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Prostaglandins: Members of a New Hormonal System

These physiologically very potent compounds of ubiquitous occurrence are formed from essential fatty acids.

Sune Bergström

In 1930 two New York gynecologists, Kurzrok and Lieb (*1*), reported that the human uterus could react with either strong contractions or relaxation on instillation of fresh human semen. We now know that these effects were due to the prostaglandins present in the seminal plasma, but not until a few years later was it clearly recognized that the effect was due to the presence of these then-unknown compounds.

Goldblatt (*2*) in England and von Euler (*3*) in Sweden independently discovered and studied the strong activity in seminal plasma that stimulates smooth muscle. The seminal plasma of several species was investigated by von Euler, who found similar effects in the seminal fluid of only monkey, sheep, and goat and in extracts of vesicular glands of male sheep. He prepared lipid extracts of these glands and found the activity to be associated with the fraction containing the lipid-soluble acids. He named the active factor prostaglandin and made extensive studies of the physiologic and pharmacologic effects of these extracts (*3*).

Isolation and Structure

At the suggestion of von Euler in 1947, I started work on the purification of a concentrate prepared (*4*) from glands of Icelandic sheep. With the small amount of material available, using primarily the Craig countercurrent procedure, I showed that the activity was associated with a fraction containing unsaturated hydroxy acids. Our present knowledge shows that the best concentrates consisted predominantly of a mixture of prostaglandins (*5*). However, for technical and personal reasons, the project was laid aside until 1956 when a program for the collection of frozen glands of sheep was organized in several countries in the Northern Hemisphere.

The bioassay used for the isolation work utilized the smooth muscle-stimulating activity of the prostaglandins on rabbit duodenum—a sensitive but rather unspecific test. With this method of assay, an improved isolation procedure (*6*) led to the isolation of two prostaglandins, PGE_1 and $PGF_{1\alpha}$, in pure crystalline form (*7*). They both showed high physiologic activity (higher than 10^{-9} gram per milliliter) on smooth muscle, but the former was the more active in reducing blood pressure in the rabbit (*8*).

Analysis of the first few milligrams by ultramicroanalysis and mass spectrometry proved that these substances were C_{20} compounds possessing a unique structure (Table 1). An early observation that proved very important was that PGE_1 was of ketonic nature and that reduction with borohydride yielded two isomeric trihydroxy compounds, one of which was identical with the isolated natural $PGF_{1\alpha}$ (*7*, *9*).

Prostaglandin PGE_1 was very sensitive to alkali, which rapidly destroyed the biologic activity, with formation of a compound absorbing at 278 nanometers. With more dilute alkali, an intermediate was first formed that absorbed at 217 nanometers. The structure finally elucidated by oxidative ozonolysis of PGE_1, dihydro-PGE_1, and the above-mentioned compounds absorbing at 217 and 278 nanometers. The reaction products were resolved by gas-liquid chromatography and identified directly by mass spectrometry (*10*).

These studies showed the PGE_1 molecule to contain a five-membered ring, with one of the hydroxyls β to the keto group, which fact explains the lability to alkali. The second hydroxyl and the *trans* double bond were located in a side chain (Fig. 1). Treatment of PGE_1 with weak base yields the α, β-unsaturated ketone absorbing at 217 nanometers, PGA_1, (PGE_1-217), that then rearranges to the doubly conjugated ketone PGB_1 (PGE_1-278).

The stereochemistry was then elucidated by complete x-ray analysis of the bromo- and iodobenzoates of $PGF_{1\beta}$ (*11*). The correct absolute stereochemical configuration has been obtained by redetermination of the optical activity of the 2-hydroxy-heptanoate formed by oxidative ozonolysis (Fig. 2) (*12*).

Two more compounds, PGE_2 and PGE_3, were subsequently isolated from the sheep glands. Mass spectrometry and nuclear magnetic resonance yielded most of the complete structure (*13*). They were identical with PGE_1 except that they contained, respectively, one and two additional *cis* double bonds (Fig. 2). The corresponding $PGF_{2\alpha}$ and $PGF_{3\alpha}$ were first isolated from sheep and bovine lung tissues, respectively (*14*). As to the structures of the six "primary" prostaglandins, the numbering system of the basic "prostanoic acid" is indicated (Fig. 2) and a general system of nomenclature is explained (Fig. 2, legend).

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Occurrence and Metabolism

After isolating the first two prostaglandins, we explored the occurrence of smooth muscle-stimulating activity in the lipid-soluble acid fractions of various organs. Some activity was found in practically all extracts investigated. However, as the rabbit duodenal test was known to be most unspecific, more careful chemical identification of the active compound was necessary for conclusion that the active compounds were indeed prostaglandins. Thus there is no point in reviewing earlier literature on the occurrence of smooth muscle-stimulating activity in crude extracts of tissue; even autoxidation products of some unsaturated fatty acids can stimulate smooth muscle.

Chromatographic procedures have now been published by which all known prostaglandins can be separated and identified (*13, 15*). A method for direct quantitative determination of the prostaglandins in human semen has been developed (*15*), but in most other issues the concentration of these relatively labile compounds is so low that isotope-dilution methods have been necessary (*16*). Thin-layer chromatography, in combination with a special micromodification of the smooth-muscle assay, has yielded a very sensitive method (*17*), but there is still great need for even more sensitive and more specific methods to reach the levels (about 10^{-6} to 10^{-9} gram per gram of tissue) that are necessary for many physiologic studies.

With these methods the presence of primary prostaglandins has been demonstrated in a number of different tissues, including lung (*14*), pancreas (*16*), brain, (*17, 18*), kidney (*19*), and iris (*20*), and in human menstrual fluid (*21*), and there are strong indications of the occurrence of prostaglandins in many other tissues. In most instances they occur in lower concentrations (0.5 to 1 microgram per gram, wet weight) than in human seminal fluid (Table 2), the richest known source. As well as some of the reported data, Table 2 shows that various tissues contain different mixtures of primary prostaglandins.

Of special interest is human seminal plasma, which contains not only the highest concentrations of the primary prostaglandins E_1, E_2, E_3 $F_{1\alpha}$ and $F_{2\alpha}$ found so far but also even higher concentrations of a number of related compounds. It was recognized early during the work on isolation of the primary prostaglandins from human seminal plasma that other compounds having similar properties were present (*22*); their structure was recently elucidated by Hamberg and Samuelsson (*23*). Among them are the two earlier-mentioned dehydration products of PGE_1 (absorbing at 217 and 278 nanometers), PGA_1, and PGB_1; also PGA_2 and PGB_2, which are related in a like manner to PGE_2. Furthermore a new type of prostaglandin has been isolated containing an additional hydroxyl group at carbon atom 19; so far the 19-hydroxyl has been found only in the four dehydrated compounds (A and B types) just mentioned and not in compounds containing the 11-hydroxyl group. All present indications are that these eight compounds are metabolites formed in vivo. It appears that at most a minor part of the A and B compounds may have formed during the isolation procedure. It is also noteworthy that seminal plasma from sheep contains mainly "primary" prostaglandins and no 19-hydroxylated compounds (*24*). All prostaglandins so far identified in human seminal plasma are shown in Fig. 3; their average concentrations appear in Table 3 (*15*). One should note that the total concentration of the 13 different prostaglandins is about 300 micrograms per milliliter of human seminal plasma; this is by far the highest concentration observed in any tissue to date.

