Cardiovascular and metabolic effects of prostaglandins in the snakehead, *Ophiocephalus maculatus* Lacépède

by

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ABSTRACT

The present investigation examined the cardiovascular and metabolic effects of various types of prostaglandin (PGA_1, PGB_1, PGE_1 and $PGF_{1\alpha}$) and arachidonic acid in snakehead fish Ophiocephalus maculatus Lacépède. Also the distribution of prostaglandin E in different tissues of snakehead was demonstrated. In order to verify the possible participation of endogenous prostaglandins in the physiology of snakehead, the prostaglandins synthetase inhibitors, indomethacin and aspirin, were applied to the fish.

Using silicic acid column chromatographic and colorimetric techniques, the presence of prostaglandin E in various tissues including gill, urinary bladder, kidney, heart, spleen, muscle, liver and gastrointestinal tract was demonstracted.

Intravenous injection of prostaglandins (PGA_1, PGB_1 and PGE_1) produced hypotensive or hypertensive effects whereas $PGF_{1\alpha}$ and arachidonic acid did not alter arterial blood pressure. Furthermore, indomethacin significantly lowered basal blood pressure.

Plasma chemistry was studied after intraperitoneal injection of PGs. All prostaglandins uniformly produced hyperglycemia. No change in liver glycogen content occurred after PGs injection except that arachidonic acid decreased glycogen level. However, only PGE_1 and $PGF_{1\alpha}$ significantly lowered plasma protein and PGA_1 and PGB_1 could not change this plasma parameter. Decrease of plasma free fatty acid and lipid was shown after injection of PGE_1. No change in K^+

and Na^{+} levels was observed.

Moreover, the results of <u>in vitro</u> studies in liver cell showed that PGs have no effect on glutamate oxaloacetate transaminase, glutamate pyruvate transaminase and glucose-6-phosphatase activities. Also PGs did not exert antilipolytic effect on fat cell except PGB_1 increased glycerol production in this preparation.

Aspirin treatment decreased plasma protein and Na^{+} levels while no observable effects were found in hematocrit, plasma osmolarity, glucose, free fatty acid, lipid, Cl^{-}, Mg^{++}, Ca^{++} and K^{+} levels.

The significance of these findings in relation to comparative physiology was discussed.

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CHAPTER I INTRODUCTION

CHAPTER I : INTRODUCTION

Prostaglandins (PGs) are unsaturated fatty acids with twenty carbon atoms, a cyclopentane ring and two aliphatic side chains; one of the side chains bears a terminal carboxyl group. There are various types of naturally-occurring prostaglandins classically categorized into four series : E, F, A, and B. Classification is based upon the type and location of the functional groups, and the degree of unsaturation (Curtis-Prior, 1976) (for details : see Chapter 2, pp.12-16).

Prostaglandins have been known to be widely distributed in nearly all tissues of various mammals (Karim et al., 1968; Karim et al., 1967), but only little information is available on lower animals. A wide-spread distribution of prostaglandins in the animal kingdom was reported by Nomura and Ogata (1975) (see also Literature Review : pp.26-32). Nomura et al. (1973) isolated prostaglandin-like substances from the testis of teleosts and the gastrointestinal tract of the shark. In the latter case, the material has been chromatographically identified as PGE_2 (Ogata and Nomura, 1974).

In mammals, the biosynthesis of prostaglandins from essential fatty acids, dihomo-γ-linolenic acid and arachidonic acid has been well documented (Bergström et al., 1964). Recently, similar studies on PG biosynthesis in

several lower animals have been published. Christ and von Dorp (1964) investigated the comparative aspects of prostaglandin biosynthesis in animal tissues. Ogata and colleagues (1978) also showed the existence of prostaglandin synthetase activity in the tissues of fish and marine invertebrates.

Among various types of prostaglandins, physiological studies on prostaglandin E is mostly studied, therefore the biological actions of E type prostaglandin is well known especially in mammals. The first examined physiological property of prostaglandin was based on the observation that extracts of mammalian seminal fluid when injected into animals characteristically lowered the blood pressure of many species including rabbit, cat and dog (von Euler, 1935a; Goldblatt, 1935). Since the separation and identification of many different naturally occurring PGs, these compounds became available for the investigation of their cardiovascular actions. E series prostaglandins (PGE_1, PGE_2, PGE_3) uniformly lower arterial blood pressure in all the laboratory animals studied. These animals included the dog (Bergström *et al.*, 1964; Sambar *et al.*, 1967; Chapple *et al.*, 1980), rat (Weiner and Kaley,1969; Ten Berg *et al.*, 1980; Kondon *et al.*, 1980), cat (Horton and Main, 1963), rabbit (Horton and Main, 1963), guinea-pig (Berti *et al.*, 1967), goose (Grande and Prigge, 1972), and chicken (Horton and Main,1967), but there are very few reports on lower vertebrates (carp: Peyraud-Waitzenegger *et al.*, 1975). Hence, the vascular effects of prostaglandin

in lower vertebrates are especially interesting from a comparative point of view. The depressor activity of PGE has been related to vasorelaxation and decrease in vascular resistance (Saunders and Moser,1972; Chapple et al., 1980). PGE has been shown to bring about tachycardia (Grande and Prigg, 1972; Peyraud-Waitzenegger et al., 1975; Hoffman and Schmid, 1979; Hornych et al., 1979) and increase cardiac output (Weeks and Wingerson, 1964; Chapple et al., 1980). PGA also elicits similar cardiovascular effects as PGE (Lee et al., 1965; Westura et al., 1970; Jones, 1972). However, effects of PGF on cardiovascular system are controversial. Karim et al. (1969) reported the cardiovascular effects of $PGF_{2\alpha}$ infusion in human volunteers. Continuous infusion of 0.01 to 2.0 µg/kg/min $PGF_{2\alpha}$ for sixty minutes did not have any effect on blood pressure and heart rate. However, other studies showed the contrary, $PGF_{2\alpha}$ in doses up to 20 µg/kg was reported to be enough to increase blood pressure of the rat and dog (Ducharme et al., 1968; Saunders and Moser, 1972; Shehadeh et al., 1969). Therefore, $PGF_{2\alpha}$ evoked species-dependent vascular responses. Moreover, Schaumen and Houvenaghel (1978) reported a dose dependent response to intra-arterial $PGF_{2\alpha}$ infusion in the pig. At higher doses (3-100 µg/min) a direct dose-dependent decrease in mesenteric blood flow occurred whereas lower doses (0.03-1 µg/min) induced a delayed increase in mesenteric blood flow. A decrease of vascular resistance and positive chronotropic effect of prostaglandin F was

reported (Scharuwen *et al.*, 1979; Kushiku and Furukawa, 1979; Saunders and Moser, 1972). However, little information regarding the cardiovascular effect of PGB has been published. A PGB_1-induced decrease in perfusion rate in the perfused kidney of the frog was observed (Chiu and Leung, unpublished). In addition, a decrease of blood pressure by PGB was demonstrated by Jones (1972).

The first observation of the effect of prostaglandins on lipid metabolism was made by Steinberg *et al.* (1963). PGE_1 depressed basal and hormone stimulated lipolysis in adipose tissue of rat (Steinberg *et al.*, 1963) and man (Carlson, 1965). The relative potencies of several PGs in blocking epinephrine-induced lipolysis were compared (Steinberg *et al.*, 1964). PGE_1 was more potent than PGE_2; PGE_3 was ineffective except at a very high concentration (16.6 μg/ml). E group prostaglandins were more potent than F group prostaglandins with respect to lipolysis. *In vivo*, PGE_1 infusion decrease plasma free fatty acids (FFA) in the dog (Sanbar *et al.*, 1967). However, many studies have reported that prostaglandins stimulated lipolysis. PGA_1 infusion (0.25 μg/kg/min) in anesthetized dog induced increase of plasma FFA (Sacca *et al.*, 1973) and PGE_1 caused an increase of plasma glycerol and FFA levels in geese and man (Grande and Prigge, 1972; Bergström *et al.*, 1965). Therefore the contradictory effects of prostaglandins on lipid metabolism may be due to species specificity, the doses employed or different experimental conditions.

Bergström and coworkers (1966) tried to clarify whether the lipolytic responses may be dose dependent. They found that the smallest dose of 0.2 µg/kg/min PGE_1 produced a reproducible increase in venous plasma FFA level. Plasma glycerol level showed the same type of response but the rise was not statistically significant. At higher doses (0.4, 0.8 and 1.6 µg/kg/min), a dose-dependent decrease of plasma FFA and glycerol levels was observed. The low dose (0.2 µg/kg/min) effect on elevation of plasma FFA level was abolished by a sympathetic ganglionic blocking agent, whereas the same drug was without influence on the high dose effects. This suggests that PGE_1 exerts not only a direct inhibitory effect on FFA mobilization from adipose tissue, but may be in addition, at low infusion rates, enhances lipid mobilization due to a stimulation of sympathetic nervous activity. Moreover, Sham and Ramwell (1968) suggested a role for prostaglandins in the regulation of basal or hormone stimulated lipolysis. Christ and Nugtell (1970) demonstrated the release of arachidonic acid and PGE_2 from rat epididymal adipose tissue during lipolysis. It has been suggested that PGs are involved in maintenance of adipose tissue homeostasis by means of a physiological negative feed-back inhibition mechanism.

Besides their actions on lipid metabolism, prostaglandins also exhibit profound influence on glucose metabolism. PGA and PGE produced hyperglycemia in animals

so far studied (dog : Bergstrom *et al.*, 1966 and Sacca *et al.*, 1973; goose : Grande and Prigge, 1972; rat : Berti *et al.*, 1965). Since catecholamines have been shown to induce hyperglycemia in vertebrates (Gordon *et al.*, 1977) in order to clarify the action of PGs and their relationship with catecholamines on glucose metabolism, studies on the effects of PG on the liver *in vitro* were undertaken. Since hyperglycemia was induced by using a continuous infusion of PGE in rat and guinea pig but adrenalectomized animals failed to show the same response led Berti *et al.* (1965) to the conclusion that this effect is mediated through catecholamines. Furthermore, Boehle and May (1967) found a decrease in glycogen levels in liver slices incubated with PGE_1. Therefore, it seems that the results of Berti *et al.* (1965) and Boehle and May (1967) are apparently in contradiction. In addition, Lemberg *et al.*(1971) found that the actions of norepinephrine (induced hyperglycemia) and PGE_1 take place at different sites in the liver cell. Norepinephrine acts on a β-receptor of the hepatic cell membrane because its effect could be blocked by a β-blocker. However, Wilson and Levine (1970) demonstrated a PGE_1 induced-decrease in labeled glucose incorporation in the perfused rat liver. More evidence shows that PGE significantly increases glycogen breakdown in low glycogen liver (Wilson and Hankewych, 1973). Curnow and Nuttall (1972) observed that PGE_1 decrease hepatic glycogen synthetase without altering glycogen concentration. This change is

compatible with increased hepatic cAMP and suggested possible direct effects on hepatic glycogen levels. However, Levine (1978) showed no effect of PGE_1 on hepatic glycogenolysis. Despite the variable effects of PGs on glucose metabolism, PGE_1 has definitely been reported to cause hyperglycemia or to inhibit glucose metabolism in the liver.

Among the various reports on physiological studies of prostaglandins, only the effects of E-type prostaglandins have been completely surveyed. On the other hand, the effects of prostaglandin A and F are less studied and investigations on prostaglandin B is scarce. A more complete study of the physiological effects of these four types of prostaglandin is especially interesting since most species employed in the examination of the physiological role of prostaglandin are mainly mammals or higher vertebrates. Therefore, the present study will focus on the effects of PGs in a lower vertebrates. The freshwater teleost, *Ophiocephalus maculatus* Lacépède (Snakehead), was chosen as the experimental animal in the present study. This is because some aspects of the physiology of the snakehead has been established in our laboratory (Tong, 1980; Woo and Cheung, 1980; Woo *et al.*, 1980; Woo and Tong, 1981) and the animals are also available in Hong Kong throughout the year. Moreover, because of easy surgical access as well as resistance to experimental manipulations, snakeheads are excellent subjects in physiological investigation.

The present study attempts to examine the cardiovascular and metabolic effects of prostaglandins (PGE_1, $PGF_{1\alpha}$, PGA_1 and PGB_1) in Ophiocephalus maculatus. The examination is divided into four sections :-

(1) Distribution of prostaglandins in various tissues

(2) A survey of the effect of aspirin (a prostaglandin biosynthesis inhibitor) on metabolism and osmoregulation

(3) Cardiovascular effects of prostaglandins

(4) In vivo and in vitro studies on metabolic effects of prostaglandins.

CHAPTER II LITERATURE REVIEW

CHAPTER II : LITERATURE REVIEW

(I) HISTORY, NOMENCLATURE, BIOSYNTHESIS AND METABOLISM OF PROSTAGLANDINS

(1) History

The first definite reference to the existence of what is now known as prostaglandins was made by Kurzrok and Lieb in 1930. They showed that fresh human semen could either inhibit or stimulate the motility of the human uterus *in vivo*. A few years after the studies of Kurzrok and Lieb, von Euler and Goldblatt independently observed and studied the marked smooth muscle stimulating activity of human seminal fluid (Euler, 1934, 1935a, b; Goldblatt, 1933, 1935). Von Euler (1934) prepared lipid soluble acid extracts of human seminal fluid and that of monkey, sheep and goat, and observed their smooth muscle stimulating and blood pressure lowering properties. Because of the source of his extracts-mammalian accessory genital glands-von Euler coined the term 'prostaglandin' for the active factor present. It was not until 1960, and with a US $100,000 grant from The Upjohn Company, that the first pure prostaglandin compounds were isolated by Bergström and Sjövall (1960). Prostaglandin had been shown to be not just a single substance but a family of several chemically related compounds derived from the hypothetical prostanoic acid (Fig. 1, pp. 11). Subsequently, large scale biosynthesis began to make these

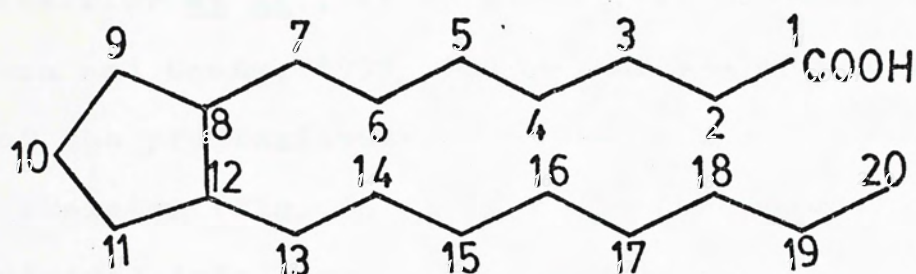

Fig. 1 Structural formula of the hypothetical prostanoic acid.

compounds available to a large number of investigators.

(2) Nomenclature

The term prostaglandin is a misnomer first defined by von Euler because these compounds are found not only in seminal fluid but also in nearly all tissues of vertebrates (Nomura and Ogata, 1976; Ogata and Nomura, 1975; Curtis-Prior et al., 1978) as well as invertebrates (Nomura and Ogata, 1975; Weinheimer and Spraggins, 1969). All of the prostaglandins have in common the prostanoic acid skeleton (Fig. 1, p. 11). The primary prostaglandins are divided into four types based on the functional groups in the cyclopentane ring : E type (11-hydroxyketones), F type (9, 11-diols), A type (10, 11-unsaturated ketones) and B type (8, 12-unsaturated ketones) (Fig. 2, p. 13). The natural prostaglandins are also grouped into the mono-, bis-, or triunsaturated classes according to the number of carbon-carbon double bonds in the two aliphatic side chains of parent E-type prostaglandin. This class designation appears as a subscript-number in the names of prostaglandins. The location of the double bonds is the same in all prostaglandins. In mono-unsaturated prostaglandins, the location of C-C double bond is between carbon 13-14. The bisunsaturated class has two double bonds between carbon 13-14 and 5-6. Moreover, the locations of unsaturation are between carbon 13-14, 5-6 and 17-18 in triunsaturated class. In addition to these features, all prostaglandin structures

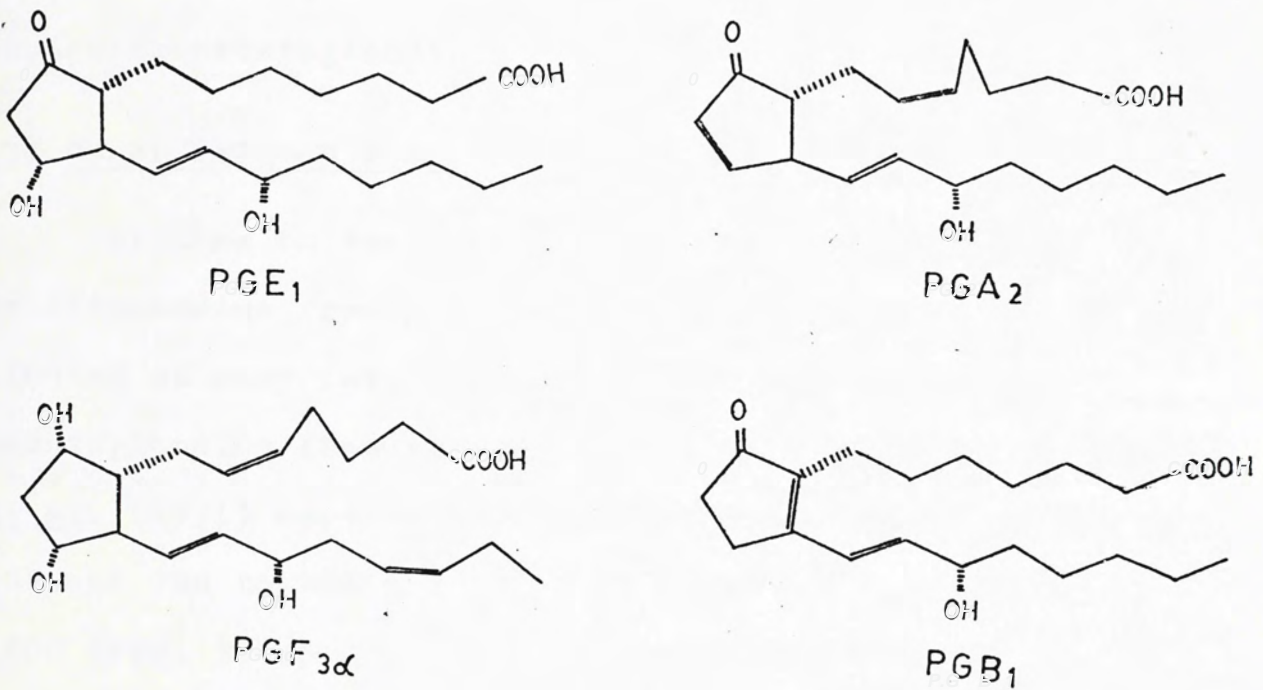

Fig. 2 Structural formulae of the classical naturally-occurring prostaglandins.

present the possibility of stereoisomers. However, only one isomer of each type has so far been isolated (Samuelsson, 1973; Ogata & Nomura, 1975; Nomura and Ogata, 1975). An alpha/beta system is employed to define the stereochemistry of functions on the cyclopentane ring. Alpha substituents are orientated on the same side of the ring as the aliphatic side chain bearing the carboxyl group whereas beta substituents are orientated on the side of the ring bearing the alkyl side chain. However, only alpha substituent is found in naturally occurring prostaglandins.

(3) Other related compounds of prostaglandins

Studies on the mechanism of the formation of prostaglandins from polyunsaturated fatty acids led to the finding of many intermediates in the biosynthesis of prostaglandins (Samuelsson, 1972). Jones (1970) and Horton et al. (1971) reported the enzymatic conversion of PGA to PGB and led to the discovery of a new prostaglandin (PGC (Fig. 3, p. 15)). A single shift of the 10, 11 double bond in PGA produces the 11, 12 double-bonded isomer (PGC) and the PGC will rapidly be converted to PGB under mildly alkaline condition (Polet and Levine, 1975). Since PGCs are unstable compounds, they exist in small amounts in the tissues. Moreover, another type of prostaglandin, PGD, contains a hydroxyl group at carbon 9 and a ketone at carbon 11 (Fig. 6, p. 21) but the two aliphatic side chains are the same as the other prostaglandins. In addition, recent

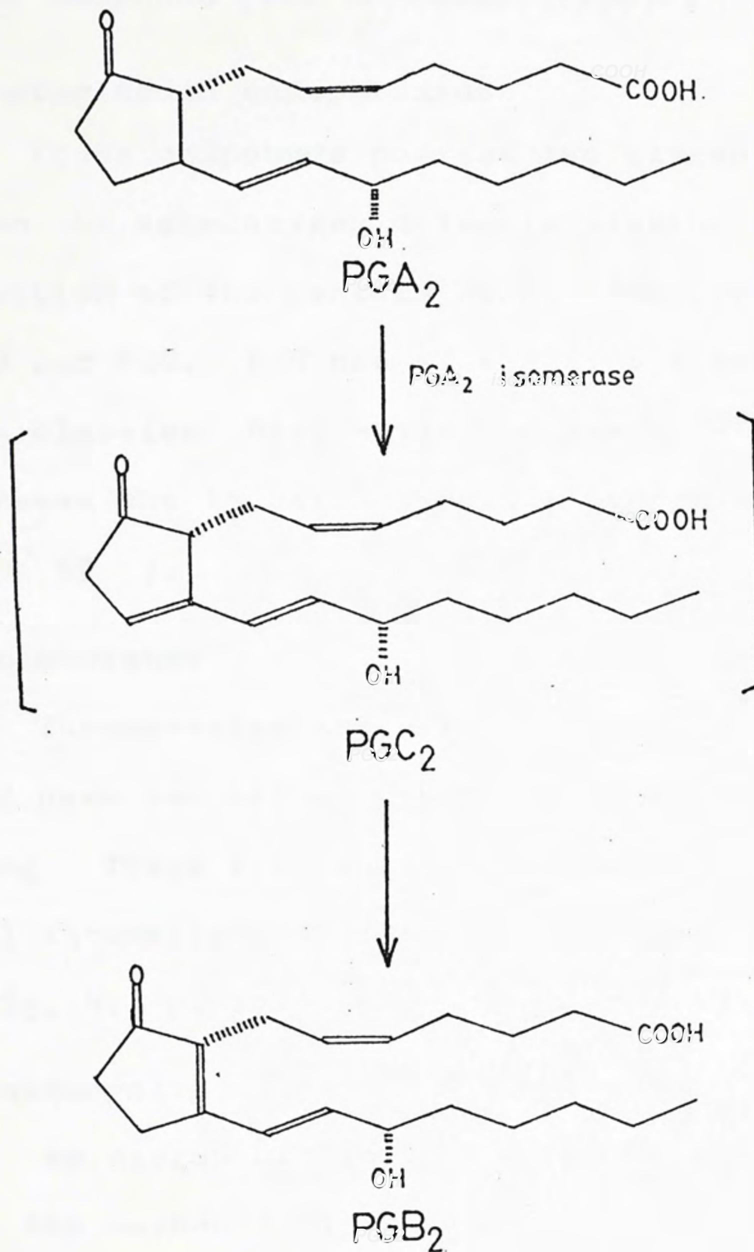

Fig. 3 Conversion of PGA_2 to PGB_2 via an intermediate PGC_2 by PGA_2 isomerase

research has led to the identification of three other types of compound (see Horrobin, 1978) :

(a) Prostaglandin endoperoxides

These compounds possess two oxygen atoms derived from the same oxygen molecule linking the 9 and 11 position of the pentane ring. Endoperoxides include PGH and PGG. PGH has side chains similar to those of the classical PGs, while PGG has an extra oxygen between the 15 carbon and its hydroxyl group (Fig. 4, p. 17).

(b) Thromboxanes

Thromboxanes are derived from the endoperoxides and have two oxygen atoms inserted into the carbon ring. There are two groups of thromboxane : (i) thromboxane A (TXA) and (ii) thromboxane B (TXB) (Fig. 4, p. 17).

(c) Prostacyclin

An oxygen bridge linking the carbon 9 of the ring to the carbon 6 of the side chain is found in this type of compound (Fig. 5, p. 18). In the past, PGX is used to denote the abbreviation of prostacyclin. These compounds have been renamed and are now called prostaglandin I to conform to the prostaglandin nomenclature.

