog, but a considerable increase in small vein pressure (140, 141). This effect is abolished by denervation and restored by electrical stimulation of the lumbar sympathetic trunk. In the whole animal, $PGF_{2\alpha}$ decreases venous capacitance. This action was clearly demonstrated in a preparation with cardiac output maintained constant by pumping blood from the right atrium to the pulmonary artery. $PGF_{2\alpha}$ still causes an increase in right atrial pressure (with a very slight effect on systemic blood pressure), or, when a pressure stabilizer is put on the venous side, it causes a shift of blood into the stabilizer reservoir (141). Hexamethonium, phenoxybenzamine, or denervation abolishes the effect of $PGF_{2\alpha}$ on the cutaneous veins of the perfused paw, but neither of these drugs prevents $PGF_{2\alpha}$ reduction of venous capacitance in the whole animal. The reason for this discrepancy has not been explained.

The pulmonary vessels likewise show a differential response to PGE_1 and $PGF_{2\alpha}$. PGE_1 decreases pulmonary resistance in the intact dog (235, 245), in the

perfused lobe of the dog lung (138), and in the isolated blood-perfused lungs of cats and rabbits (185). On the other hand, $PGF_{2\alpha}$ increases pulmonary arterial pressure in both cats (13) and dogs (140, 141).

Injection of either PGE_1, PGE_2, PGA_1 or $PGF_{1\alpha}$ into the carotid artery of anesthetized dogs causes a dose-related constriction of the blood vessels of the nasal mucosa and decrease in resistance of the nasal airway (313). PGE_1 and PGE_2 are the most potent, the threshold dose ranging, in different dogs, from 1 to 50 ng. The maximum effect produced by these two compounds is about the same as an equal dose of epinephrine, but their duration of action is more than seven times as long. Nasal mucosa has not been examined for the presence of prostaglandins, but a smooth-muscle stimulating unsaturated fatty acid has been reported in dog and sheep nasal mucosa (319).

An old observation of von Euler (158) of the effect of crude prostaglandin (probably mostly PGE_1) on the portal circulation of cats merits reinvestigation. He found that prostaglandin caused a rise in portal pressure and blanching of the liver. Since clamping of the abdominal aorta (but not the hepatic artery only) prevented the fall in carotid blood pressure after an intravenous injection of prostaglandin, pooling of blood in the portal vessels probably contributed to the depressor action.

Another old observation of von Euler, that prostaglandin increased resistance of human placental vessels (157), becomes more interesting since prostaglandins have been found in human umbilical cord, amniotic fluid, and decidua (208, 209, 211).

Isolated heart and coronary blood flow. Again there are species differences in the reaction of isolated perfused (Langendorff) mammalian hearts. PGE_1 has no effect on contractile force and rate of hearts from the cat (70, 158, 233), and rabbit (70, 156, 233), but increases them in the rat (70, 233, 328), guinea pig (70, 233) and chicken (199). In the frog, heart rate is not increased, but contractile force is (70, 156). In guinea pig hearts, 1 μg of PGE_1 had the same effect as 1 μg of epinephrine (70), and the effects produced were related to the Ca^{++} content of the medium (233). With normal Ca^{++} concentration, PGE_1 increased heart rate and coronary blood flow, but the inotropic responses were inconsistent, whereas in the presence of ½ the usual Ca^{++} concentration PGE_1 consistently increased coronary flow and contractile force. Studies on calcium exchange rate by adding ^{45}Ca to the medium indicate that there is no net effect on the total exchangeable calcium, yet the rate at which tissues come to equilibrium is accelerated. The period of inotropic action roughly parallels the time of more rapid exchange of Ca^{++} (22, 216). When a compound increases rate and force of contraction, there is the possibility that the force effects may be only secondary to rate changes. However, in the frog heart the inotropic action of PGE_1 was not associated with an increased rate (22).

PGA_2, as medullin (section VI C), was without effect on the rabbit heart (228). $PGF_{1\alpha}$, at concentrations as low as 50 ng/ml, increased contractile force of the perfused rat heart without affecting rate or coronary flow (328); but $PGF_{2\alpha}$ had no action on the isolated chicken heart (199). PGE_1 did not affect contraction of

the isolated heart of *Venus mercenaria* (118). In cultured chick hearts, both PGE_2 and $PGF_{2\alpha}$ were inactive (302).

It is unfortunate that the prostaglandins have not been tested in the dog heart-lung preparation, since in the intact dog with a Walton-Brodie strain gauge on the ventricle, intravenous PGE_1 increases the force (245) and $PGF_{2\alpha}$ is without effect (140).

B. Human studies

A short infusion of PGE (0.2 to 0.7 μg/kg/min) over 4 to 10 minutes in 2 persons was reported in 1959 to produce tachycardia, moderate hypotension, and decreased cardiac output (50). The results did not suggest any gross change in total peripheral resistance. PGE_1 given intravenously at 0.1 to 0.2 μg/kg/min to 3 healthy subjects for 20 minutes (39, 40) increased the heart rate about 20 beats/min, while systolic, diastolic, and mean arterial pressure remained unchanged. In more detailed studies with heart catheterization, PGE_1 was infused intravenously for 30-minute periods to 8 normal men at doses that were increased stepwise from 0.058 to a maximum of 0.58 μg/kg/min. Up to 0.1 μg/kg/min the peripheral resistance decreased (average 61 %), cardiac output increased (average 60 %), and there was an increase in both heart rate (average 27 beats/min) and stroke volume average 19 %). Average mean brachial artery pressure decreased 9 mm Hg. The oxygen uptake increased 15 % (107). The circulatory changes induced by 0.058 to 0.1 μg/kg/min of PGE_1 in normal man are thus like the condition seen with arteriovenous shunts, cardiac output being elevated in relation to oxygen consumption. The increased heart rate may be due to sympathetic stimulation (114) or to a direct effect of PGE_1 on the heart (245). The increase in stroke volume in the presence of an increased heart rate reflects an increased venous return (107). Infusion of doses higher than 0.1 μg/kg/min further increased the heart rate and decreased the mean arterial pressure and stroke volume (107).

During infusion of PGE_1, right ventricular systolic pressure remained unchanged while the end-diastolic pressure decreased in spite of the increased stroke volume, cardiac output, and heart rate (107). This suggests a change in the myocardial function curve as was observed in dogs after single injections of PGE_1 (245).

Peripheral blood flow in the human limbs was increased by intra-arterial infusion of PGE_1. Measured by venous occlusion plethysmography, brachial artery infusion of as little as 0.01 ng/kg/min doubled blood flow and 1 ng/kg/min increased blood flow 10-fold (73). Likewise, infusion of 1 ng/kg/min into the femoral artery markedly increased femoral venous oxygen content without changes in heart rate or blood pressure (108). These observations suggest an increased blood flow in human limbs as seen after single injections in dogs (245).

The effect of PGE_2, PGA_1, $PGF_{1\alpha}$, $PGF_{1\beta}$ and $PGF_{2\alpha}$ in normal man was studied by intravenous infusion of 0.1 to 0.58 μg/kg/min for 30-minute periods. PGE_2 increased heart rate but to a lesser extent than PGE_1, and PGA_1 caused only a slight increase. Neither PGE_2 nor PGA_1 affected blood pressure. $PGF_{1\alpha}$, $PGF_{1\beta}$ and $PGF_{2\alpha}$ had no effect on these parameters (109).

C. Renal vasodepressor lipids

Crude lipid extracts of the medullary portion of the kidney of the dog, pig (188, 240), rabbit (188, 230, 312), and rat (188) and of man (188, 240, 312) have an acute vasodepressor action. The actions of these lipids are compatible with those of PGE_1 or PGE_2. PGE_1 has been reported present in rabbit renal medullary extracts (228, 312), but more recent work indicates that PGE_2 is the major vasodepressor lipid of the rabbit kidney (130). The isolation procedure used in one of the studies reporting PGE_1 (312) would not differentiate PGE_1 from PGE_2 (172). The name "medullin" was applied to a lipid extracted from rabbit kidney which was subsequently identified as PGA_2 (228, 229). This PGA_2 apparently was formed by acid conversion of PGE_2 to PGA_2 during the extraction process (312).

Experimental hypertension. Muirhead and co-workers (240, 242) have prepared a neutral renal lipid from hog kidney that was without acute depressor action but, when given by mouth, suppressed renoprival hypertension in the dog. This lipid, even though still impure, was effective against dog renoprival hypertension at doses of less than 4 μg/kg/day and decreased blood pressure when given daily by mouth after about a 10-day latent period in renal hypertension in the dog (239) and rabbit (241). Because of the close association of prostaglandins with these neutral renal lipids, certain prostaglandins were tested under blind code in canine renoprival hypertension. PGE_1, PGA_2 and $PGF_{2\alpha}$ were not tested sufficiently for definitive conclusions, but PGE_2, PGA_1 and $PGF_{1\alpha}$ were active (242). The doses used, in μg/kg/day orally, were PGE_2 15 to 29, PGA_1 5 to 100, and $PGF_{1\alpha}$ 15 to 30.

In contrast to the neutral renal lipids, PGA_1 has not shown clear activity in experimental renal hypertension. Muirhead *et al.* (242) reported a suggestion of activity in canine renal hypertension, but further testing failed to confirm this impression (238). Contrary to one preliminary report (187), PGA_1, in single daily doses up to 0.32 mg/kg intraperitoneally for 2 weeks, did not lower blood pressure in rats with renal hypertension (337). PGE_2, in massive daily doses up to 3 mg/kg intramuscularly, lowers blood pressure in rat renal hypertension (243). Whether this action is related to the anti-renoprival action in the dog is conjectural.

PGE_1, infused into the renal artery, did not affect renin release from the dog kidney (323).

Renal function. When subhypotensive doses of PGE_1 are infused directly into one renal artery of anesthetized dogs, urine flow, sodium excretion, and free water clearance increase, glomerular filtration rate remains unchanged, and *p*-aminohippurate extraction decreases (204, 323). The results are the same whether prostaglandin is given during mannitol diuresis, vasopressin infusion, or water loading. There were no effects on the contralateral kidney, blood pressure or heart rate. Threshold effects were evident in some dogs at 20 ng/min. PGA_1 was infused intravenously to dogs during mannitol, vasopressin and desoxycorticosterone infusion (115). The results are not strictly comparable because blood pressure and glomerular filtration rate were decreased, but PGA_1 still caused an increased blood flow and decreased TcH_2O (sometimes progressing to free water clearance). It is tempting to attribute these diuretic effects to an inhibition of the action of

vasopressin, as seen in the isolated toad bladder (251) and in the isolated perfused collecting tubules of the rabbit kidney (249). However, these same effects are produced by a variety of chemically unrelated vasodilators (see 204 for references). Redistribution of renal blood flow and an inhibition of tubular sodium reabsorption are adequate alternate explanations. Since prostaglandins occur in the kidney and are effective in very small amounts, they may serve in the intrarenal regulation of sodium and water excretion.

VII. RELATIONSHIPS TO LIPID AND CARBOHYDRATE METABOLISM

A. Metabolism of free fatty acids

In the last decade the metabolism of plasma free fatty acids (FFA) has been elucidated. FFA are continuously mobilized from adipose tissue into blood plasma and transported to various tissues, where they are esterified and eventually oxidized to generate energy. The mobilization of FFA from adipose tissue is a complex process regulated by a variety of hormonal, nervous, and nutritional factors. It was against this background of fundamental interest that the inhibition of the mobilization of FFA by PGE_1 was demonstrated *in vitro* (306) and *in vivo* (41, 306). The metabolism and physiology of FFA has recently been reviewed extensively (271).

Studies in vitro in adipose tissue. Steinberg *et al.* (306, 307) first showed that PGE_1 reduces the release of glycerol into the medium during incubation of rat epididymal fat. Since glycerol release measures the rate of lipolysis independent of the rate of re-esterification (271), this finding indicates that PGE_1 inhibits the breakdown of triglycerides (figure 3). Inhibition by PGE_1 of nonstimulated (basal) glycerol release from adipose tissue of fed rats has been repeatedly confirmed (38, 45, 67, 78, 79, 101–103, 220, 232, 253, 305, 308, 309, 326). PGE_1 is one of the most potent inhibitors of lipolysis known: as little as 1 ng/ml reduces basal glycerol release and 100 ng/ml inhibits it maximally (102). Several authors have reported that in adipose tissue from fasted rats PGE_1 does not inhibit lipolysis (38, 102, 308, 309). Re-feeding fasted rats with glucose restores the sensitivity to PGE_1 in 1 hour (105). Yet PGE_1 inhibits lipolysis in isolated parametrial fat cells from fasted rats (161), and Kupiecki (222) found that PGE_1, in comparable concentrations, inhibits lipolysis when added to adipose tissue from fasted rats and that in tissue taken from fasted rats pretreated with PGE_1 the FFA concentration is decreased. Haessler and Crawford (176) recently reported that in fat from fasted rats the inhibition by PGE_1 of both basal lipolysis and lipolysis stimulated by epinephrine was dependent upon the presence of glucose in the incubation medium; but Bergström and Carlson (38) used a glucose-containing medium and found PGE_1 to be ineffective. Perhaps differences between strains of rat may explain these discrepancies.