Another type of metabolic pattern

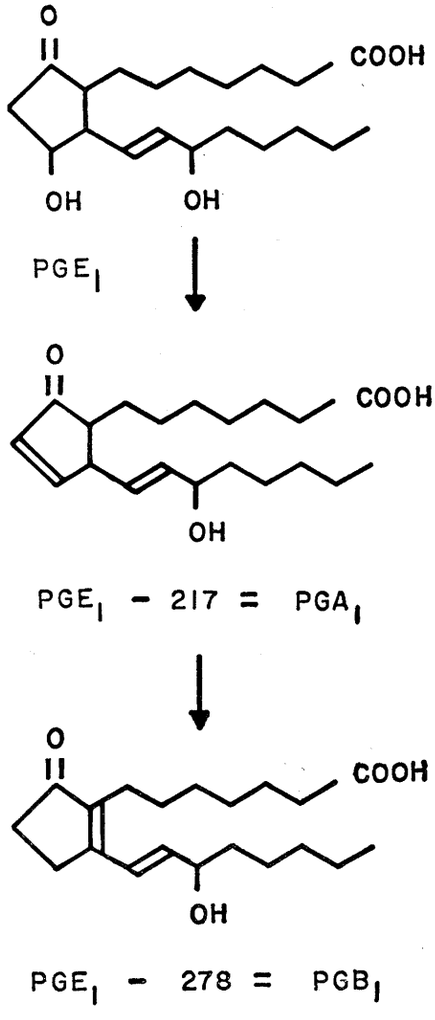

Fig. 1. Dehydration and rearrangement of PGE_1 when treated with mild alkali.

Table 1. Chemical details of two prostaglandins; mw, molecular weight.

Prostaglandin	Melting point (°C)	CHO analysis	Mass spectr. (mw)	Carboxyl	Keto group	Hydroxyls	*Trans* double bond	Ring
PGE_1	115–17	$C_{20}H_{34}O_5$	354	1	1	2	1	1*
$PGF_{1\alpha}$	102–03	$C_{20}H_{36}O_6$	356	1		3	1	1

* Cyclopentanone.

Table 2. Occurrence (+) of six primary prostaglandins in various tissues.

Source	PGE_1	PGE_2	PGE_3	$PGF_{1\alpha}$	$PGF_{2\alpha}$	$PGF_{3\alpha}$
Vesicular gland, sheep	+	+	+	+		
Seminal plasma, human	+	+	+	+	+	
Seminal plasma, sheep	+	+	+	+	+	
Menstrual fluid, human		+			+	
Lungs, sheep		+			+	
Lungs, bovine					+	+
Lungs, pig, guinea pig, monkey, man					+	
Iris, sheep					+	
Brain, bovine					+	
Thymus, calf	+					
Pancreas, bovine		+			+	
Kidney, pig		+			+	

Table 3. Approximate concentrations of prostaglandins in human seminal plasma and their actions on human myometrium in vitro. According to a recent nomenclature proposal, PGA_1 and A_2 are used instead of PGE_1-217 and PGE_2-217, respectively; PGB_1 and B_2, instead of PGE_1-278 and PGE_2-278.

Substance	Concentration (μg/ml)	Effect on human myometrium		
		Nature	Threshold conc. (μg/ml)	
			Nonpregnant	Pregnant
PGE_1	25	Inhibition	0.01–0.1	0.03–0.3
PGE_2	23 } 53.5			
PGE_3	5.5			
PGA_1, PGA_2, PGB_1, PGB_2	50	Inhibition	.3–1.0	
19-Hydroxy-PGA_1, 19-Hydroxy-PGA_2, 19-Hydroxy-PGB_1, 19-Hydroxy-PGB_2	200	Inhibition	1.0–3.0	
$PGF_{1\alpha}$	3.6 } 8	Stimulation	0.3–0.5	.01– .03
$PGF_{2\alpha}$	4.4			

is shown by lung tissue, in which PGF_2 has been found (*13*) in all species investigated (Table 2) (*25*). Early physiologic studies indicated that the lungs might have a relatively high capacity to inactivate injected PGF_1. Extensive studies (*26*) with labeled compounds have shown that PGE_1, PGE_2, and PGE_3 are metabolized by the supernatant fraction from lung homogenates (Fig. 4); there is either a reduction of the 13–14 double bond or a dehydrogenation of the 15-hydroxyl group, or sometimes a combination of both reactions. In guinea pig lung both reactions occur; in pig lung, only the dehydrogenation. Therefore pig lung was used as the source for the purification of the 15-hydroxydehydrogenase (*27*). This enzyme showed a high specificity for the 15-hydroxyl in compounds of the E, F, and A types; this finding obviously may be of great value in the development of a sensitive and specific enzymic method for determination of prostaglandins.

Both PGE_2 and the corresponding dehydration product, PGA_2 (PGE_2-217), have been found in rabbit-kidney extracts (*19*). The latter compound, also isolated from human semen (*23*), is apparently primarily responsible for the activity ascribed to the factor "medullin" (*28*). Whether PGA_2 occurs preformed in the tissue or is formed during the isolation procedure is not certain (*29*).

Studies of the fate of small amounts of tritium-labeled PGE_1, intravenously injected into rats, showed that 40 hours later about 50 percent of the isotope had been excreted in the urine and 10 percent in the feces, by way of the bile. The metabolites found in the blood were those previously shown to be formed by lung tissue (Fig. 4), whereas the metabolites in urine and bile were more polar and remain unidentified (*30*). High concentrations of radioisotope were found in kidneys and liver, with a maximum about 20 minutes after the injection. The concentrations were low in brain, muscle, and adipose tissue; intermediate in lung, uterus, heart, and a number of endocrine glands.

Autoradiographs of mice that had been injected intravenously with tritium-labeled PGE_1 (2 to 7 micrograms) also showed transient high concentrations in kidney, liver, and connective tissue, with somewhat lower concentrations in the lungs. Intestinal smooth muscle was almost devoid of activity, whereas the myometrium of the uterus showed a significant uptake (*31*). The unexpectedly high concentrations in connective tissue are unexplained.