(4) Biosynthesis of prostaglandin

In recent years, there is a rapid research on the

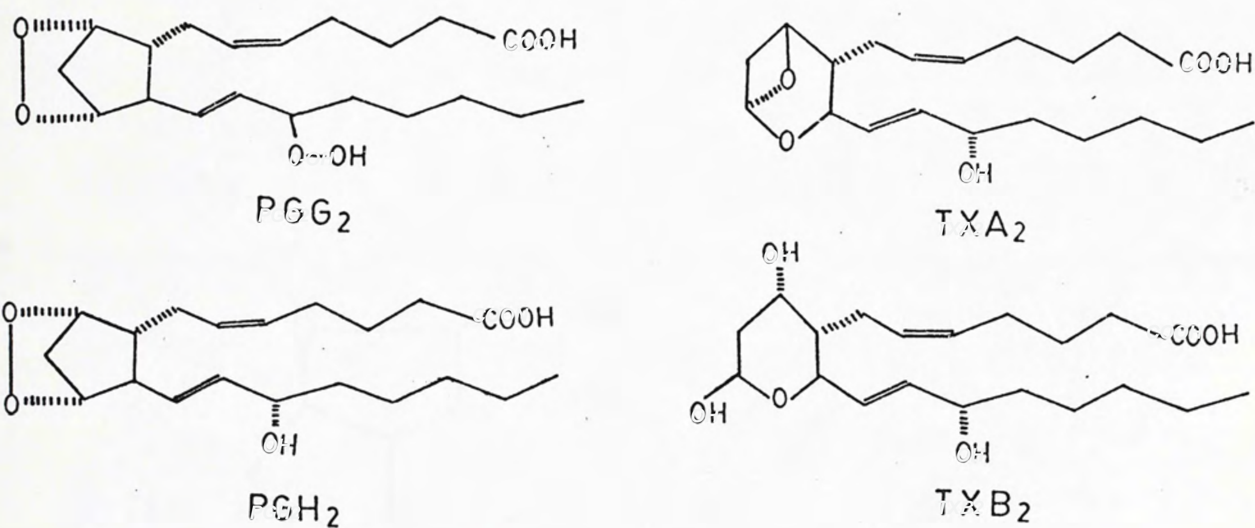

Fig. 4 Structural formulae of PGG_2, PGH_2, TXA_2 and TXB_2.

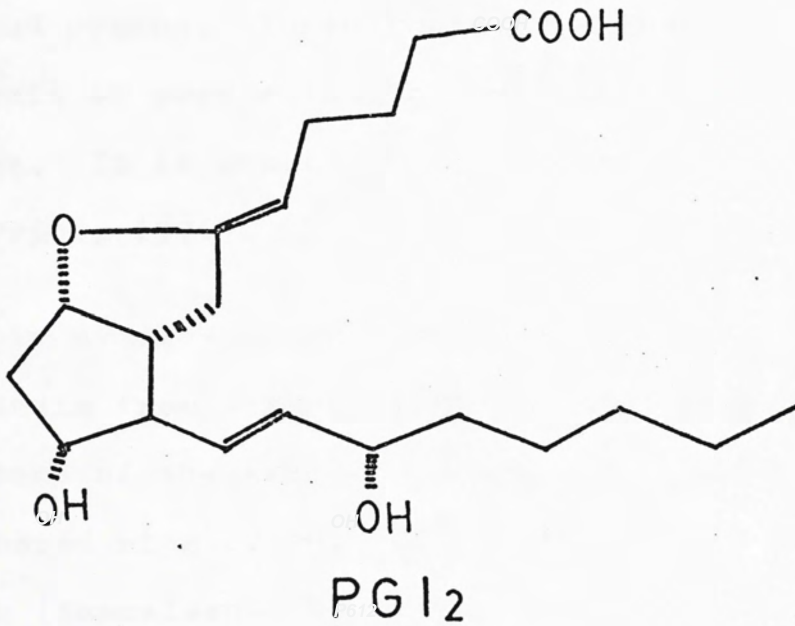

Fig. 5 Structural formula of PGI_2.

biosynthesis of prostaglandin and this leads to the new finding of many intermediates and products. Bergström et al. (1964) and Van Dorp et al. (1964) found that homogenates of sheep seminal vesicles could convert enzymatically arachidonic acid to prostaglandin E_2. Moreover, formation of the 1 series of prostaglandins from dihomo-gamma-linolenic acid has been demonstrated (Beerthuis et al., 1968). This suggests that dietary fatty acids, arachidonic acid and dihomo-gamma-linolenic acid may be the precursors of prostaglandins in mammalian tissues and organs. Furthermore, the enzyme complex of biosynthesis of prostaglandins was called prostaglandin synthetase. It is generally considered to be membrane-bound (Curtis-Prior, 1976).

Early studies on the mechanism of the formation of prostaglandin from polyunsaturated fatty acids showed that oxygen atoms of the keto group and the hydroxyl group in the five-membered ring of PGE_1 originated in the same molecule of oxygen (Samuelsson, 1965). On the basis of this result and other considerations, it was proposed that an endoperoxide structure is formed as an intermediate in the biosynthesis of prostaglandins (Samuelsson, 1965). Later in 1973, the isolation, characterization and examination of biological activity of two endoperoxide intermediates, PGG and PGH has been successfully demonstrated (Hamberg and Samuelsson, 1973; Nugteren and Hazelhof, 1973). PGH has now been shown to be a common precursor of PGE, PGF, PGD, PGI and thromboxane (for details see Schneider, 1976; Samuelsson, 1978). In addition,

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PGA_1, PGA_2, and their 19-hydroxy analogues as found in kidney medulla, semen and other tissues are largely enzymatic products (Attallah et al., 1974). Furthermore, PGC has been shown to result from the action of an isomerase present in blood plasma (Jones et al., 1972), and this unstable prostaglandin will rapidly be converted into PGB. An outline of the biosynthetic pathway of 2 series of prostaglandins and related compounds from arachidonic acid is shown in Fig. 6 (p. 21).

(5) Catabolism of prostaglandins

Prostaglandins are rapidly catabolised by a variety of animal tissues to products possessing reduced biological activity. Lung (Ferreira and Vane, 1967; Piper et al., 1970), kidney (Larsson and Änggard, 1973), liver (Vane, 1969) and human placenta (Jarabak, 1972) appear to be major sites of prostaglandin degradation. Samuelsson and coworkers (1971) has reviewed the metabolism of prostaglandins and found out a number of metabolites of prostaglandins. The most important metabolic pathway are summarized in the following (for details see also Curtis-Prior, 1976) :

(a) Dehydrogenation of the C15 hydroxyl group by 15-hydroxy-dehydrogenase to give 15-keto compounds.

This enzyme is widely distributed in lung homogenate (Änggard and Samuelsson, 1966), human placenta (Jarabak, 1972), kidney of rabbit and rat (Curtis-Prior, 1976b,c) and lung and spleen (Änggard et al., 1971). It is probably the main enzymes responsible for the extremely effective

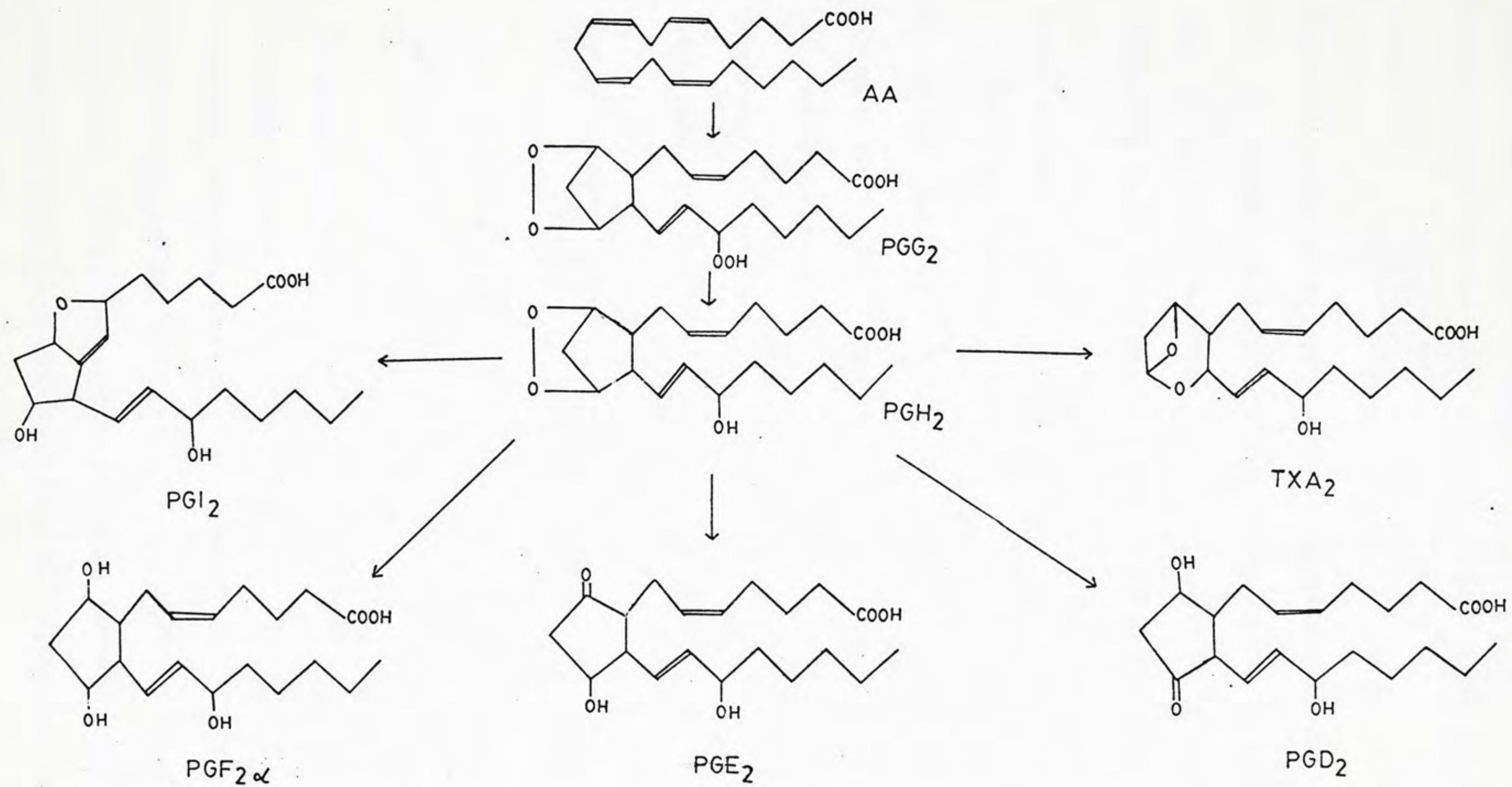

Fig. 6 Transformation of arachidonic acid (AA) to various prostaglandins.

destruction of the biological activity of E and F prostaglandins by the lungs. (see also Marrazzi and Andersen, 1974).

(b) Reduction of the C-13 double bond by PG Δ 13-reductase

It seems probable that in many situations this reduction follows the dehydrogenation although this is probably not always the case (Curtis-Prior, 1976 f).

(c) Beta oxidation

This is the non-specific removal of 2 carbon fragment from the carboxylic end of the molecule. This oxidation occurs as in all other fatty acid metabolism. Removal of one 2-carbon fragment gives a dinor derivative while removal of two 2-carbon fragments give a tetranor derivative (Samuelsson et al., 1971).

(d) Omega hydroxylation at C-19 or C-20

A hydroxyl group is inserted into carbon 19 or 20. This omega-hydroxyl metabolite may undergo beta oxidation (McDonald-Gibson et al., 1973).

(e) Omega oxidation of alkyl side chain

The omega hydroxyl group is converted into a carboxyl group. Metabolism of the E prostaglandins by these five stages gives 7α-hydroxyl-5, 11-diketotetranorprosta-1, 16-dioic acid, the main urinary metabolite in man (Hamberg and Samuelsson, 1971). Similar pathways metabolize the F prostaglandins (Powell, 1980).

However, there are probably many differences in metabolism between tissues and between species and much more work is required in this field.

(f) Conversion of thromboxane A to thromboxane B

Thromboxane A is a highly unstable compound with a half life of around 30 seconds in buffer solution and it is probably non-enzymatically converted to thromboxane B, a much more stable and relatively inert substance (Horrobin, 1978).

(g) Conversion of PGI_2 to 6-keto $PGF_{1\alpha}$

PGI_2 is also unstable and is converted partly enzymatically to 6-keto $PGF_{1\alpha}$ (Sun and Tayler, 1978; Pace-Asciak <u>et al</u>., 1977).

(h) Prostaglandin A-isomerase

Relatively little work has been carried out on the metabolism of prostaglandins of the A series. Whereas they are not inactivated by passing through the lungs. (Horton and Jones, 1969), they are metabolized by an enzyme in plasma (Jones, 1970). This enzyme is called PGA-isomerase which catalyses PGA to PGC and finally to PGB (Fig. 3 , p. 15). The details have been mentioned earlier on p. 14 .

The catabolism of prostaglandin usually does not follow a definite sequence of pathway, therefore, for example, dehydrogenation of the C15 hydroxyl group may precede reduction of the C-13 double bond or vice versa.

Moreover, the metabolism of prostaglandins may not occur in all of these pathways and they may be metabolized by only one or two of these enzyme systems. In addition, most of the metabolites of PGs are excreted in the urine (Hamberg and Samuelsson, 1971). A summary of the destructive reactions occurred on $PGF_{2\alpha}$, as an example of catabolism of prostaglandin is outlined in Fig. 7 (p. 25).

(6) Interconversion of Prostaglandins

PGs often can be converted to each other, sometimes by enzymatic or non-enzymatic reactions. The most important and frequently occurred interconversion are the followings :

(a) Conversion between PGE and PGFα

This conversion is catalyzed by an NADPH-dependent 9-keto-reductase. This reaction is potentially reversible (Lee and Levine, 1974).

(b) Formation of PGA from PGE

Conversion of PGE to PGA by loss of water from the ring may take place enzymatically but may also occur non-enzymatically particularly during isolation procedures. PGAs are relatively resistant to degradation by the lungs and so could act as circulating hormones (Horton and Jones, 1969).

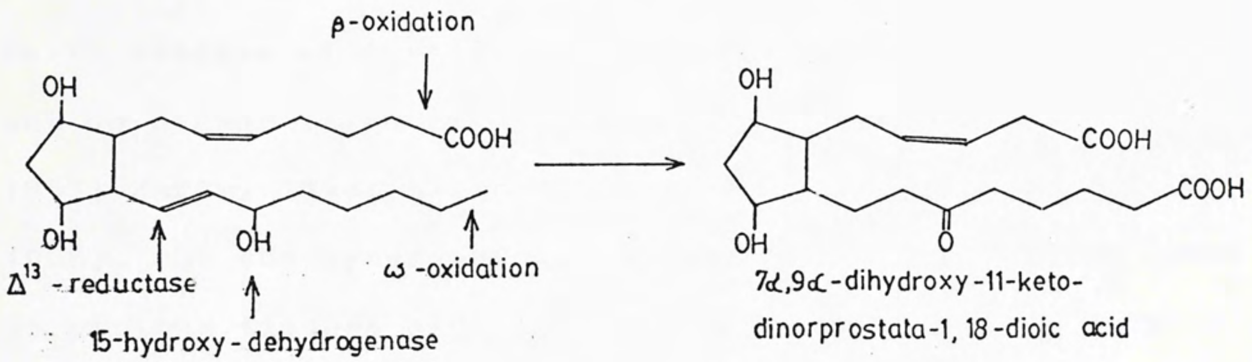

Fig. 7 Summary of the degradative reactions undergone by prostaglandin F series, resulting a urinary end-product.

(II) DISTRIBUTION OF PROSTAGLANDINS

Although prostaglandins were first discovered in male accessory sexual glands (Goldblatt, 1933 and 1935; von Euler, 1934), virtually, prostaglandins are widely distributed in various tissues and body fluids of animals (Nomura and Ogata, 1975; Pace-Asciak and Rangaraj, 1977; Weber et al., 1979; Nomura et al., 1973; Karim et al., 1967 Weinheimer and Spraggins, 1969; Curtis-Prior et al., 1978). Early studies of distribution of prostaglandins were carried out on higher vertebrates especially in mammals (Karim et al., 1967; Karim, 1968; Karim et al., 1967; Bergström et al., 1968), but the types and quantities of prostaglandins found in various tissues and different mammalian species vary considerably (Table I).

Among various tissues of mammals, semen is found to possess largest amount of prostaglandins E_2, (38500 ng/g wet tissue : Bergström & Samuelsson, 1962; Nomura and Ogata, 1976). Kidney also contains large quantities of PGA_2 and PGE_2 (Vance, 1973) and the gastrointestinal tract and lung possess large amounts of PGE_2 (Nomura and Ogata, 1976; Bennett et al. (1968). However, skin, testis, brain, heart, thymus and thyroid only contain small amounts of prostaglandin E_2 (Nomura and Ogata, 1976). Moreover, PGE_2 and $PGF_{2\alpha}$ can be found in most tissues, whereas the occurrence of PGE_1, PGE_3, $PGF_{1\alpha}$ and $PGF_{3\alpha}$ is less prevalent. Besides seminal and renal PGA, haemal and seminal PGB have been demonstrated; but no

Table 1. Distribution of prostaglandins in Mammals.

Animal	Source	Prostaglandins	References
Human	Semen fluid	$E_1, E_2, E_3, F_{1\alpha}$	Bergström & Samuelsson (1962)
		$F_{2\alpha}, A_1, A_2, B_1$	Samuelsson (1963a; 1963b)
	Menstrual fluid Endometrium	$E_2, F_{2\alpha}$	Eglinton et al. (1963); Pickles et al. (1965)
	Amniotic fluid during prenancy and labour	$E_1, E_2, F_{1\alpha}, F_{2\alpha}$	Karim (1968)
	Lung	$E_2, F_{2\alpha}$	Karim et al. (1967)
	Thymus	E_1	Karim et al. (1967)
	Thyroid	$E_2, F_{2\alpha}$	
	Vagus nerve	$E_2, F_{2\alpha}$	
	Cervical sympathetic Nerve	$E_2, F_{2\alpha}$	
	Cardiac muscle	E_2	
	Bronchi	$E_2, F_{2\alpha}$	
	Stomach mucosa	E_2	Bennett et al. (1968)
	Blood	$F_{2\alpha}$, E, A, F, B	Jones, (1972); Unger et al. (1971); William (1971); Jubiz et al. (1972); Wolfe et al. (1972); Jaffe et al. (1973); Pletka and Hickler (1974); Lijnen et al. (1979)
	Platelet	$E_2, F_{2\alpha}$	Smith and Willis (1970)
	Urine	$F_{1\alpha}, F_{2\alpha}, E_1, E_2$	Frölich et al. (1973)
	Kidney	A, E,	Vance (1973)
Sheep	Vesicular gland	$E_1, E_2, E_3, F_{1\alpha}$	Bergström & Sjövall (1957) Bergström (1966)
	Iris	$F_{2\alpha}$	Änggard and Samuelsson (1964b)
	Lung	$E_2, F_{2\alpha}$	Bergström (1966)
	Serum	$E_2, F_{2\alpha}$	Hwang et al. (1980)
Monkey	Lung	$F_{2\alpha}$	Änggard (1964)
	Serum	$E_2, F_{2\alpha}$	Hwang et al. (1980)
Rat	Stomach	E_1	Bennett et al. (1967)
	Gastrointestinal tract	E_2	Nomura & Ogata (1976)
	Skin	E_2	
	Testis	E_2	
	Lung	E_2	
	Brain	E_2	
	Pancreas	E_2	
	Heart	E_2	
	Kidney	E_2	
	Serum	E_2, F_2	Hwang et al. (1980)
Cat	Adrenal gland	$F_{2\alpha}$	Shaw & Ramwell (1967)
	Serum	$E_2, F_{2\alpha}$	Hwang et al. (1980)
Bovine	Lung	$F_{2\alpha}, F_{3\alpha}$	Bergström (1966)
	Brain	$F_{2\alpha}$	
	Pancreas	$E_2, F_{2\alpha}$	
	Serum	$E_2, F_{2\alpha}$	Hwang et al. (1980)
Calf	Thymus	E_1	Bergström (1966)
Pig	Kidney	$E_2, F_{2\alpha}$	Bergström (1966)
	Lung	$F_{2\alpha}$	
	Serum	$E_2, F_{2\alpha}$	Hwang et al. (1980)
Guinea pig	Lung	$F_{2\alpha}$	Änggard (1964)

other tissue has been reported to contain PGA and PGB. This may be due to the less abundance of these prostaglandins in tissues or negligence in most studies.

Later in 1969, Weinheimer and Spraggins found that a remarkably high concentration of prostaglandin isomers in the gorgonian, Plexaura homomalla. This led the investigators to realize that prostaglandins are distributed not only in higher animals but also in lower animals. To elucidate the occurrence and the biological significance of PGs in the reproductive system of marine animals, Nomura et al. (1973) had made a pioneer survey of PGs in fish testes and semen. PGE_2, $PGF_{1\alpha}$ and $PGF_{2\alpha}$ were shown in fish testes (Nomura et al., 1973; Nomura and Ogata, 1976). Ogata and Nomura (1975) reported that only E type but not F type prostaglandin are found in the gastrointestinal tract of shark. The distribution of prostaglandins in tissues of fish and frog is listed (Table 2). Gastrointestinal tract, air bladder, heart, gill and kidney are inclined to have a higher level of prostaglandins than other tissues (Nomura et al., 1973; Nomura and Ogata, 1976). Nevertheless, little information is available for the distribution of PGF, PGA and PGB in fish and frog.

Prostaglandins are found also in invertebrates; e.g. sea-squirt, mussel, scallop, crayfish, blue crab, sea-anemone and gorgonia (Table 3). It should be noted that the gills of invertebrates possess a large amount of prostaglandin E (Nomura and Ogata, 1976).

Table 2. Distribution of Prostaglandins in Fish and Frog.

Animal	Source	Prostaglandins	References
Carp	Ovary	E	Nomura & Ogata (1976)
	Liver	E	
	Gastrointestinal tracts	E	
	Fin	E	
	Air bladder	E	
	Gills	E	
	Testis	E	
	Heart	E	
Carp	Brain	E	Nomura & Ogata (1976)
	Skeletal muscle	E	
	Skin	E	
Sheat-fish	Brain	E	Nomura & Ogata (1976)
	Intestine	E	
	Gill	E	
	Air bladder	E	
	Liver	E	
	Ovary	E	
	Heart	E	
	Kidney	E	
	Stomach	E	
	Skeletal muscle	E	
Leopard Shark	Gastrointestinal tract	E_2, E_3	Ogata & Nomura (1975); Nomura & Ogata (1976)
	Liver	E	Nomura & Ogata (1976)
	Skin & fin	E	
	Gill	E	
	Brain	E	
	Kidney	E	
	Testis	E	
	Heart	E	
Flounder	Testis	E_2	Nomura et al. (1973)
Chum salmon	Testis	$F_{2\alpha}$	Nomura et al. (1973)
Bluefin Tuna	Testis	$E_2, F_{2\alpha}$	Nomura et al. (1973)
Frog	Intestine	$E_1, E_2, F_{1\alpha}$	Nomura & Ogata (1976); Vogt et al. (1967)
	Liver	E_2	Nomura & Ogata (1976)
	Ovary	E_2	
	Lung	E_2	
	Skin	E_2	

Table 3. Distribution of Prostaglandins in Invertebrates.

Animal	Source	Prostaglandins	References
Sea-squirt	Testis Ovary Muscle	E E E	Nomura & Ogata (1976)
Mussel	Gonad Digestive diverticula Gill Feet Mantle	E E E E E	Nomura & Ogata (1976)
Scallop	Gill Feet	E E	Nomura & Ogata (1976)
Crawfish	Liver Gill	E E	Nomura & Ogata (1976)
Blue crab	Gill	E	Nomura & Ogata (1976)
Sea-anemone	Whole animal	E	
Gorgonian	Whole animal	15-epi-PGA_2 and its diester	Weinheimer & Spraggins (1969)

In addition, there is a scarcity of reports on the distribution of prostaglandins in reptiles. In order to complete a thorough survey on the distribution of prostaglandins in the Animal Kingdom, further studies on this aspect should be pursued.

(III) PROSTAGLANDIN BIOSYNTHESIS IN ANIMAL TISSUES

Since the ubiquitous distribution of prostaglandins in animal tissues has been shown, it is interesting to demonstrate the biosynthesis of prostaglandins in those tissues. After a detailed examination of prostaglandin biosynthesis in tissue homogenates of animals has been studied (Christ and Van Dorp, 1972; Pace-Asciak and Rangaraj, 1977, 1977; Weber et al., 1979; Ogata et al., 1978; Herman et al., 1978), prostaglandins are virtually found to be locally synthesized in tissues. Prostaglandin synthetase activity was found in almost every mammalian tissue investigated as well as in tissues from other vertebrate and invertebrate species.

Comparative aspects of prostaglandin biosynthesis in animal tissues was studied by Christ and Van Dorp (1972). Vesicular gland, kidney medulla, lung and stomach of mammals have a high capacity of conversion of prostaglandin precursor to PGE (Christ and Van Dorp, 1972). In birds (Cock, hen and duck), lung, kidney, intestine, and seminal vesicles possess high PGE_1 biosynthetic activity whereas $PGF_{1\alpha}$ synthetic activity is low (Christ and Van Dorp, 1972). The activities of PG synthesis found in birds were generally lower than those found in similar mammalian tissues (Christ and Van Dorp, 1972). Gill, kidney, spleen and intestine of carp, trout and tench; lung, skin and urinary bladder of frog and toad; whole animal

of horny coral, jelly fish, earthworm and houseefly; whole animal and gill of lobster and mussel (Christ and Van Dorp, 1972) all can synthesize prostaglandin E_1. The synthetic activities of prostaglandins from dihomo-γ-linolenic acid in lower vertebrates and invertebrates were generally low compared with those of tissues of higher vertebrate (Ogata et al., 1978).