PGE_1 reduces lipolysis in rat adipose tissue treated with a variety of lipolytic hormones, such as epinephrine and norepinephrine (76, 79, 102, 161, 306, 307, 309, 310), ACTH and TSH (161, 306, 307), tri-iodothyronine (232), glucagon (306), and growth hormone (161, 76, 79). As with basal lipolysis, PGE_1 is a potent inhibitor: 3.2 ng/ml significantly inhibits the effect of 100 ng/ml of epinephrine

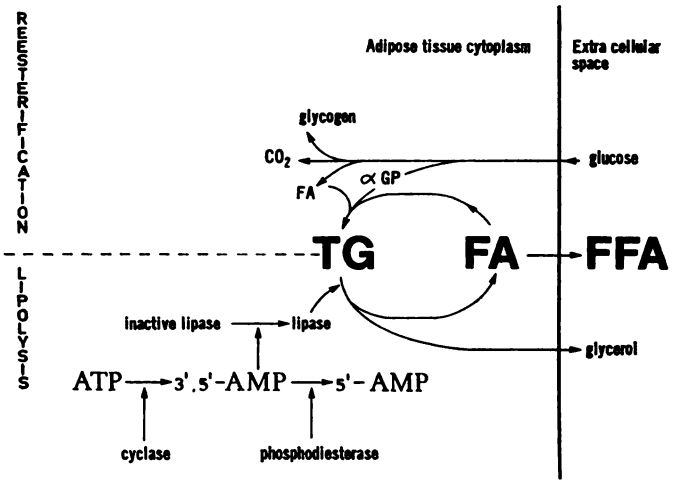

FIG. 3. Schematic illustration of lipolysis and re-esterification in adipose tissue, the two major processes that regulate the mobilization of free fatty acids. The effect of PGE_1 on these processes is reviewed in the text. FA, fatty acid; FFA, free fatty acid; TG, triglyceride; α-GP, α-glycerophosphate; 3′,5′-AMP, 3′,5′-adenosine monophosphate.

and the maximal effect (41 % inhibition) occurs at 100 ng/ml (340). The antagonism of PGE_1 and catecholamines has been described as competitive (161), noncompetitive (237) and mixed, the noncompetitive component being more prominent in a medium with ½ the usual calcium (162).

FFA release evoked by electrical stimulation of the sympathetic nerves to the rat epididymal fat pad *in vitro* was abolished by 1 ng/ml of PGE_1 (71). Since prostaglandin release from the fat pad can also be evoked by nerve stimulation in comparable concentrations (266, 298) (section V A), a physiological role as feedback regulation of FFA mobilization is suggested. It must be recognized, however, that rat adipose tissue has been studied only in the epididymal fat pad.

The basal release of glycerol from excised human subcutaneous adipose tissue is reduced by 10 ng/ml but not 1 ng/ml of PGE_1 (101), and 1 μg/ml reduces lipolysis stimulated by norepinephrine (36). Recently, the responses of human subcutaneous and omental adipose tissue to PGE_1 and norepinephrine were compared (111). PGE_1 at 1 μg/ml depressed norepinephrine-stimulated glycerol release in tissue from both sites but at 100 ng/ml it reduced basal glycerol release from omental fat in only 3 of 13 cases compared to 9 of 11 for subcutaneous tissue. Possibly prostaglandin-sensitive lipolytic systems are more active under basal conditions in the subcutaneous tissue.

Surprisingly, little has been done *in vitro* on adipose tissue from species other than man and the rat. In rabbit omental and perirenal adipose tissue, PGE_1 has no effect on basal glycerol release but reduces lipolysis stimulated by norepinephrine or ACTH (113).

The antilipolytic potencies of several other prostaglandins have been determined on rat adipose tissue. PGA_1 is virtually inactive (45, 129, 338). $PGF_{1\alpha}$ and

$PGF_{2\alpha}$ are much weaker than their PGE analogs (83, 105, 307) and $PGF_{1\beta}$ is completely inactive (83, 85, 307). The results of a parallel-line assay comparing prostaglandins E_1, E_2, A_1 and A_2 are given in table 1. PGE_2 is almost 3 times more potent than PGE_1. Of several other prostaglandin analogs tested, only *dihomo* PGE_1 (the C-22 analog) has activity roughly equal to that of PGE_1 (104).

Studies in vivo. Continuous infusion of norepinephrine in anesthetized dogs raises plasma FFA and glycerol by mobilizing them from adipose tissue. Either a single intravenous injection or continuous infusion of PGE_1 lowers elevated plasma FFA and glycerol in such dogs (41, 42); the result suggests an effect on lipolysis in adipose tissue. Studies with labeled palmitate showed that PGE_1 inhibited the movement of FFA into the plasma. Likewise, PGE_1 inhibits the effect on FFA mobilization of either epinephrine or the sympathetic ganglion stimulant dimethylphenylpiperazinium bromide (DMPP) (43, 44, 307). In rats, PGE_1 infusion reduces the elevated FFA induced by norepinephrine, ACTH, or exposure to cold (67). These results are compatible with those obtained *in vitro* on the inhibition of catecholamine lipolysis by PGE_1 in adipose tissue.

The picture became more complex when the effect of PGE_1 was studied on unstimulated FFA mobilization. A relatively large single parenteral dose or the intravenous or intra-aortic infusion of PGE_1 lowers plasma FFA in rabbits (77, 78, 80), guinea pigs (68) and rats (67, 68, 222). However, the intravenous infusion of the relatively small dose of 0.2 μg/kg/min into man or the dog increases plasma glycerol and FFA (39, 40, 43, 44). In man, this dose of PGE_1 only slightly reduces the elevation in glycerol and plasma FFA induced by simultaneous norepinephrine infusion (39, 40). However, when PGE_1 is infused in dogs in larger doses, comparable to those used to inhibit the effects of norepinephrine infusion (0.4 to 1.6 μg/kg/min), plasma glycerol and FFA decrease (44). A ganglion blocking agent prevents the increase in plasma FFA induced by low doses of PGE_1 (44). This implies that the increase was secondary to activation of the sympathetic nervous system by lowered blood pressure. Indeed, nitroglycerine and a variety of unrelated hypotensive drugs also increase plasma FFA in the dog (287, 304). In the same dose in unanesthetized dogs, PGE_1 lowered but PGA_1 raised plasma FFA (304). PGA_1 has no antilipolytic effect but is at least equal to PGE_1 as a vasodepressor agent. In fasted (but not in fed) rats, intravenous PGE_1 had a biphasic effect on plasma FFA: first a fall, followed by a rise, and then return to normal after 30 minutes (222). It was suggested that in rats, contrary to dogs (42, 304), the antilipolytic action was brief compared to sympathetic activation.

All of these studies suggest that intravenous PGE_1 acts by two independent mechanisms (44, 102): 1) at low doses stimulation of FFA mobilization by way of the sympathetic nervous system, probably as a compensatory reaction to lowered blood pressure; and 2) at high doses reduction of FFA mobilization by inhibition of lipolysis enough to overcome the sympathetic effect.

The actions of other prostaglandins *in vivo* are what would have been expected from their effects *in vitro*. Single injections of PGE_2 and PGE_3 lowered norepinephrine-induced elevation of plasma FFA in dogs and $PGF_{1\alpha}$ was without effect (42). PGA_1 either raised or did not change plasma FFA (45, 304). In man,

infusion of PGE_2 (0.056 to 0.32 μg/kg/min for 30 min) tended to raise FFA as in the case of PGE_1, while the same doses of PGA_1, $PGF_{1\alpha}$ and $PGF_{1\beta}$, and $PGF_{2\alpha}$ had no clear effect (106).

Mechanism of action. The two major processes that regulate FFA mobilization are lipolysis and re-esterification, as outlined in a simplified form in figure 3. Several lipases may participate in the lipolytic process, but a triglyceride lipase called "the hormone-sensitive lipase" appears to be rate-limiting (327). Available evidence indicates an important role of cyclic 3′,5′-AMP in mediating the lipolytic actions of catecholamines and other agents by activating this lipase (see 87, 272). Glucose metabolism has a key position in the control of re-esterification by providing the obligatory α-glycerophosphate, which cannot be formed from glycerol in adipose tissue (figure 3). When considering the site of action of PGE_1, one must recognize that effects on the lipase system may have secondary effects on re-esterification and *vice versa*.

Since PGE_1 opposes the catecholamine-induced elevations of plasma FFA and glycerol in a parallel fashion in intact dogs (42), an action on the lipolytic process appeared reasonable. PGE_1 decreases the epinephrine-induced activation of the lipase of rat adipose tissue (307), and in rats treated with PGE_1 the basal rate of lipolysis of their isolated adipose tissue is reduced (222). Epinephrine increases the accumulation of cyclic AMP in adipose tissue *in vitro* (84), and this increase is inhibited by PGE_1 in concentrations that also inhibit lipolysis (85, 87). These data do not show whether the PGE_1 reduces the formation of cyclic AMP by inhibition of the cyclase or accelerates its destruction by stimulating the phosphodiesterase (see figure 3). Since PGE_1 does not inhibit stimulation of lipolysis by either cyclic AMP itself or its dibutyryl derivative, it probably acts before activation of the lipase system by cyclic AMP (305).

Theophylline stimulates lipolysis presumably by inhibiting phosphodiesterase (86). PGE_1 inhibits theophylline-induced lipolysis in rat adipose tissue (237, 301, 305), apparently in a competitive manner (237). At high concentrations of theophylline, PGE_1 may be relatively ineffective, either because of the competitive nature of the inhibition or because in the presence of virtually complete inhibition of phosphodiesterase the rate of formation of cyclic AMP is no longer rate-limiting (303, 305). At equivalent concentrations, PGE_1 inhibits theophylline-induced lipolysis to a greater degree than norepinephrine and also the maximum inhibition is greater (237).

Vasopressin is considered to have its actions by way of cyclic AMP (250). PGE_1 inhibits the lipolysis induced by vasopressin in rat adipose tissue (325) and the decrease of water permeability induced by vasopressin in isolated collecting tubules of the rabbit (249) and in the toad bladder (251). In the latter tissue PGE_1 inhibits the change of permeability caused by theophylline but not that caused by cyclic AMP itself. Theophylline stimulation on the toad bladder is extremely sensitive to PGE_1 inhibition, significant activity being detected at 0.06 ng/ml (1.7×10^{-10} M) (251).

In whole epididymal fat tissue of the rat, PGE_1 inhibits both the increase in lipolysis and the increase in tissue cyclic AMP induced by epinephrine. Para-

doxically, PGE_1 alone increases cyclic AMP to levels which would have maximally activated lipolysis; nevertheless there is no effect on lipolysis (83, 84). In isolated fat cells, PGE_1 similarly inhibits the epinephrine-induced increase in cyclic AMP but has no effect when added alone. This result implies that some other type of cell in the fat pad was responsible for the increased cyclic AMP. Indeed, PGE_1 also increases cyclic AMP levels in isolated rat lung, diaphragm and spleen, but does not decrease the epinephrine effect in these tissues. The significance of this action of PGE_1 on cyclic AMP is not yet known.

Intravenous heparin in intact rabbits releases lipoprotein lipase into plasma, and this leads to elevated plasma FFA and glycerol. Simultaneous infusion of PGE_1 antagonizes only the rise in FFA, but does not affect the rise in glycerol (80). PGE_1 does not affect lipoprotein lipase activity of human or rabbit plasma (74, 80). This selective action on FFA was interpreted as stimulation of re-esterification in tissues by PGE_1, leading to an increased efflux of FFA from plasma (80). It has been reported that in isolated rat adipose tissue, PGE_1 does not affect incorporation of ^{3}H-palmitate (301); this result would imply that there was no effect on re-esterification, but there is also a report that re-esterification was promoted (176).

B. Deficiency of essential fatty acids

Since the precursors of prostaglandins are essential fatty acids (EFA), animals deficient in EFA might also become deficient in prostaglandins. This lack of prostaglandin, by removing a natural inhibitor to lipolysis, could result in chronic hypermobilization of FFA. In line with this hypothesis, EFA deficient rats have elevated plasma FFA (37, 132, 223) [but one observer reported no elevation (253)], the rise in their plasma FFA after subcutaneous norepinephrine is greater (253), and their adipose tissue *in vitro* has an elevated basal lipolytic rate (37, 253). Also basal lipolysis *in vitro* is not inhibited by a concentration of PGE_1 which inhibits it in normal tissue (37), and liver lipids are elevated in both deficient rats and deficient mice (4, 37, 132, 223). Norepinephrine stimulation of lipolysis *in vitro* in adipose tissue from deficient rats is inhibited by PGE_1 (37).

However, the overt signs of EFA deficiency (decreased growth rate, scaly tail and feet, increased water permeability of the skin) were not influenced in EFA deficient rats or mice by PGE_1 either by mouth or intravenously (up to 1 mg/kg/day for 8 weeks by continuous intravenous infusion) (133, 168, 223, 224). Similarly, a 4-week continuous intravenous infusion of a mixture of PGE_2 and $PGF_{2\alpha}$ (0.5 mg/kg/day of each) was without effect on the dermal signs in EFA deficient rats (223).

C. Metabolism of carbohydrate

Blood glucose. PGE_1, given by either single injection or infusion, elevates blood sugar in dogs (44, 287), rabbits (78, 253), rats, and guinea pigs (67–69). Since this hyperglycemia does not occur in rats after adrenal demedullation, it is probably mediated by a reflex release of adrenal epinephrine in response to lowered blood pressure. Several hypotensive drugs cause hyperglycemia in dogs

(287). In dogs given a catecholamine or the ganglion stimulant DMPP, PGE_1 blocks the rise in plasma fatty acids but has no effect upon the simultaneous hyperglycemia (42, 43, 304). It further increases theophylline-induced hyperglycemia in the rabbit (253).