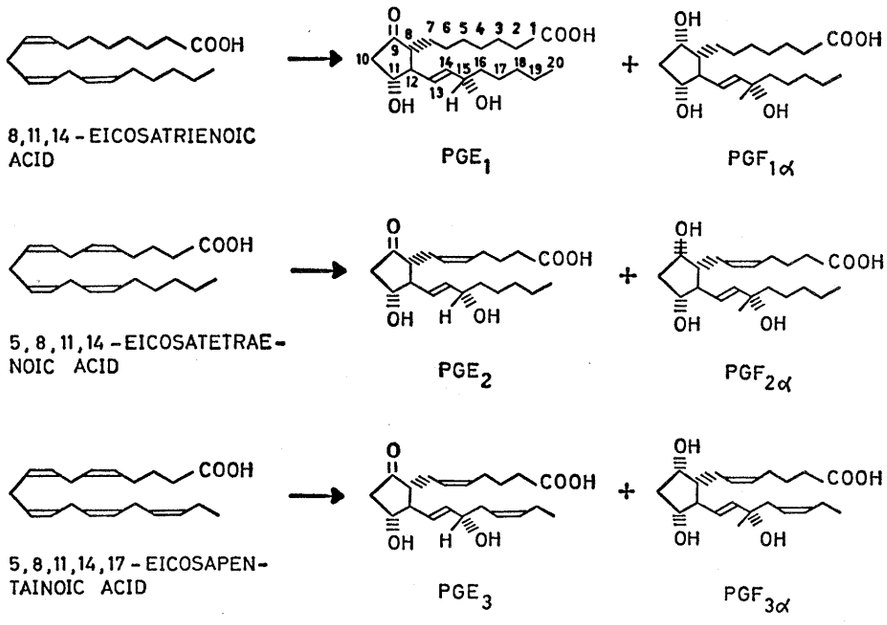

Fig. 2. The primary prostaglandins and their precursors. A general system for nomenclature of the prostaglandins has been proposed (*12, 75*). The basic structure of a fully saturated C_{20} acid, with C-8 to C-12 closed to form a five-membered ring, is designated prostanoic acid; PGE_1 (top middle) then becomes 9-keto-11α,15α-dihydroxyprost-13-enoic acid.

Biosynthesis

When the structures of the primary prostaglandins had been elucidated, it occurred to several groups of investigators that the appropriate C_{20} polyunsaturated fatty acids, with methylene-interrupted double bonds, might be the precursors of the prostaglandins. It was simultaneously shown by two groups that prostaglandin PGE_2 was formed in high yield as the main product when tritium-labeled arachidonic acid (*32*) was incubated with whole homogenates of sheep vesicular glands (*33, 34*). Wallach (*35*) found independently that this biosynthesis also could be effected by an acetone powder of vesicular glands of the bull; this finding is of interest since this species

does not have detectable levels of prostaglandins in the seminal plasma.

The work has subsequently expanded rapidly in various directions. Dihomo-γ-linolenic acid (all-*cis*-eicosa-8,11,14-trienoic acid) and eicosa-5,8,-11,14,17-pentaenoic acid were shown to be precursors of PGE_1 (*33, 35*) and PGE_3 (*36*), respectively (see Figs. 2 and 4).

Biosynthesis, with the use of homogenate of sheep vesicular glands, yielded only small amounts of the corresponding PGF compounds, but incubations of homogenate of guinea pig lung with arachidonic acid yielded predominantly $PGF_{2\alpha}$, together with PGF_2 and its two metabolites (Fig. 4) (*37*). It is of special interest that the PGE and PGF compounds do not seem to be interconvertible in any tissue homogenates investigated so far (see below).

The prostaglandins are thus formed, in a facile enzymic sequence of reactions in several different tissues, from members of the group of essential fatty acids. The concept of "essential fatty acids" is based on the discovery in 1929 (*38*) that linoleic acid is a dietary constituent necessary for the normal growth and health of rats. In the absence of essential fatty acids a series of typical symptoms of deficiency develop, including poor growth, skin lesions ("scaly tail") with excessive evaporation of water through the skin, kidney damage, and impairment of fertility. These observations have since been extended to other species: certain skin lesions in children, ascribed to lack of essential fatty acids, have been reportedly cured by administration of linoleate.

Subsequently γ-linolenic, dihomo-γ-linolenic, and arachidonic acids have proved to be even more active than linoleate in curing the deficiency symptoms in rats. These acids are, however, normal metabolites of dietary linoleic acid that are formed according to the pathway outlined in Fig. 5. The two last members of this reaction sequence, bishomo-γ-linolenic and arachidonic acid, are thus, respectively, the precursors of PGE_1 and PGE_2. Dietary linolenic acid, reported to be much less active than linoleic acid in preventing signs of deficiency, likewise undergoes similar reactions to yield the fatty acid with 20 carbon atoms and five double bonds that is the precursor of PGE_3 (Fig. 2).

Very little is known about the mechanism underlying the deficiency of es-

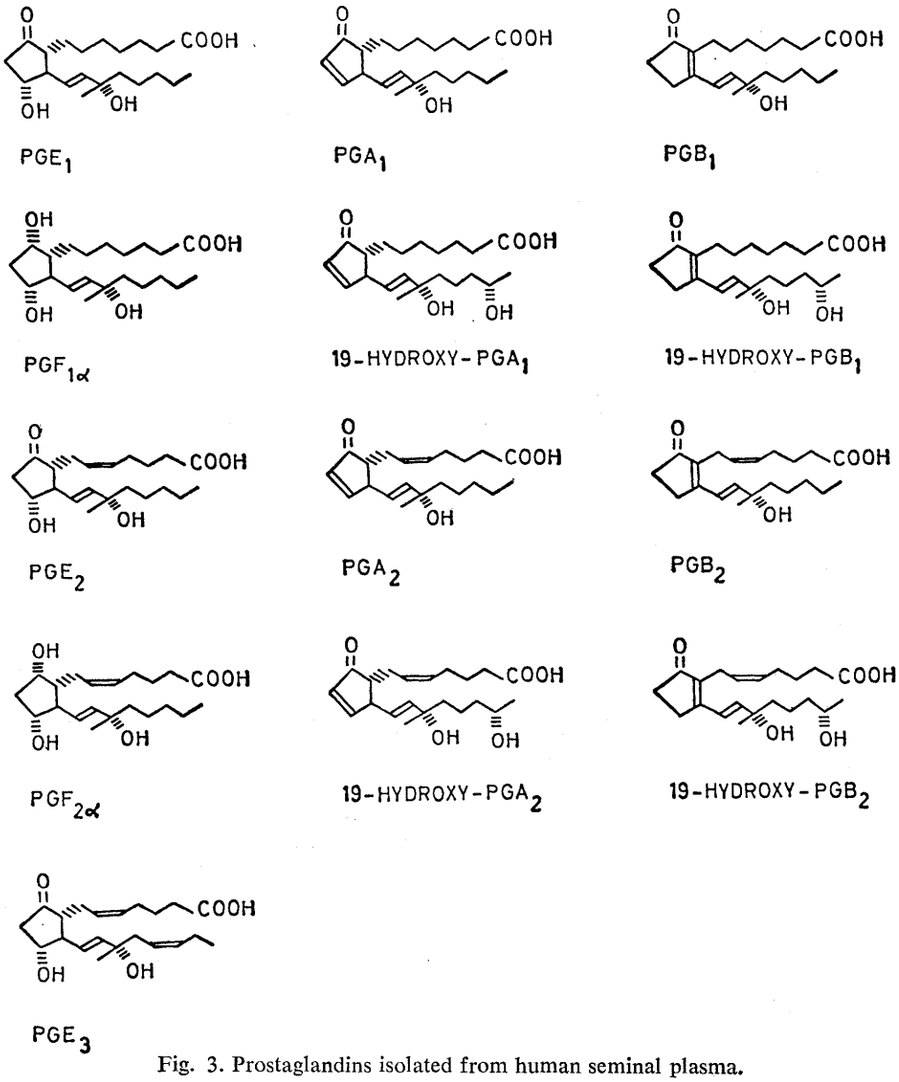

Fig. 3. Prostaglandins isolated from human seminal plasma.