Moreover, rabbit kidney has a high capacity to produce a spectrum of different prostaglandins (PGE_2, $PGF_{2\alpha}$, 6-keto-$PGF_{1\alpha}$, TXB_2) (Weber et al., 1979). In addition, homogenates of rat and fetal lamb brain, lung, liver, spleen, kidney ductus arteriosus, aorta and pulmonary artery produced different amounts of prostaglandin E_2, $F_{2\alpha}$, D_2, 6-keto-prostaglandin $F_{1\alpha}$ and thromboxane B_2 (Pace-Asciak and Rangaraj, 1977). The main prostaglandin product in fetal lamb and rat organs was PGE_2, arterial tissue formed mostly 6-keto-$PGF_{2\alpha}$. (Pace-Asciak and Rangaraj, 1977) whereas PGD_2 and TXB_2 formations in these organs were relatively low. These results demonstrate significant differences between organs and tissues in the relative direction of the 'prostaglandin synthesis' enzyme complex.

Furthermore, the diversification of the prostaglandin synthetase in different non-mammalian tissues to form one or several of these products has not yet been fully explored, probably because of the difficulties encountered in the measurement of each of these products. Another possible reason may be that little information is yet available on the

biological properties and therefore on the biological importance of products other than the classical prostaglandins E and Fα .

(IV) PROSTAGLANDIN SYNTHETASE SYSTEM

Prostaglandin synthetase is a multiple enzyme complex catalyzing the conversion of certain unsaturated fatty acids into a wide array of prostaglandins and prostaglandin-like materials. The reaction is initiated by the incorporation of two oxygen molecules at C-11 of the unsaturated eicosanoic acid, followed by the oxygenation at C-15 and concomitant linkage of the carbon chain at C-8 and C-12 (Samuelsson, 1965, 1972). This intermediate is PGG (Hamberg et al., 1974) and the hydroperoxy group at C-15 is then converted to a hydroxy group, and this product is found to be PGH (Hamberg et al., 1974). Finally reduction or isomerization of PGH gives rise to PGE or PGF.

Prostaglandin synthetase activity was first demonstrated in sheep seminal vesicle glands and subsequently in a variety of tissues, but tissue activities were low compared to that of seminal vesicle glands (van Dorp et al., 1964; Bergstrom et al., 1964; Christ and van Dorp, 1972). Moreover, the prostaglandin synthetase activity is associated with the microsomal fraction (Nugteren et al., 1966).

PG synthetase is found in the microsomes of bovine vesicular gland, it contains two fractions (Fraction I and Fraction II) after ultracentrifugation. Fraction I is PG endoperoxide synthetase, which is responsible for the formation of PG endoperoxide (PG intermediate) from PG

precursor (Yamamoto et al., 1977). Fraction II can convert PG endoperoxide to prostaglandin and it is called PG endoperoxide E-isomerase (Yamamoto et al., 1977) (For details see also Yamamoto et al., 1977; Pong and Levine, 1977).

(V) EXTRACTION, SEPARATION AND QUANTITATION OF PROSTAGLANDINS

After the first discovery of the acidic, lipid-soluble, smooth muscle stimulating compounds (prostaglandins) in seminal fluid (Goldblatt, 1933; von Euler, 1934, 1935), there was a long period of inactive pursuit on the properties of these compounds. This may probably be due to the lack of suitable techniques for qualitative and quantitative determination of prostaglandins. In the early 1960s, the first isolation of crystalline prostaglandin was achieved by Bergström and Sjövall (1960) and its chemical structure was subsequently elucidated (Bergström et al., 1962). Recently, using sophisticated instrumentation, analysts are able to develop sensitive and specific physico-chemical and biochemical methods for analysis of prostaglandins.

(1) Extraction of Prostaglandins

Numerous methods for the extraction of PGs have been developed. These include extraction by adsorption to Amberlite XAD-2 (Gréen, 1971) and solvent extraction (Unger et al., 1971). The advantage of the former method is that it is useful for the extraction of all the primary prostaglandins as well as for the major circulating and urinary metabolites of PGE_2 and $PGF_{2\alpha}$. Solvent extraction is useful for the extraction of PGA, PGB, PGE, PGF and thromboxane B (Frölich, 1973). In most extractions, the biological sample is acidified to pH 3-4 with either dilute

mineral acid (HCl) or acetic acid and then partitioned twice with one, two or three volumes of a polar organic solvent such as ethyl acetate or diethyl ether (Samuelsson, 1963; Unger et al., 1971). In certain instances the initial extraction may be with ethanol or n-butanol, and followed by concentration and extraction as described above. Alternative procedures are employed for the extraction of prostaglandins from a range of biological sources including the uterus, the kidney, urine and the blood (Horton, 1972; Salmon and Karim, 1976).

(2) Separation of Prostaglandins

During the past decade a variety of chromatographic procedures have been developed to improve the purifications of prostaglandins by chromatographic techniques. These techniques will be discussed in the following paragraphs.

(a) Thin-layer Chromatography

This chromatographic procedure has become increasingly popular primarily because of its wide range of applicability and ease of operation. Many of the solvent and support systemsin use are modifications of those reported previously (Gréen and Samuelsson, 1964; Andersen, 1969a). Gréen and Samuelsson (1964) were able to separate individual PGs with a different number of double bonds using silica gel plates impregnated with silver nitrate. Recently, Wickramasinghe and Shaw (1973) reported the separation of PGs A, B and C by thin layer chromatography on silica gel impregnated with ferric

chloride; the resolution obtained was far superior to that on silica gel plates impregnated with silver nitrate.

After development of thin layer plates, the PGs can be visualised by spraying with a variety of reagents (Shaw and Ramwell, 1969). Finally, PGs must be scraped from the plate, extracted and assayed by other means.

(b) Silicic Acid and Silica Gel Column Chromatography

The use of silicic acid and silica gel column chromatography is almost as popular as thin-layer chromatography. Again, the procedures involved are highly flexible, reproducible, simple to operate and also allow the efficient separation of the various prostaglandins.

Silicic acid has to be activated prior to use and this is performed by heating 115° - 120° C for at least 1 hour (Samuelsson, 1963b). The silicic acid is then slurried in ethyl acetate-toluene (1:9 v/v) and poured into glass columns under free flow. In general, PGA(PGB and PGC), PGE and PGF$_\alpha$ are sequentially eluted in steps of increasing ethyl acetate in toluene. For certain batches of silicic acid it may be necessary to add small quantities of methanol to each eluant to ensure complete recoveries. This chromatographic technique is used for separation of PGs in the present study, therefore detailed discussion will be described later (see also pp. 87-88).

Although silica gel column chromatography is not as popular as silicic acid, it also has a wide range of application. It can be used for purification of blood prostaglandins and prostaglandin intermediates (Corey et al., 71; 71).

(c) High Pressure Liquid Chromatography

Recently, high-pressure liquid chromatography has found numerous applications and taken its place alongside thin-layer and gas chromatography. Modern column-packing methods and pumps result in highly efficient separations on columns packed with very small particles which are eluted under high pressure and flow (for review, see Heftmann, 1975). A high reproducibility and recovery of using high-pressure liquid chromatography technique was reported (Frölich, 1977). Therefore, this method is useful and convenient for analyzing PGE_2, $PGF_{2\alpha}$ and their major circulating metabolites in the same plasma sample or in kidney incubation (Gréen et al., 1973).

(d) Reversed Phase Partition Chromatography

This system employs hyflo supercel (hydrophobic celite) as the support medium for separation of PGs. The chromatographic process has proved to be highly versatile for purification of PGs and their metabolites (Hensby, 1977).

Preparation of the hydrophobic celite has been described elsewhere (Shaw and Ramwell, 1969). In practical

operation, hyflo supercel reversed phase chromatography systems, when used in combination with other chromatographic techniques such as silicic acid and thin layer chromatography, have been extensively used for the purification of prostaglandins and metabolites from a wide variety of sources (Samuelsson, 1964).

(e) Lipophilic Sephadex Gel Column Chromatography

In this chromatographic system, a variety of column supports are available from Sephadex and its derivates. Due to the high recovery of separation, reusability, mild chromatography condition employed (reducing chemical instability) and simple technique, this method becomes popular as analytical tools for prostaglandin research (for details, see Hensby, 1977).

(3) Measurement of prostaglandins

(a) Bioassay

Bioassays have been extensively used for the detection and quantitative estimation of PGs. Although the main disadvantage associated with bioassay is lack of specificity of tissues to PGs and interference due to the presence of other substances in tissues and fluid extracts. This limitation can be overcome by proper methods of PG separation and specific antagonists to other biological active substances that may be present in assay extracts.

However, the use of bioassay methods is still widespread because of its rapidity in estimation and

easy evaluation of the physiological potencies of PGs.

In most cases of PG bioassay, various gastrointestinal smooth muscle preparations or mesenteric vascular beds from mammals are often used (Weeks et al., 1968; Vane, 1969; Ambache, 1966; Bergström et al., 1968). Full accounts of bioassay methods is described by Salmon and Karim (1976).

(b) Radioimmunoassay

Antibodies against a prostaglandin-protein conjugate have been successfully raised in mammals. The resultant circulating antibodies are obtained (antisera) and used for the assay of the prostaglandin concerned. The assay itself is based upon isotope dilution; radioactively labeled prostaglandin (usually ^{3}H or ^{14}C) to which the antisera were raised is used to construct binding curves for the antisera against the non-labelled prostaglandin. The antisera is then used to compare the effects of various biological samples on the binding of the radioactive label to the antisera. In this manner, a sensitive and relatively selective assay for a given prostaglandin is obtained (Jaffe and Behrman, 1974; Caldwell et al., 1971). However, it must be mentioned that any compound that will inhibit the binding of the radioactive label will be assayed as that given prostaglandin, and thus the results should be expressed as prostaglandin equivalents unless confirmed by two or more assay methods.

(c) Detection of Prostaglandins by Gas Liquid Chromatography with Flame Ionization, Electron Capture and Mass Spectrometry

Many detector systems are available to measure the effluent from a gas-liquid chromatography but only three have been used extensively for PG analysis, namely: (i) flame ionization detector (FID); (ii) electron capture detector (ECD); (iii) mass spectrometer (MS). The sample preparation is basically the same for each system (see Salmon and Karim, 1976).

Both flame ionization and electron capture detectors are relatively non-specific by comparison with a mass spectrometer. With flame ionization detection, any compound that elutes from gas-liquid chromatography column and combusts in the presence of hydrogen and oxygen to produce ions will be detected. This is a feature which prostaglandins share with almost all other organic compounds. Nevertheless, both flame ionization and electron capture detection have been successfully applied to the gas-liquid chromatography of prostaglandins and their metabolites (Albro and Fishbein, 1969; Pace-Asciak and Wolfe, 1971; Keirse and Turnbull, 1973).

The use of a mass spectrometer as a method of detecting prostaglandin derivatives is increasingly proving to be one of the most sensitive and selective developments in the physical analysis of prostaglandins.

However, its sophistication, time consumption and high expense make this method unsuitable for routine use.

Moreover, the mass spectrometer has a greater degree of specificity to the analysis of compounds being eluted from the gas chromatograph. Hence, applications of mass spectrophotometry are of great importance in the structure elucidation of the prostaglandins and their derivatives. (see also Frölish, 1977).

(d) Ultra-violet Spectrophotometry

The dienone structure present in PGB compounds has a characteristic ultra-violet (UV) absorption with a λ max (in ethanol) at 278nm. The Beer-Lambert law accurately represents the relationship between concentration and absorbance so that PGB may be quantitatively estimated using a spectrophotometer (Shaw and Ramwell, 1969).

PGE and PGA compounds are converted to the corresponding PGB by treatment with alkali (Samuelsson, 1964; Zusman, 1972) which can then be measured.

(e) Zimmermann Reaction

The basic principle of Zimmermann reaction is the formation of a coloured product by the reaction of ketone with m-dinitrobenzene in alkaline condition. This coloured product has an absorption peak at 580 mm (Cork et al., 1962).

The procedure is simple and relatively inexpensive but the sensitivity of the method is low.

(VI) INHIBITION OF PROSTAGLANDIN BIOSYNTHESIS BY ASPIRIN-LIKE DRUGS

'Aspirin-like' drugs are pharmacological agents with diverse chemical structures. However, they all share (to a varying degree) antipyretic, analgesic and anti-inflammatory actions which are characteristic of aspirin. The aspirin-like drugs include : meclofenamic acid, niflumic acid, indomethacin, mefenamic acid, flufenamic acid, naproxan, phenylbutazone, aspirin and ibuprofen, which are frequently studied in recent research.

In 1971, aspirin-like drugs were shown to inhibit prostaglandin release from human platelets by Smith and Willis. Aspirin also inhibits prostaglandin release from perfused dog spleen (Ferreira et al., 1971) and prostaglandin synthesis in cell-free homogenates of guinea-pig lungs (Vane, 1971).

(1) Inhibition of Synthesis

Inhibition of prostaglandin synthesis by aspirin-like drugs has now been demonstrated in many different systems (see Table 4). For the purpose of review, studies on the inhibition of aspirin-like drugs are divided into three categories :

(a) Homogenates and subcellular fractions

'Microsomal' preparations of tissue homogenates have been widely used, since the synthesising enzymes

Table 4. Summary of systems in which prostaglandin synthesis is inhibited by aspirin-like drugs.

Species	Tissue	References
Human	Platelet	Smith and Willis (1971)
	Semen	Collier and Flower (1971)
	Whole body	Hamberg (1972)
	Skin	Ziboh et al. (1973)
	Urinary metabolite	
	Prostate gland	Cavanaugh et al (1980)
Sheep	Seminal vesicle	Smith and Lands (1971)
Dog	Kidney	Aiken and Vane (1971)
	Spleen	Ferreira et al. (1971)
	Brain	Flower and Vane (1972)
	Platelet	Flower et al. (1972)
Cat	Spleen	Ferreira and Moncada (1971)
	Kidney	Somova (1973)
	CNS	Milton (1973)
Guinea-pig	Uterus	Poyser (1972)
	Lungs	Palmer et al. (1973)
	Whole body	Hamberg and Samuelsson (1972)
Rabbit	Jejunum	Ferreira et al. (1972)
	Brain	Flower and Vane (1972)
	Kidney	Aiken and Vane (1971)
	Eye	Eakins et al. (1972b)
Rat	Pregnant uterus	Aiken (1972)
	Inflammatory exudate	Greaves and McDonald-Gibson (1972)
Toad	Bladder	Wong et al. (1972)

are located in this fractions.

From comparison of inhibition action of aspirin-like drugs, it is possible to deduce the order of (decreasing) potency (Flower, 1974); meclofenamic acid > niflumic acid or indomethacin > mefenamic acid flufenamic acid > naproxen > phenylbutazone > aspirin or ibuprofen. This order of potency is, generally speaking, consistent with all the data so far published regardless of the source of the synthetase although some minor variations have been reported (Ham _et al._, 1972).

Aspirin or indomethacin have been found to be effective in inhibition of PGE_2 and $PGF_{2\alpha}$ production in guinea-pig uterus (Poyser, 1972). Indomethacin was a strong inhibitor of E_2 and $F_{2\alpha}$ production by microsomal preparations or homogenates of rabbit spleen (Bhattacherjee and Eakins, 1973) and kidney medulla (Tai and Hollander, 1973).

(b) Isolated tissues

The ability of aspirin-like drugs to inhibit release in tissues was first shown by Smith and Willis (1971) and by Ferriera _et al._ (1971). Smith and Willis (1971) found that aspirin-like drugs inhibited production of PGs in human platelet _in vitro_. Indomethacin was about 10 times more potent than aspirin which was in turn more potent than other salicylate. Gryglewski and Vane (1972) found that PGs are released when slices of

rabbit spleen are mechanical stimulated, and used this preparation to test the potency of inhibition of PG synthesis caused by aspirin-like drugs. The order of decreasing potency was found to be: meclofenamic acid > indomethacin > oxyphenbutazone > aspirin.

(c) Whole animals and man

Collier and Flower (1971) as well as Horton et al. (1973) showed that the amount of PGE_2 and $PGF_{2\alpha}$ in human semen was decreased by oral intake of aspirin.

Inhibition of prostaglandin synthesis in whole animals has been shown in other species. Administration of indomethacin to dogs, cats and rabbits abolishes the release of PGs from kidney in response to angiotensin injections (Aiken and Vane, 1971) haemorrhage or endotoxin-induced hypotension (Collier et al., 1973).

(2) Mechanism of Inhibition Action

The chemical diversity of aspirin-like drugs indicates the possibility of more than one mode of action. Several possibilities have been suggested (Flower, 1974) : competition with substrate or cofactor on the enzyme site, irreversible inactivation of enzyme, chelation, or a free radical destruction mechanism.

The majority of the aspirin-like drugs are organic acids and so the simplest hypothesis would envisage a straight competition between drug and substrate at the catalytic site. Indeed, some results appear to support this

concept. Flower et al. (1973) found that the degree of indomethacin inhibition was dependent on the substrate concentration.

However, other workers (Smith and Lands, 1971 and Lands et al., 1973) showed that the majority of the common aspirin-like drugs are 'competitive irreversible' inhibitors. Two possible situations may prevail (Curtis-Prior, 1976):-

(a) Inhibitor irreversibly binds to the dioxygenase system catalytic site. In the presence of substrate the velocity of this combination is reduced. Since the initial degree of inhibition is a function of substrate concentration, competition arises.

(b) This is similar to the first case but the inhibitor binds adjacent to the catalytic site and reduces it affinity for the substrate, allosterically.

In summary, most of the aspirin-like drugs block the initial stages of the synthetase reaction in a 'competitive-irreversible' fashion. They probably exert this effect by combining slowly with a site which, although not the substrate site, is sufficiently close to reduce the catalytic activity of the enzyme in a time-dependent fashion. Some compounds may affect endoperoxide breakdown but this cannot be regarded as definitely proven.

(3) Sensitivity of Aspirin-like Drugs

Due to a wide variation in sensitivity to aspirin in different tissues, Vane (1972) therefore, proposed that the

PG generating system exists in multiple molecular forms within the organism. The synthetase enzymes from each tissue type have a different pharmacological profile.

Bhattacherjee and Eakins (1973) tested the inhibitory potency of indomethacin against various rabbit tissues. The drug showed good activity against enzyme preparations from spleen, but was less active against kidney enzyme and evenmore, relatively inactive against the synthetase prepared from retina.

Furthermore, different potencies of these drugs against the same tissue taken from different species were reported. For example, doses of indomethacin to produce the same potency of inhibition in dog (0.17 μm) and rabbit spleen (0.14 μm) are different (Flower et al., 1972).

In addition, PG synthetases prepared from different tissues show different sensitivities to aspirin-like drugs. This reflects the possible existence of a series of isoenzymes and can explain the variations in activity within the group of compounds. However, a regional pattern of PGE_2 biosynthesis inhibition by indomethacin in rabbit kidney was reported (Attallah and Stahl, 1980). Cortical inhibition is found to be more profound than medullary and papillary response. They suggest that uneven distribution of blood flow and/or organic acid transport could lead to a higher concentration of the drug in the cortical region.

Moreover, potent prostaglandin synthetase inhibitors

are useful tools for defining the role that PGs play in the body. The evidence so far shows that PGs, as well as being important mediators of inflammation (Ferrira and Vane, 1974), fever (Milton and Wendlant, 1971), pain (Bergstrom _et_ _al_., 1959; Collier _et_ _al_., 1972) maintenance of isolated smooth muscle tone (Ono _et_ _al_., 1979), contribution to the explusion of the fetus (Aiken, 1972), modulation of lipolysis (Illiano and Cuatrecasas, 1971) and catecholamine release (Güllner _et_ _al_., 1979) (For details, see Ferreira and Vane, 1974)

In the present study, aspirin was used as a PGs-synthesis inhibitor for the investigation of the function of PGs in the snakehead.

(VII) EFFECTS OF PROSTAGLANDINS

The ubiquitous distribution and local biosynthesis of prostaglandins in various animal tissues have been previously reported (see also p. 22). Because of this, one would deduce that PGs may have versatile physiological actions on body. Actually, PGs have a wide spectrum of activities and individual compounds differ in their actions.

The physiological and pharmacological effects of PGs in mammals have been well documented. They stimulate as well as relax smooth muscles; they affect the cardiovascular system, acting as pressor agents under some circumstances and as depressor agents under others. PGs exert effects on adipose tissue metabolism and glucose homeostasis. Furthermore, PGs have influence on reproduction, inflammation, pulmonary physiology, renal function, digestion, the immune response, blood cells and hypothalamus-pituitary-endocrine system. Since PGs possess a lot of physiological and pharmacological activities, only those effects which are relevant to the present study will be discussed in detail.

(1) Cardiovascular effects of prostaglandins

In 1930s, Goldblatt(1933, 1935) and von Euler (1935) independently observed the biological actions of extracts of human seminal fluid and of sheep vesicular gland. These extracts produced a fall in blood pressure

and stimulated a variety of smooth muscle organs. However, the comprehensive study of the pharmacology of the prostaglandins only began with the isolation and characterization by Bergström and Sjövall (1957) of small quantities of two crystalline prostaglandins from vesicular glands. These were designated PGE and PGF. After this discovery, primary prostaglandins have become readily available to numerous biologists.

Among various biological actions of prostaglandins, their effects on cardiovascular system are first discovered and most studied. In this section, the actions of PGs on cardiovascular system will be discussed in detail (for details, see also Malik and Mcgill, 1976).

(a) Prostaglandins E

The intravenous administration of an E-type prostaglandin lowers the arterial blood pressure of all species examined (rat : Holmes et al., 1963; Weeks and Wingerson, 1964, cat : Holmes et al., 1963, mouse : Weeks, 1969, dog : Chapple et al., 1980, Sambar et al., 1967; Glaviano and Master, 1968, rabbit : Bergström and Euler, 1963, man : Bergström et al., 1959a; Carlson et al., 1969, Chicken : Horton and Main, 1967, goose : Grande and Prigge, 1972, carp : Peyraud-Waitzenegger et al., 1975). In most of these species the dose of PGE_1 or PGE_2 required to lower arterial blood pressure ranged from 0.1 to 10 µg/kg. Moreover, the

action of PGE_1, PGE_2 and PGE_3 on arterial blood pressure was qualitatively similar. However, the potencies of PGE varied in different animal species. Hence, the threshold infusion dose of PGE_1 required to lower arterial blood pressure was 180 ng/kg/min in cat, 440 ng/kg/min in dog and 1 μg/kg/min in rabbit (Horton and Jones, 1969). Prostaglandins E_1 and E_2 were equipotent in most animal species. However, in rat and dog, Weeks et al. (1969) found PGE_2 to be somewhat more active in lowering blood pressure than PGE_1 .

The cardiovascular effects of E-type prostaglandins were dependent upon the route of administration. Thus, intra-aortic injections produced more pronounced changes than intravenous injections (Bergström et al., 1964a). Similar observations have been made by a number of investigators (Carlson and Orö, 1966; McGill et al., 1969b; Nakano, 1971a) in rats and dogs. The difference is explained by rapid uptake and/or metabolism of E prostaglandins by the lungs and liver as well as rapid diffusion from the blood (Ferreira and Vane, 1967; Horton and Jones, 1969; McGill et al., 1969b).

The fall in arterial blood pressure caused by PGEs is most probably due to a decrease in total peripheral resistance as a result of their direct vasodilator action on resistance vessels. Arterial injections of PGE_1 have been shown to decrease the total

peripheral resistance, the resistance in carotid, femoral, brachial, coronary and renal vascular beds of the dog (Nakano and McCurdy, 1967a; Chapple et al., 1980), the hindlimb vasculature of the cat (Horton and Jones, 1969), pancreatic vascular bed of rat (Saunders and Moser, 1972) and mesenteric vascular bed of pig (Hovenaghel et al., 1979).

Bergström et al. (1959) reported that the depressor action of PGE_1 was not prevented by doses of atropine and antihistaminics sufficient to block acetylcholine and histamine-induced vasodepression, respectively. PG-induced fall in blood pressure was also not abolished by vagotomy or after the administration of various anaesthetic drugs (Giles et al., 1969), β-adrenergic blockade (Carlson and Orö, 1966), ganglionic blocking agents (Carlson, 1967; DuCharme and Weeks, 1967) and anticholinergic substance (Chapple et al., 1980). From these results, we can conclude that vasodilation and the associated decrease in arterial blood pressure produced by PGEs was not mediated through cholinergic mechanism, release of histamine, stimulation of β-adrenergic receptors or through blockade of α-adrenergic receptors.

Moreover, PGE_1 diminished the angiotensin, epinephrine-, norepinephrine- and vasopressin-induced increase in blood pressure (Weiner and Kaley, 1969). It suggests

that the abolishment of vasopressor substances induced blood pressure elevation may be due to the summation of two oppositive effects.

The PGE-induced decrease in blood pressure was accompanied by positive chronotropic and inotropic effects in dog (Nakano and McCurdy, 1967). However, PGE_1 did not increase the heart rate in vagotomized and intact dogs pretreated with pronethalol (adrenergic blocker), reserpine (β-adrenergic blocker) and ganglionic drugs (Carlson and Orö, 1966; Nakano and McCurdy, 1967), but these treatments did not affect the vasopression. It would, therefore, show that the chronotropic effect of PGE in intact dogs is mediated through reflex sympathetic stimulation as a consequence of the decreased systemic arterial pressure.

However, cardiovascular response to intracerebroventricular injection (IVT) of PGE_2 showed the contrary. This treatment resulted in elevation of blood pressure and tachycardia in rat (Hoffman and Schmid, 1979; Anderson and Leskell, 1975; Leskell, 1976; Kondo et al., 1979). α-adrenergic blockade inhibited pressor response of IVT of PGE_2 but could not alter the effect on heart rate (Hoffman and Schmid, 1979). Moreover, β-adrenergic blockade inhibited tachycardia but not pressor effect and atropine attenuated heart and blood pressure responses to PGE_2. These results indicate that

IVT PGE_2 injection produces pressor and tachycardia response in the unanaesthetized rat which are mediated primarily by centrally mediated sympathetic outflow.