Insulin-like actions. PGE_1 not only affects the lipolytic process in adipose tissue but also influences some aspects of glucose metabolism in a way similar to insulin. In isolated rat adipose tissue, PGE_1 stimulates glucose uptake, glucose oxidation, and triglyceride synthesis from both glucose and acetate (75, 76, 79, 176, 326). As with insulin, the increased incorporation of label is primarily into the fatty acid part of the triglycerides (75, 76, 176, 326). PGE_1 given to intact rats at 10 μg/kg intraperitoneally increases the incorporation of labeled glucose into the glycogen of the diaphragm and of epididymal fat (76). *In vitro*, PGE_1 stimulates glycogen synthesis in rat adipose tissue, but it does not affect glucose uptake by the diaphragm; PGA_1 had neither of these effects (326). Intravenous infusion of PGE_1 in anesthetized dogs did not change the insulin-like activity of blood, even though in this study blood glucose as well as FFA decreased (235). This effect on blood glucose is contrary to previous studies in dogs (43, 44) and man (106). It is not known if these effects on glucose metabolism reflect primary actions of PGE_1 or if they occur secondarily as a result of inhibited lipolysis and a lowered intracellular fatty acid pool.

D. Other aspects

Adipose tissue from rats with experimental hypothalamic obesity fails to release FFA on stimulation with epinephrine *in vitro* (174). The adipose tissue from such rats contains an inhibitor with some properties similar to those of the prostaglandins (175). This finding is of especial interest in that prostaglandins are also normally found in adipose tissue and are released upon either nerve stimulation or addition of catecholamines (296, 298).

PGE_1 reduces lipolysis in adipose tissue from alloxan diabetic rats *in vitro* (309) (see also section VII A). Another observation possibly related to diabetes is that the sensitivity of rat adipose tissue *in vitro* to inhibition of basal lipolysis by PGE_1 decreases with increasing age of the animals (110).

Although PGE_1 seems neither to inhibit adipose tissue lipase itself (305), nor to affect plasma lipoprotein lipase (74, 80), intravenous infusion of PGE_1 in rats and guinea pigs increased the activity of cardiac lipoprotein lipase (68) and cardiac phosphorylase (255). It was suggested this effect might have been a response to a simultaneous reduction in plasma FFA.

A 2-week infusion of PGE_1 (1 mg/kg/day) to unanesthetized rats had no effect on the baseline lipolysis of their epididymal fat tissue *in vitro*, but the lipolysis induced by epinephrine was significantly greater than in controls (223). Limited tests showed an even greater effect after an 8-week infusion (224).

VII. MISCELLANEOUS

Clinical effects of prostaglandin infusions. Relatively large intravenous infusions (0.2 to 0.7 μg/kg/min) of PGE_1 cause flushing of the face, headache, and an

oppressive feeling in the chest (50). Recently, observations were made on a total of 11 healthy male volunteers with smaller rates of infusion (39, 103, 106). Whereas infusion of 0.03 μg/kg/min had no effect, at 0.05 or 0.1 μg/kg/min flushing of the face appeared in all subjects, and most reported a headache of a pulsating character. Intermittent abdominal cramps occurred at infusion rates of 0.1 to 0.3 μg/kg/min. The commonest reason to stop the infusion was this cramping, and headache was second. The symptoms disappeared within 15 to 30 minutes after stopping the infusion. One subject complained of seeing flashes of light and then getting a unilateral headache. Similar visual symptoms were described by another subject. Although these symptoms resembled migraine, none of the subjects had any history of this disease.

Oxygen consumption increased about 15% with 0.1 μg/kg/min of PGE_1, and with higher infusion rates to an average of 42%. Total ventilation increased in all subjects and in 3 respiratory alkalosis developed. These observations are in accord with the increased respiration and oxygen consumption observed in anesthetized dogs (235).

Acute and chronic toxicity. Because of the limited amounts of these compounds and their relatively low toxicity, few acute toxicity studies have been reported. In mice the intraperiteonal LD50 for PGE_1 was about 70 mg/kg with deaths being delayed several hours. Mice survived 100 mg/kg of PGA_1 intraperitoneally. For both prostaglandins, only loss of the righting reflex was noticed (337). Of 6 rats that received 100 mg/kg by intravenous infusion over periods ranging from 32 to 86 minutes, 4 died between 80 minutes and 24 hours later. Abnormal electrocardiographic changes were not observed; the outstanding signs were prostration (but the rats responded to pinching), a precipitous fall in blood pressure to 25 to 40 mm Hg, and some cyanosis in the feet and ears (339).

Continuous intravenous infusion of 3 mg/kg/day of PGE_1 to unanesthetized unrestrained rats led to a swelling of the hind feet after about 3 days. This swelling subsided a day or two after stopping the infusion. Neither PGA_1 nor $PGF_{2\alpha}$ at the same dose caused this reaction after 7 days of infusion (339).

Blood platelets and coagulation. Platelet-to-glass adhesiveness and platelet aggregation induced by ADP, ATP, serotonin, thrombin, or connective tissue extract are inhibited by PGE_1 in pig, rat, and human plasma (152, 218). PGE_1 disperses aggregates induced by ADP but not those induced by collagen, even though it inhibits the formation of the latter (116). PGE_1 suppresses platelet thrombus formation in injured cerebral arteries in rabbits after single intravenous injections of 5 to 30 μg/kg or infusion of 0.4 to 1.6 μg/kg/min (152). A massive dose (2 mg/kg intravenously) in rats prevents platelet aggregation for at least 30 minutes; lower doses for a shorter time (116). Intravenous infusion of 0.05 to 0.1 μg/kg/min for 30 minutes to 3 healthy human subjects had no apparent effect on platelet aggregation (112).

In his initial study, Kloeze (218) reported that PGE_2, in contrast to PGE_1, stimulated platelet aggregation at about the same concentration. Chandrasekhar (117), using both the revolving plastic loop technique (299) and an optical density method similar to that used by Kloeze, found PGE_2 to be qualitatively like

PGE_1. The differences between the two laboratories are yet to be resolved. Relative potencies are given in table 1.

Although Emmons *et al.* (152) reported that PGE_1 did not affect recalcification time in whole rabbit blood, Ferri *et al.* (164) found that PGE_1 restored coagulation time to normal in a calcium deficient system.

Other. PGE_1 increases the short circuit current in the frog skin; this effect implies a stimulation of sodium ion transport (162). Intradermal injection of PGE_1 increases capillary permeability to pontamine sky blue in guinea pigs but has no effect on production of pain in the human blister base (191). PGE_1 (10 μg/ml) does not affect ciliary activity of rabbit trachea (193). PGE_1 or PGB_1, but not $PGF_{1\alpha}$, in cultured skin of the chick embryo, blocks development of the down feather organ and stimulates epidermal proliferation and keratizination (215). PGE_1 and $PGF_{1\alpha}$ in low (0.04 to 0.4 μg/ml) but not high (40 μg/ml) concentrations inhibited protein synthesis in a cell-free system (275).

IX. SUMMARY

The prostaglandins are a family of lipids, originally discovered over 30 years ago in human seminal fluid, which have since been found not only to have a wide variety of striking pharmacological actions, but also to be present in many if not all mammalian tissues. They have an unusual chemical structure, being 20-carbon fatty acids derived enzymically from the essential fatty acids by cyclization and oxidation. Converting enzymes have been demonstrated in many tissues; they are especially active in the vesicular glands of the sheep, which are used for a practical method of biosynthesis.

The individual prostaglandins differ among themselves both qualitatively and quantitatively. Prostaglandins have a wide spectrum of biological action: They are smooth muscle stimulants, depressor peripheral vasodilators (except the PGFs which are pressor and venoconstrictor in dogs), and inhibitors of lipolysis, platelet aggregation and gastric secretion. In these areas, they are among the most potent compounds known, activity being present in some systems at concentrations of 0.01 ng/ml *in vitro*, and activity of 10 ng/kg *in vivo*. Prostaglandin formation and release is brought about by nerve activity, both central and peripheral. Their presence in biologically large concentrations in menstrual fluid and amniotic fluid at term is intriguing.

Physiological roles for these recently rediscovered compounds are yet to be established, but whenever substances are found in tissues which in very small doses can affect the function of these tissues, there is the possibility that they are regulators of physiological activity. Each effect of one or another prostaglandin suggests a corresponding physiological role, whether stimulatory or inhibitory, on such systems as smooth muscle, nerves, the circulation, and the reproductive organs. In the last named, roles in relation to fertility and coitus and later possible action in relation to labor and postpartum uterine contraction have been proposed. Prostaglandins liberated by nerve stimulation, which then have actions opposite to that of the nerve stimulation, suggests a role as feed-back inhibitors. Thus, sympathetic nerve stimulation to adipose tissue induces both lipolysis and

the release of antilipolytic prostaglandins, and vagal stimulation to the stomach, both secretion and the release of prostaglandins with powerful antisecretory actions. On the other hand, the ability of minute amounts of certain prostaglandins, inactive in their own right, to potentiate other agonists, suggests a more general role on ion transport or membrane function.

REFERENCES

1. Abrahams, O. L. and Hawkins, D. F.: Lipid-soluble uterine muscle stimulants in human amniotic fluid. J. Obstet. Gynaecol. Brit. Commonw. 74: 235–246, 1967.
2. Abrahamsson, S., Bergström, S. and Samuelsson, B.: The absolute configuration of prostaglandin $F_{2\alpha-1}$ Proc. Chem. Soc. 332, 1962.
3. Adamson, U., Eliasson, R. and Wiklund, B.: Tachyphylaxis in rat uterus to some prostaglandins. Acta Physiol. Scand. 70: 451–453, 1967.
4. Allman, D. W. and Gibson, D. M.: Fatty acid synthesis during early linoleic acid deficiency in the mouse. J. Lipid Res. 6: 51–62, 1965.
5. Ambache, N.: Further studies on the preparation, purification and nature of irin. J. Physiol. (London) 146: 255–294, 1959.
6. Ambache, N.: Irin and a hydroxy-acid from brain. Biochem. Pharmacol. 12: 421–428, 1963.
7. Ambache, N.: Biological characterisation of, and structure-action studies on, smooth-muscle-contracting hydroxy-acids. Mem. Soc. Endocrinol. 14: 19–28, 1966.
8. Ambache, N., Brummer, H. C., Rose, J. G. and Whiting, J.: Thin-layer chromatography of spasmogenic unsaturated hydroxy-acids from various tissues. J. Physiol. (London) 185: 77P–78P, 1966.
9. Ambache, N., Brummer, H. C., Whiting, J. and Wood, M.: Atropine-resistant substances in extracts of plexus-containing longitudinal muscle (PC-LM) from guinea-pig ileum. J. Physiol. (London) 186: 32P–33P, 1966.
10. Ambache, N. and Lessin, A. W.: Classification of intestinomotor drugs by means of type D botulinum toxin. J. Physiol. (London) 127: 449–478, 1955.
11. Änggård, E.: The isolation and determination of prostaglandins in lungs of sheep, guinea pig, monkey and man. Biochem. Pharmacol. 14: 1507–1516, 1965.
12. Änggård, E.: The biological activities of three metabolites of prostaglandin E_1. Acta Physiol. Scand. 66: 509–510, 1966.
13. Änggård, E. and Bergström, S.: Biological effects of an unsaturated trihydroxy acid ($PGF_{1\alpha}$) from normal swine lung. Acta Physiol. Scand. 58: 1–12, 1963.
14. Änggård, E., Gréen, K. and Samuelsson, B.: Synthesis of tritium-labeled prostaglandin E_2 and studies on its metabolism in guinea pig lung. J. Biol. Chem. 240: 1932–1940, 1965.
15. Änggård, E. and Samuelsson, B.: Smooth muscle stimulating lipids in sheep iris. The identification of prostaglandin $F_{2\alpha}$. Biochem. Pharmacol. 13: 281–283, 1964.
16. Änggård, E. and Samuelsson, B.: Metabolism of prostaglandin E_1 in guinea pig lung: The structure of two metabolites. J. Biol. Chem. 239: 4097–4102, 1964.
17. Änggård, E. and Samuelsson, B.: The metabolism of prostaglandin E_2 in guinea pig lung. Biochemistry (N. Y.) 4: 1864–1871, 1965.
18. Änggård, E. and Samuelsson, B.: Biosynthesis of prostaglandins from arachidonic acid in guinea pig lung. J. Biol. Chem. 240: 3518–3521, 1965.
19. Änggård, E. and Samuelsson, B.: Purification and properties of a 15-hydroxyprostaglandin dehydrogenase from swine lung. Arkiv Kem. 25: 293–300, 1966.
20. Änggård, E. and Samuelsson, B.: The metabolism of prostaglandins in lung tissue. *In* Prostaglandins, Proc. 2nd Nobel Symp., Stockholm, June 1966, ed. by S. Bergström and B. Samuelsson, pp. 97–106, Almqvist and Wiksell, Stockholm; Interscience, New York, 1967.
21. Änggård, E. and Samuelsson, B.: Unpublished observations, cited in ref. (19).
22. Arienti, S., Piccinini, F. e Pomarelli, P.: Prostaglandina E_1 (PGE_1) escambi del calcio a livello della cellula miocardica. Boll. Soc. Ital. Biol. Sperim. 43: 521–524, 1967.
23. Asplund, J.: A quantitative determination of the content of contractive substances in human sperm and their significance for the motility and vitality of the spermatozoa. Acta Physiol. Scand. 13: 103–108, 1947.
24. Asplund, J.: Some preliminary experiments in connection with the effect of prostaglandin on the uterus and tubes *in vivo*. Acta Physiol. Scand. 13: 109–114, 1947.
25. Avanzino, G. L., Bradley, P. B. and Wolstencroft, J. H.: Actions of prostaglandins E_1, E_2 and $F_{2\alpha}$ on brain stem neurones. Brit. J. Pharmacol. Chemotherap. 27: 157–163, 1966.
26. Baker, J. L., Clitherow, H. J. and Pickles, V. R.: The menstrual stimulant in primary dysmenorrhoea. J. Endocrinol. 25: i–ii, 1962.
27. Bass, P. and Bennett, D. R.: Local chemical regulation of motor action of the bowel—substance P and lipid soluble acids. *In* Handbook of Physiology, section 6, Alimentary canal, ed. by C. F. Code, American Physiological Society, Washington, D. C., in press.
28. Beck, L. and Pollard, A. A.: Selective reduction of sustained dilatation by prostaglandin E_1 and phenoxybenzamine. Proc. Can. Federation Biol. Sci. 10: 23, 1967.
29. Beck, L., Pollard, A. A., Kayaalp, S. O. and Weiner, L. M.: Sustained dilatation elicited by sympathetic nerve stimulation. Federation Proc. 25: 1596–1606, 1966.