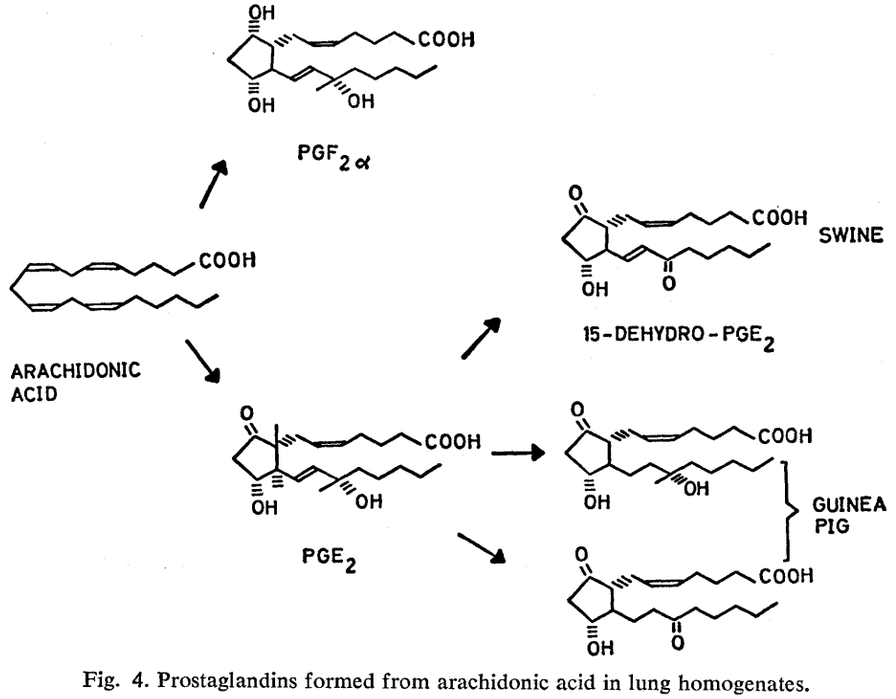

Fig. 4. Prostaglandins formed from arachidonic acid in lung homogenates.

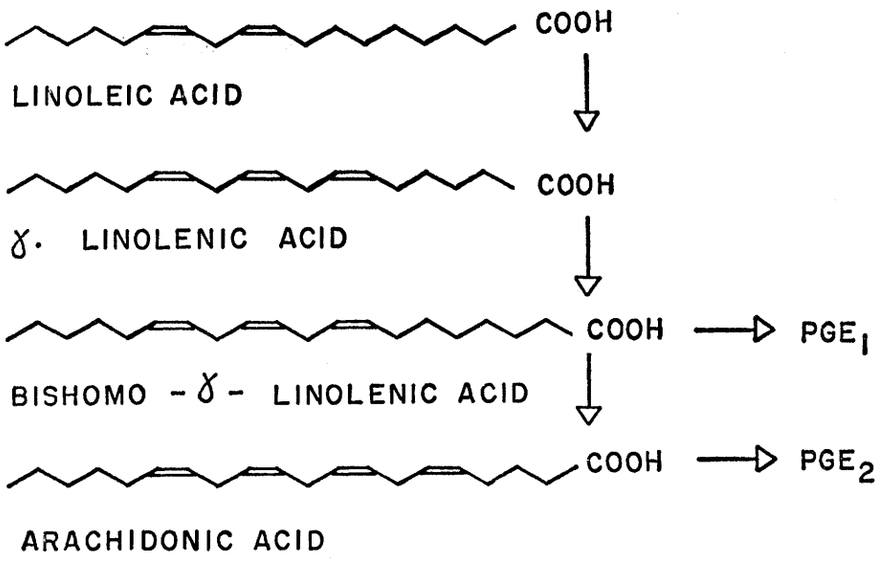

Fig. 5. Formation of arachidonic acid from linoleic acid in animals.

sential fatty acids. The lack of precursors of prostaglandins presumably cannot explain all the symptoms of deficiency. Attempts to alleviate with prostaglandins the skin symptoms of rats deficient in essential fatty acids have not succeeded (*39*).

Mechanism of Biosynthesis

The biosynthesis of prostaglandins is effected by an enzyme system associated with the microsome fraction, together with a heat-stable cofactor present in the supernatant fraction (*40*). The cofactor requirement can also be met by reduced glutathione, tetrahydrofolate, or 6,7-dimethyltetrahydropteridine, but not by reduced nicotinamide-adenine dinucleotide or dinucleotide phosphate. Under optimal conditions a yield of 60 percent of PGE_1 can be obtained. Samuelsson *et al.* (*40*) recently solubilized and partially purified the enzyme system from the microsomes with a nonionic detergent.

With the aid of $18o_2$, all three oxygen atoms present at carbon atoms 9, 11, and 15 have been shown to derive from molecular oxygen (*41*). Of special interest is the finding that the two oxygens present in the ring derive from the same oxygen molecule. This point was first demonstrated by Samuelsson by incubation in a mixture of equal parts of $18o_2$ (18o-18o) and $16o_2$ (16o-16o); the product contained exclusively either 18o or 16o in the two oxygens at C-9 and C-11 of the ring (*41*). Through the study of several stereospecifically tritium-labeled precursors, the reaction mechanism has been elucidated further and can now be summarized (Fig. 6) (*41*).

The initial reaction appears to be stereospecific removal of a hydrogen atom at C-13, followed by formation of a lipoxidase-type reaction yielding 11-peroxy-8,12,14-eicosatrienoic acid. The formation of the cyclic peroxide is visualized as involving a concerted reaction, with addition of oxygen at C-15, isomerization of the 12,13 double bond, formation of the ring through a new carbon-carbon bond joining C-8 and C-12, and attack on the 11-peroxy radical at C-9 (Fig. 6). Among the by-products are 11-hydroxy-8,12,14-eicosatrienoic and 12-hydroxy-8(*trans*)-10(*trans*)heptadecadienoic acid (Fig. 6). The latter compound is formed by an interesting reaction in which carbon atoms 9, 10, and 11 are eliminated, as malonaldehyde, from the cyclic peroxide (*40, 42*).

Analysis is in accordance with the following formulas:

$$C_{20}H_{34}O_2+2O_2+RH_2 \longrightarrow \underset{(PGE_1)}{C_{20}H_{34}O_5}+R+H_2O$$

$$C_{20}H_{34}O_2+2O_2+2RH_2 \longrightarrow \underset{(PGF_{1\alpha})}{C_{20}H_{36}O_5}+2R+H_2O$$

When a PGE compound is formed, reduction occurs only at C-15, whereas the rearrangement of the cyclic peroxide does not entail any overall change in the oxidative state of the molecule. When a PGF compound is formed, there is instead a reductive opening of the endoperotide, yielding the two hydroxyl groups at C-9 and C-11. The ratio of PGE and PGF compounds formed can be varied in vitro and presumably also in vivo; regulation of ratio must be of physiologic importance because the two types of compound have quite different biologic properties.