The cardiovascular actions of PGE have been well documented in mammals. However, to the best of the author's knowledge, there are only three reports on non-mammals. Chiu and Leung (unpublished) studied the PGE_1 effect on perfusion flow rate in the perfused frog kidney. PGE_1 did not alter the perfusion flow rate in this preparation. In addition, PGE_2 intra-arterial injection induced a prolonged depression of arterial pressure associated with an initial bradycardia followed by tachycardia and hypertension in carp (Peyraud-Waitzenegger et al., 1975).

(b) Prostaglandins A

Prostaglandins of the A series are derived from E prostaglandins by dehydration which results in a marked relaxation on smooth muscles of the gastrointestinal, respiratory and reproductive tracts, but an increase in the dilatory activity of cardiovascular smooth muscles (Bygdeman et al., 1966; Weeks et al., 1969). Cardiovascular effects of A prostaglandins are qualitatively similar to E prostaglandins. PGA_1 and PGA_2 have a potent blood pressure lowering effect in both conscious and anaesthetized dogs and rats (Lee et al., 1965; Weeks, 1969; Higgins et al., 1970,

1972; Greenberg et al., 1974), anaesthetized cats (Kannegiesser and Lee, 1971) and rabbits (Horton and Jones, 1969). Similar to E prostaglandins, the activity of A prostaglandins varies in different animal species (Horton and Jones, 1969). PGE_1 and E_2 were more potent than PGA_1 or PGA_2 on intra-aortic administration to the cat, whereas a positive result of intravenous injection was reported (Kannegiesser and Lee, 1971). This difference is due to the fact that intravenous injection necessitates passage through the lungs which selectively inactivates E prostaglandins but not A prostaglandins which pass freely across the pulmonary circulation (Ferreira and Vane, 1967; McGill et al., 1969). The inability of the pulmonary circulation to inactivate A prostaglandins is most likely due to their lesser specificity for 15-hydroxy-prostaglandin dehydrogenase than those of the E series (Nakano et al., 1969).

Like PGE, the hypotensive effect of A prostaglandins is most likely due to marked dilatation of the peripheral vasculature resulting in a decrease in total peripheral resistance (Horton and Jones, 1969; Weeks et al., 1969). Similar to PGE, PGA also promotes heart rate and myocardial contractile force. β-adrenergic blockade prevented PGA_1-induced increase in heart rate whereas it could not change the vasodepressor action by PGA_1 (Higgins et al., 1971).

(c) Prostaglandins B and C

Prostaglandins of the B and C series are formed from A prostaglandins by the action of a prostaglandin isomerase which has been shown to be present in the plasma of a number of animal species (Jones, 1972 ; Jones and Cammock, 1973). The vasodepressor potencies of PGE_1, PGA_1, PGB_1 and PGC_1 was compared by Jones (1972). Relative to PGE_1 (= 100), PGA_1, PGB_1 and PGC_1 had potencies of 16, 0.9 and 44 respectively. Similarly, PGA_2, PGB_2 and PGC_2 possessed 16, 1.2 and 47% of the activity of PGE_2. From this observation, it showed that PGB_1 and B_2 are relatively impotent in cardiovascular action. PGC_1 and C_2 are comparatively potent than PGA_1 and A_2. PGC_1 also lowers vascular resistance and possesses an inotropic action (Jones et al., 1974). However, PGB_1 was a more potent depressant of left ventricular pressure than PGB_2. PGB_1 and B_2 also decrease the heart rate in the dog, PGB_1 being less potent than PGB_2 (Greenberg et al., 1973). These agents constricted the perfused hindlimb and hindpaw vasculature (Greenberg et al., 1973).

(d) Prostaglandins F

There are considerable species variation in the effects of $PGF_{2\alpha}$ and related F compounds on the cardiovascular system. In rat and dog, $PGF_{2\alpha}$ is a pressor agent (DuCharme et al., 1968). It was shown that $PGF_{2\alpha}$

has a potent constrictor action on small veins in the dog hindlimb and that cardiac output was raised with no change in calculated peripheral resistance (DuCharme et al., 1968). It was suggested that $PGF_{2\alpha}$ raised the blood pressure primarily by peripheral venoconstriction. Other workers disagree with this conclusion. Nakano and Cole (1969) have shown that $PGF_{2\alpha}$ increased cardiac output and total and regional peripheral resistances and decrease systemic venous return by sequestration of blood in the splanchnic vascular beds. They suggested that an increase in cardiac contractility and constriction in arterial beds are mainly responsible for the pressor effect of $PGF_{2\alpha}$. Emerson and co-workers (1971) also emphasized the arteriolar constrictor activity of $PGF_{2\alpha}$ in the dog. Furthermore, $PGF_{2\alpha}$ increased vascular resistance in the isolated perfused rat pancreas (Saunders and Moser, 1972) and mesenteric vascular bed in pig (Schrauwen et al., 1979).

In the isolated rat heart, $PGF_{1\alpha}$ increases the contractile force of the ventricles at low concentrations in the perfusate (Vergroesen et al., 1967). Thus, it is possible that the pressor effect in the rat is partly due to direct cardiac stimulation.

In addition, the vascular effects of PGE and PGF on different tissue preparations have been fully described (Nakano, 1973).

(e) Prostaglandins I (prostacyclin)

The studies of cardiovascular effects of PGI really

began in the late 1970s. PGI_2 is a potent vasodepressor substance (Ten Berg et al., 1980; Kondo et al., 1980) which survives passage through the lungs (Pace-Asciak et al., 1978, 1979; Chapple et al., 1980). While it was about twice as active as prostaglandin E_2 in lowering the arterial blood pressure of the normotensive rat, it was 3-4 times more active in the spontaneously hypertensive rat (Pace-Asciak et al., 1978). PGI_2 also reduced aortic pressure and total peripheral resistance and induced bradycardia (Chapple et al., 1980). Atropine (Cholinergic blocker) reduced bradycardia and hypotensive effects induced by PGI_2 (Chapple et al., 1980). After vagotomy, the hypotensive effects of PGI_2 was also reduced. These results reflects that hypotension induced by prostacyclin is due to two components : (1) a direct relaxation of vascular smooth muscles and (2) a reflex, non-cholinergic vasodilatation. The bradycardia is reflex in nature and is partially mediated by the vagus pathway.

PGI_2 attenuated pressor responses to norepinephrine in conscious rats (Okuno et al., 1980). A dose dependent antihypertensive effect caused by PGI_2 in conscious rats with spontaneous and chronic renal hypertension was reported (Schölken, 1978 and Pace-Asciak et al., 1979). However, intracerebroventricular administration of PGI_2 and PGE_2 elevated arterial blood pressure (Kondo et al., 1979).

An age-dependent increase in PGI_2 biosynthesis capacity was observed and reached maximum by three months of age in the spontaneously developing hypertensive rat

(Pace-Asciak and Carrara, 1979). This indicates that enhanced synthesis of the potent vasodepressor PGI_2 in the rat is likely to be an adaptive mechanism for the attenuation of the substained elevation in blood pressure in this animal model. Moreover, PGI_2 survives pulmonary transit for sufficient time to elicit a biological action (Pace-Asciak et al., 1979). Thus, its continuous systemic vascular synthesis could play an important role in the control of hypertension.

(f) Prostaglandin precursors

The PG precursors, arachidonic acid (AA), is a potent vasoactive agent. The AA infusion-induced decrease blood pressure and increase vascular resistance in dog (Mullane et al., 1979), rat (Damas and Troquet, 1978; Damas, 1978; Okuno et al., 1980; Ten Berg et al., 1980) and pig were reported (Houvenaghel et al., 1979; Schrauwen and Houvenaghel, 1978). However, cardiac effects of AA are variable. Administration of AA produced positive inotropic effects on the dog heart (Rose et al., 1974), whereas AA-induced bradycardia was reported by (Mullane et al., 1979). Irrespective of AA-induced positive inotropia or bradycardia, all of the cardiovascular effects of AA are abolished by indomethacin (Mullane et al., 1979; Okuno et al., 1980; Damas and Volon, 1978). These results indicate that AA-induced cardiovascular effects is mediated by biotransformation to active PGs. Besides, inhibition of prostaglandin

synthesis by indomethacin did not change the blood pressure of conscious rats. The endogenous prostaglandins seem to play a minor role in direct regulation of systemic blood pressure in conscious rats (Kondo et al., 1980). However, indomethacin potentiated the vascular reactivity to norepinephrine (Kondo et al., 1980) suggesting that the endogenous prostaglandins act to inhibit the norepinephrine response in the systemic circulation.

Other PG precursors, such as Dihomo-γ-linolenic acid exerts similar cardiovascular effects as AA and their effects are abolished by PG synthetase inhibitors (Rose and Kot, 1977).

Finally, which AA intermediate(s) is/are responsible for the obserable cardiovascular effects remains unknown. Data suggest that AA circulatory effects are not due to the primary prostaglandins PGE_2 and $PGF_{2\alpha}$, but rather to potent intermediates in PG synthesis or other end products of AA metabolism (Rose and Kot, 1977).

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(2) Metabolism Effects of Prostaglandins

In early 1960s, there was a great development on the chemistry of prostaglandins. Since the isolation and purification of prostaglandins (Bergström and Sjövall, 1960), these compounds became available for study of their biological actions. Later, the well-known effects of PGs on blood pressure and on smooth muscle had been published. Due to their remarkable biological potency, this led Steinberg et al. (1963) to propose that PGs might also play hormonal or regulatory roles beyond the actions on blood pressure and smooth muscle. Therefore, they tested the effects of prostaglandins on fatty acid release in adipose tissue. The outcome showed that PGs at extremely low concentrations, suppressed basal lipolysis in rat epididymal fat pads in vitro. After this finding, many biologists began to study the metabolic effects of PGs. However, only the effects of PGs on free fatty acid and glucose metabolism have been well documented. This section will mainly discuss these two aspects of metabolic effects of PGs.

(a) Free fatty acid (FFA) Metabolism

(i) In vitro effects

The first observations of the effects of prostaglandins on lipid metabolism were made by Steinberg et al. (1963, 1964) and by Bergström et al. (1965). Steinberg and co-workers (1964) showed that PGE_1 reduced basal lipolysis in pieces of epididymal

adipose tissue and those incubated with adrenaline, noradrenaline, ACTH, TSH and glucagon. At that time, of course, the mechanism of this antagonism could not be explained.

The release of free fatty acid from adipose tissue triglycerides (TG) is regulated by the sympathetic nervous system and by a variety of hormones activating the hormone sensitive lipase.

Lipase can be activated and inhibited at different levels. Activation is induced by hormones stimulating adenyl cyclase (AC), an enzyme cycling ATP into cyclic adenosine 3', 5'-monophosphate (cyclic AMP) which is a nucleotide acting as a secondary messenger in various organs (Fig. 9).

Butcher _et al_. (1967, 1968) suggested that anti-lipolysis action of PGE_1 was associated with formation of cyclic AMP which in turn activated intracellular triglyceride lipase (Rizack, 1964). Haessler and Crawford (1967) independently noted the similar action of PGE_1 and insulin in anti-lipolysis.

The relative potencies of several PGEs in blocking epinephrine-induced lipolysis were compared (Handler _et al_., 1965). PGE_1 was more potent than PGE_2; PGE_3 was ineffective even at 1 μg/ml, although an effect could be demonstrated at a very high concentration (16.6 μg/ml). PGE_1 was more

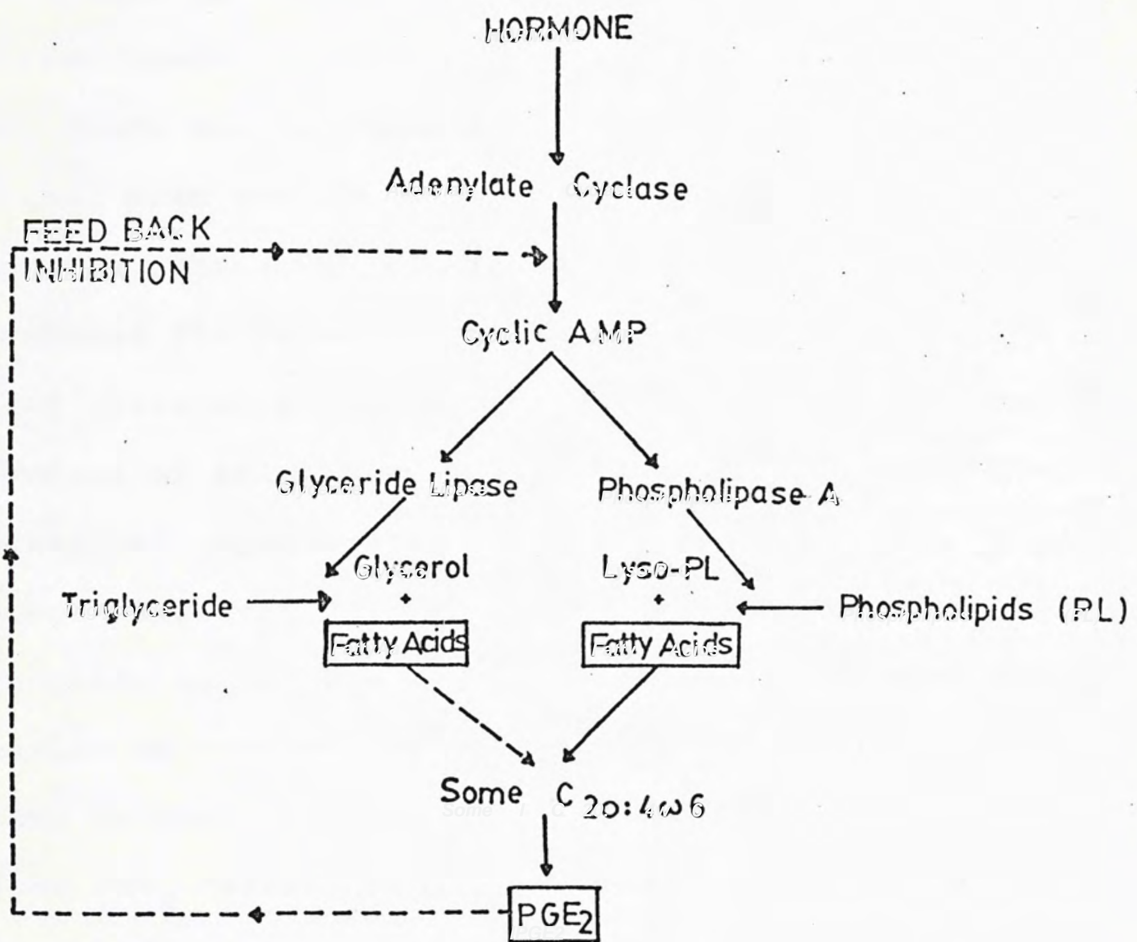


Fig. 8 A hypothetical scheme of negative feed-back inhibition of adipose cell lipolysis by prostaglandins.

potent than $PGF_{1\alpha}$ or $PGF_{1\beta}$ and PGE_2 more potent than $PGF_{2\alpha}$. These relative potencies resemble those for depression of blood pressure.

Shaw and Ramwell (1968) extended and correlated these findings and showed that when epididymal adipose tissue was stimulated _in vitro_ by lipolytic hormones (eg. adrenaline, noradrenaline and ATCH) or when the epididymal nerve was stimulated electrically, or when animals were previously fasted, there was an increased efflux of prostaglandins associated with the enhanced free fatty acid release. Furthermore, there was a reduced release of PGs (concomitant with reduced FFA release) in the presence of insulin. This led these workers to propose that PGs are involved in maintenance of adipose tissue homeostasis by way of a physiological negative feed-back control mechanism (Fig. 8). Colin and William (1974) also reported the existence of PG biosynthesis in rat isolated fat cells and negative feed-back regulation of synthetic activity. Similar findings were obtained by Christ and Nugteren (1970) who also showed a decrease PGE_2 release and an increased rate of adrenaline- and theophylline-stimulated lipolysis in essential fatty acid-deficient rats.

Aktories _et al_. (1979) demonstrated that PGs and α-adrenergic agonists (e.g. nicotinic acid) reduced hamster fat cell adenylate cyclase activity only in the concurrent presence of GTP and Na^+ ion (GTP 10 μm and Na Cl 120 mM)

in hamster adipocyte ghost. GTP has recently been shown in various tissues to be absolutely necessary for the inhibition of adenylate cyclase by α-adrenergic and cholinergic agonists (Watanabe et al., 1979). The exact mechanism of requirement of Na^+ in inhibition of hamster fat cell adenylate cyclase by PGE_1 remains unclear. It suggests that Na^+ may be involved in the transduction of the inhibitory signal to the adenylate cyclase or on the hormone binding.

However, Kather and Simon (1979) showed that adenylate cyclase of human fat cell ghosts shows a biphasic response towards PGE_2 with inhibition occurring at nanomolar concentrations of the hormone and stimulation at concentrations beyond 10^{-6} mol/liter. The expression of the inhibitory effect is critically dependent on GTP. This result is thus compatible with the concept that the antagonistic effects of the PGE on lipolysis are mediated via interaction with the membrane-bound adenylate cyclase thereby suggesting that the role of these hormones is more complex than assumed by the negative feedback concept. They proposed that the actual effects of PGs are the result of a delicately regulated balance between two opposing PG effects, which is not only dependent on the concentration of these C-20 fatty acids. However, this suggestion still requires to be elucidated.

(ii) _In vivo_ effects

The effects of PGs on plasma lipid are dependent upon the dose of PGs and the kinds of animals. PGA_1 infusion in anesthetized dog produced an increase in plasma FFA level (Sacca et al., 1973) and PGE injection also elevated the

plasma FFA and glycerol level in man (Bergström et al., 1965) and in goose (Grande and Prigge, 1972). However, Sanbar et al. (1967) reported a contradictory result and plasma FFA level was lowered by injection of PGE_1.

Moreover, intravenous infusion of PGE_1 in non-anaesthetized dog showed a dose-dependent response in plasma FFA level : low doses increased whereas high doses decreased plasma FFA level (Bergström et al., 1966). Bergström et al. suggested that low dose of PGE_1 stimulated the sympathetic nervous activity (enhanced lipid mobilization) while high dose of PGE_1 has a direct inhibitory effect on FFA mobilization from adipose tissue.

Carlson et al. (1970a) showed no PGE_1 antagonism of noradrenaline-induced FFA release in vivo in man but explained their findings by the suggestion that this PG is not normally transported by the blood to adipose tissue in vivo. At the same time, human adipose tissue incubated in vitro showed a significant inhibition of lipolysis by PGE_1. In more recent experiments in man, several PGs (A_1, E_2, $F_{1\alpha}$, $F_{1\beta}$ and $F_{2\alpha}$) have been infused into healthy subjects in doses ranging from 0.056 to 0.56 μg/kg/min (Carlson et al., 1970b). PGA_1 and PGE_2 induced a modest increase in heart rate and plasma FFA but F series of PG produced no significant changes in the heart rate and plasma FFA level.

The finding that insulin has similar actions as PGE_1 in counteracting the effects of epinephrine on cyclic AMP

accummlation and on lipolysis in adipose tissue has been discussed (p. 65).

Bressler (1968) first reported that PGE_1 induced an increase in plasma insulin level in mice. Later, Rossini _et al_ .(1971) showed that PGE_1 and PGA_1 could not induce release of insulin in isolated rat islets. They postulated that the increase in PG-induced insulin release _in vivo_ is the result of regional increase in pancreatic blood flow. However, Lefebvre and Luyckx (1973) showed that _in vivo_ intravenous administration of PGE_1 in dogs caused a significant drop in arterial blood pressure and in pancreatic duodenal vein pressure. At cessation of infusion there was a very significant increase in insulin release accompanied by slight hypoglycemia.

Others have found that prostaglandin E_1 and E_2 lowered circulating insulin levels and blunted insulin responses following intravenous glucose loading (Robertson, 1973). Similar results have been shown for PGA_1 in fasting dog (Sacca _et al_., 1973). However, it is still not possible to establish whether such an effect depends on a direct action of PGA_1 on pancreatic beta cells or it results from an indirect mechanism, eg., a sympathetic activity.

(b) Glucose metabolism

Besides PGs have effects on lipolysis, they also affect glucose metabolism.

(i) In vivo effects

PGE infusion produces hyperglycemic effects in anaesthetized and conscious dogs (Bergström et al., 1966; Sanbar et al., 1967), goose (Grande and Prigge, 1972), rat (Berti et al., 1965) and guinea-pig (Berti et al., 1965). Intravenous PGA_1 infusion also elevated plasma glucose level in anaesthetized dog (Sacca et al., 1973).

Moreover, PGE_1 infusion produced an increase in plasma glucose and decrease in plasma FFA level in intact rat while the hyperglycemic effect was abolished in adrenal medullectomized rat (Berti et al., 1967). In these treated rats, PGE_1 fully retains its effect on plasma FFA. These data indicate that the PGE_1 effects on plasma FFA and blood glucose are due to different mechanisms and that hyperglycemia depends on hypersecretion of medullary catecholamines. Therefore PGE_1 releases endogenous catecholamines and is able to antagonize the effects of these hormones on lipolysis but not on glucose mobilization.

(ii) In vitro effects

Vaughan (1966) observed that PGE_1 has a slight but significant effect on glucose uptake by rat epididymal adipose tissue whereas PGA_1 was ineffective. PGE_1 was also insulin-like in stimulating synthesis of fatty acids and glycogen from glucose.

In experiments on incubation of liver slices with PGE_1, Bohle and May (1967) found a decrease in glycogen levels.

However, Berti et al. (1967) suggested that the hyperglycemic effect of PG is mediated via catecholamines. Therefore, the results of Berti et al. and Boehle and May's conclusion are apparently in contradiction. Furthermore, Lemberg et al. (1971) reported that PGE_1 infusion induced a significant increment in the average glucose levels in the perfusate of the isolated perfused rat liver. On the other hand, Wilson and Levine (1970) described a decrease in labelled glucose incorporation in rat liver perfusions in the presence of PGE_1. In addition, PGE_1 decreases the basal rate of glucose production and abolishes the increase usually caused by glucagon or cyclic AMP (Wheeler and Epand., 1975). This effect is mimicked by insulin and indomethacin, both of which also decrease PG levels. It suggests that inhibition of the synthesis of a reactive metabolic intermediate in PG biosynthesis may explain these apparently anomalous effects.

Since PGE infusion induces hyperglycemia in most animals so far studied and liver provides the ample source of glucose in the body, a study of PG action on liver enzyme systems has been repeatedly emphasized.

PGE_1 has been shown to have insulin-like effects (Vaughan, 1966; Bohle et al., 1967) and to alter cAMP levels, either increasing or decreasing them in various tissues (Ramwell and Shaw, 1970). Two enzyme systems are involved: glycogen synthetase (glycogen synthesis key enzymes) and glycogen phosphorylase (glycogen degradation enzymes) of rat

liver which are known to be influenced by alternations in concentration of cAMP and by insulin. Both enzymes exist in physiological active and inactive forms. It was found that PGE_1 caused a significant decrease in glycogen synthetase activity regardless of nutritional status or presence or absence of adrenal glands (Curnow and Nuttall, 1972). Furthermore, β adrenergic and ganglionic blockade did not diminish the response of the synthetase system to PGE_1. On the contrary, PGE_1 promoted a significant increase in liver phosphophosphorylase activity in fasted intact rats which could be completely inhibited by ganglionic blockade and partially inhibited by β-adrenergic blockade. It was concluded that the <u>in vivo</u> effects of PGE_1 on the synthetase and phosphophosphorylase are mediated through different mechanisms. These changes in enzyme activities are compatible with increased hepatic cAMP and suggested possible direct effects on hepatic glycogen level. On the other hand, Exton <u>et al.</u> (1971) and Levine (1974; 1979) showed that PGE_1 and PGE_2 perfusion in rat liver failed to alter cAMP levels or glucose production. PGE_1 did not promote hyperglycemia, glycogenolysis, lipolysis or prevent epinephrine-induced hyperglycemia in the isolated perfused rat liver (Levine, 1979). Moreover, PGE_2 alone does not influence hepatic gluconeogenesis from lactate but does suppress glucagon mediated gluconeogensis and cAMP production in

isolated rat liver (Debubertis et al., 1974; Levine and Schwartzel, 1980).

From the above discussion of in vivo and in vitro effects of PGE on glucose metabolism, there is no general conclusion on the mechanism of PG actions. Until now, the other PGs, such as PGA, PGF and PGB, their actions on fatty acid and glucose metabolism has seldom been examined. Further studies on the mechanism of PGE action and on the metabolic effects of other PGs are required.

(3) Osmoregulatory Effects of Prostaglandins

Prostaglandins have been well known in modulating blood pressure. In the kidney, they are produced locally (Weber et al., 1979) and seem to increase renal blood flow and to promote natriuresis (Scherer et al., 1980; Oliw, 1979). However, the role of renal PGs in the handling of sodium-and potassium-chloride is still under debate. For PGE_2, both natriuretic (Papanicolaon et al., 1976) as well as antinatriuretic (Scherer et al., 1977) actions have been proposed, while sometimes no clear relationship could be found (Scherer and Weber, 1979). A review of the role of prostaglandins in the control of sodium excretion has been published (Kirschenbaum and Serros, 1980). Kirschenbaum and Serros suggested that prostaglandins may not have one consistent, uniform role in sodium excretion and they act not by themselves but rather in concert with numerous other natriuretic and antinatriuretic factors to control sodium excretion.