30. Bennett, A., Friedmann, C. A. and Vane, J. R.: The release of prostaglandin E_1 from the rat stomach. Nature (London), 216: 868–873, 1967.
31. Bergström, S.: Prostaglandinets kemi. Nord. Med. 42: 1465–1466, 1949.
32. Bergström, S.: The prostaglandins. Recent Progr. Hormone Res. 22: 153–175, 1966.
33. Bergström, S.: Prostaglandins: Members of a new hormonal system. Science (N. Y.) 157: 383–391, 1967.
34. Bergström, S.: Isolation, structure and action of the prostaglandins. *In* Prostaglandins, Proc. 2nd Nobel Symp., Stockholm, June 1966, ed. by S. Bergström and B. Samuelsson, pp. 21–30, Almqvist and Wiksell, Stockholm; Interscience, New York, 1967.
35. Bergström, S.: Unpublished observations.
36. Bergström, S. and Carlson, L. A.: Inhibitory action of prostaglandin E_1 on the mobilisation of free fatty acids and glycerol from human adipose tissue *in vitro*. Acta Physiol. Scand. 63: 195–196, 1965.
37. Bergström, S. and Carlson, L. A.: Lipid mobilisation in essential fatty acid deficient rats. A preliminary note. Acta Physiol. Scand. 64: 479–480, 1965.
38. Bergström, S. and Carlson, L. A.: Influence of the nutritional state on the inhibition of lipolysis in adipose tissue by prostaglandin E_1 and nicotinic acid. Acta Physiol. Scand. 65: 383–384, 1965.
39. Bergström, S., Carlson, L. A., Ekelund, L.-G. and Orö, L.: Cardiovascular and metabolic response to infusions of prostaglandin E_1 and to simultaneous infusions of noradrenaline and prostaglandin E_1 in man. Acta Physiol. Scand. 64: 332–339, 1965.
40. Bergström, S., Carlson, L. A., Ekelund, L.-G. and Orö, L.: Effect of prostaglandin E_1 on blood pressure, heart rate and concentration of free fatty acids of plasma in man. Proc. Soc. Exp. Biol. Med. 118: 110–112, 1965.
41. Bergström, S., Carlson, L. A. and Orö, L.: Effects of prostaglandins on catecholamine-induced changes in the free fatty acids of plasma in the dog. Biochem. J. 89: 27P–28P, 1963.
42. Bergström, S., Carlson, L. A. and Orö, L.: Effect of prostaglandins on catecholamine induced changes in the free fatty acids of plasma and in blood pressure in the dog. Acta Physiol. Scand. 60: 170–180, 1964.
43. Bergström, S., Carlson, L. A. and Orö, L.: Effect of prostaglandin E_1 on plasma free fatty acids and blood glucose in the dog. Acta Physiol. Scand. 67: 141–151, 1966.
44. Bergström, S., Carlson, L. A. and Orö, L.: Effect of different doses of prostaglandin E_1 on free fatty acids of plasma, blood glucose and heart rate in the nonanesthetised dog. Acta Physiol. Scand. 67: 185–193, 1966.
45. Bergström, S., Carlson, L. A. and Orö, L.: A note on the cardiovascular and metabolic effects of prostaglandin A_1. Life Sci. 6: 449–455, 1967.
46. Bergström, S., Danielsson, H., Klenberg, D. and Samuelsson, B.: The enzymatic conversion of essential fatty acids into prostaglandins. J. Biol. Chem. 239: PC4006–PC4008, 1964.
47. Bergström, S., Danielsson, H. and Samuelsson, B.: The enzymatic formation of prostaglandin E_2 from arachidonic acid. Biochim. Biophys. Acta 90: 207–210, 1964.
48. Bergström, S., Dressler, F., Krabisch, L., Rythagr, R. and Sjövall, J.: The isolation and structure of a smooth muscle stimulating factor in normal sheep and pig lungs. Arkiv Kem. 20: 63–66, 1962.
49. Bergström, S., Dressler, F., Rythagr, R., Samuelsson, B. and Sjövall, J.: The isolation of two further prostaglandins from sheep prostate glands. Arkiv Kem. 19: 563–567, 1962.
50. Bergström, S., Dunér, H., Euler, U. S. von, Pernow, B. and Sjövall, J.: Observations on the effects of infusion of prostaglandin E in man. Acta Physiol. Scand. 45: 145–151, 1959.
51. Bergström, S., Eliasson, R., Euler, U. S. von and Sjövall, J.: Some biological effects of two crystalline prostaglandin factors. Acta Physiol. Scand. 45: 133–144, 1959.
52. Bergström, S. and Euler, U. S. von: The biological activity of prostaglandin E_1, E_2 and E_3. Acta Physiol. Scand. 59: 493–494, 1963.
53. Bergström, S., Krabisch, L., Samuelsson, B. and Sjövall, J.: Preparation of prostaglandin F from prostaglandin E. Acta Chem. Scand. 16: 969–974, 1962.
54. Bergström, S., Krabisch, L. and Sjövall, J.: Smooth muscle stimulating factors in ram semen. Acta Chem. Scand. 14: 1706–1710, 1960.
55. Bergström, S., Rythagr, R., Samuelsson, B. and Sjövall, J.: The structure of prostaglandin E, F_1 and F_2. Acta Chem. Scand. 16: 501–502, 1962.
56. Bergström, S., Rythagr, R., Samuelsson, B. and Sjövall, J.: Degradation studies on prostaglandins. Acta Chem. Scand. 17: 2271–2280, 1963.
57. Bergström, S., Rythagr, R., Samuelsson, B. and Sjövall, J.: The structures of prostaglandin E_1, $F_{1\alpha}$ and $F_{1\beta}$. J. Biol. Chem. 238: 3555–3564, 1963.
58. Bergström, S. and Samuelsson, B.: Isolation of prostaglandin E_1 from human seminal plasma. J. Biol. Chem. 237: PC3005–PC3006, 1962.
59. Bergström, S. and Samuelsson, B.: Isolation of prostaglandin E_1 from calf thymus. Acta Chem. Scand. 17: S283–S287, 1963.
60. Bergström, S. and Samuelsson, B.: Prostaglandins. Annu. Rev. Biochem. 34: 101–108, 1965.
61. Bergström, S. and Sjövall, J.: The isolation of prostaglandin. Acta Chem. Scand. 11: 1086, 1957.
62. Bergström, S. and Sjövall, J.: The isolation of prostaglandin F from sheep prostate glands. Acta Chem. Scand. 14: 1693–1700, 1960.
63. Bergström, S. and Sjövall, J.: The isolation of prostaglandin E from sheep prostate glands. Acta Chem. Scand. 14: 1701–1705, 1960.
64. Berry, P. A. and Collier, H. O. J.: Bronchoconstrictor action and antagonism of a slow-reacting substance from anaphylaxis of guinea-pig isolated lung. Brit. J. Pharmacol. Chemotherap. 23: 201–216, 1964.
65. Berti, F., Borroni, V., Fumagalli, R., Marchi, P. and Zocchr, G. P.: Sull' attività pressoria della prostaglandina. Atti Accad. Med. Lomb. 19: 397–403, 1964.

66. Berti, F. & Kabir Naimzada, M.: Spiccata attività' della prostaglandina E_1 (PGE_1) sull' utero di cavia. Boll. Soc. Ital. Biol. Sperim. 41: 1324–1326, 1965.
67. Berti, F., Kabir Naimzada, M., Lentati, R., Mantegazza, P. and Paoletti, R.: Relations between some *in vitro* and *in vivo* effects of prostaglandin E_1. Progr. Biochem. Pharmacol. 3: 110–121, 1967.
68. Berti, F., Lentati, R. & Grafnetter, D.: Effetti della sommistrasione di prostaglandina E_1 sulla lipasi lipoproteica cardiaca. Boll. Soc. Ital. Biol. Sperim. 43: 515–518, 1967.
69. Berti, F., Lentati, R. & Usardi, M. M.: La prostaglandina E_1 (PGE_1) spiega attivita' iperglicemizzante. Boll. Soc. Ital. Biol. Sperim. 41: 1327–1329, 1965.
70. Berti, F., Lentati, R. and Usardi, M. M.: The species specificity of prostaglandin E_1 effects on isolated heart. Med. Pharmacol. Exp. 13: 233–240, 1965.
71. Berti, F. and Usardi, M. M.: Investigations on a new inhibitor of free fatty acid mobilisation. Gior. Arteriosci. 2: 261–265, 1964.
72. Best, F. A. and Pickles, V. R.: A myometrial effect of oestradiol, imitated by high Mg^{2+} concentrations. J. Physiol. (London) 166: 12P–13P, 1963.
73. Bevegård, S. and Orö, L.: Unpublished observations.
74. Borberg, J., Carlson, L. A., Ekelund, L.-G. and Orö, L.: Lipoprotein lipase activity in plasma before and during prostaglandin E_1 infusion. In preparation.
75. Böhle, E., Ditschuneit, H., Ammon, J. und Döbbert, R.: Über die Wirkung von Prostaglandin E_1 (PGE_1) auf den Fett- und Kohlenhydratstoffwechsel des isolierten Rattenfettgewebes. Verhandl. Deut. Ges. Inn. Med. 72: 465–469, 1966.
76. Böhle, E., Rettberg, H., Ditschuneit, H. H., Döbbert, R. und Ditschuneit, H.: Tierexperimentelle Untersuchungen über die Beeinflussung des Fett- und Kohlenhydratstoffwechsels durch Prostaglandin E_1. Verhandl. Deut. Ges. Inn. Med. 73: 797–801, 1967.
77. Böhle, E., Ditschuneit, H., Melani, F., Beyer, J., Schöpflin, K. und Pfeiffer, E. F.: Über die Regulation der unveresterten Fettsauren unter besonderer Berücksichtigung der Prostaglandine. Deut. Med. Wochensch. 91: 1083–1090, 1966.
78. Böhle, E., Döbbert, R., Ammon, J. und Beyer, J.: Untersuchungen über die Wirkungen von Prostaglandin E_1 (PGE_1) auf den Fettstoffwechsel. *In* Fette in der Medizin, Series 7, ed. by N. Henning and G. Berg, pp. 20–25, Pallas Verlag, Munich, 1966.
79. Böhle, E., Döbbert, E., Ammon, J. und Ditschuneit, H.: Über Stoffwechselwirkungen von Prostaglandinen. I. Der Einfluss von Prostaglandin E_1 auf den Glucose- und Fettstoffwechsel des epididymalen Fettgewebes der Ratte. Diabetologia 2: 163–168, 1966.
80. Böhle, E., Döbbert, R. und Merkl, I. M.: Über die Wirkung von Prostaglandin E_1 und Insulin auf die heparininduzierte Triglyceridhydrolyse. Z. Gesamte Exp. Med. 144: 285–299, 1967.
81. Boyarsky, S., Labay, P. and Gerber, C.: Prostaglandin inhibition of ureteral peristalsis. Invest. Urol. 4: 9–11, 1966.
82. Brudin, J.: Distribution and function of adrenergic nerves in the rabbit fallopian tube. Acta Physiol. Scand. 66 (Suppl. 259): 40–41, 1965.
83. Butcher, R. W. and Baird, C. E.: Effects of prostaglandins on cyclic AMP levels in fat and other tissues. J. Biol. Chem., in press.
84. Butcher, R. W., Ho, R. J., Meng, H. C. and Sutherland, E. W.: Adenosine 3',5'-monophosphate in biological materials. II. The measurement of adenosine 3',5'-monophosphate in tissues and the role of the cyclic nucleotide in the lipolytic response of fat to epinephrine. J. Biol. Chem. 240: 4515–4523, 1965.
85. Butcher, R. W., Pike, J. E. and Sutherland, E. W.: The effect of prostaglandin E_1 on adenosine 3',5',-monophosphate levels in adipose tissue. *In* Prostaglandins, Proc. 2nd Nobel Symp., Stockholm, June 1966, edited by S. Bergström and B. Samuelsson, pp. 133–138, Almqvist and Wiksell, Stockholm; Interscience, New York, 1967.
86. Butcher, R. W. and Sutherland, E. W.: Adenosine 3',5'-phosphate in biological materials. I. Purification and properties of cyclic 3',5'-nucleotide phosphodiesterase and use of this enzyme to characterise adenosine 3',5'-phosphate in human urine. J. Biol. Chem. 237: 1244–1250, 1962.
87. Butcher, R. W. and Sutherland, E. W.: The effects of the catecholamines, adrenergic blocking agents, prostaglandin E_1 and insulin on cyclic AMP levels in the rat epididymal fat pad in vitro. Ann. N. Y. Acad. Sci. 139: 849–850, 1967.
88. Bygdeman, M.: The effect of different prostaglandins on the human myometrium in vitro. Acta Physiol. Scand. 63: (Suppl. 242): 1–78, 1964.
89. Bygdeman, M. Studies of the effects of prostaglandins in seminal plasma on human myometrium in vitro. *In* Prostaglandins, Proc. 2nd Nobel Symp., Stockholm, June 1966, ed. by S. Bergström and B. Samuelsson, pp. 93–96, Almqvist and Wiksell, Stockholm; Interscience, New York, 1967.
90. Bygdeman, M. and Eliasson, R.: The effect of prostaglandin from human seminal fluid on the motility of the non-pregnant human uterus in vitro. Acta Physiol. Scand. 59: 43–51, 1963.
91. Bygdeman, M. and Eliasson, R.: A comparative study on the effect of different prostaglandin compounds on the motility of the isolated human myometrium. Medicina Exp. 9: 409–415, 1963.
92. Bygdeman, M. and Hamberg, M.: The effect of eight new prostaglandins on human myometrium. Acta Physiol. Scand. 69: 320–326, 1967.
93. Bygdeman, M., Hamberg, M. and Samuelsson, B.: The content of different prostaglandins in human seminal fluid and their threshold doses on the human myometrium. Mem. Soc. Endocrinol. 14: 49–64, 1966.
94. Bygdeman, M. and Holmberg, O.: Isolation and identification of prostaglandins from ram seminal plasma. Acta Chem. Scand. 20: 2306–2310, 1966.