The substrate specificity of the enzyme system is not absolute for the C-20 acids discussed so far. Preliminary work showed that C_{19} and C_{22} homologues of dihomo-γ-linolenic acid could be converted into a nor- and a dihomo-PGE_1, respectively (*33, 36*). This work has been greatly extended by the Dutch group (*40*), who found that both dihomo-γ-linolenic and arachidonic acids could be elongated at the carboxyl end by one to four carbon atoms, and shortened by one, and still yield prostaglandin homologues, whereas practically no reaction occurred with a C_{18} acid. The double-bond system of all these acids starts at C-6 counted from the methyl end of the molecule—that is, at ω-6. A number of isomeric acids have been tested as precursors, in which the double-bond system had been moved to new positions in the molecules. Biosynthesis of a prostaglandin-type compound only occurred after relocation to ω-7, but not in ω-3, -5, -8, or -9 systems. Reduction or esterification also precludes biosynthesis (*40, 43*). Thus the specificity of the enzyme system is not very high, but preliminary reports indicate that the biologic action on smooth muscle is limited to the C_{20} compounds (*40*).

Chemical Synthesis

Work on total synthesis of the prostaglandins is under way in may laboratories. So far only one synthesis of a naturally occurring prostaglandin, dihydro-PGE_1, has been reported (*44, 46*).

Physiologic Action on the Circulatory System

The early work on the physiologic properties of prostaglandins was done with extracts of vesicular glands of sheep or with human seminal plasma; it was mainly limited to the cardiovascular system and to the reactions of smooth muscle in the intestine and in the female reproductive tract (*2, 3, 47*). After isolation of PGE_1 and $PGF_{1\alpha}$ (*7*),

both proved very potent physiologically; both were very active in stimulating intestinal smooth muscle of rabbits, but they showed different activities in several systems. The former was at least ten times more active in reducing blood pressure.

The physiological properties of the many prostaglandin compounds that were subsequently isolated are now under intensive study in many laboratories. I shall limit this article to certain aspects of recent work on their action on the cardiovascular system and the female reproductive tract, and on some metabolic reactions. Comprehensive reviews are available (*44, 48–50*).

Most of the prostaglandins have potent biologic activity of some kind. The data in Table 4 illustrate the different relative biologic activities of some of these closely related compounds both in the same and in different species. Reduction of the double bond decreases the effect on the blood pressure of rabbits but increases it in the guinea pig. Reduction of the keto group increases the effect on the intestine in the rabbit but drastically decreases it in the guinea pig. This reduction of PGE compound to a PGF compound always decreases the effect of lowering the blood pressure. In fact $PGF_{2\alpha}$ even increases the blood pressure in dogs, apparently by a unique peripheral venoconstriction (DuCharme and Weeks in *44*). The typical effect of the PGE compounds, however, is peripheral vasodilation of the small arteries.

The first studies of infusion of PGE_1 in humans in 1959 (*51*) showed an increase in heart rate and a slight fall in arterial blood pressure. Subsequent work both on dogs and humans has shown that infusion of PGE_1 causes a typical and reproducible increase in heart rate even before any fall in blood pressure is seen. After treatment with sympathetic ganglionic blocking agents there is no increase in heart rate whereas the blood-pressure response remains. This and other evidence strongly suggests that the activity of the sympathetic nervous system is required for both the heart-rate response and the stimulation of mobilization of free fatty acids (see below) observed after low doses of PGE_1 (*52–55*). An example of cardiovascular response in humans to infusion of increasing doses of PGE_1 is shown in Fig. 7 (*56*).

Table 4. Relative activities of a number of prostaglandins in five test systems.

Test system	PGE_1	PGE_2	PGE_3	PGE_1·H_2	$PGF_{1\alpha}$
Rabbit, duodenum	1	3	0.4	0.4	1.5
Guinea pig, ileum	1		.2	.1	>0.05
Rabbit, blood pressure	1	0.6	.3	.6	> .1
Guinea pig, blood pressure	1			1.6	
Rat, fat pad	1	~.1	~.2	~0.1	< .1

Compounds that may prove of special medical interest are the dehydration products absorbing at 217 nanometers—PGA_1 and PGA_2 ("medullin"). They are at least as active as the corresponding PGE compounds in reducing the blood pressure, but have a weak effect on both intestinal smooth muscles and lipid metabolism (*28*).

According to a recent report (*28, 44*), several prostaglandins also show antihypertensive action in nephrectomized dogs. Paoletti *et al.* (*57*) especially have made extensive studies in vitro of the influence of prostaglandins on heart rate and coronary blood flow in many species. These pharmacologic effects are more evident when isolated tissues are incubated in media deficient in calcium ions (*44, 57*). Considerable data are now available on the action of various prostaglandins on the cardiovascular system both in vitro and in vivo; there are special review articles (*44, 47–50*).