It has been reported that renal PGE_2 formation is reduced by high NaCl intake and enhanced by low NaCl intake and in hypokalemic states (Weber et al., 1979). These findings make renal PGE's good candidates for participation in the regulation of salt and water balance and in the control of blood pressure. Moreover, a participation of PGs in the control of renin release has been reported (Oates et al., 1979; Hockel and Cowley, 1980; Okahara et al., 1980;

Sato et al., 1980). PGI_2 and PGE_2 has been shown to increase renin activity in mammals (Oates et al., 1979). Indomethacin, a blocker of prostaglandin synthesis, lowered plasma renin activity, renal PG synthesis and causes sodium retention in man (Frölich, 1980). A number of laboratories have reported almost simultaneously on effects of prostaglandins on renin release (Donker et al., 1976; Frölich et al., 1976; Larsson et al., 1974; Romero et al., 1976). A more precise mechanism whereby prostaglandins influence renin release is given showing that the baroreceptor and the macula densa mechanisms of renin release are affected by prostaglandins (Frölich, 1980).

Since the collecting tubule has been regarded as playing a crucial role in regulation of Na excretion in the final urine, the inhibiting effects of PGE_1, PGE_2 and $PGF_{2\alpha}$ on Na absorption in the isolated collecting tubules by acting on the peritubular membrane were studied (Iino and Imal, 1978).

Moreover, the osmoregulatory effects of PGs on the other organs with osmotic participation have been published. In isolated bullfrog small intestine preparation, addition of PGE_1 to the serosal medium elicited small increases in transmural potential difference and short-circuit current while addition of PGE_1 to the mucosal medium caused no change in the electrical parameters (Gerencser et al., 1977).

A number of reports have been published that PGs are involved in water and ion transport in the isolated toad urinary bladder (Halushka *et al.*, 1980; Urakabe *et al.*, 1975; Lipson *et al.*, 1971). PGE_1 alone induced no significant change in osmotic water flow, but stimulated the short circuit current and inhibited vasopressin and theophylline-induced osmotic water flow across the urinary bladder of the toad (Lipson *et al.*, 1971; Urakabel *et al.*, 1975) and PGE_1 could not inhibit cAMP induced increase in water flow (Urakabe *et al.*, 1975). These results suggest that PGE_1 inhibits vasopressin and theophylline-induced water flow by inhibiting adenyl cyclase activity in the toad urinary bladder (Lipson et al., 1971). In contrast to the effects on water flow, PGE_1 stimulated Na transport across the bladder. This stimulation of transport can be potentiated by theophylline, a finding that suggests an action of PGE_1 on adenyl cyclase and consequent stimulation of Na transport by cAMP. Furthermore, a comparative study of different PGs inhibition on vasopressin induced osmotic flow in the isolated toad urinary bladder has been reported (Urakabe *et al.*, 1975). The potency of this effect was PGE > PGF > PGA > PGB and 1 series > 2 series.

Many types of invertebrate tissue contain (Nomura and Ogata, 1976) or synthesize (Christ and Van Dorp, 1972) prostaglandins. Therefore, PGs probably have many physiological actions in invertebrate. PGE_2 was identified in *Modiolus demissus* (Bivalve) gill tissue and sea water

incubated surrounding the tissue (Fereas and Grollman, 1980). Hyposmotic stress significantly increased release of prostaglandins into sea water and increase PG synthesis. This indicates that PGs may have a physiological role in the acclimation of <u>Modiolus</u> <u>demissus</u> to salinity stress. Furthermore, PGE_2 injected into the mussel (<u>Ligumia</u> <u>subrestrata</u>) caused an 80% reduction of Na influx and the animals experienced a net loss of Na^+. (Graves and Dietz, 1979). Injection of indomethacin caused a doubling of Na^+ influx relative to the control animals. It is concluded that Na transport in freshwater mussels is apparently regulated, in part, by prostaglandins.

In fish, Pic(1975) observed that either $PGF_{2\alpha}$ or PGE_1 could inhibit branchial Na^+ and Cl^- outflow in the sea water acclimated mullet (<u>Mugil</u> <u>capito</u>). Immersion of <u>Ophiocephalus</u> <u>maculatus</u> in fresh water containing aspirin for 24 hours significantly lowered plasma osmolality and plasma Na^+ and Cl^- concentrations (Woo <u>et</u> <u>al</u>., 1980). Horseman and Meier (1978) reported similar findings in <u>Fundulus</u> <u>grandis</u>. These findings strongly suggest that PGs participate in maintaining proper ionic balance in fish.

The subsequent investigations examined the function of exogenous and endogenous PGs in ionic regulation in snakeheads.

(4) Miscellaneous

Prostaglandins have a wide spectrum of activities and individual compounds differ in their actions. Their effects on cardiovascular system, metabolism and osmoregulation have been described earlier. Besides, other manifestations of prostaglandin effects on smooth muscle are represented by their oxytocic actions on the gravid uterus and by their abilities to stimulate intestinal motility. Prostaglandin effects on reproduction are quite pronounced. High levels of prostaglandins are associated with a breakdown of the corpus luteum and a depression in progesterone secretion. They are also implicated in the release of LH by mediating the release of LRF in the hypothalamus. Prostaglandins are implicated in the inflammatory response, in various aspects of the immune response, in the hematopoietic system, in gastro-intestinal secretion, in bone resorption, in digestive system, in respiratory system and in the central nervous system, where certain PGs have been implicated as neurotransmitters (further information, see Ramwell, 1973, 1974, 1977).

Perhaps the most important activity of prostaglandins is their involvement with the actions of cAMP, the second messenger in so many trophic hormone activities. This is evidenced by the fact that PGs significantly increase the tissue content of cAMP in many tissues and organs including lung, spleen, diaphragm, blood platelets, liver,

pituitary, aorta, bone, gastric mucosa, kidney, heart, corpus luteum, thyroid and erythrocyte (Ramwell, 1977).

CHAPTER III EXPERIMENTAL

CHAPTER III EXPERIMENTAL

(I) DISTRIBUTION OF PROSTAGLANDIN E IN THE TISSUES OF THE SNAKEHEAD

(1) Introduction

The distribution of various prostaglandins in the tissues of various animals has been reviewed (for details, see pp. 26-31). Among the mammals, a comparison of the distribution of different types of PGs shows that E series of prostaglandin is the most abundant and is present in almost every tissue. The existence of $PGF_{\alpha}s$ in the tissues is less abundant and the amount of PGA and PGB in the tissues are found to be least.

Due to the scarcity of information on the distribution of prostaglandins in lower vertebrates (Ogata and Nomura, 1975; Nomura et al., 1973; Vogt et al., 1967) and in invertebrates (Nomura and Ogata, 1976; Weinheimer and Spraggins, 1969), a comparison of the distribution of PGs cannot be stated. From all these reports on occurrence of PGs in tissues of lower vertebrates and invertebrates, PGEs are generally distributed in every tissue so far studied. $PGF_{\alpha}s$ are less commonly found in tissues. No information about the existence of PGAs (except Gorgonian has 15-epi-PGA_2 and its diester) and PGBs in the tissues of lower animals has been published.

In general, the amount of PGEs in the tissues varies with the type of tissues (1 to 2000 ng/g of wet tissue, except human semen : 38500 ng/g of wet tissue) (Nomura and Ogata, 1976).

In fishes (Carp, sheat-fish and leopard shark), the alimentary canal possesses a large amount of PGE (75-1350 ng/g of wet tissue) when compared with other tissues in the same fish. Heart, kidney, gill and air bladder also have an abundant amount of PGE (100 to 500 ng/g of wet tissue). Skeletal muscle, ovary, testis, fin and brain contain about 1 to 60 ng PGE/g of wet tissue (Nomura and Ogata, 1976).

It is well known that prostaglandins have an extensive diversity of biological effects in mammals. However, their physiological importance in lower animals has not been reported. It is quite interesting to decipher this puzzle in the comparative point of view. Therefore in the present study, experiments designed to investigate the effects of PGs in a lower vertebrate, the snakehead, were performed.

Before studying the effects of prostaglandins in the snakehead, a confirmation of the occurrence of PGs in the tissues of the snakehead seems to be necessary. Besides, a study of the distribution of PGE in the snakehead will at least indicate that PG may play a physiological role in this animal. Such a study also provides more information on the occurrence of PGs in lower vertebrate. Hence, the present experiment was designed to investigate the possible existence of PGs in the snakehead.

(2) Materials and Methods

(a) The Experimental Animal

The snakehead fish, Ophiocephalus maculatus Lacépède (Teleostei : Ophiocephalidae) was used in the present study. This fish is indigenous to South-East Asia. Ophiocephalidae has a marked ability to withstand hypoxia and a high growth rate (Chow et al., 1962; Hu and Chen, 1964). It is carnivorous and inhabits ponds and rivers. It can also tolerate a certain range of salinity change (from 1-450 mOsm/kg) (Tong, 1980).

In order to ensure that the same species of fish (see Plate 1) was used in all of the experiments, a careful identification of the snakehead has been undertaken. It is known that different species of snakeheads have different number of fin spines. The number of fin spines of the experimental fish were counted :

Pectoral fin	:	15
Pelvic fin	:	5
Dorsal fin	:	45
Anal fin	:	29

A count of the number of spines in addition to the general morphology of the fish contributes to the identification work. The experimental animal in the present study corresponds to the species Ophiocephalus maculatus Lacépède reported by Ng and Kam (1977).

(b) Keeping of the Snakeheads in the Laboratory

Snakeheads were purchased from a local commercial supplier and transported to the laboratory without

Plate 1 Photograph of snakehead, *Ophiocephalus maculatus* Lacépède.

anaesthetic. All snakeheads were adapted to fresh water at 18-23°C without food for at least two weeks before further treatment. Since it is known that body size of fish affects the metabolic rate and other physiological processes greatly (Chan and Woo, 1978), snakeheads with similar size were chosen in order to eliminate the body size effect. The body weights of the animals were in the range of 110-140g.

(c) Sampling Techniques

Snakehead was sacrificed and the tail was cut and the blood was collected from the exposed caudal vein as quickly as possible. Heparinized microhematocrit tubes were used to collect blood and then one of its end was sealed. After centrifugation plasma was saved and then frozen for later use.

Various organs including : liver, kidney, spleen, gastrointestinal tract, epaxial muscle, urinary bladder, heart and gill were removed from the fish. Then the gastro-intestinal tract was placed in cold 0.9% NaCl for washing out the food particles present in the tract. The tract was then lightly blotted on filter paper. The whole liver was removed and weighed. Muscle tissue samples were taken from the left side below the dorsal fin of each fish. Individual gills were removed by cutting through each gill arch and washed in cold 0.9% NaCl and excess fluid removed. Only gill filaments were saved for extraction of PGE. Urinary bladder was collected and washed in the cold 0.9% saline. All these tissues were subjected to homogenization

immediately after removal without freezing.

(d) Extraction, Separation and Measurement of Prostaglandins

(i) Extraction of prostaglandins

The present methods were modified after the guide for the radioimmunoassay of prostaglandin E (Calbiochem).

For Tissue Sample

1. Tissues were homogenized (500-2000 mg) with glass homogenizer in a 1 ml solution of 0.9% NaCl and 0.4 ml 0.1 N HCl
2. The homogenate was transferred into an extraction tube. The homogenizer was rinsed with 2.6 ml of ethyl acetate (Unilab)/isopropanol (Mallinckrodt) (1:1 vol/vol) and added to the homogenate. The tube was stoppered and shaken vigorously for 15 min
3. 3 ml of 0.9% saline and 2 ml ethyl acetate were added and mixed well
4. The tube was centrifuged and the upper ethyl acetate phase was saved
5. The upper ethyl acetate phase was evaporated under a stream of air

For Plasma Sample

1. 3 ml of ethyl acetate/isopranol/0.1 N HCl (3:3:1; vol/vol/vol) was added to 1 ml of plasma
2. The contents were shaken vigorously for 15 min
3. The procedure was repeated as from step 3 for tissue sample

(ii) Separation of Prostaglandins

This method also followed the guide for the radioimmunoassay of prostaglandin E (Calbiochem)

Solvents for PG Separation

The four solvent mixtures listed below were used for separation of PG's into subclasses. Reagent grade benzene (Fisher), ethyl acetate (Unilab) and methanol (Merck) were used for preparing the separation mixtures. The nomenclature S-1, S-2, S-3 and S-4 were used to denote the four solvent mixtures as follows (Orczyk and Behrman, 1972):-

S-1 : Benzene/Ethyl acetate 60:40 (vol/vol)

S-2 : Benzene/Ethyl acetate/Methanol
60:40:20 (vol/vol/vol)

S-3 : Benzene/Ethyl acetate/Methanol
60:40:10 (vol/vol/vol)

S-4 : Benzene/Ethyl acetate/Methanol
60:40: 2 (vol/vol/vol)

Preparation and Use of Silicic Acid Columns

1. A 10 ml glass pipet was used for the preparation of silicic acid column
2. A small plug of glass wool was inserted into the tip of the pipet
3. For every 0.25 g silicic acid (100 mesh, BG, ref: FLUKA 60780), 1 ml S-1 was added. 2 ml of this suspension was carefully added to the column
4. The column was allowed to drain. The following solvents : 5 ml of S-2; 1 ml of S-1; 0.5 ml of S-1

were successively added one by one and then was allowed to drain

5. 0.2 ml of S-2 was added to the evaporated crude lipid extract and then 0.6 ml of S-1 was added. This solution was carefully applied to the prepared column with a Pasteur pipet
6. The column was allowed to drain, then eluted with 6 ml of S-1. The fraction contained neutral lipids, fatty acid, steroids, PGB and PGA
7. The column was then eluted with 12 ml of S-4. This fraction contained PGE
8. The final elution was with 3 ml of S-2. This fraction contained PGF

(iii) Measurement of PGEs

The fraction containing PGE was evaporated under a stream of air. The quantity of PGE was estimated by the colorimetric method based on the Zimmermann reaction (Corker et al., 1962). This reaction is based on the formation of a colored product by the reaction of ketones with m-dinitrobenzene in alkaline condition. Since PGEs also contain a ketone group at carbon-9, therefore they react with m-dinitrobenzene to form a colored product in alkaline condition.

Solvents for PGEs assay

1. All components of the reaction mixture were made

up of absolute ethanol (Unilab)

2. 2.5 N KOH in ethanol was stabilized with N_2

3. 1% m-dinitrobenzene in absolute ethanol was prepared

Procedure

1. 0.1 ml ethanol was added into the dry fraction containing PGE
2. 0.3 ml of 1% m-dinitrobenzene in absolute ethanol was added
3. The contents were mixed well and the tubes were cooled to $0^{\circ}C$ in an ice-salt bath
4. 0.3 ml of 2.5 N KOH was added and was immediately mixed by shaking briskly. Bright light was avoided
5. The tubes were stood in a covered ice-salt bath for 20 min
6. The sample was diluted with 2.5 ml of cooled 80% ethanol
7. The optical density was read at 580 nm within a 10 min period

Standard curve of PGE

PGE_1 (No. P-5515) was purchased from Sigma Chemical company. PGE_1 with amount of 2,4,10,20 µg were used for preparing the standard curve. PGE_1 is initially dissolved in ethanol (Unilab) (PGE_1 is very stable in ethanol and is stored at $0^{\circ}C$) and suitable amount of PGE_1 solution was taken and was evaporated

to dryness. 0.1 ml of ethanol was added and colorimetric determination of PGE_1 processed as listed above.

Data are present as Means ± Standard Error of the Mean (M± S.E.M.). Statistical analysis of the results were carried out using Student's t test (Gray, 1971).

(3) Results

A standard curve of PGE_1 was prepared (Fig. 9). Since the result of standard curve of PGE_1 could be repeatable, it is quite reliable to use the colorimetric method of Zimmermann reaction to estimate pure PGEs.

Other possible interfering substances in the assay of PGEs were also tested (Table 5). The compounds included cholesterol, cholecalciferol, palmitric acid, retinal palmitate, vitamin E acetate, hydrocortisone (sodium succinate), triolein and snakehead plasma lipid. Some of these substances are commonly found in various tissues and most of them are soluble in organic solvent. Therefore they may be present in the PGEs tissue and plasma extracts and hence might interfere with the estimation of PGEs in the colorimetric method. The amounts of these substances used in the test were far more than the actual quantity found in the tissues and plasma (Krupp et al., 1979). Cholesterol (1.46 mg/3.2 ml), vitamin E acetate (15mg/3.2 ml) and triolein (0.6 mg/3.2 ml) had absorbance of 0.08, 0.015 and 0.035 repectively. Since the amounts of these substances were much more than the actual occurrence in the tissues and plasma, therefore, these substance would not interfere with the present method for assay of prostaglandin. Other compounds such as cholecaliferol, palmitic acid, retinol palmitate, hydrocortisone and snakehead plasma lipid in alkaline

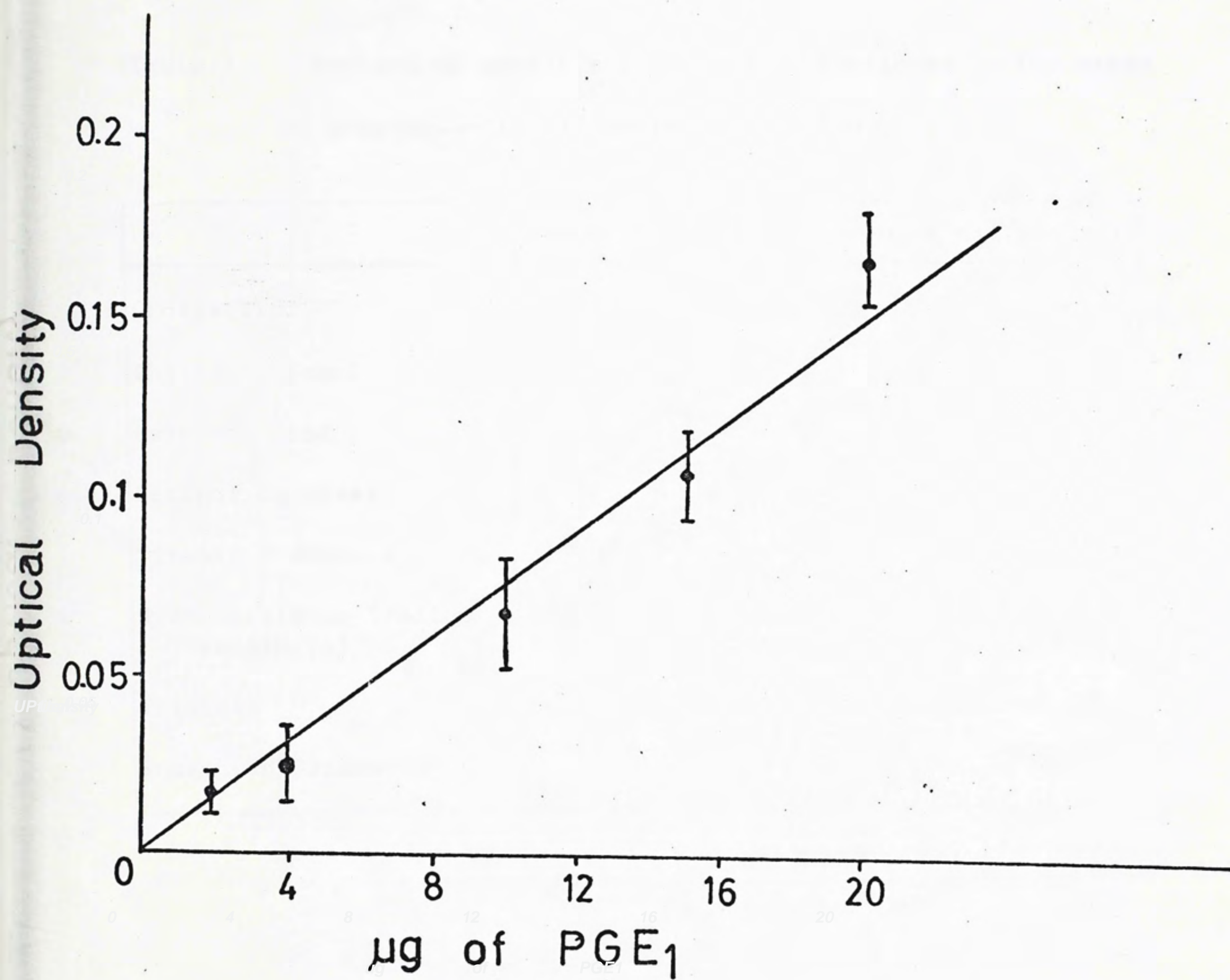


Fig. 9 Standard curve of PGE_1 in Zimmermann reaction.

Table 5. A summary of possible interfering substances in the assay of prostaglandin (Zimmermann Reaction).

	Amount (mg)	Absorbance (at 580 nm)
Cholesterol	1.46	0.08
Cholecalciferol	7.2	0
Palmitic Acid	2.1	0
Retinol Palmitate	2.94	0
Vitamin E acetate	15	0.015
Hydrocortisone (Sodium succinate)	13.5	0
Triolein	0.6	0.035
Snakehead Plasma Lipid	0.04	0

m-dinitrobenzene solution did not have any absorbance at 580 mm.

A recovery control of authentic PGE_1 after running the silicic acid column was done. Recovery of PGE_1 after the column was found to be 42.7%.

Although the qualitative analysis of PGE was not demonstrated in this experiment, the present result indicates that PGE-like substances were found in the tissues and plasma of snakehead. The occurrence of PGE-like substances in various tissues of snakehead was listed in Table 6. The present result shows that the comparatively significant amount of PGE-like substances in each tissue and plasma. The concentrations of PGE-like substances in μg/g of wet tissue were estimated and PGE_1 was used as standard. All the tissues in the present study contain PGE-like substances. The concentration of PGE-like substances varies with different tissues and they were in the range of 0.078 to 1.31 μg/g of wor tissue. It appears that liver (1.31 μg/g of wet tissue) and urinary bladder (1.154 μg/g of wet tissue) are inclined to have a higher level of PGE-like substances. Gill, Spleen, kidney, heart and plasma have a moderate amount of PGE-like substances (0.117-0.167 μg/g of wet tissue) whereas muscle (0.078 μg/g of wet tissue) and gastrointestinal tract (0.083 μg/g of wet tissue) contain a significantly low concentration of PGE-like substances when compared with the other tissues.

Table 6. Occurrence of PGE-like substance in various tissues and plasma of the snakeheads.

	Plasma# (7)	liver (7)	Gastrointestinal tract (7)	Spleen (7)	Kidney (7)
PGEs* (µg/g wet tissue)	0.120±0.019	1.310±0.337	0.083±0.012	0.140±0.032	0.131±0.004

	Skeletal muscle (7)	Gill+ (6)	Heart (6)	Urinary bladder (6)
PGEs* (µg/g wet tissue)	0.078±0.043	0.117	0.167±0.023	1.154±0.119

The number in parenthesis represents the number of animals.

* The amount of PGE-like substance was estimated by using PGE_1 as standard. All values had been corrected to 100% recovery by standard PGE_1 recovery control. The values are expressed in Mean±Standard error of the mean.

+ Gill filaments were pooled from six animals.

The amount of PGE-like substance in plasma was expressed in µg PGE_1/ml.

(4) Discussion

The present extraction method has been well established and widely used (Lijnen et al., 1979; Godard et al., 1976). Although a minor modification involving the use of chloroform/methanol (2:1 by vol) (Samuelsson, 1973; Ogata and Nomura, 1975) instead of ethyl acetate/isopropanol (1:1 by vol) has been employed, there is no change in the general extraction condition (acidified to pH 3.0 with HCl solution). Since PGs are very soluble in organic solvent, a change in organic solvent system will not cause a significant change in extraction efficiency. Therefore, the present extraction method provides a reliable method for extraction of PGEs in plasma and tissues.

For the separation of PGs, silicic acid column chromatography (SACC) and thin layer chromatography (TLC) have gained popularity in the laboratory (Eastman and Dowsett, 1976; Ogata and Nomura, 1975, 1976; Ogata et al., 1978; Christ and van Dorp, 1972; Caldwell et al., 1971; Godard et al., 1976; Lijnen et al., 1979). However, only silicic acid column chromatography was employed in the present study since there are many advantages to use SACC instead of TLC. First of all, using SACC consumes less time in the separation procedure especially for analysis of a great number of samples. Also SACC is a simpler process than TLC and the running cost of SACC is less than TLC. Besides, similar recovery for the separation of PGs

were reported for both TLC and SACC. Hence, SACC is preferred in this study.

Recovery control of PGE_1 after silicic acid column chromatography was done. The recovery after the extraction and separation procedure is 42.7%. The recovery in this study shows a good agreement with the recovery (42.08%) of William's experiment (1971) using the silicic acid column chromatography for separation of PGE_1.