95. Bygdeman, M., Kwon, S. U., Muhkerjee, T. and Wiqvist, N.: The effect of intravenous infusion of prostaglandin E_1 and E_2 on the motility of the pregnant human uterus. Amer. J. Obstet. Gynecol., in press.
96. Bygdeman, M., Kwon, S. and Wiqvist, N.: The effect of prostaglandin E_1 on human pregnant myometrium invivo. *In* Prostaglandins, Proc. 2nd Nobel Symp., Stockholm, June 1966, ed. by S. Bergström and B. Samuelsson, pp. 93–96, Almqvist and Wiksell, Stockholm; Interscience, New York, 1967.
97. Bygdeman, M. and Samuelsson, B.: Quantitative determination of prostaglandins in human semen. Clin. Chim. Acta 10: 566–568, 1964.
98. Bygdeman, M. and Samuelsson, B.: Analyses of prostaglandins in human semen. Clin. Chim. Acta 13: 465–474, 1966.
99. Caldeyro-Barcia, R. and Alvarez H.,: Abnormal uterine action in labour. J. Obstet. Gynaecol. Brit. Emp. 59: 646–656, 1952.
100. Caldeyro-Barcia, R., Sica-Blanco, Y., Poseiro, J. J., González-Panizza, V., Méndez-Bauer, C., Fielitz, C., Alvarez, H., Pose, S. V. and Hendricks, C. H.: A quantitative study of the action of synthetic oxytocin on the pregnant human uterus. J. Pharmacol. Exp. Therap. 121: 18–31, 1957.
101. Carlson, L. A.: Inhibition of the mobilization of free fatty acids from adipose tissue. Ann. N. Y. Acad. Sci. 131: 119–142, 1965.
102. Carlson, L. A.: Cardiovascular and metabolic effects of prostaglandins. Progr. Biochem. Pharmacol. 3: 94–100, 1967.
103. Carlson, L. A.: Metabolic and cardio-vascular effects in vivo of prostaglandins. *In* Prostaglandins, Proc. 2nd Nobel Symp., Stockholm, June 1966, ed. by S. Bergström and B. Samuelsson, pp. 123–132, Almqvist and Wiksell, Stockholm; Interscience, New York, 1967.
104. Carlson, L. A.: Unpublished observations, cited in ref. (134).
105. Carlson, L. A.: Unpublished observations.
106. Carlson, L. A., Ekelund, L. -G. and Orö, L.: Clinical and metabolic effects of different doses of prostaglandin E_1 in man. Acta Med. Scand., in press.
107. Carlson, L. A., Ekelund, L. -G. and Orö, L.: Circulatory and respiratory effects of different doses of prostaglandin E_1 in man. In preparation.
108. Carlson, L. A., Ekelund, L. -G. and Orö, L.: Effect of infusion of prostaglandin E_1 into the femoral artery of man. In preparation.
109. Carlson, L. A., Ekelund, L. -G. and Orö, L.: Metabolic and cardiovascular effects of different prostaglandins. In preparation.
110. Carlson, L. A. and Fröberg, S. O.: Unpublished observations.
111. Carlson, L. A. and Hallberg, D.: Basal lipolysis and effects of noradrenaline and prostaglandin E_1 on lipolysis in human subcutaneous and omental adipose tissue. J. Lab. Clin. Med. 71: 368–377, 1968.
112. Carlson, L. A., Ikton, E. and Orö, L.: Effect of infusion of prostaglandin E_1 on the aggregation of blood platelets in man. Life Sci. 7: 85–90, 1968.
113. Carlson, L. A. and Mechelli, H.: Unpublished observations.
114. Carlson, L. A. and Orö, L.: Effect of prostaglandin E_1 on blood pressure and heart rate in the dog. Acta Physiol. Scand. 67: 89–99, 1966.
115. Carr, A. A.: The effect of a prostaglandin on renal function. Clin. Res. 15: 354, 1967.
116. Chandrasekhar, N.: Inhibition of platelet aggregation by prostaglandins. Blood 30: 554, 1967.
117. Chandrasekhar, N.: Inhibition of platelet aggregation by prostaglandins. In preparation.
118. Clayton, J. A. and Sergo, C. M.: Depletion of rat thyroid serotonin accompanied by increased blood flow as an acute response to thyroid-stimulating hormone. Endocrinology 80: 689–698, 1967.
119. Clegg, P. C.: The effect of prostaglandins on the response of isolated smooth-muscle preparations to sympathomimetic substances. Mem. Soc. Endocrinol. 14: 119–136, 1966.
120. Clegg, P. C.: Antagonism by prostaglandins of the responses of various smooth muscle preparations to sympathomimetics. Nature (London) 209: 1137–1139, 1966.
121. Clegg, P. C., Hall, W. J. and Pickles, V. R.: The action of ketonic prostaglandins on the guinea pig myometrium. J. Physiol. (London) 183: 123–144, 1966.
122. Clitherow, H. J. and Pickles, V. R.: The separation of the smooth-muscle stimulants in menstrual fluid. J. Physiol. (London) 156: 225–237, 1961.
123. Cochani, F., Page-Aschak, C., Volta, F. and Wolfe, L. S.: Effect of nerve stimulation on prostaglandin formation and release from the rat stomach. Amer. J. Physiol. 213: 1056–1064, 1967.
124. Cochani, F. and Wolfe, L. S.: Prostaglandins in brain and the release of prostaglandin-like compounds from the cat cerebellar cortex. Can. J. Physiol. Pharmacol. 43: 445–450, 1965.
125. Cochani, F. and Wolfe, L. S.: On the action of prostaglandin E_1 and prostaglandins from brain on the isolated rat stomach. Can. J. Physiol. Pharmacol. 44: 933–950, 1966.
126. Cockrill, J. R., Miller, E. G, Jr. and Kurrok, R.: The substance in human seminal fluid affecting uterine muscle. Amer. J. Physiol. 112: 577–580, 1935.
127. Crowshaw, K.: Potential errors in the isolation and identification of millimicrogram quantities of prostaglandins. Federation Proc. 25: 765, 1966.
128. Dakhil, T. und Vogt, W.: Hydroperoxide als Träger der darmerregenden Wirkung hochungesättigter Fettsäuren. Arch. Exp. Pathol. Pharmakol. 243: 174–186, 1962.
129. Daniels, E. G., Hinman, J. W., Johnson, B. A., Kupiecki, F. P., Nelson, J. W. and Pike, J. E.: The isolation of an additional prostaglandin derivative from the enzymic cyclisation of homo-γ-linolenic acid. Biochem. Biophys. Res. Commun. 21: 413–417, 1965.

130. Daniels, E. G., Hinman, J. W., Leach, B. E. and Muirhead, E. E.: Identification of prostaglandin F_2 as the principal vasodepressor lipid of rabbit renal medulla. Nature (London) 215: 1298–1299, 1967.
131. Davies, B. N., Horton, E. W. and Witherington, P. G.: The occurrence of prostaglandin E_2 in splenic venous blood of the dog following nerve stimulation. J. Physiol. (London) 188: 38P–39P, 1966.
132. De Pury, G. G. and Collins, F. D.: A raised level of free fatty acids in serum of rats deficient in essential fatty acids as a contributing cause of their fatty livers. Biochim. Biophys. Acta 106: 213–214, 1965.
133. Dorp, D. A. van: The biosynthesis of prostaglandins. Mem. Soc. Endocrinol. 14: 39–47, 1966.
134. Dorp, D. A. van: Aspects of the biosynthesis of prostaglandins. Progr. Biochem. Pharmacol. 3: 71–82, 1967.
135. Dorp, D. A. van, Beerthuis, R. K., Nugteren, D. H. and Vonkeman, H.: The biosynthesis of prostaglandins. Biochim. Biophys. Acta 90: 204–207, 1964.
136. Dorp, D. A. van, Beerthuis, R. K., Nugteren, D. H. and Vonkeman, H.: Enzymatic conversion of all-*cis*-polyunsaturated fatty acids into prostaglandins. Nature (London) 203: 839–841, 1964.
137. Dorp, D. A. van, Jouvenaz, G. H. and Strulik, C. B.: The biosynthesis of prostaglandin in pig eye iris. Biochim. Biophys. Acta 137: 396–399, 1967.
138. DuCharme, D. W.: Unpublished observations.
139. DuCharme, D. W. and Weeks, J. R.: Prostaglandin $F_{2\alpha}$, a unique pressor substance. Federation Proc. 26:1 681, 1967.
140. DuCharme, D. W. and Weeks, J. R.: Cardiovascular pharmacology of prostaglandin $F_{2\alpha}$, a unique pressor agent. *In* Prostaglandins, Proc. 2nd Nobel Symp., Stockholm, June 1966, ed. by S. Bergström and B. Samuelsson, pp. 173–182, Almqvist and Wiksell, Stockholm; Interscience, New York, 1967.
141. DuCharme, D. W., Weeks, J. R. and Montgomery, R. G.: Studies on the mechanism of the hypertensive effect of prostaglandin $F_{2\alpha}$. J. Pharmacol. Exp. Therap. 160: 1–10, 1968.
142. Eglinton, G., Raphael, R. A., Smith, G. N., Hall, W. J. and Pickles, V. R.: The isolation and identification of two smooth muscle stimulants from menstrual fluid. Nature (London) 200: 960, 992–995, 1963.
143. Eliasson, R.: A comparative study of prostaglandin from human seminal fluid and from prostate gland of sheep. Acta Physiol. Scand. 39: 141–146, 1957.
144. Eliasson, R.: Studies on prostaglandin. Occurrence, formation and biological actions. Acta Physiol. Scand. 46: (Suppl. 158): 1–73, 1959.
145. Eliasson, R.: Mode of action of prostaglandins on the human non-pregnant uterus. Biochem. Pharmacol. 15: 755, 1966.
146. Eliasson, R.: The effect of different prostaglandins on the motility of the human myometrium. Mem. Soc. Endocrinol. 14: 77–88, 1966.
147. Eliasson, R. and Posse, N.: The effect of prostaglandin on the non-pregnant human uterus *in vivo*. Acta Obstet. Gynecol. Scand. 39: 113–126, 1960.
148. Eliasson, R. and Posse, N.: Rubin's test before and after intravaginal application of prostaglandin. Int. J. Fert. 10: 373–377, 1965.
149. Eliasson, R. and Risley, P. L.: Potentiated response of isolated seminal vesicles to catecholamines and acetylcholine in the presence of PGE_1. Acta Physiol. Scand. 67: 253–254, 1966.
150. Eliasson, R. and Risley, P. L.: Potentiated response of isolated seminal vesicles to catecholamines and acetylcholine in the presence of prostaglandins. *In* Prostaglandins, Proc. 2nd Nobel Symp., Stockholm, June 1966, ed. by S. Bergström and B. Samuelsson, pp. 85–90, Almqvist and Wiksell, Stockholm; Interscience, New York, 1967.
151. Embrey, M. P.: Unpublished observations, cited in ref. (263).
152. Emmons, P. R., Hampton, J. R., Harrison, M. J. G., Honour, A. J. and Mitchell, J. R. A.: Effect of prostaglandin E_1 on platelet behaviour in vitro and in vivo. Brit. Med. J. 2: 468–472, 1967.
153. Euler, U. S. von: Zur Kenntnis der pharmakologischen Wirkungen von Nativsekreten und Extrackten männlicher accessorischer Geschlechtsdrüsen. Arch. Exp. Pathol. Pharmakol. 175: 78–84, 1934.
154. Euler, U. S. von: A depressor substance in the vesicular gland. J. Physiol. (London) 84: 21P, 1935.
155. Euler, U. S. von: Über die spezifische blutdrucksenkende Substanz des menschlichen Prostata- und Samenblasensekretes. Klin. Wochensch. 14: 1182–1183, 1935.
156. Euler, U. S. von: On the specific vasodilating and plain muscle stimulating substances from accessory genital glands in man and certain animals (prostaglandin and vesiglandin). J. Physiol. (London) 88: 213–234, 1937.
157. Euler, U. S. von: Action of adrenaline, acetylcholine and other substances on nerve-free vessels (human placenta). J. Physiol. (London) 93: 129–143, 1938.
158. Euler, U. S. von: Weitere Untersuchungen über Prostaglandin, die physiologisch aktive Substanz gewisser Genitaldrüsen. Skand. Arch. Physiol. 81: 65–80, 1939.
159. Euler, U. S. von: Introductory survey: prostaglandin. Mem. Soc. Endocrinol. 14: 3–18, 1966.
160. Euler, U. S. von und Hammarström, S.: Über das Vorkommen des Prostaglandins in Tierorganen. Skand. Arch. Physiol. 77: 96–99, 1937.
161. Fain, J. N.: Adrenergic blockade of hormone-induced lipolysis in isolated fat cells. Ann. N. Y. Acad. Sci. 139: 879–890, 1967.
162. Fassina, G. and Contessa, A. R.: Digitoxin and prostaglandin E_1 as inhibitors of catecholamine-stimulated lipolysis and their interaction with Ca^{2+} in the process. Biochem. Pharmacol. 16: 1447–1453, 1967.
163. Ferreira, S. H. and Vane, J. R.: Prostaglandins: Their disappearance from and release into the circulation. Nature (London) 216: 873–876, 1967.
164. Ferri, S., Galatulas, I. e Piccinini, F.: Azione della prostaglandina E_1 sulla coagulazione del sangue. Boll. Soc. Ital. Biol. Sperim. 41: 1243–1245, 1965.
165. Gaddum, J. H. and Szerb, J. C.: Assay of substance P on goldfish intestine in a microbath. Brit. J. Pharmacol. Chemotherap. 17: 451–463, 1961.