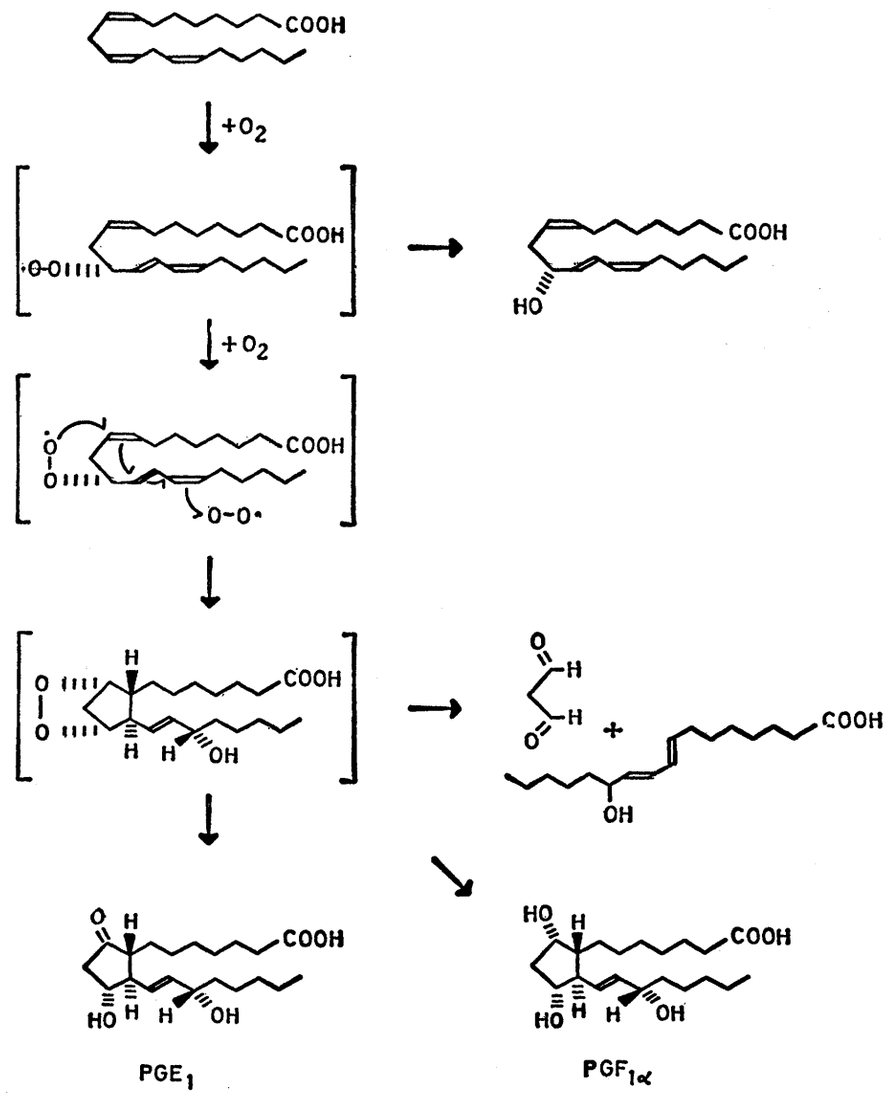

Fig. 6. Mechanism of biosynthesis of prostaglandins E_1 and $F_{1\alpha}$.

Action on Female Reproductive Organs

All prostaglandins of the PGE type have strong inhibitory action in vitro on strips of myometrial muscle strips from nonpregnant females. The PGF compounds, on the other hand, show stimulatory action that varies in degree during the menstrual cycle (Table 3). Prostaglandin PGE_1 has a complex effect on the motility of pregnant-human myometrium in vitro: small doses (0.01 microgram per milliliter of bath fluid) stimulate the motility, with an increase in tonus, whereas higher doses (0.03 microgram per milliliter) inhibit the motility—the effect always observed with nonpregnant myometrium (*29*). Study in vitro of the action in the fallopian tube (*58*) showed contractions of the inner third and relaxation of the outer part. As I have mentioned, small amounts of prostaglandins have been identified in human menstrual fluid, and the variations in endometrial tissue during the menstrual cycle are under study (*49*).

Normal human seminal plasma has the highest concentration of prostaglandins found so far (Table 3). A few of a number of samples, from the males of infertile couples, lacked prostaglandins; the generality and implications of this finding are not yet clear (*15*), but obviously the action in vivo of prostaglandins in the human female is of special interest. Deposition of semen and seminal extracts in the vaginas of infertile (*59*) or fertile (*47*) young women affected the motility of the uterus only at the time of ovulation: after stimulation of the motility for a few minutes, a period of relaxation followed.

These results have recently been extended by use of pure compounds and more sensitive techniques (*60*). In some instances, continuous intravenous injection of as little as 0.01 microgram of PGE_1 per minute per kilogram into pregnant women causes the onset of uterine activity similar to normal activity during labor. At such dosage no subjective side effects are observed and the blood pressure remains constant. Deposition of pure prostaglandins in the vagina has similar effects. In view of the considerable amounts of PGE's present in an ejaculation (150 to 200 micrograms) it seems possible that sufficient prostaglandin can be absorbed from the vagina to influence strongly in some instances the motility of the pregnant uterus (*44, 61*). There has been much speculation in this field; more information is needed for evaluation of the possible roles of the prostaglandins in conception and delivery.

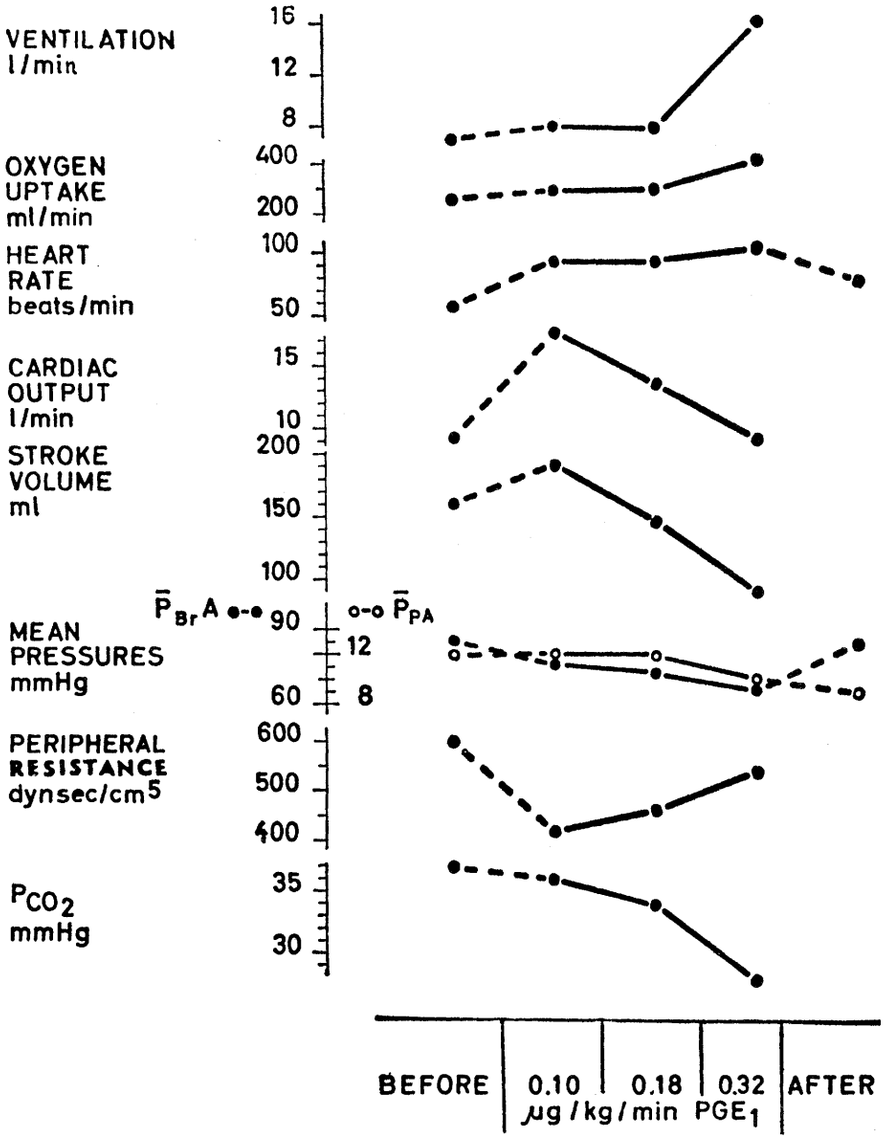

Fig. 7. Cardiovascular effects on man of intravenous infusion of increasing doses of PGE_1.

Metabolic Actions

In vitro, PGE_1 strongly antagonizes the stimulatory effects of a number of hormonal compounds on the release of free fatty acids and glycerol from epididymal fat pads of fed rats (*53*). In this tissue preparation there is a certain small, continuing, net hydrolysis of triglycerides. Part of the fatty acids that are set free inside the fat cells are, however, utilized in resynthesis of triglycerides to varying degrees, whereas practically all the glycerol of the hydrolyzed triglycerides is released from the cells. The amount of glycerol found in the medium therefore constitutes a better indicator of the extent of the lipolysis than does the amount of free fatty acids released. The rate of lipolysis is determined by the activity of a triglyceride lipase that hydrolyzes only one ester bond, with the formation of a diglyceride, which is then totally hydrolyzed by other lipases present. The triglyceride lipase is present largely in an inactive form, but it can be rapidly activated by the addition of one of a number of hormonal compounds such as epinephrine, norepinephrine, glucagon, adrenocorticotropic hormone, thyroid-stimulating hormone, or vasopressin. The rate of lipolysis and release of glycerol and free fatty acids is rapidly increased by addition

of any of these compounds to the medium (Fig. 8).