From the standard curve of PGE_1 (Fig. 9 , p. 92) only more than 7 µg of PGE_1 in the assay sample would reliably be determined. Therefore, it is better to use the other methods such as radioimmunoassay, or mass spectrophotometry rather than Zimmermann reaction method. However, those methods require sophisticated techniques and are time consuming. Ultra violet spectrometry is also not a very sensitive method to measure PGE. Also the present assay method cannot differentiate different types of PGEs and all types of PGEs are detected by present method. Moreover, the formation of colored product in the Zimmermann reaction depends on the amount of substances which contain a ketone group in their configuration. However silicic acid column chromatography can provide a good technique to purify PGE from the other lipid and fatty acid. Therefore, the substance detected by the Zimmermann reaction in the present study is PGE. Furthermore, PGFs, PGAs and PGBs have not been estimated in this experiment. Since PGAs

and PGBs are eluted in the same fraction in silicic acid column chromatography method, a further separation is required before assaying these compounds. In addition, PGFs, PGAs and PGBs can only be detected by radioimmunoassay and mass spectrophotometry. These methods require sophisticated techniques and hence these compounds have not been measured. Furthermore, the main purpose of present experiment is to elucidate the occurrence of PGE-like substances in the snakehead and the Zimmermann reaction method provides a convenient assay for this purpose.

The distribution of PGE-like substances in the snakehead was listed in Table 6. All the tissues and plasma studied contained PGE-like substances. When compared with other fish species, such as carp, sheat-fish, leopard shark (Nomura and Ogata, 1976) the amounts of PGE-like substances in the tissues of the snakeheads are much higher. This is possibly due to the low sensitivity of the present method. However, the amount of PGE-like substances in gill and gastrointestinal tract of the snakehead had similar content as the carp, sheat-fish and leopard shark (Nomura and Ogata, 1976).

The existence of high concentration of PGE-like substances in urinary bladder suggests that PGE may be involved in biological functions in the urinary bladder (Lipson <u>et al.</u>, 1971). It has already been shown that in the toad urinary bladder, PGE_1 inhibits vasopressin-induced water transport by inhibition of adenyl cyclase activity (Orloff <u>et al.</u>, 1965).

It is tempting to speculate that the regulation of ion and/ or water transport by PGs is a more general phenomenon, in view of the high PG synthetase activity found in gills of fishes, mollusca and arthopoda and in frog urinary bladder and mammalian renal medullar tissue (Chirst and van Dorp, 1972).

The variable levels of PGE-like substances in different tissues of the snakeheads indicates that a relative importance of PGE in biological functions in these tissues. High amount of PGE-like substances in liver suggests PGEs may play an important role in the physiology of liver and it is proposed that these compounds may regulate glycogen metabolism in liver. Many reports have been published to show that PGs are involved in the metabolic processes (Curnow and Nuttall, 1972; Exton et al., 1971; Levine and Schwartzel, 1980). Therefore, in this study, metabolic effects of PGs in snakeheads were investigated in later experiments.

The present finding of the occurrence of PGE-like substances in gastrointestinal tract is in accordance with the report that PGs synthetase activity was found in carp intestine (Christ and van Dorp, 1972; Ogata and Nomura, 1975). Since PGs also exist in lower vertebrates, it is thought that PGs have some physiological significance in the smooth muscle motility of lower vertebrates as well as higher vertebrates.

Furthermore, the present data show that PGEs are

found in kidney, gill, urinary bladder and gastrointestinal tract of the snakehead. These organs have been reported to be involved in the osmoregulatory processes in fish (Conte, 1969). The existence of PGE-like substances in these may indicate the possible participation of prostaglandins in osmoregulation of the snakeheads. It has been reported that renal PGs formation is affected by NaCl uptake in rabbit (Weber et al., 1979). This suggests renal PGs may be involved in salt and water regulation. When the gill tissue of a marine bivalve, Modiolus demissus was incubated in hypoosmotic solution, a significant increase PGEs synthesis was observed (Freas and Grollman, 1980). This result suggests that PGE synthesis in the gill is dependent on ambient osmolarity. In addition, Horseman and Meier (1978) reported that some actions of prolactin with regard to teleostean osmoregulation are mediated by stimulation of prostaglandin synthesis.

Therefore, in subsequent experiments, the possibility of the involvement of PGs in osmoregulation of snakehead was investigated. The approach was to measure plasma ions levels in the snakeheads after PGs injection and aspirin treatment (PGs synthesis inhibitor).

In the present experiment, other types of PGs in the tissues have not been estimated and further studies should be pursued.

II) CARDIOVASCULAR EFFECTS OF PROSTAGLANDINS

(1) Introduction

A wide spectrum of physiological and pharmacological actions of prostaglandins in mammals have been well documented. Since the first discovered effect of prostaglandins was found to be their blood pressure lowering property, hence, many reports on the cardiovascular effects of prostaglandins have been published (for details, see also Literature Review, pp. 41-53). Generally, PGEs exhibit a blood pressure lowering action and is accompanied by positive chronotropic and inotropic effects. PGA and PGB also decrease blood pressure in animals whereas PGF elicits an animal-dependent cardiovascular response. As well as PGs, their precusors such as arachidonic acid also depresses blood pressure in animals.

However, these studies of prostaglandins effects on the cardiovascular system were mainly carried out in mammals. The author has conducted a literature survey on the topic and found that similar reports on lower vertebrates are lacking (except Peyraud-Waitzenegger et al., 1974, 1975; Leffler et al., 1980; Chiu and Leung, unpublished, 1980). Therefore, the present experiments were attempted to elucidate whether the cardiovascular responses to prostaglandins in snakehead is similar to those in mammals. Studies of the effects of PGA_1, PGB_1, PGE_1, $PGF_{1\alpha}$ as well as their precursor,

arachidonic acid, on blood pressure in snakehead were carried out. Breathing rate, heart rate and oxygen consumption were also measured in these studies. Moreover, possible interactions between prostaglandins and some drugs such as indomethacin (PG synthesis inhibitor), reserpine (α-adrenergic receptor blocker) and pentolinium tartrate (sympathatic and parasympathetic ganglionic blocker) were also investigated.

(2) Materials and Methods

(a) Experimental Animal

Snakeheads were purchased from a local commerical supplier and kept as previously described (pp. 83-85). Fish with body weights between 190-240 g were chosen in these experiments. They were kept in freshwater at 20-25°C without food for 2 weeks before experimentation.

(b) Surgery

(i) Catheterization of the gonadal vein

Snakehead was anaesthetized by total immersion in a MS-222 (triacaine methanesulfonate, Sandoz) solution. While the snakehead was still anaesthetized, the abdominal body wall of the animal was cut open and the incision was kept open by a pair of retractors (Plate 2). The gonadal vein was exposed by clearing the adherent fatty tissues. The vein was tied distally with a piece of surgical thread and clamped proximally with a bulldog clip. A PE-10 (Intramedic Polyethylene Tubing, Clay Adams) cannula tube filled with heparinized isotonic saline (0.9% NaCl) was inserted through a puncture into the gonadal vein and the tube was tightly fastened in place. The gonadal vein was unclamped.

(ii) Catheterization of pneumogastric artery

After the catheterization of the gonadal vein, the pneumogastric artery was also cannulated by

Plate 2 Cannulation of the gonadal vein. The abdominal body wall of snakehead was cut open and the incision was kept open by a pair of retractors.

inserting a PE-60 (Clay Adams) polyethylene tube (Plate 3) as described above. The body wall was sutured up with silk thread and the fish was then returned to a black respirometer containing 3 litres of well aerated fresh water. The fish was allowed to recover from anaesthesia without disturbance.

(c) Injection of Prostaglandins

After recovery for two hours from anaesthesia, different types of prostaglandins was injected separately into the fish through the gonadal vein cannula. Different doses of PGA_1, PGB_1, PGE_1, $PGF_{1\alpha}$ and arachidonic acid (5, 15, 30 and 60 μg/kg of body weight) were injected intravenously. Before injection, suitable amounts of prostaglandins (Upjohn) and arachidonic acid (Sigma) (dissolved in absolute ethanol; 0.5 mg/ml) was diluted five fold with 0.9% NaCl. Injections of appropriate amounts of ethanol and 0.9% NaCl served as controls.

(d) Recording of Blood Pressure

Before and after the injection of PGs and arachidonic acid, the arterial blood pressure of the fish was measured through the pneumogastric artery cannula by means of a pressure transducer (Harvard Apparatus) connected to a series of amplifiers (Harvard Apparatus) and the final output was displayed on a Harvard Biograph System.

Plate 3 Catheterization of pneumogastric artery. The abdominal body wall of snakehead was cut open and the incision was kept open by a pair of retractors.

(e) Counting of Heart Beat and Breathing Rate

The heart rate of snakehead were estimated by the number of pen deflection of the blood pressure tracing. Breathing rate was estimated by counting the number of opercular movements.

(f) Measurement of Oxygen Consumption Rate

The oxygen consumption rate of snakehead was measured by following the rate of oxygen depletion in a closed chamber. Polyethylene container (3 litre) with removable lid served as the respiratory chamber. Before measurement, the fish was kept in the respiratory chamber (fresh water) at a constant temperature $20\pm1^{o}C$ for two hours to allow it to adapt to the surroundings. The water was well aerated during the period. The chamber was then filled with water and the experiment started by removing aeration. The water inside the respirometer was mixed using a varistaltic pump (Manostatic, Advance Model). The decline in pO_2 of the water was continuously monitored using a Gilson oxygraph system coupled to an oxygen electrode (Yellow Stone Instruments).

(g) Injection of drugs : Indomethacin, Reserpine and Pentolinium tartrate

Reserpine, pentolinium tartrate and indomethacin were purchased from (Sigma). Reserpine and pentolinium tartrate were dissolved in 0.9% NaCl and indomethacin was

suspend in the 0.9% saline. Indomethacin (10 mg/kg of body weight), reserpine (0.5 mg/kg) and pentolinium tartrate (5 mg/kg) were injected intra-peritoneally into the fish daily for 3 consecutive days. A fourth dose was injected just before surgery. The cannulation procedure and measurement of blood pressure was similar to that previous described.

(3) Results

(a) Effects of Prostaglandins and Arachidonic acid on Blood Pressure

The basal arterial blood pressure of snakehead is 20.5±1.0 mmHg. Injection of ethanol (diluted to five fold with 0.9% NaCl) as a control into the fish produced no change in arterial blood pressure (Fig. 10). PGE_1 and PGA_1 elicited a dose-dependent decrease in arterial pressure (Fig. 11). PGE_1 was more potent than PGA_1 in lowering arterial pressure. On the contrary, PGB_1 elevated the arterial pressure in a dose-dependent manner in snakeheads (Fig. 11). The minimum dose of producing blood pressure effects of PGA_1, PGB_1 and PGE_1 are approximately equal to 2 μg/kg of body weight. Hence the doses which are below 2 μg/kg cannot produce any effects on blood pressure in snakehead. Also the effects of the three PGs reach the plateau at dose 90 μg/kg (Fig. 10, PGA_1 injection with 90 and 120 μg/kg), therefore, higher doses cannot exert a stronger action on blood pressure.

The most striking finding was that $PGF_{1\alpha}$ and arachidonic acid produced no response on arterial blood pressure in snakeheads (Fig. 10) regardless of the dose. These results are in contrast to those reported on mammals (for details, see also pp. 59-60).

A plot of the time course of changes in arterial pressure following PGB_1 injection is shown in Fig. 12.

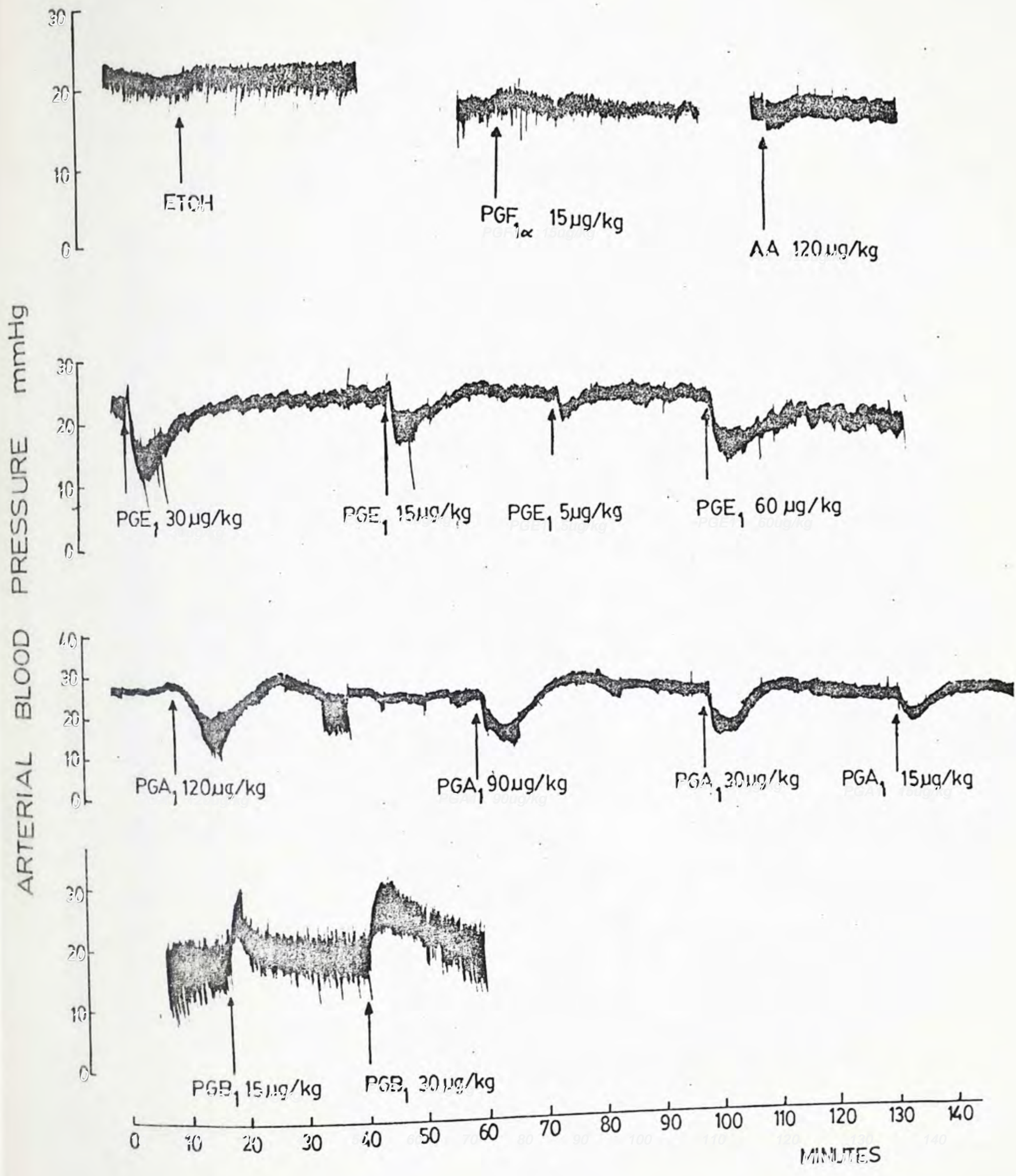


Fig. 10 Effects of intravenous injection of arachidonic acid (AA), PGs and ethanol (ETOH) in arterial blood pressure of snakeheads. The blood pressure was measured through the pneumogastric artery cannula.

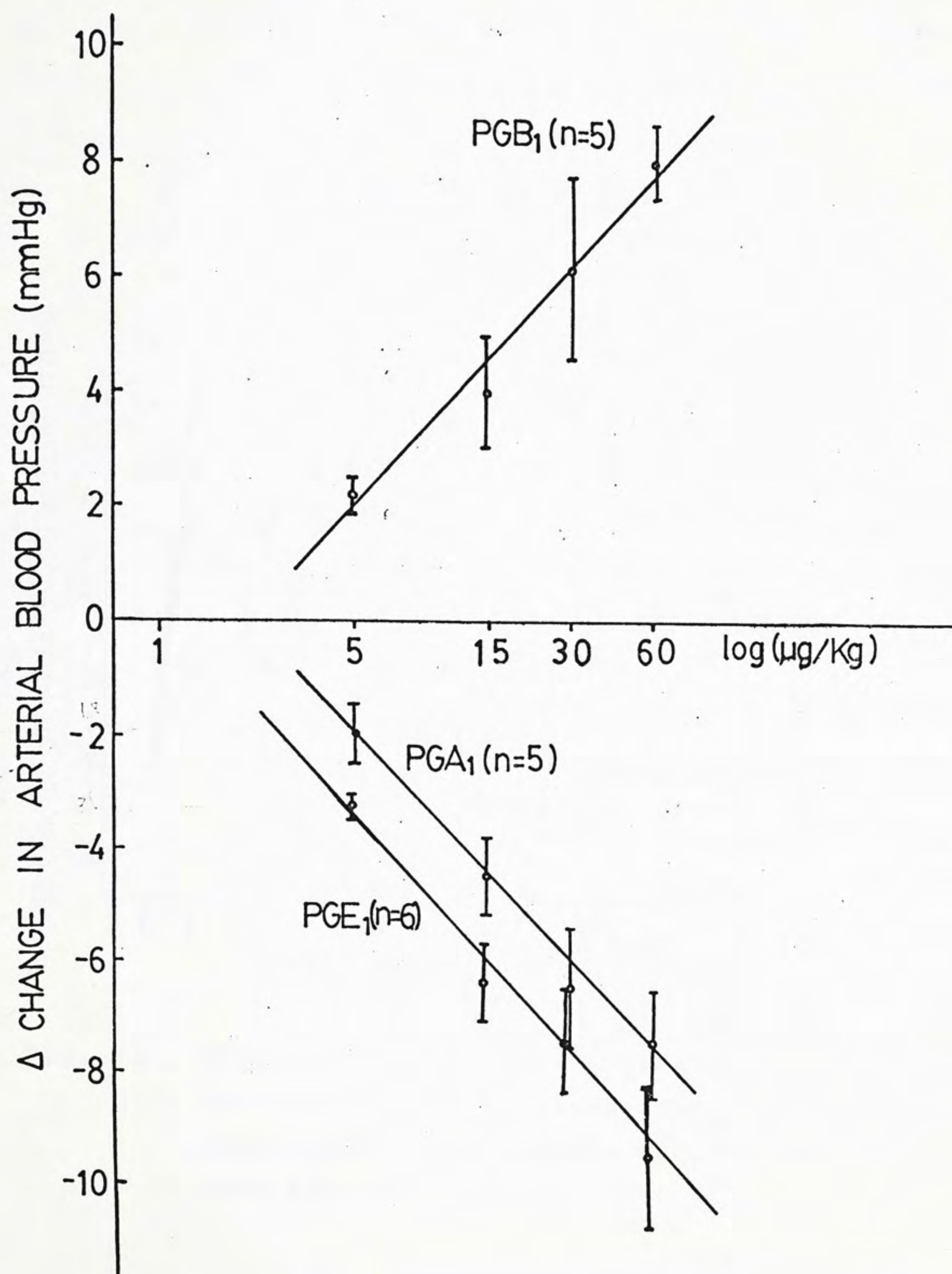


Fig. 11 Dose-dependent responses to PGA_1, PGE_1 and PGB_1 in arterial blood pressure of snakeheads. Data were expressed in the Mean±SEM. n denotes the number of fish.

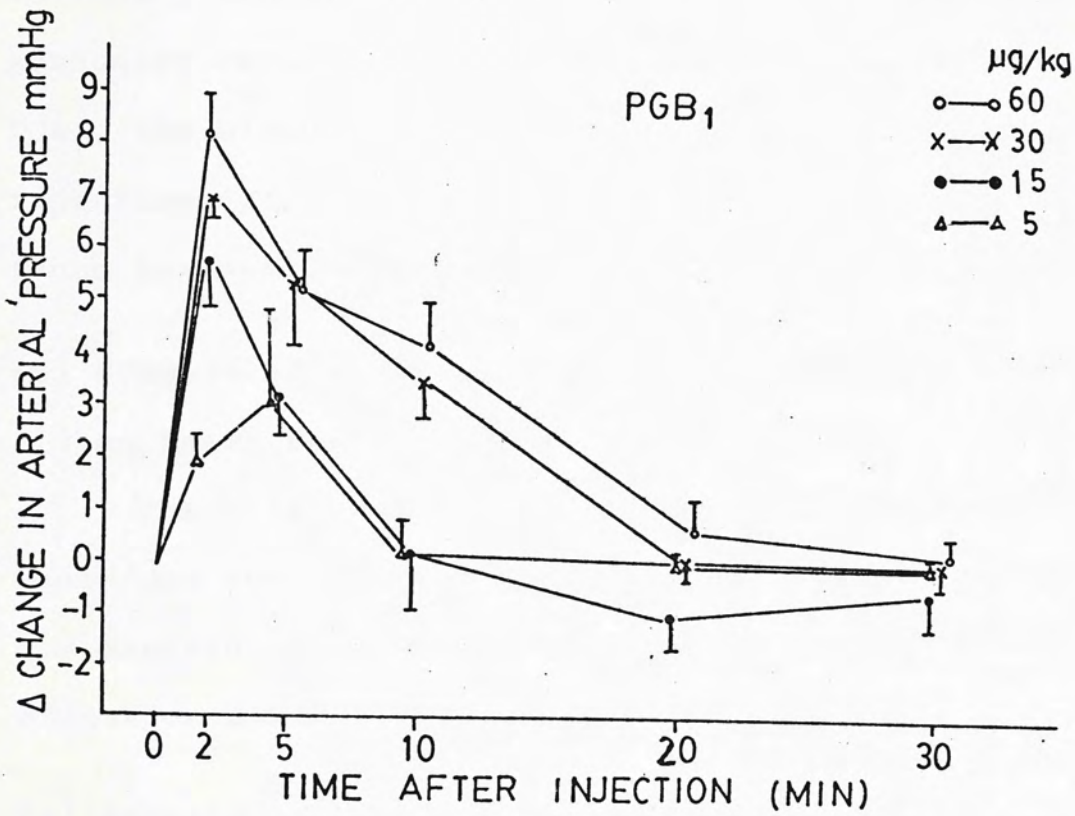


Fig. 12 Effect of intravenous injection of PGB_1 in arterial blood pressure of five snakeheads. Each symbol denotes the Mean and SEM.

After PGB_1 injection, an immediate and prompt increase in arterial pressure was observed. This increase reached its peak about 2 minutes after receiving PGB_1 and was followed by a gradual and prolonged decrease which returned to normal after 20 minutes.

PGE_1-induced decrease in blood pressure reached maximum 5 min after injection and then the blood pressure gradually returned to normal (Fig. 13). After the injection PGA_1, the blood pressure reached the maximum 1.8 min after injection (Fig. 14). This increase in blood pressure was found to occur more quickly than PGE_1 effect.

(b) Effects of Prostaglandins and Arachidonic acid on Heart Beat and Breathing Rate

The basal heart beat and breathing rate were 42 ± 6 beat /min and 25 ± 3 beat /min respectively. Prostaglandins and arachidonic acid did not produce any chronotropic effect or alter breathing rate of snakeheads.

(c) Effects of Drugs on Blood Pressure

Four daily intraperitoneal injections of indomethacin (10 mg/kg) and pentolium tartrate (5 mg/kg) significantly lowered the blood pressure when compared with their corresponding controls (Fig. 15). However, reserpine (0.5 mg/kg) did not change the basal blood pressure (Fig. 15).

When snakehead received pentolinium tartrate

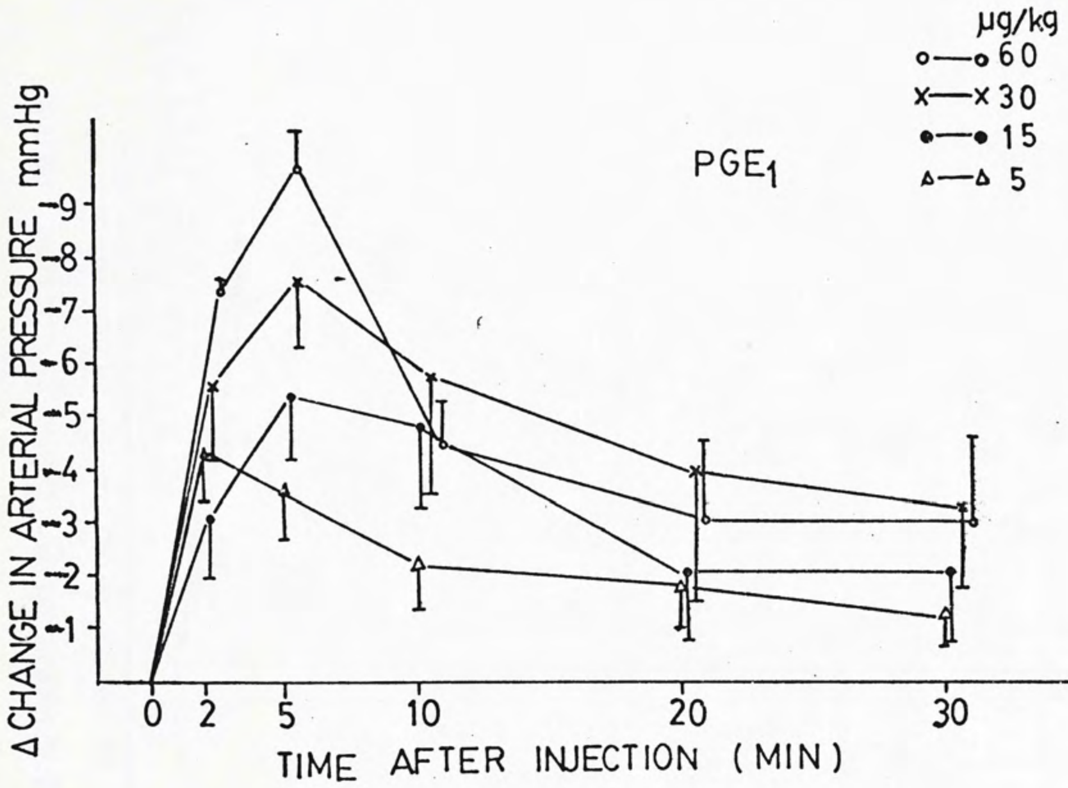


Fig. 13 Effect of PGE_1 injection in arterial blood pressure of six snakeheads. Each symbol denotes the Mean and SEM.