166. Goldblatt, M. W.: A depressor substance in seminal fluid. J. Soc. Chem. Ind. (London) 52: 1056–1057, 1933.
167. Goldblatt, M. W.: Properties of human seminal plasma. J. Physiol. (London) 84: 208–218, 1935.
168. Gottenboe, J. J., Beerthuis, R. K. and Dorp, D. A. van: Essential fatty acid bio-assay of prostaglandin E_1 in rats and mice. *In* Prostaglandins, Proc. 2nd Nobel Symp., Stockholm, June 1966, ed. by S. Bergström and B. Samuelsson, pp. 57–62, Almqvist and Wiksell, Stockholm; Interscience, New York, 1967.
169. Granström, E., Ingber, U. and Samuelsson, B.: The structure of a urinary metabolite of prostaglandin $F_{1\alpha}$ in the rat. J. Biol. Chem. 240: 457–461, 1965.
170. Granström, E.: On the metabolism of prostaglandins in man. Progr. Biochem. Pharmacol. 3: 89–93, 1967.
171. Grén, K., Hansson, E. and Samuelsson, B.: Synthesis of tritium labeled prostaglandin $F_{1\alpha}$ and studies of its distribution by autoradiography. Progr. Biochem. Pharmacol. 3: 85–88, 1967.
172. Grén, K. and Samuelsson, B.: Thin layer chromatography of the prostaglandins. J. Lipid Res. 5: 117–120, 1964.
173. Haddy, F. J., Molnar, J. I. and Campbell, R. W.: Effects of denervation and vasoactive agents on vascular pressures and weight of dog forelimb. Amer. J. Physiol. 201: 631–638, 1961.
174. Hagedler, H. A. and Crawford, J. D.: Alterations in the fatty acid composition of depot fat associated with obesity. Ann. N. Y. Acad. Sci. 131: 476–484, 1965.
175. Hagedler, H. A. and Crawford, J. D.: Lipolysis in homogenates of adipose tissue: An inhibitor found in fat from obese rats. Science (N. Y.) 154: 909–910, 1966.
176. Hagedler, H. A. and Crawford, J. D.: Insulin-like inhibition of lipolysis and stimulation of lipogenesis by prostaglandin E_1 (PGE_1). J. Clin. Invest. 46: 1065, 1967.
177. Hall, W. J. and Pickles, V. R.: The dual action of menstural stimulant A2 (prostaglandin E_2). J. Physiol. (London) 169: 90P–91P, 1963.
178. Hamberg, M. and Samuelsson, B.: Isolation and structure of a new prostaglandin from human seminal plasma. Biochim. Biophys. Acta 106: 215–217, 1965.
179. Hamberg, M. and Samuelsson, B.: Novel biological transformations of 8, 11, 14-eicosatrienoic acid. J. Amer. Chem. Soc. 88: 2349–2350, 1966.
180. Hamberg, M. and Samuelsson, B.: Prostaglandins in human seminal plasma. J. Biol. Chem. 241: 257–263, 1966.
181. Hamberg, M. and Samuelsson, B.: New groups of naturally occurring prostaglandins. *In* Prostaglandins, Proc. 2nd Nobel Symp., Stockholm, June 1966, ed. by S. Bergström and B. Samuelsson, pp. 63–70, Almqvist and Wiksell, Stockholm; Interscience, New York, 1967.
182. Hamberg, M. and Samuelsson, B.: Oxygenation of unsaturated fatty acids by the vesicular gland of sheep. J. Biol. Chem. 242: 5344–5354, 1967.
183. Hamberg, M. and Samuelsson, B.: On the mechanism of the biosynthesis of prostaglandins E_1 and $F_{1\alpha}$. J. Biol. Chem. 242: 5336–5343, 1967.
184. Hansson, E. and Samuelsson, B.: Autoradiographic distribution studies of ^3H-labeled prostaglandin E_1 in mice. Biochim. Biophys. Acta 106: 379–385, 1965.
185. Haugh, A., Lunde, P. K. M. and Waaler, B. A.: Effects of prostaglandin E_1 and adrenaline on the pulmonary vascular resistance (PVR) in isolated rabbit lungs. Life Sci. 6: 673–680, 1967.
186. Hawkins, D. F. and Labrum, A. H.: Semen prostaglandin levels in fifty patients attending a fertility clinic. J. Reprod. Fert. 2: 1–10, 1961.
187. Hickler, R. B., Birbari, A. E., Qureshi, E. U. and Karnovsky, M. L.: Purification and characterization of the vasodilator and antihypertensive lipid of rabbit renal medulla. Trans. Ass. Amer. Physicians 79: 278–288, 1966.
188. Hickler, R. B., Lauler, D. P., Saravis, C. A., Vagnucci, A. I., Steiner, G. and Thorn, G. W.: Vasodepressor lipid from the renal medulla. Can. Med. Ass. J. 90: 280–287, 1964.
189. Holmes, S. W. and Horton, E. W.: The nature and distribution of prostaglandins in the central nervous system of the dog. J. Physiol. (London) 191: 134P–135P, 1967.
190. Holmes, S. W., Horton, E. W. and Main, I. H. M.: The effect of prostaglandin E_1 on responses of smooth muscle to catecholamines, angiotensin and vasopressin. Brit. J. Pharmacol. Chemotherap. 21: 538–543, 1963.
191. Horton, E. W.: Action of prostaglandin E_1 on tissues which respond to bradykinin. Nature (London) 200: 892–893, 1963.
192. Horton, E. W.: Actions of prostaglandins E_1, E_2 and E_3 on the central nervous system. Brit. J. Pharmacol. Chemotherap. 22: 189–192, 1964.
193. Horton, E. W.: Biological activities of pure prostaglandins. Experientia 21: 113–118, 1965.
194. Horton, E. W. and Main, I. H. M.: A comparison of the biological activities of four prostaglandins. Brit. J. Pharmacol. Chemotherap. 21: 182–189, 1963.
195. Horton, E. W. and Main, I. H. M.: A comparison of the actions of prostaglandins $F_{1\alpha}$ and E_1 on smooth muscle. Brit. J. Pharmacol. Chemotherap. 24: 470–476, 1965.
196. Horton, E. W. and Main, I. H. M.: Effects of prostaglandins on unanesthetised animals. J. Physiol. (London) 177: 34P, 1965.
197. Horton, E. W. and Main, I. H. M.: Actions of prostaglandins on the spinal cord of the chick. J. Physiol. (London) 179: 18P–20P, 1965.
198. Horton, E. W. and Main, I. H. M.: The identification of prostaglandins in central nervous tissues of the cat and the fowl. J. Physiol. (London) 185: 36P–37P, 1966.
199. Horton, E. W. and Main, I. H. M.: Further observations on the central nervous actions of prostaglandins $F_{1\alpha}$ and E_1. Brit. J. Pharmacol. Chemotherap. 30: 568–581, 1967.
200. Horton, E. W. and Main, I. H. M.: Identification of prostaglandins in central nervous tissues of the cat and chicken. Brit. J. Pharmacol. Chemotherap. 30: 582–602, 1967.
201. Horton, E. W. and Main, I. H. M.: Central nervous actions of the prostaglandins and their identification in the

brain and spinal cord. *In* Prostaglandins, Proc. 2nd Nobel Symp., Stockholm, June 1966, ed. by S. Bergström and B. Samuelsson, pp. 253–260, Almqvist and Wiksell, Stockholm; Interscience, New York, 1967.

202. Horton, E. W., Main, I. H. M.: and Thompson, C. J.: Effects of prostaglandins on the oviduct, studied in rabbits and ewes. J. Physiol. (London) 180: 514–528. 1965.

203. Horton, E. W. and Thompson, C. J.: Thin-layer chromatography and bioassay of prostaglandins in extracts of semen and tissues of the male reproductive tract. Brit. J. Pharmacol. Chemotherap. 22: 183–188, 1964.

204. Johnston, H. H., Herzog, J. P., and Lauler, D. P.: Effect of prostaglandin E_1 on renal hemodynamics, sodium and water excretion. Amer. J. Physiol. 213: 939–946, 1967.

205. Kabir Naimzada, M.: Azione della prostaglandina E_1 sulla vesica urinaria (esperienza nella cavia nel ratto). Boll. Soc. Ital. Biol. Sperim. 43: 518–521, 1967.

206. Kadar, D. and Sunahara, F. A.: The influence of prostaglandins on the effect of autonomic drugs in the superior mesenteric vein. Proc. Can. Federation Biol. Sci. 10: 24, 1967.

207. Kaplan, H. R., Grega, G. J. and Buckley, J. P.: Evidence of the central cardiovascular action for prostaglandin E_1. Pharmacologist 9: 223, 1967.

208. Karim, S. M. M.: Identification of prostaglandins in human amniotic fluid. J. Obstet. Gynaecol. Brit. Commonw. 73: 903–906, 1966.

209. Karim, S. M. M.: A smooth muscle contracting substance in extracts of human umbilical cord. J. Pharm. Pharmacol. 18: 519–530, 1966.

210. Karim, S. M. M.: The identification of prostaglandins in human umbilical cord. Brit. J. Pharmacol. Chemotherap. 29: 230–237, 1967.

211. Karim, S. M. M. and Devlin, J.: Prostaglandin content of amniotic fluid during pregnancy and labour. J. Obstet Gynaecol. Brit. Commonw. 74: 230–234, 1967.

212. Karlson, S.: The influence of seminal fluid on the motility of the nonpregnant human uterus. Acta Obstet. Gynecol. Scand. 38: 503–521, 1959.

213. Kataoka, K., Ramwell, P. W. and Jessup, S.: Prostaglandins: localisation in subcellular particles of rat cerebral cortex. Science (N. Y.) 157: 1187–1189, 1967.

214. Khairallah, P. A., Page, I. H. and Türker, R. K.: Some properties of prostaglandin E_1 action on muscle. Arch. Int. Pharmacodyn. Therap. 169: 328–341, 1967.

215. Kircher, C. W.: Effects of specific prostaglandins on development of chick embryo skin and down feather organ *in vitro*. Developmental Biol. 16: 203–215, 1967.

216. Klaus, W. und Piccinini, F.: Über die Wirkung von Prostaglandin E_1 auf den Ca-Haushalt isolierter Meerschweinchenherzen. Experientia 23: 556–557, 1967.

217. Klenberg, D. and Samuelsson, B.: The biosynthesis of prostaglandin E_1 studied with specifically ^{3}H-labelled 8,11,14-eicosatrienoic acids. Acta Chem. Scand. 19: 534–535, 1965.

218. Klöber, J.: Influence of prostaglandins on platelet adhesiveness and platelet aggregation. *In* Prostaglandins, Proc. 2nd Nobel Symp., Stockholm, June 1966, ed. by S. Bergström and B. Samuelsson, pp. 241–252, Almqvist and Wiksell, Stockholm; Interscience, New York, 1967.

219. Konzett, H. und Rössler, R.: Versuchsanordnung zu Untersuchungen an der Bronchialmuskulatur. Arch. Exp. Pathol. Pharmakol. 195: 71–74, 1940.

220. Korolkiewicz, Z. and Lentati, R. L.: An investigation of the points of action of nicotinic acid and PGE_1 on lipid mobilization using as a tool theophylline. *In* Symposium summary, Italo-Francese sui Problemi Dell Aterosclerosi, Saint Vincent, 1966, pp. 8–9.

221. Krnjević, K.: Actions of drugs on single neurones in the cerebral cortex. Brit. Med. Bull. 21: 10–14, 1956.

222. Kupiecki, F. P.: Effects of prostaglandin E_1 on lipolysis and plasma free fatty acids in the fasted rat. J. Lipid Res. 8: 577–580, 1967.