The ability of PGE_1 (0.1 microgram per milliliter; $2.8 \times 10^{-7}M$) to inhibit strongly the lipolytic effect of epinephrine (0.1 microgram per milliliter; $5.5 \times 10^{-7}M$) and a number of other hormonal compounds is illustrated in Fig. 8; PGE_1 is slightly more active than PGE_3, $PGF_{1\alpha}$, or $PGF_{1\beta}$ (*44*, *53*). The lipolysis in human adipose tissue in vitro is inhibited similarly by PGE_1 (*62*). Even lower concentrations of PGE_1 can completely block the release of free fatty acids from fat pads, induced by electrical stimulation of the postganglionic sympathetic fibers innervating the tissue (*63*).

The effect of PGE_1 in epinephrine-stimulated adipose tissue is due to suppression of the activation of the hormone-sensitive triglyceride lipase (*44*). I shall return later to the question of the mechanism by which so many different compounds elicit the same response that can be counteracted by prostaglandins.

The action of prostaglandins on metabolism of free fatty acids has also been studied in vivo in dogs and humans (*53*, *54*, *56*). Injection of epinephrine induces increase both of glucose and of glycerol and free fatty acids in blood; PGE_1 counteracts the increase of the latter two compounds, whereas the glucose response is unchanged. As an example, Fig. 9 shows in a dog the continuous high level of plasma free fatty acids induced by continuous infusion of norepinephrine. A single injection of PGE_1 during this period causes a transient reduction in the level of free fatty acids, whereas a continuous infusion of PGE_1 brings this level back to normal.

Table 5. Tissues in which cyclic 3′,5′-AMP is affected by a hormone other than a catecholamine (*70*). ACTH, adrenocorticotrophic hormone.

Tissue	Hormone
Liver	Glucagon
Adrenal cortex	ACTH
Toad bladder	Vasopressin
Kidney	Vasopressin
Frog skin	Vasopressin
Corpus luteum	Luteinizing
Thyroid	Thyroid-stimulating
Adipose	Glucagon, ACTH, vasopressin, others
Fasciola hepatica	Serotonin
Heart	Acetylcholine
Brain	Histamine
Gastric mucosa	Histamine

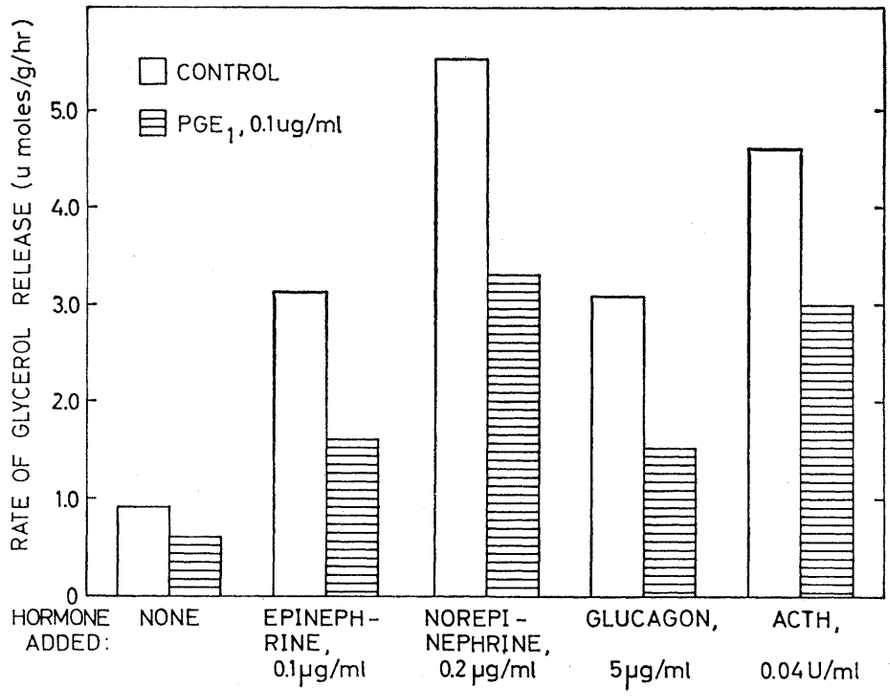

Fig. 8. Influence of PGE_1 on the hormone-stimulated release of glycerol from epididymal fat pads of rats.

It is found (*54*), however, that at low dosage (0.2 microgram per kilogram) constant infusion of PGE_1 alone, in either conscious or anesthetized dogs, increases free fatty acids, but their level is constant or decreased at higher dosage. This effect is apparently due to a certain stimulatory effect resulting in sympathetic discharge at concentrations below those that are directly inhibitory on adipose tissue (*64*). Because of this effect, intact animals also show hyperglycemia during infusion of PGE_1. The effect in humans is similar when PGE_1 is infused at 0.1 to 0.4 microgram per kilogram (*54*).

The content of cyclic AMP (adenosine-3′,5′-monophosphate) increases in the fat pad on addition of epinephrine to the medium, the cyclic-AMP response is elicited before the increase in lipolysis, and the concentration reached correlates with the extent of lipolysis (*65*, *66*). And low concentrations of PGE_1 antagonize both the accumulation of cyclic AMP and the release of free fatty acids (*67*). An even more clearcut effect on epinephrine-stimulated formation of cyclic AMP is found in isolated fat cells. As Fig. 10 shows, PGE_1 completely blocks the effect of an equal amount of epinephrine (*68*). Furthermore, the dibutyryl derivative of cyclic AMP, which penetrates into cells, stimulates lipolysis in both perfused and incubated fat

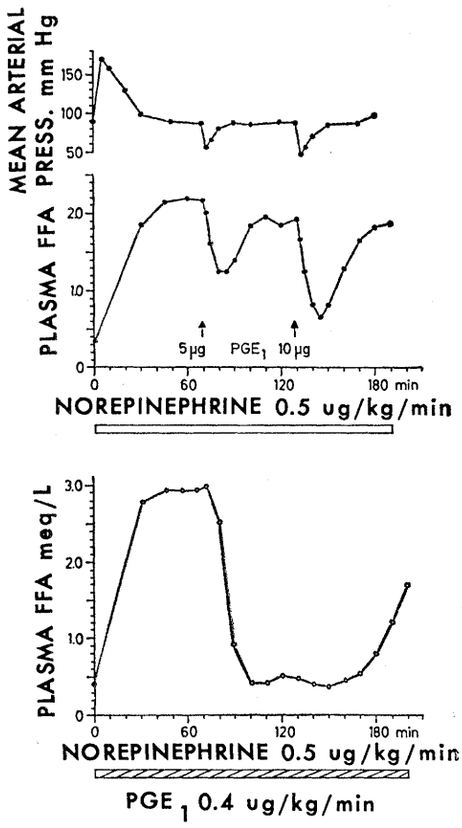

Fig. 9. (Top) Effect of single, intravenous injections of PGE_1 on the level in plasma of free fatty acids in an anesthetized dog during infusion of noradrenaline (*54*). (Bottom) Effect of infusion of PGE_1 during noradrenaline-induced increase in concentration of free fatty acids in arterial blood in an anesthetized dog (*55*); PGE_1 injected between 70 and 150 minutes.