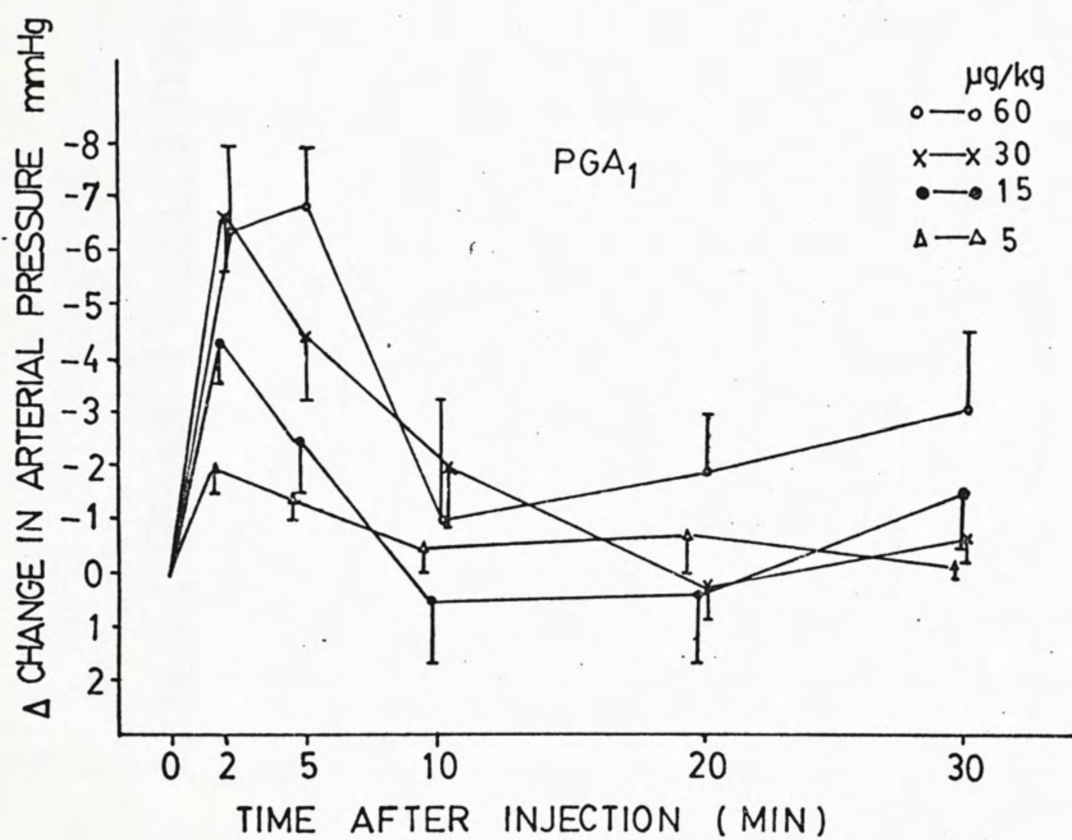


Fig. 14 Effect of PGA_1 in arterial blood pressure of five snakeheads. Each symbol denotes the Mean and SEM.

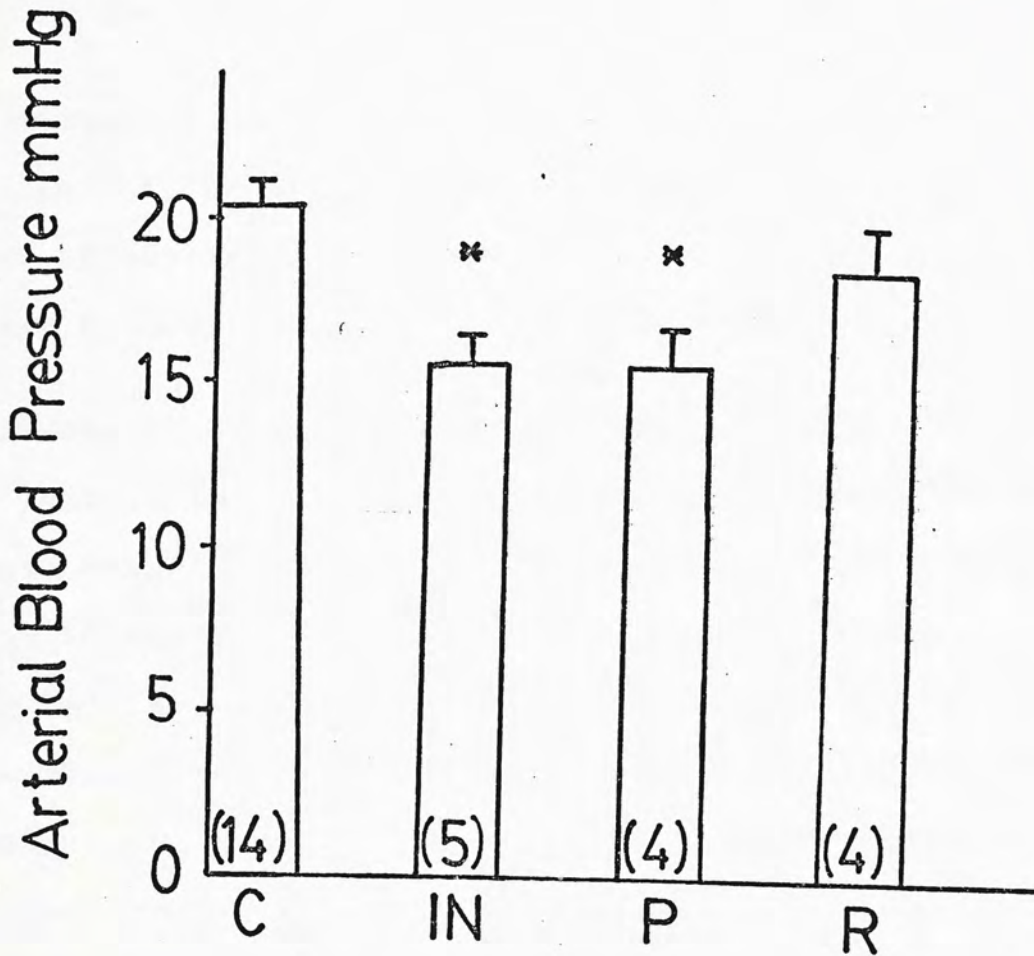


Fig. 15 Arterial blood pressure in snakeheads after indomethacin (IN), pentolinium tartrate (P) and reserpine (R) treatment. Results were expressed in the Mean and SEM. C=contol. Number in parenthesis represents the number of fish. * $P < 0.05$ when compared with controls.

(ganglionic blocker) treatment, the PGA_1-induced hypotensive effect was partially abolished (Table 7). Moreover, pentolinium tartrate also partially reduced the pressor response to high dose of PGB_1 (60 μg/kg), whereas no effect could be observed on the response to low dose of PGB_1 (30 μg/kg). On the other hand, pentolinium tartrate did not alter the PGE_1-induced decrease in arterial pressure.

Reserpine has been found to be an antihypertensive drug. In this experiment, reserpine did not change the pressor effect induced by PGB_1 at high dose (60 μg/kg) or low dose (30 μg/kg).

(d) Effects on Oxygen Consumption Rate by PGEs

Control injection (Ethanol : 0.9% NaCl; 1:4 by vol) did not change the oxygen consumption rate of snakehead, whereas 10 min after intravenous injection of PGE_1 (30 μg/kg), a statistically significant decrease in oxygen consumption rate (Fig. 16) was observed. This PGE_1-induced decrease in O_2 consumption rate gradually returned to pre-injection level after 3 hours.

able 7 : Effects of PGs injection in arterial blood pressure on snakeheads.

Treatment mg/kg		N	PG‡ µg/kg		Δ Change A.B.P. mmHg	S.D.
--		6	PGA_1	60	-7.5 ± 0.56	
--		6	PGA_1	30	-6.5 ± 1.17	
--		6	PGE_1	60	-9.5 ± 1.15	
--		6	PGE_1	30	-7.4 ± 0.9	
--		5	PGB_1	60	$+8.1 \pm 0.64$	
--		5	PGB_1	30	$+6.2 \pm 1.63$	
Pentolinium tartrate i.p.	5	5	PGA_1	60	-3.6 ± 0.9	$P < 0.01$
Pentolinium tartrate i.p.	5	5	PGA_1	30	-3.3 ± 0.25	$P < 0.01$
Pentolinium tartrate i.p.	5	3	PGE_1	60	-12 ± 2.6	No
Pentolinium tartrate i.p.	5	5	PGB_1	60	$+5.0 \pm 0.5$	$P < 0.01$
Pentolinium tartrate i.p.	5	1	PGB_1	30	+5	No
Reserpine i.v.	0.5	4	PGB_1	60	$+4.8 \pm 1.59$	No
Reserpine i.v.	0.5	3	PGB_1	30	$+4.4 \pm 0.36$	No

N=number of fish ; A.B.P.=arterial blood pressure

S.D.=statistical significant difference

i.p.=intraperitoneal injection ; i.v.=intravenous injection

‡ PGs injected intravenously

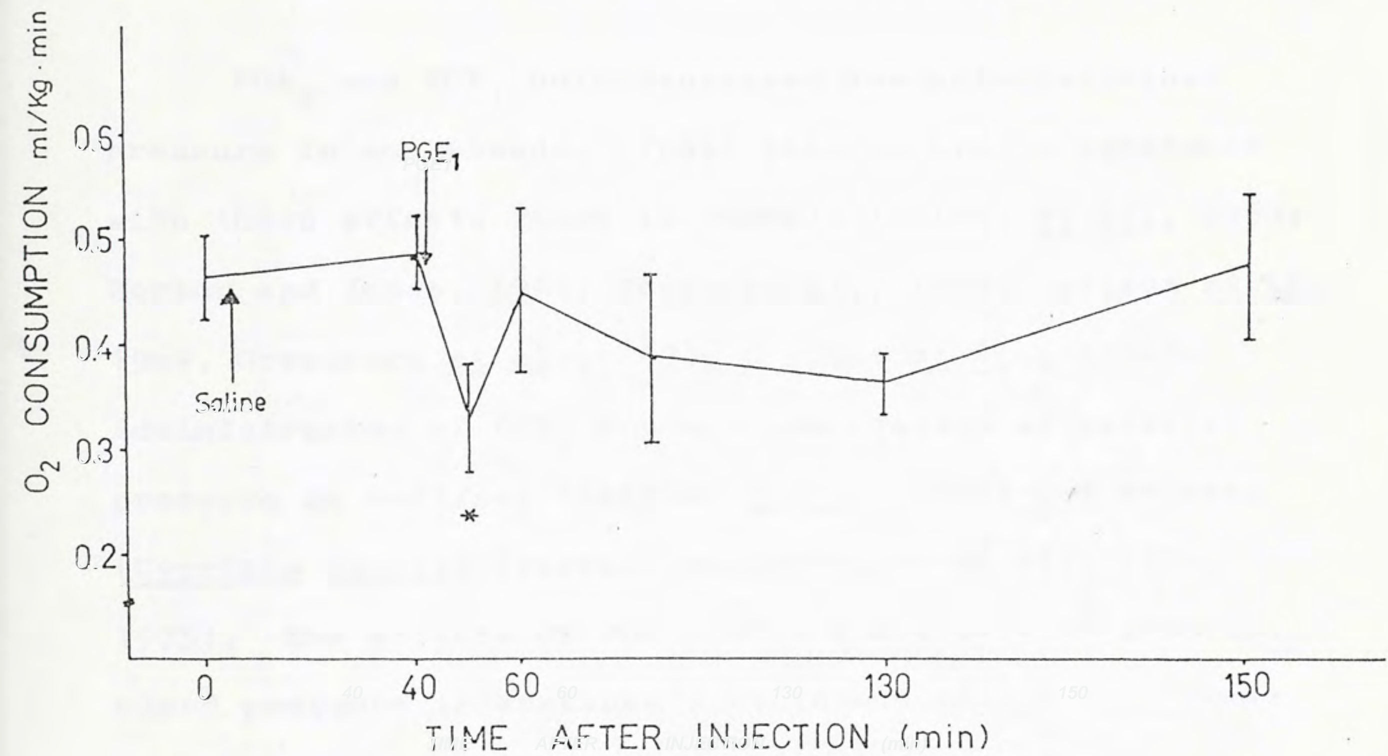


Fig. 16 Effect of intravenous injection of PGE_1 (30 μg/kg) in O_2 consumption of five snakeheads. Appropriate amount of ethanol+saline was injected into the fish as controls. Results were expressed in Mean± SEM. * $P < 0.05$ when compared with controls.

(4) Discussion

The present results strongly suggest that prostaglandins exert cardiovascular effects on snakeheads. However, only PGA_1, PGB_1 and PGE_1 had profound vascular actions in snakeheads, while $PGF_{1\alpha}$ and arachidonic acid did not alter the arterial blood pressure.

PGA_1 and PGE_1 both decreased the arterial blood pressure in snakeheads. These results are in agreement with those effects found in mammals (Holmes et al., 1963; Horton and Jones, 1969; Weeks et al., 1969; Carlson et al., 1969, Greenberg et al., 1974; Chapple et al., 1980). Administration of PGE_2 produced depression of arterial pressure in bullfrog (Leffler et al., 1980) and in carp (*Cyprinus carpio*) (Peyraud-Waitzenegger et al., 1974; 1975). The effects of PGE_1-induced decrease in arterial blood pressure in snakeheads is in accordance with those effects on carp.

However, the mechanism of action of the hypotensive effect of PGA_1 and PGE_1 in snakehead has not been investigated in this experiment. In mammals, the hypotensive effect of PGAs and PGEs are most likely due to marked dilatation of peripheral vasculature resulting in a decrease in total peripheral resistance (Horton and Jones, 1969; Weeks et al., 1969; Saunders and Moser, 1972; Hovenaghel et al., 1979). In frog kidney perfusion, PGE_1 has been

shown to increase perfusion rate (Chiu and Leung, unpublished). Therefore, it is suggested that PGA_1-and PGE_1-induced hypotensive effect in snakeheads may also be caused by a decrease in total peripheral resistance in this fish.

Prostaglandin Bs are formed from prostaglandin A by the action of a prostaglandin isomerase which is present in plasma (Jones, 1972 ; Jones and Cammock, 1973). PGAs and PGBs both have similar structure except the location of double bond in the ring (for details, see also p. 14). Due to the similarity of these two compounds, they both exert similar actions on cardiovascular system. It has been published that PGA_1 and PGB_1 both have vasodepressor potencies in mammals (Jones, 1972; Greenberg et al., 1973). Furthermore, the vasodepressor action of PGBs is less potent than PGAs. However, the present finding in snakehead is in discordance with those found in mammals. PGB_1 induced vasopressor effect in snakehead instead of vasodepressor action. The present experiment cannot explain this aberrant result of PGB_1 in snakehead.

The cardiovascular effects of $PGF_{\alpha}s$ and arachidonic acid in mammals have been previously described in pp. 59-63. Chiu and Leung (unpublished) showed that $PGF_{1\alpha}$ constricted renal vascular system to reduce the perfusion rate in frog kidney perfusion. However, Leffler et al. (1980) showed that $PGF_{2\alpha}$ and arachidonic acid decreased arterial pressure

in bullfrog. In this study, however, both $PGF_{\alpha s}$ and arachidonic acid had no effect on cardiovascular system in snakeheads. Moreover, the cardiovascular effects of arachidonic acid in mammals and bullfrog is mediated by biotransformation to active PGs (since indomethacin abolished all of the cardiovascular effects of arachidonic acid) (Okuno et al., 1980; Damas and Volon, 1978; Leffler et al., 1980). Due to no observable arachidonic acid-induced cardiovascular effects in snakeheads, arachidonic acid may not be converted immediately to an active PG(s) in the vascular system of snakeheads. The inability to synthesize PG(s) from arachidonic acid may be caused by low biosynthetic activity of PGs in the vascular system or lack of PGs synthesis enzymes. The latter possibility is eliminated by the result obtained from indomethacin-treated fish since indomethacin significantly decreased the basal arterial blood pressure in snakeheads. Although this indomethacin-induced decrease was only one fifth of the basal arterial pressure, the endogenous prostaglandins seem to play a role in direct regulation of systemic blood pressure in this fish. Hence, this indicates the existence of prostaglandin biosynthesis enzymes in the vascular system of snakeheads.

When compared with the dosage of PGs which can produce cardiovascular effects in mammals, snakeheads seem to require a much higher dose to produce the same effects.

Whereas a dose of PGE_1 (0.1 μg/kg) can induce vasodepressor effect in mammals, a minimal dose of 2 μg PGE_1/kg is required to produce the same effects in snakeheads. Moreover, a high dose of PGE_2 (about 9 μg/kg) is required to induce a measurable decrease in blood pressure of bullfrog (Leffler et al., 1980). It has been reported that the metabolic rate of poikilothermic animal increases as the ambient temperature increases (Bartholomew, 1977). Hence the mammals have a higher metabolic rate than in poikilothermic fish (acclimated at 20°C). Therefore, in snakehead, the higher doses of PGs required to elicit effects can be explained by a lower metabolic rate in this fish.

In addition to the low body temperature in snakeheads, a long biological half life of PGs in snakehead also indicate a low metabolic rate in this fish. The biological half life of prostaglandins in snakehead can be shown by the long duration of the vasodepressor or vasopressor actions. In dog, only 3 min was required to recover from the PGE_1 (4 μg/kg)-induced hypotensive effect (Nakano and McCurdy, 1967) whereas a similar fall in blood pressure in bullfrog required 7 min to recover (Leffler et al., 1980) Moreover, the present results showed that snakeheads required 20 min to recover from the effect. As well as PGE_1, PGA_1 and PGB_1 also produced long duration vasoactive effects in the fish. Therefore, this suggests a long

biological half life of PGs in snakehead. In mammals, 90% of PGE_1 was inactivated after one single passage through the pulmonary circulation (Ferreira and Vane, 1967). In contrast, the biological effects of infused PGA_1 and PGA_2 were not lost after one passage through the lungs of dog and cat (McGill et al., 1969; Horton and Jones, 1969), suggesting that PGAs may act as circulating hormones. However, the possible ways of inactivation of prostaglandins in fish have not been reported and cannot be demonstrated in the present experiment. Further studies should be required in order to decipher this problem. Moreover, the long biological half life may be due to the intrinsic property of snakehead and or low metabolic rate. Of course, the long duration effects can also be explained by a prolonged action of the activated PG receptors. Such a proposal of course requires further experimental evidence.

The PGE-induced decrease in blood pressure was accompanied by positive chronotropic and inotropic effects in dog (Nakano and McCurdy, 1967). However, the present results show the contrary, PGE_1 exerted no effect on the heart beat in fish. Moreover, the present experiment cannot demonstrate the possible effect of PGE_1 on cardiac contractility. Moreover, Peyraud-Waitzenegger et al., 1974; 1975) studied the effect of PGE_2 in carp. They showed that PGE_2 induced a prolonged depression of arterial pressure associated with an initial bradycardia followed by tachycardia. Efferent vagal blockade by atropine or bilateral

section of cardiac branches of vagus prevents the initial bradycardia and the correlative drop in arterial pressure. Nevertheless, the prolonged vasodepressor effect was not abolished. This suggests that the hypotensive effect caused by PGE_2 seems to result from 2 components : (1) an indirect action mediated by vagus (2) direct action on smooth vascular muscle.

Pentolinium tartrate has been reported to have the inhibitory effects on both sympathetic and parasympathetic ganglia (Meyer et al., 1972). Its mechanism of action is to prevent depolarization of the postsynaptic membrane of the postganglionic fiber. Therefore the fish treated with pentolinium tartrate exhibited a decline in ganglionic activation and hypotension. However, in snakeheads, ganglionic blockade (pentolinium tartrate) did not reduce the PGE_1-induced hypotension. This shows that PGE_1-induced vasodepressor effect is not mediated through the autonomic nervous system. From other reports (see also p. 56), we can conclude that vasodilation and the associated decrease in arterial blood pressure produced by PGEs was not mediated through cholinergic mechanism, release of histamine, stimulation of β-adrenergic receptors or through blockade of α-adrenergic receptors. Furthermore, pentolinium tartrate can partially abolish PGA_1 vasodepressor effect. This suggests ganglionic activation may be at least involved in the hypotensive action induced by PGA_1 in the snakeheads. Pentolinium tartrate also partially

abolished the PGB_1-induced hypertension while reserpine did not modify the vasopressor action of PGB_1. This indicates that PGB_1-induced vasopressor effect is independent of the cholinergeric elements but at least ganglionic components are involved.

From these results, it can be inferred that the vascular effects of PGA_1, PGE_1 and PGB_1 are mediated through different mechanisms in the snakehead. In order to clarify these mechanisms, detail studies are required.

There is considerable evidence to suggest that the diverse actions of prostaglandins are mediated by interaction with different receptor types. However, pharmacologists do not at present talk in terms of named receptors since the specific receptor antagonists, essential to this approach, are not as yet available. In addition, the sheep arterial system has been reported to contain a PGE_2-sensitive receptor mediating vasodilation (Jones, 1976). Fat cell and corpus luteum have been shown to have PGE-sensitive receptor (mediating antilipolytic action) (Kuehl et al., 1973) and $PGF_{2\alpha}$-sensitive binding receptor (Powell et al., 1974) respectively. These receptors have been found to have different relative dissociation constants for the binding of different prostaglandins (Jones, 1976). From the previous paragraphs, the findings of cardiovascular effects of PGA_1, PGE_1 and PGB_1 suggest that they seem to have prostaglandin receptors in the vascular system of this

fish. Evenmore, they each may have their independent receptors in snakehead.

In carp, hyperventilation caused by PGE_2 was observed (Peyraud-Waitzenegger et al., 1975). However no observable hyperventilation can be induced by PGE_1, PGA_1 and PGB_1 in snakeheads.

Besides the vasodepressor effect induced by PGE_1, a decrease in O_2 consumption rate after intravenous injection of PGE_1 was demonstrated. A statistical significant decrease in O_2 consumption rate was found only at about 10-15 min after injection of PGE_1. An alteration of oxygen consumption may result from change(s) in heart rate, stroke volume, gill vasculature and metabolic necessity (Bartholomew, 1977). In the present results, no PGE_1-induced change in heart rate was observed, hence this eliminates the possible contribution of heart rate in decrease in O_2 consumption. Since many reports have been published that PGE_1 can significantly increase stroke volume and cardiac output in dogs and rats (Weeks et al., 1969; Carlson et al., 1969; Maxwell, 1967), therefore, PGE_1-induced decrease in O_2 consumption rate is unlikely due to a decrease in stroke volume and cardiac output in snakehead. Moreover, PGE_1 has been reported to decrease peripheral vascular resistance in mammals (see also pp. 54-55), therefore a decrease in O_2 consumption may not presumably be caused by a decrease of gill vascular resistance in snakehead. The last factor

involved in a change in O_2 consumption rate is the metabolic requirement. Therefore, subsequent experiments were designed to investigate the possible involvement of prostaglandins on the metabolism of snakehead (for detail, see also pp. 129-195).

In the present experiments, the dose applied to snakeheads were very high, it ranged from 5 μg/kg to 60 μg/kg of prostaglandins. They are presumably within the pharmacological range rather than physiological doses. Such high doses were used because at least 2 μg/kg of prostaglandins is required to induce measurable cardiovascular effects in snakehead. This phenomenon may be due to the low basal blood pressure and low basal metabolic rate or can be an intrinsic property of the snakeheads. Although high doses of PGs are required to elicit blood pressure changes in the snakehead, the observation that indomethacin treatment (PG synthesis blocker) lowers basal blood pressure indicates an involvement of PGs in the control of normal vascular function.

(III) METABOLIC EFFECTS OF PROSTAGLANDINS

(1) Introduction

The actions of PGE_1 on glucose metabolism have been well decumented. In in vivo studies injection of PGE_1 produced hyperglycemia and decreased plasma FFA level in mammals (Berti et al., 1965; 1967; Sacca et al., 1973) and goose (Grande and Prigge, 1972). In experiments on incubation of liver slices with PGE_1, a decrease in glycogen level was observed (Boehle and May, 1967). Furthermore, Lemberg et al. (1971) reported that PGE_1 infusion induced a significant increment in the average glucose levels in the perfusate of the isolated perfused rat liver. Also two glucose metabolic key enzymes (glycogen synthetase and glycogen phosphorylase) activities in liver were altered by PGE_1 and increased glucose release (Curnow and Nuttall, 1972). On the contrary, Exton et al. (1971) and Levine (1974; 1979) showed that PGE_1 and PGE_2 perfusion in rat liver failed to alter cAMP and glucose production.