223. Kupiecki, F. P., Chandrasekhar, N. and Weeks, J. R.: Effects of infusion of some prostaglandins in essential fatty acid deficient and normal rats. In preparation.

224. Kupiecki, F. P. and Weeks, J. R.: Prolonged intravenous infusion of prostaglandin E_1 (PGE_1) on lipid metabolism in the rat. Federation Proc. 25: 719, 1966.

225. Kurzrok, R. and Lieb, C. C.: Biochemical studies of human semen. II. The action of semen on the human uterus. Proc. Soc. Exp. Biol. Med. 28: 268–272, 1930.

226. Labay, P. and Boyarsky, S.: Ureteral effect of prostaglandins in the dog. Clin. Res. 15: 362, 1967.

227. Landesman, R. and Wilson, K.: Smooth muscle active substances in the amniotic fluid. Clin. Obstet. Gynecol. 9: 554–564, 1966.

228. Lee, J. B., Covino, B. G., Takman, B. H. and Smith, E. R.: Renomedullary vasodepressor substance, medullin: Isolation, chemical characterization and physiological properties. Circulation Res. 17: 57–77, 1965.

229. Lee, J. B., Gougoutas, J. Z., Takman, B. H., Daniels, E. G., Grostic, M. F., Pike, J. E., Hinman, J. W. and Muirhead, E. E.: Vasodepressor and antihypertensive prostaglandins of PGE type with emphasis on the identification of medullin as $PGE_{3\text{-}217}$. J. Clin. Invest. 45: 1036. 1966.

230. Lee, J. B., Hecklee, R. B., Saravis, C. A. and Thorn, G. W.: Sustained depressor effect of renal medullary extract in the normotensive rat. Circulation Res. 13: 350–366, 1963.

231. Main, I. H. M.: The inhibitory actions of prostaglandins on respiratory smooth muscle. Brit. J. Pharmacol. Chemotherap. 22: 511–519, 1964.

232. Mandel, L. R. and Kuehl, F. A., Jr.: Lipolytic action of 3,3'5-tri-iodo-l-thyronine, a cyclic AMP phosphodiesterase inhibitor. Biochem. Biophys. Res. Commun. 26: 13–18, 1967.

233. Mantegazza, P.: La prostaglandina E_1 come sostanza sensibilizzatrice per il calcio a livello del cuore isolato di cavia. Atti Accad. Med. Lomb. 20: 66–72, 1965.

234. Mantegazza, P. e Kabir Naimzada, M.: Attività della prostaglandina E_1 sul preparato nervo ipogastrico-deferente di varie specie animali. Atti Accad. Med. Lomb. 20: 58–64, 1965.

235. Maxwell, G. M.: The effect of prostaglandin E_1 upon the general and coronary haemodynamics and metabolism of the intact dog. Brit. J. Pharmacol. Chemotherap. 31: 163–168, 1967.
236. Miyasaki, E., Ishikawa, M., Sunano, S., Syuto, B. and Sakagami, T.: Stimulating action of prostaglandin on the rabbit duodenal muscle. *In* Prostaglandins, Proc. 2nd Nobel Symp., Stockholm, June 1966, ed. by S. Bergström and B. Samuelsson, pp. 277–282, Almqvist and Wiksell, Stockholm; Interscience, New York, 1967.
237. Mühlbachová, E., Sólyom, A. and Puglisi, L.: Investigations on the mechanism of the prostaglandin E_1 antagonism to norepinephrine and theophylline-induced lipolysis. European J. Pharmacol. 1: 331–335, 1967.
238. Muirhead, E. E.: Personal communication.
239. Muirhead, E. E., Brooks, B., Kosinski, M., Daniels, E. G. and Hinman, J. W.: Renomedullary antihypertensive principle in renal hypertension. J. Lab. Clin. Med. 67: 778–791, 1966.
240. Muirhead, E. E., Daniels, E. G., Booth, E., Freyburger, W. A. and Hinman, J. W.: Renomedullary vasodepression and antihypertensive function. Arch. Pathol. 80: 43–49, 1965.
241. Muirhead, E. E., Daniels, E. G. and Hinman, J. W.: Renomedullary antihypertensive principle. *In* Club Internationale sur L'Hypertension Arterielle, Paris, July 1965, ed. by P. Millies and P. Teberdakoff, vol. 1, pp. 177–187, L'Expansion Scientifique Francaise, Paris, 1966.
242. Muirhead, E. E., Daniels, E. G., Pike, J. E. and Hinman, J. W.: Renomedullary antihypertensive lipids and the prostaglandins. *In* Prostaglandins, Proc. 2nd Nobel Symp., Stockholm, June 1966, ed. by S. Bergström and and B. Samuelsson, pp. 183–196, Almqvist and Wiksell, Stockholm; Interscience, New York, 1967.
243. Muirhead, E. E., Leach, B. E., Brown, G. B., Daniels, E. G. and Hinman, J. W.: Antihypertensive effect of prostaglandin E_2 (PGE_2) in renovascular hypertension. J. Lab. Clin. Med. 70: 986–987, 1967.
244. Nakano, J.: Effect of changes in coronary arterial blood flow on the myocardial contractile force. Jap. Heart J. 7: 78–86, 1966.
245. Nakano, J. and McCurdy, J. R.: Cardiovascular effects of prostaglandin E_1. J. Pharmacol. Exp. Therap. 156: 538–547, 1967.
246. Nugteren, D. H., Beerthuis, R. K. and Dorp, D. A. van: The enzymic conversion of *all-cis* 8,11,14-eicosatrienoic acid into prostaglandin E_1. Recueil Trav. Chim. Pays-Bas 85: 405–419, 1966.
247. Nugteren, D. H. and Dorp, D. A. van: The participation of molecular oxygen in the biosynthesis of prostaglandins. Biochim. Biophys. Acta 98: 654–656, 1965.
248. Nugteren, D. H., Dorp, D. A. van, Bergström, S., Hamberg, M. and Samuelsson, B.: Absolute configuration of the prostaglandins. Nature (London) 212: 38–39, 1966.
249. Orloff, J. and Grantham, J.: The effect of prostaglandin (PGE_1) on the permeability response of rabbit collecting tubules to vasopressin. *In* Prostaglandins, Proc. 2nd Nobel Symp., Stockholm, June 1966, ed. by S. Bergström and B. Samuelsson, pp. 143–146, Almqvist and Wiksell, Stockholm; Interscience, New York, 1967.
250. Orloff, J. and Handler, J.: The role of adenosine 3′,5′-phosphate in the action of antidiuretic hormone. Amer. J. Med. 42: 757–768, 1967.
251. Orloff, J., Handler, J. S. and Bergström, S.: Effect of prostaglandin (PGE_1) on the permeability response of the toad bladder to vasopressin, theophylline and adenosine 3′,5′-monophosphate. Nature (London) 205: 397–398, 1965.
252. Pace-Asciak, C., Cocrani, F. and Wolfe, L. S.: Comparison of the conversion of arachidonic acid into prostaglandins by some tissues. Proc. Can. Federation Biol. Sci. 10: 80, 1967.
253. Paoletti, R., Lenfant, R. L. and Korolkiewicz, Z.: Pharmacological investigations on the prostaglandin E_1 effect on lipolysis. *In* Prostaglandins, Proc. 2nd Nobel Symp., Stockholm, June 1966, ed. by S. Bergström and and B. Samuelsson, pp. 147–160, Almqvist and Wiksell, Stockholm; Interscience, New York, 1967.
254. Paton, D. M. and Daniel, E. E.: On the contractile response of the isolated rat uterus to prostaglandin E_1. Can. J. Physiol. Pharmacol. 45: 795–804, 1967.
255. Picchini, F.: Sull'azione della prostaglandina E_1 (PGE_1) sulla fosforilasi cardiaca. Boll. Soc. Ital. Biol. Sperim. 41: 1233–1234, 1965.
256. Pickles, V. R.: Myometrial responses to the menstrual plain-muscle stimulant. J. Endocrinol. 19: 150–157, 1959.
257. Pickles, V. R.: Active lipids in menstrual fluid. Biochem. Pharmacol. 12: 429–430, 1963.
258. Pickles, V. R.: The menstrual stimulant in puberty. J. Physiol. (London) 183: 69P–70P, 1966.
259. Pickles, V. R.: Prostaglandins in the human endometrium. Int. J. Fert. 12: 335–338, 1967.
260. Pickles, V. R.: The myometrial actions of six prostaglandins: consideration of a receptor hypothesis. *In* Prostaglandins, Proc. 2nd Nobel Symp., Stockholm, June 1966, ed. by S. Bergström and B. Samuelsson, pp. 79–84, Almqvist and Wiksell, Stockholm; Interscience, New York, 1967.
261. Pickles, V. R. and Hall, W. J.: Some physiological properties of the 'menstrual stimulant' substances A1 and A2. J. Reprod. Fert. 6: 315–317, 1963.
262. Pickles, V. R., Hall, W. J., Best, F. A. and Smith, G. N.: Prostaglandins in endometrium and menstrual fluid from normal and dysmenorrhoeic subjects. J. Obstet. Gynaecol. Brit. Commonw. 72: 185–192, 1965.
263. Pickles, V. R., Hall, W. J., Clegg, P. C. and Sullivan, T. J.: Some experiments on the mechanism of action of prostaglandins on the guinea-pig and rat myometrium. Mem. Soc. Endocrinol. 14: 89–103, 1966.
264. Pike, J. E., Kupiecki, F. P. and Weeks, J. R.: Biological activity of the prostaglandins and related analogs. *In* Prostaglandins, Proc. 2nd Nobel Symp., Stockholm, June 1966, ed. by S. Bergström and B. Samuelsson, pp. 161–172, Almqvist and Wiksell, Stockholm; Interscience, New York, 1967.
265. Ramwell, P. W. and Shaw, J. E.: Spontaneous and evoked release of prostaglandins from the cerebral cortex of anesthetised cats. Amer. J. Physiol. 211: 125–134, 1966.
266. Ramwell, P. W. and Shaw, J. E.: Prostaglandin release from tissues by drug, nerve and hormone stimulation. *In* Prostaglandins, Proc. 2nd Nobel Symp., Stockholm, June 1966, ed. by S. Bergström and B. Samuelsson, pp. 283–292, Almqvist and Wiksell, Stockholm; Interscience, New York, 1967.
267. Ramwell, P. W., Shaw, J. E., Clarke, G. B., Grostic, M. F., Kaiser, D. G. and Pike, J. E.: Prostaglandins.

In Progress in the Chemistry of Fats and Other Lipids, vol. 9, ed. by R. Holman, pp. 73–115, Pergamon Press, Oxford, 1967.