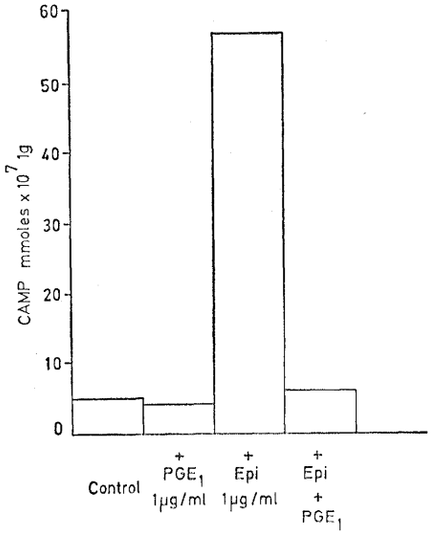

Fig. 10. Influence of epinephrine and PGE_1 on the formation of cyclic AMP in isolated fat cells [after Butcher (*44*)].

pads (*65*), but PGE_1 does not antagonize this effect (*53*, *67*). The conclusion is that the site of action is on the cyclase enzyme involved in the formation of cyclic AMP from adenosine triphosphate (Fig. 11).

Several lines of evidence thus indicate that cyclic AMP functions as the intracellular mediator of the lipolytic effect of epinephrine in adipose tissue. The same conclusion was reached for another system by Orloff *et al.* (*69*), who found that PGE_1 blocked the vasopressin-induced increase in water permeability of the toad bladder in vitro but did not block the similar action of cyclic AMP.

Vasopressin also, together with a number of hormones that I have mentioned, stimulates lipolysis in fat pads. Sutherland and Robinson (*70*) have listed the hormones (Table 5) that influence the level of cyclic AMP in certain tissues. According to Sutherland's "second messenger" concept, the actions of the different hormones on their specific target tissues are mediated by way of the "cyclase" that catalyzes the formation of cyclic AMP. The concentration of this compound in turn regulates the specific metabolic reactions in that type of cell—that is, the response to the hormone in question.

Now prostaglandins have been found to antagonize every one of the hormones listed (Table 5), under at least one set of experimental conditions. Thus it is tempting to speculate that prostaglandins can act as general modulators of the cyclase reaction in different tissues. However, it must be realized that addition of PGE_1 (alone) can increase the level of cyclic AMP in certain tissues. Thus a marked increase occurs in platelets in which the other hormones just mentioned do not seem to influence the level of cyclic AMP (*68*), a finding that is of interest in view of reports that prostaglandins can greatly influence the platelet adhesiveness and aggregation (*71*). This finding could also explain the many different biologic effects of prostaglandins that have been observed, only a few of which I have discussed.

The speculations could be extended further in relation to interesting observations (*44*, *72*) that prostaglandins can be released from nerves, brain, adrenals, diaphragm preparations, and fat pads by stimulation of the efferent nerves or by hormones or drugs. If it is assumed that the stimulation is mediated by way of the cyclase reaction, the cyclic AMP could effect a certain activation of an inactive lipase (in tissues other than adipose tissue) as a minor side reaction to the main metabolic reaction. Such activation would release some fatty acids, precursors to prostaglandins, that would rapidly lead to formation of corresponding prostaglandins by the microsomal prostaglandin-synthesizing system that seems to occur in all cells investigated. The prostaglandins formed would then influence the cyclase reaction and possibly function as a general feedback regulator (Fig. 11). Some of the prostaglandins formed would then be expected to be found in the venous effluent from the tissue in question, in the superperfusate of stimulated brain tissue, or in the medium of incubations in vitro.

The dramatic effect of intraventricular injections of PGE_1 that Horton (*48*, *73*) has observed may be mediated possibly by way of the cyclase reaction, as the brain is known to have a high level of cyclic AMP. The iontophoretic application of various prostaglandins in the neighborhood of single neurons in the brain stems of cats has been found to cause excitation of, inhibition of, or no effect on the spontaneous firing rate (*74*). Whether or not the prostaglandins are involved as transmittors in certain types of neurons is not established, but it seems likely that they have some role in relation to neuronal activity.

This short review should make it obvious that prostaglandins can exert marked effects on various important physiologic processes. More work is needed to ascertain their specific roles in the living organism. Are they mainly intracellular metabolic modulators or systemic hormonal regulators?

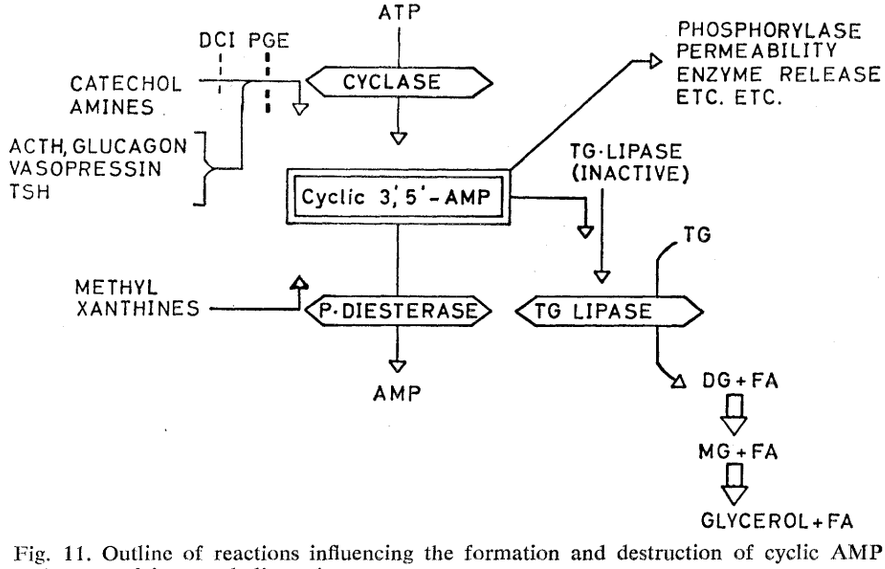

Fig. 11. Outline of reactions influencing the formation and destruction of cyclic AMP and some of its metabolic actions.

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76. Work supported by the Swedish Medical Research Council, the Andersson and Svensson Foundations of the Karolinska Institute, and the Wallenberg Foundation, Stockholm. I also thank the Upjohn Company, Kalamazoo, Michigan, Prostaglandins for experimental work are available in limited amounts from the author, the Upjohn Co. (J. Pike), Kalamazoo, Mich., and Unilever Research Laboratories (D. A. van Dorp), Vlaardingen, Holland.