268. Ramwell, P. W., Shaw, J. E., Douglas, W. W. and Poisner, A. M.: Efflux of prostaglandin from adrenal glands stimulated with acetylcholine. Nature (London) 210: 273–274, 1966.
269. Ramwell, P. W., Shaw, J. E. and Jesup, R.: Spontaneous and evoked release of prostaglandins from frog spinal cord. Amer. J. Physiol. 211: 998–1004, 1966.
270. Ramwell, P. W., Shaw, J. E. and Kucharski, J.: Prostaglandin release from the rat phrenic nerve-diaphragm preparation. Science (N. Y.) 149: 1390–1391, 1965.
271. Renold, A. E. and Cahill, G. F., Jr. (eds.): Handbook of Physiology, section 5, Adipose tissue. American Physiological Society, Washington, D. C., 1965.
272. Robison, G. A., Butcher, R. W. and Sutherland, E. W.: Adenyl cyclase as an adrenergic receptor. Ann. N. Y. Acad. Sci. 139: 703–723, 1967.
273. Robert, A., Nezamis, J. E. and Phillips, J. P.: Inhibition of gastric secretion by prostaglandins. Amer. J. Dig. Dis. 12: 1073–1076, 1967.
274. Rosell, S.: Release of free fatty acids (FFA) from adipose tissue during sympathetic stimulation. Acta Physiol. Scand. 68 (Suppl. 277): 173, 1967.
275. Ruddon, R. W. and Johnson, J. M.: The effect of prostaglandins on protein and nucleic acid synthesis in a cell-free system. Life Sci. 6: 1245–1252, 1967.
276. Rythagr, R. and Samuelsson, B.: The origin of oxygen incorporated during the biosynthesis of prostaglandin E_1. Biochem. Biophys. Res. Commun. 19: 279–282, 1965.
277. Samuelsson, B.: Prostaglandins of human seminal plasma. Biochem. J. 89: 34P, 1963.
278. Samuelsson, B.: The structure of prostaglandin E_2. J. Amer. Chem. Soc. 85: 1878–1879, 1963.
279. Samuelsson, B.: Isolation and identification of prostaglandins from human seminal plasma. J. Biol. Chem. 238: 3229–3234, 1963.
280. Samuelsson, B.: Identification of a smooth muscle-stimulating factor in bovine brain. Biochim. Biophys. Acta 84: 218–219, 1964.
281. Samuelsson, B.: The identification of prostaglandin $F_{2\alpha}$ in bovine lung. Biochim. Biophys. Acta 84: 707–713, 1964.
282. Samuelsson, B.: Synthesis of tritium-labeled prostaglandin E_1 and studies on its distribution and excretion in the rat. J. Biol. Chem. 239: 4091–4096, 1964.
283. Samuelsson, B.: The prostaglandins. Angew. Chem. Int. Ed. 4: 410–416, 1965; and Angew. Chem. 77: 445–452, 1965.
284. Samuelsson, B.: On the incorporation of oxygen in the conversion of 8,11,14-eicosatrienoic acid to prostaglandin E_1. J. Amer. Chem. Soc. 87: 3011–3013, 1965.
285. Samuelsson, B.: Biosynthesis and metabolism of prostaglandins. Progr. Biochem. Pharmacol. 3: 59–70, 1967.
286. Samuelsson, B., Granström, E. and Hamberg, M.: On the mechanism of the biosynthesis of prostaglandins. *In* Prostaglandins, Proc. 2nd Nobel Symp., Stockholm, June 1966, ed. by S. Bergström and B. Samuelsson, pp. 31–44, Almqvist and Wiksell, Stockholm; Interscience, New York, 1967.
287. Sanbar, S. S., Mitchell, S. A., Lockey, R. F., Vlcek, E. A. and Greene, Jr., J. A.: Metabolic effects of hypotension induced by hemorrhage and by hypotensive drugs. Clin. Res. 15: 410, 1967.
288. Sandberg, F., Ingelman-Sundberg, A., Lindgren, L. and Rydén, G.: *In vitro* effects of prostaglandin on different parts of the human fallopian tube. Nature (London) 193: 781–782, 1962.
289. Sandberg, F., Ingelman-Sundberg, A. and Rydén, G.: The effect of prostaglandin E_1 on the human uterus and the fallopian tubes in vitro. Acta Obstet. Gynecol. Scand. 42: 269–278, 1963.
290. Sandberg, F., Ingelman-Sundberg, A. and Rydén, G.: The specific effect of prostaglandin on different parts of the human fallopian tube. J. Obstet. Gynaecol. Brit. Commonw. 70: 130–134, 1963.
291. Sandberg, F., Ingelman-Sundberg, A. and Rydén, G.: The effect of prostaglandin E_2 and E_3 on the human uterus and fallopian tubes in vitro. Acta Obstet. Gynecol. Scand. 43: 95–102, 1964.
292. Sandberg, F., Ingelman-Sundberg, A. and Rydén, G.: The effect of prostaglandin $F_{1\alpha}$, $F_{1\beta}$, $F_{2\alpha}$ and $F_{3\beta}$, on the human uterus and the fallopian tubes *in vitro*. Acta Obstet. Gynecol. Scand. 44: 585–594, 1965.
293. Sandberg, F., Ingelman-Sundberg, A., Johlsson, I. and Rydén, G.: Preliminary investigation on the absorption of prostaglandin E_1 from the human vagina. *In* Prostaglandins, Proc. 2nd Nobel Symp., Stockholm, June 1966, ed. by S. Bergström and B. Samuelsson, pp. 91–92, Almqvist and Wiksell, Stockholm; Interscience, New York, 1967.
294. Sasamori, S.: Isolation of prostaglandins from human seminal fluid and studies on their stimulating effects on the intestinal smooth muscles. Sapporo Med. J. 28: 286–299, 1965.
295. Shaw, J. E.: A study of the release of noncholinergic substances from the cerebral cortex. Ph.D. Thesis, Birmingham University, 231 pp., 1964.
296. Shaw, J. E.: Prostaglandin release from adipose tissue *in vitro* evoked by nerve stimulation or catecholamines. Federation Proc. 25: 770, 1966.
297. Shaw, J. E. and Ramwell, P. W.: Prostaglandin release from the adrenal gland. *In* Prostaglandins, Proc. 2nd Nobel Symp., Stockholm, June 1966, ed. by S. Bergström and B. Samuelsson, pp. 291–299, Almqvist and Wiksell, Stockholm; Interscience, New York, 1967.
298. Shaw, J. E. and Ramwell, P. W.: Prostaglandin release from rat epididymal fat pad on nerve and hormone stimulation. J. Biol. Chem., in press.
299. Silver, M. J.: Role of calcium ions and phospholipids in platelet aggregation and plug formation. Amer. J. Physiol. 209: 1128–1136, 1965.
300. Smith, E. R., McMorrow, J. V., Jr., Covino, B. G. and Lee, J. B.: Mechanism of the hypotensive and vasodilator action of prostaglandin E_1. Clin. Res. 15: 222, 1967.

301. Sólyom, A., Puglisi, L. and Mühlbachová, E.: Effect of *in vitro* theophylline and prostaglandin E_1 on free fatty acid release and on triglyceride synthesis in rat adipose tissue. Biochem. Pharmacol. 16: 521–525, 1967.
302. Sperelakis, N. and Lehmkuhl, D.: Insensitivity of cultured chick heart cells to autonomic agents and tetrodotoxin. Amer. J. Physiol. 209: 693–698, 1965.
303. Steinberg, D.: Prostaglandins as adrenergic antagonists. Ann. N. Y. Acad. Sci. 139: 897–909, 1967.
304. Steinberg, D. and Pittman, R.: Depression of plasma FFA levels in unanesthetised dogs by single intravenous doses of prostaglandin E_1. Proc. Soc. Exp. Biol. Med. 123: 192–196, 1966.
305. Steinberg, D. and Vaughan, M.: In vitro and in vivo effects of prostaglandins on free fatty acid metabolism. *In* Prostaglandins, Proc. 2nd Nobel Symp., Stockholm, June 1966, ed. by S. Bergström and B. Samuelsson, pp. 109–122, Almqvist and Wiksell, Stockholm; Interscience, New York, 1967.
306. Steinberg, D., Vaughan, M., Nestel, P. and Bergström, S.: Effects of prostaglandin E opposing those of catecholamines on blood pressure and on triglyceride breakdown in adipose tissue. Biochem. Pharmacol. 12: 764–766, 1963.
307. Steinberg, D., Vaughan, M., Nestel, P. J., Strand, O. and Bergström, S.: Effects of the prostaglandins on hormone-induced mobilisation of free fatty acids. J. Clin. Invest. 43: 1533–1540, 1964.
308. Stock, K., Böhle, E. and Westermann, E.: Differential effects of prostaglandin E_1 on lipolysis. Progr. Biochem. Pharmacol. 3: 122–128, 1967.
309. Stock, K. und Westermann, E.: Hemmung der Lipolyse durch α- und β-Sympathicolytica, Nicotinsaure und Prostaglandin E_1. Arch. Pharmakol. Exp. Pathol. 254: 334–354, 1966.
310. Stock, K. and Westermann, E.: Competitive and non-competitive inhibition of lipolysis by α- and β- adrenergic blocking agents, methoxamine derivatives and prostaglandin E_1. Life Sci. 5: 1667–1678, 1966.
311. Strong, C. G. and Bohr, D. F.: Effects of prostaglandins E_1, E_2, A_1 and $F_{1\alpha}$ on isolated vascular smooth muscle Amer. J. Physiol., 213: 725–733, 1967.
312. Strong, C. G., Bouchier, R., Nowacynski, W. and Genest, J.: Renal vasodepressor lipid. Proc. Staff Meetings Mayo Clin. 41: 433–452, 1966.
313. Stovall, R. and Jackson, R. T.: Prostaglandins and nasal blood flow. Ann. Otol. Rhinol. Laryngol. 76: 1051–1059, 1967.
314. Strulik, C. B., Beerthuis, R. K. and Dorp, D. A. van: Specificity in the enzymatic conversion of poly-unsaturated fatty acids into prostaglandins. *In* Prostaglandins, Proc. 2nd Nobel Symp., Stockholm, June 1966, ed. by S. Bergström and B. Samuelsson, pp. 51–56, Almqvist and Wiksell, Stockholm; Interscience, New York, 1967.
315. Strulik, C. B., Beerthuis, R. K., Pabon, H. J. J. and Dorp, D. A. van: Specificity in the enzymic conversion of polyunsaturated fatty acids into prostaglandins. Recueil Trav. Chim. Pays-Bas 85: 1233–1250, 1966.
316. Sullivan, T. J.: Response of the mammalian uterus to prostaglandins under differing hormonal conditions. Brit. J. Pharmacol. Chemotherap. 26: 678–685, 1966.
317. Sunahara, F. A. and Sterne, V. E.: Effects of prostaglandins on isolated ureters. Proc. Can. Federation Biol. Sci. 10: 21, 1967.
318. Suzuki, T. und Vogt, W.: Prostaglandine in einem Darmstoffpräparat aus Froschdarm. Arch. Exp. Pathol. Pharmakol. 252: 68–78, 1965.
319. Toh, C. C. and Mohuiddin, A.: Vasoactive substances in the nasal mucosa. Brit. J. Pharmacol. Chemotherap. 13: 113–117, 1958.
320. Trendelenburg, U.: Supersensitivity and subsensitivity to sympathomimetic amines. Pharmacol. Rev. 15: 225–276, 1963.
321. Tuttle, R. S. and Skelly, M. M.: Interactions of prostaglandin E_1 and ouabain on contractility of isolated rabbit atria and intracellular cation concentration. In preparation.
322. Vandelli, I.: E possibile differenziare l'attavità della istamine da quella della prostaglandina sullo intestino isolato di cavia. Boll. Soc. Ital. Biol. Sperim. 18: 74–75, 1943.
323. Vander, A. J.: Direct effects of prostaglandin on renal function and renal release in anesthetised dog. Amer. J. Physiol., 214: 218–221, 1968.
324. Vane, J. R.: The use of isolated organs for detecting active substances in the circulating blood. Brit. J. Pharmacol. Chemotherap. 23: 360–373, 1964.
325. Vaughan, M.: Effect of pitressin on lipolysis and on phosphorylase activity in rat adipose tissue. Amer. J. Physiol. 207: 1166–1168, 1964.
326. Vaughan, M.: An effect of prostaglandin E_1 on glucose metabolism in rat adipose tissue. *In* Prostaglandins, Proc. 2nd Nobel Symp., Stockholm, June 1966, ed. by S. Bergström and B. Samuelsson, pp. 139–142, Almqvist and Wiksell, Stockholm; Interscience, New York, 1967.
327. Vaughan, M. and Steinberg, D.: Glyceride biosynthesis, glyceride breakdown and glycogen breakdown in adipose tissue: Mechanisms and regulation. *In* Handbook of Physiology, section 5, Adipose tissue, ed. by A. E. Renold and G. F. Cahill, Jr., pp. 239–252, American Physiological Society, Washington, D. C., 1965.
328. Vergroesen, A. J., De Boer, J. and Gottenbos, J. J.: Effects of prostaglandins on perfused isolated rat hearts. *In* Prostaglandins, Proc. 2nd Nobel Symp., Stockholm, June 1966, ed. by S. Bergström and B. Samuelsson, pp. 211–218, Almqvist and Wiksell, Stockholm; Interscience, New York, 1967.
329. Vogt, W.: Pharmacologically active acidic phospholipids and glycolipids. Biochem. Pharmacol. 12: 415–420, 1963.
330. Vogt, W., Suzuki, T. and Babilli, S.: Prostaglandins in SRS-C and in darmstoff preparation from frog intestinal dialysates. Mem. Soc. Endocrinol. 14: 137–142, 1966.
331. Vonkeman, H.: De biosynthese van prostaglandines. Chem. Weekbl. 62: 361–366, 1966.
332. Waitzman, M. B., Bailey, W. R., Jr. and Kirby, C. G.: Chromatographic analysis of biologically active lipids from rabbit irides. Exp. Eye Res. 6: 130–137, 1967.

333. WAITZMAN, M. B. AND KING, C. D.: Prostaglandin influences on intraocular pressure and pupil size. Amer. J. Physiol. **212**: 329–334, 1967.
334. WAITZMAN, M. B. AND KING, C. D.: Mechanisms of prostaglandin influences on ocular pressure and pupil size. Federation Proc. **26**: 655, 1967.
335. WAITZMAN, M. B. AND KIRBY, C. G.: Effects of chromatographically pure compounds extracted from iris on intraocular pressure and pupil size. Invest. Ophthalmol. **5**: 329, 1966.
336. WALLACH, D. P.: The enzymic conversion of arachidonic acid to prostaglandin E_2 with acetone powder preparations of bovine seminal vesicles. Life Sci. **4**: 361–364, 1965.
337. WEEKS, J. R.: Unpublished observations.
338. WEEKS, J. R., CHANDRASEKHAR, N. AND DUCHARME, D. W.: Relative activity of prostaglandins E_1, E_2, A_1 and A_2 on lipolysis, platelet aggregation, smooth muscle and the cardiovascular system. In preparation.
339. WEEKS, J. R. AND LAWSON, C. F.: Unpublished observations.
340. WEEKS, J. R. AND WALK, R. A.: Reduction of variability between replicates in assay of hormone stimulated lipolysis: A study on prostaglandin E_1 (PGE_1) inhibition. Pharmacologist **9**: 254, 1967.
341. WEEKS, J. R. AND WINGERSON, F.: Cardiovascular action of prostaglandin E_1 evaluated using unanesthetized relatively unrestrained rats. Federation Proc. **23**: 327, 1964.
342. WIQVIST, N., BYGDEMAN, M., KWON, S. U. AND MUHKERJEE, T.: The effect of prostaglandin E_1 on the mid-pregnant human uterus. Intravenous, intramuscular, intraamniotic and vaginal administration. Amer. J. Obstet. Gynecol., in press.
343. WOLFE, L. S., COCEANI, F. AND PACE-ASCIAK, C.: Brain prostaglandins and studies of the action of prostaglandins on the isolated rat stomach. *In* Prostaglandins, Proc. 2nd Nobel Symp., Stockholm, June 1966, ed. by S. Bergström and B. Samuelsson, pp. 265–276, Almqvist and Wiksell, Stockholm; Interscience, New York, 1967.
344. ZIMMERMAN, B. G.: Personal communication.