Besides the actions on glucose metabolism, PGs have also been shown to affect lipid metabolism. Steinberg et al. (1964) showed that PGE_1 reduced basal lipolysis in pieces of epididymal adipose tissue. Butcher et al. (1967, 1968) suggested that antilipolysis action of PGE_1 was associated with formation of cAMP which in turn activated intracellular

triglyceride lipase.

Although the metabolic effects of prostaglandins have been studied extensively, these experiments only focused on mammalian systems. The metabolic effects of prostaglandins in lower animals have never been published before. Therefore the present experiments attempted to investigate the metabolic effects of prostaglandins in snakeheads. Besides, a comparative study of the effects of PGA_1, PGB_1, PGE_1, $PGF_{1\alpha}$ and PGs precursor, arachidonic acid on metabolism were also carried out in the present experiments.

The effects of intraperitoneal injection of PGs and arachidonic acid on plasma metabolites : glucose, lipid, free fatty acid and protein (total, albumin and globulin) levels and liver glycogen and some liver enzymes were studied. Plasma Na^+, K^+, osmolality and hematocrit were also measured after PGs injection.

PGE_1 has been well known to have pronounced metabolic effects. Hence, an experiment involving intravenous injection of PGE_1 was undertaken and plasma metabolites were estimated. This provided more evidence on the metabolic effects of PGE_1.

Since the metabolic studies of exogenous prostaglandins have been established, a possible effect of endogenous prostaglandins in metabolism is indicated.

In order to elucidate the possibilities of endogenous PGs involvements in metabolic control, a PGs biosynthetic blocker-aspirin was employed in snakeheads. The plasma and liver metabolites were measured after aspirin treatment and PGE_1 injection in aspirin treated fish.

(2) *In vivo* studies

3. Materials and Methods

(a) Experimental Animals

Snakeheads were bought from a local supplier and transported to the laboratory without anaesthesia. Fish were kept in plastic tanks with fresh water (20-23°C). They were unfed for at least two weeks before experimentation. Fish with weight 100-140g were chosen for the present studies.

(b) Preparation of Prostaglandins Solution

PGA_1, PGB_1, PGE_1, $PGF_{1\alpha}$ were gifts from Dr. John E. Pike (Upjohn Company, Kamalazoo) and arachidonic acid was purchased from Sigma Company. Prostaglandins and arachidonic acid were dissolved in absolute ethanol (0.5 mg/ml) and the solutions were stored in refrigerator at 4°C. PG solutions were warmed up to room temperature before injection.

(c) Intraperitoneal Injection of Prostaglandins and Sampling techniques

Prostaglandin in ethanol solution was injected intraperitoneally into the fish and the dose used in this experiment was uniformly 0.75 mg/kg of body weight. A control experiment was undertaken by intraperitoneal injection of ethanol (1.5 ml/kg). The fish were killed at zero time, 30, 60, 120 and 180 min after injection. The caudal vertebral column was cut and blood was collected immediately from caudal artery by heparinized

microhematocrit tubes (Dade). Then the microhematocrit tubes were centrifuged and then hematocrit was measured. The plasma was separated from the packed blood cells and was collected in a plastic vial. The plasma was frozen for later determination of plasma osomlality and other parameters.

The whole liver was amputated and wrapped with parafilm and then was stored in the freezer for later analysis.

(d) Intravenous Injection of Prostaglandin E_1 and Sampling techniques

In some experiments, the pneumogastric artery and gonadal vein were cannulated (methods as previous described, pp. 103-105). After surgery, fish were allowed to recover in a respiratory chamber for 2 hours before injection. PGE_1 (30 µg/kg) was prepared as previous described (p. 105) and was injected intravenously. Then the blood samples were collected through the arterial cannula with heparinized microhematocrit tubes at 15, 30, 60, 120 and 180 min after injection. Before PGE_1 injection, an appropriate volume of ethanol-saline mixture was injected as controls. Then two blood samples were collected at 30 and 60 min after vehicle injection. Hematocrit value, plasma glucose and protein were determined as previously described.

(e) Aspirin Treatment

In some experiments, aspirin (acetylsalicyclic acid, Sigma) was used to block the biosynthesis of prostaglandin in snakeheads. The fish were kept in aspirin solution (1g/25l freshwater) for 24 hours before receiving PGE_1 (0.75 mg/kg). All aspirin-treated fish were sacrificed 2 hours after injection. The plasma and liver were sampled as previously described.

(f) Plasma Composition Analysis

(i) Plasma osmolality

Plasma osmolality expressed in mOsm/kg, was measured in 50 μg plasma samples by determining the freezing point depression in a Knauer Osmometer.

(ii) Plasma glucose

Plasma glucose was determined by coupled glucose oxidase-peroxidase reaction (Sigma Technical Bulletin No. 510). As the plasma contains considerable amounts of protein which will interfere with the reaction, the plasma was first deproteinized by adding 0.3N $Ba(OH)_2$ & 0.3N $ZnSO_4 \cdot 7H_2O$.

(iii) Plasma protein (Total, albumin and globulin)

The total plasma protein was estimated by the modified Lowry et al. method according to Hartree(1972). The reagents used were as following :

a. Standard solution : Bovine Serum Albumin (2mg/ml)

b. Solution A : 2g potassium sodium tartrate and 100g Na_2CO_3 were dissolved in 500ml 1N NaOH and diluted with distilled water to 1 litre

c. Solution B : 2g potassium sodium tartrate and 1g $CuSO_4 \cdot 5H_2O$ are dissolved in 90ml distilled water and 19ml 1N NaOH was added

d. Solution C : 1 volume Folin-Ciocalteu reagent (Merck) was diluted with 15 volume water. This solution must be freshly prepared.

In this test, a micromodification was employed. 5µl plasma was diluted with 1ml of distilled H_2O. Then 0.9ml of solution A was added. The content was heated in water both at 50°C for 10 min. Then it was cooled to room temperature. 0.1ml of solution B was added and standed for at least 10 min. 3ml of solution C was injected into the sample and was immediately mixed throughly. The content was then placed in 50°C water for 10 min. After cooling to room temperature, the optical density was read at 650 nm.

Plasma albumin and globulin levels were measured according to the Sigma Technical Bulletins No, 630 and 560 respectively.

(iv) Plasma α-amino acids (FAA)

The concentration of plasma free α-amino acids was determined by the method of Mathews et al. (1964) with micromodification. In the present study, all reagents

and samples were scaled down by a factor of 3. The optical density was read at 570 nm.

(v) Plasma total lipid

Method of Woodman and Price (1972) was employed for the determination of plasma total lipid. 190 µl of distilled water was added to 10 µl plasma sample. Then, 1ml concentrated H_2SO_4 was added into this diluant. Then the content was boiled in water both for 20 min. After cooling, 2ml color reagent (6gm anhydrous KH_2PO_4+ 0.3g vanillin +100ml H_2O; freshly prepared). The content was read exactly after 10 min at 530 nm against reagent blank. The standard was prepared by extraction of snakehead plasma lipid using petroleum spirit.

(vi) Plasma free fatty acids (FFA)

Plasma free fatty acids was determined by method of Pinelli (1973). In this test, a micromodification was used. The volumes of sample and reagents were scaled down to a factor of 5. Zinc dibenzyldithiocarbamate was used as color reagent and was read at 440 nm.

(vii) Plasma K^+, Na^+, Mg^{++} and Ca^{++} levels

Plasma K^+ and Na^+ levels were determined by Flame Photometer (Evans Electroselenium Ltd.) with appropriate dilution. Since plasma contained a high concentration of Na^+, appropriate amount of NaCl was added to the potassium ion standard solution. Mg^{++} and Ca^{++} concentration were measured by atomic absorption (Shandon Southern A 3600) with appropriate dilution.

Lanthanum chloride (finial concentration 0.5%) was added to the sample for Ca^{++} and Mg^{++} mesaurements.

(viii) Plasma Cl^-

Plasma Cl^- level was determined by a modification of the method of Tietz (1973). 25 µl of plasma was mixed with 1ml distilled water, 25 µl 0.72N H_2SO_4 and 25 µl 10% sodium tungstate. After centrifugation, 100 µl of clear supernatant was added to 0.1ml diphenylcarbarbazone and titrated with 0.01N mercuric nitrate (faint blue-violet color at end point).

(g) Liver Composition Analysis

Liver was homogenized in distilled water by glass homogenizer with 20 folds dilution. The sample was stored in freezer for later analysis.

(i) Liver glycogen

The liver glycogen content was determined by method of Murat and Serfaty (1974). The liver glycogen was hydrolized by amyloglucosidase to glucose. Then glucose was estimated by coupled glucose oxidase-peroxidase reaction (Sigma Technical Bulletin No,510) (see also plasma glucose determination p. 134). The liver glycogen content was expressed in mg of glucose/ g of wet tissue.

(ii) Liver soluble protein

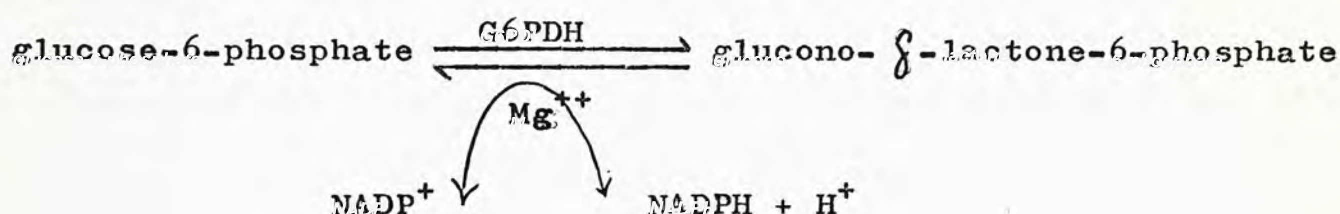
An appropriate volume (50-100 µl) of liver homogenate as previous prepared was taken out for determination of liver soluble protein. The

homogenate was centrifuged and then the protein content of supernatant was estimated by the modified Lowry method (Hartree, 1972) as described previously in plasma protein determination.

(iii) Liver enzymes activities

a. Glucose-6-phosphate dehydrogenase (G6PDH)

G6PDH catalyzes the following reaction:



Liver homogenates diluted 20 fold were centrifuged at 5000g for 5 min. A series of reagents was prepared.

Tris buffer : 50 mM (6 mg/ml) (6 mg/ml) EDTA 5 mM (2 mg/ml)pH was adjusted to 7.6 with 5N NaOH

Glucose-6-phosphate (Monosodium salt) : 30 mM (8,463 mg/ml)

NADP : 10 mM (8 mg/ml)

The reagent was pre-incubated at 20°C for about 10 min. A standard 1 cm curette was used. 2 ml buffer, 0.1 ml NADP and 0.1 ml of supernatant were added into the curette. After thoroughly mixing the reaction was started by adding 50 µl of G-6-P. The curette was pushed immediately into the spectrophotometer (Spectronic 70). The increase in optical density at 366 nm over a period of 10 min was noted. Throughout

the test, temperature was kept at 20±0.5°C. Enzyme activity was calculated using the following relationship and was expressed as µ mole NADPH produced/g of soluble protein^{-1} min^{-1}.

$$\Delta \text{O.D.} \times 0.295 = \Delta \mu \text{ mole ml}^{-1}$$

b. Glucose-6-phosphatase (G6 Pase, E.C.3.1.3.9)

The activity of G6 Pase of the liver homogenate (20 fold) was assayed by a modification of the method of Harper (1963). The reagents used were as following :

Cacodylate buffer (0.1 M, pH 6.5)

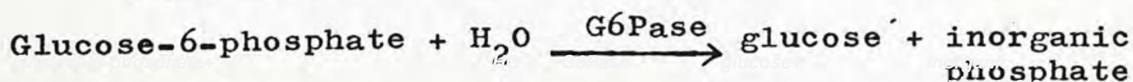
2.141 g sodium cacodylate·$3H_2O$ dissolved in 50 ml distilled water and pH was adjusted to 6.5 with 5N HCl, the buffer was diluted to 100 ml with distilled water.

Glucose-6-phosphate (0.08 M)

This reagent should be freshly prepared.

The G-6-Pase assay temperature was maintained at 20°C. 0.1 ml of the cacodylate buffer and 0.1 ml of 0.08M glucose-6-phosphate were mixed together in test tube. The reaction was started by adding 0.1 ml liver homogenate and allowed to proceed for 30 min. The reaction was terminated by the addition of 2 ml 10% trichloroacetic acid.

Since G6Pase catalyze the following reaction :



$$\text{Glucose-6-phosphate} + H_2O \xrightarrow{\text{G6Pase}} \text{glucose} + \text{inorganic phosphate}$$

The activity of this enzyme was determined by the amount of inorganic phosphate formation. Inorganic phosphate was assayed by the procedure of Fiske and Subbarow (1925).

Tissue controls as well as reagent controls for G6Pase assay was also carried out. The enzyme activity was expressed as μ mole phosphate released mg soluble protein^{-1} min^{-1}.

(h) Tissue water

Pieces of muscle (1-2g) and gill filaments (0.2-0.5g) were put in preweighted tubes and weighed. After drying in oven at 80°-100°C for 48 hours, the samples were cooled in desiccator. Then, the samples were reweighed and the percentage of tissue water was calculated by weight difference.

(i) Statistical analysis

All data were expressed as means ± standard error of the mean (M ±S.E.M.). Student's t test was performed to assess the differences between groups.

2. Results

(a) Plasma hematocrit and osmolality

Intraperitoneal injection of arachidonic acid (0.75 mg/kg) significantly decreased plasma osmolality (Fig. 17) 2 and 3 hours after injection in snakehead. $PGF_{1\alpha}$ also lowered plasma osmolality 3 hours after intraperitoneal injection whereas no effect was observed after PGA_1, PGB_1 and PGE_1 injection when compared with their corresponding controls (Fig. 17).

Plasma hematocrit value was lowered 1 hour after intraperitoneal injection of PGE_1 (Fig. 18). However, intravenous injection of PGE_1 could not alter plasma hematocrit value. Intraperitoneal injection of $PGF_{1\alpha}$ significantly increased hematocrit value from 40.6% to 45% (Fig. 18).

Moreover, PGE_1 and aspirin treatment did not produce any effect on hematocrit values (Fig. 19). Although PGE_1 injection did not affect the plasma osmolality, aspirin and aspirin+ PGE_1 treatment significantly fell plasma osmolality in this fish.

(b) Carbohydrate metabolism

Intact Fish + Intraperitoneal Injection of PGs

Intraperitoneal ethanol injection increased plasma glucose level in snakeheads. However, only the increase in plasma glucose level at 2 hours after injection was

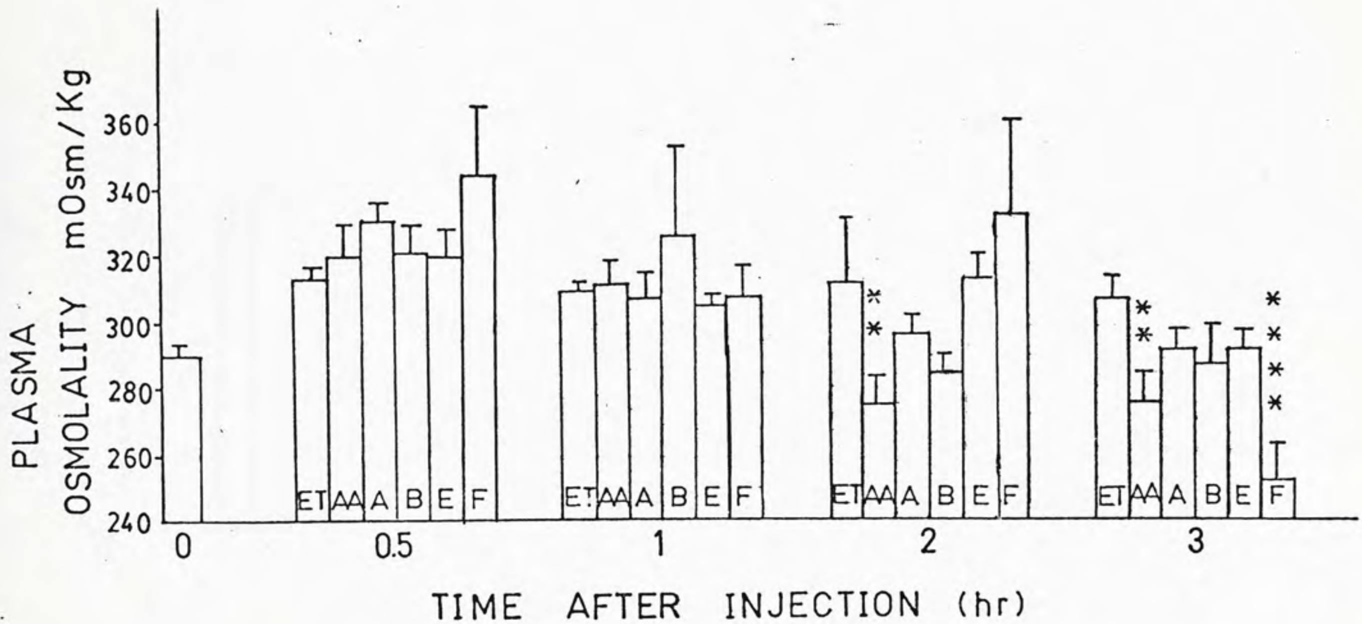


Fig. 17 Effect of intraperitoneal injection of PGs, arachidonic acid (AA) and ethanol (ET) in plasma osmolality of 6 snakeheads. Data were presented in the Mean and SEM. A=PGA_1, B=PGB_1, E=PGE_1 and F=$PGF_{1\alpha}$. ** $p < 0.02$ and **** $P < 0.001$ when compared with controls.

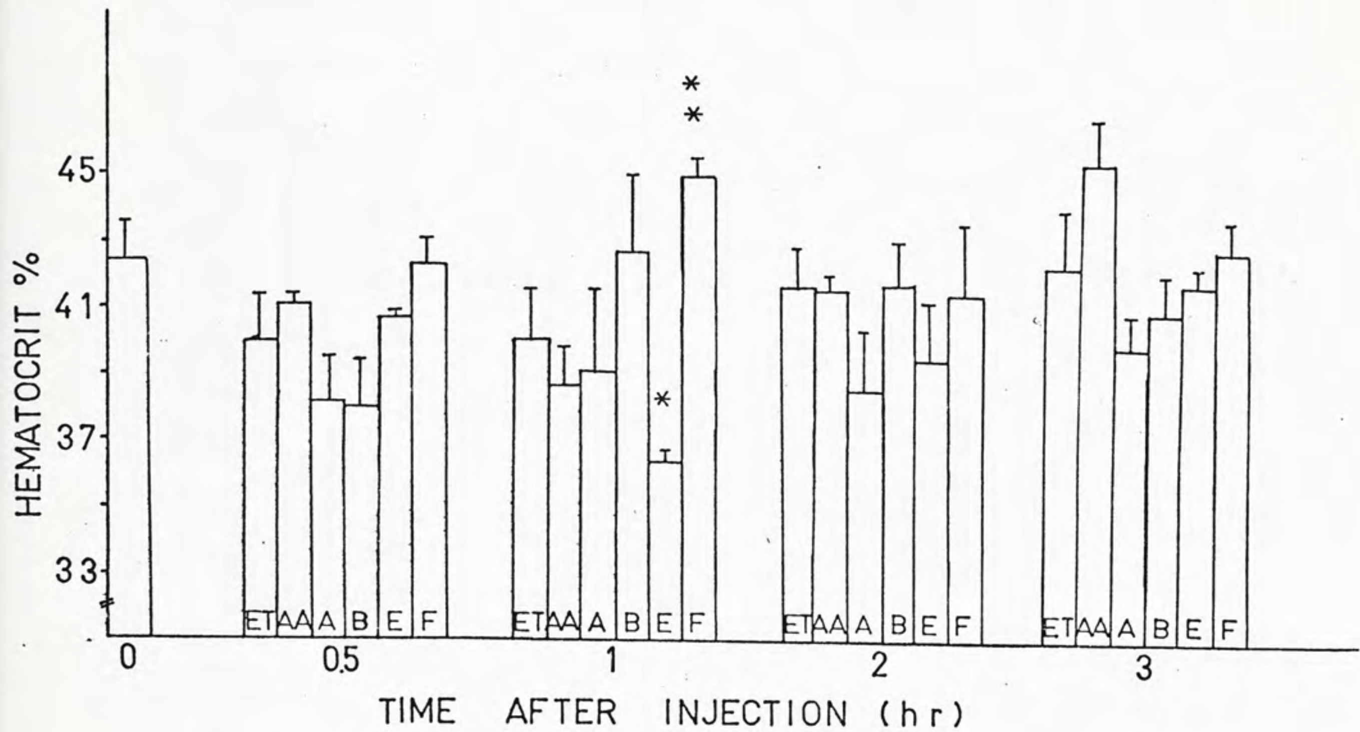


Fig. 18 Effect of intraperitoneal injection of PGs, arachdonic acid (AA) and ethanol (ET) in hematocrit of six snakeheads. A=PGA_1, B=PGB_1, E=PGE_1 and F=$PGF_{1\alpha}$ Results were presented in the Mean and SEM. * $P < 0.05$ and ** $P < 0.02$ when compared with controls.

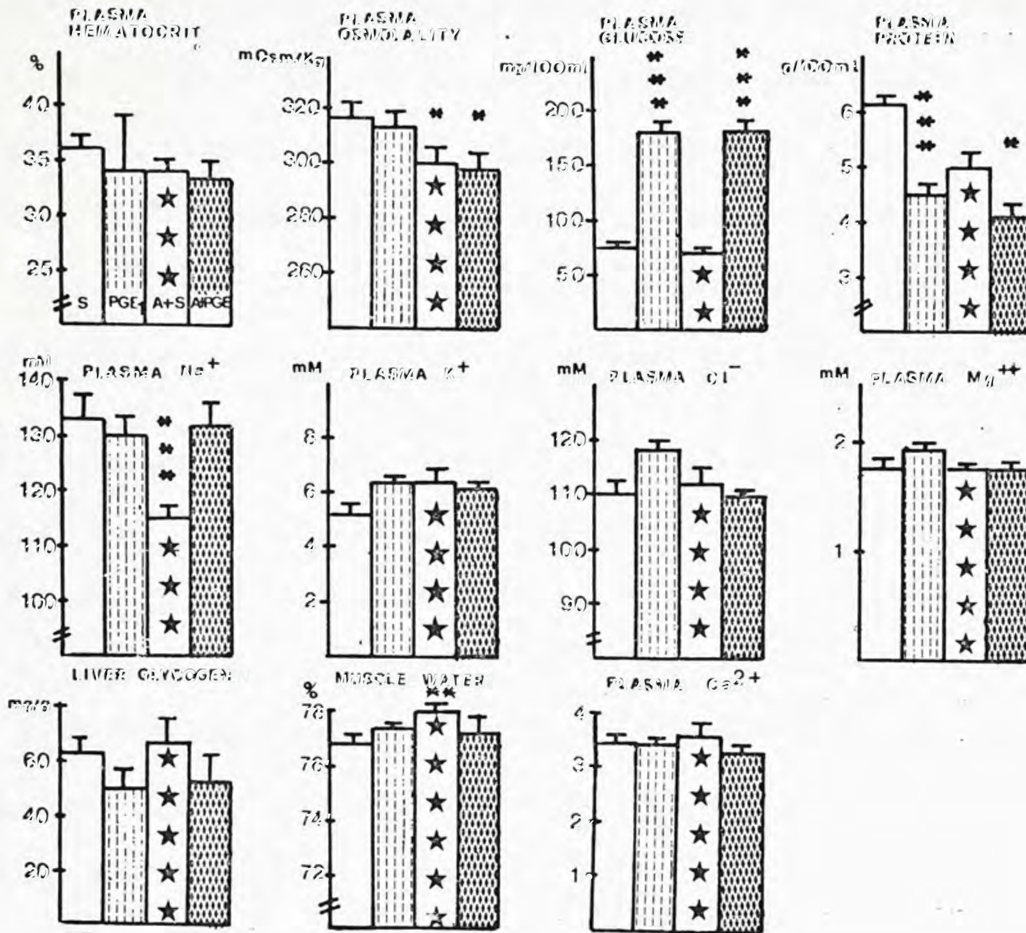


Fig. 19 Changes in plasma parameters, liver glycogen, and muscle water in intact and aspirin treated fish (6) after injection of saline and PGE_1. S=saline, A+S=saline injected into aspirin treated fish and A+PGE=PGE_1 injected into aspirin treated fish. Data were expressed in the Mean and vertical bar denotes SEM. $* P < 0.05$, $** P < 0.02$, $*** P < 0.01$ and $**** P < 0.001$ when compared with controls.

statistically significant when compared with zero time controls (Fig. 20). Arachidonic acid and PGA_1 increased plasma glucose 30 min after injection and PGB_1 significantly increased plasma glucose 1 and 2 hours after injection. Whereas PGE_1 induced hyperglycemia 1 hours after injection, the hyperglycemia elicited by $PGF_{1\alpha}$ only occurred 3 hours after injection (Fig. 20).

When the fish were injected with arachidonic acid, the liver glycogen content were depleted 1, 2 and 3 hours after injection (Fig. 21). Other PGs such as PGA_1, PGB_1, PGE_1 and $PGF_{1\alpha}$ also decreased glycogen level 1 hour after injection, however these decreases were not statistically significant. Also, PGE_1 injection slightly decreased glycogen content in normal and aspirin-treated fish. However, these decreases were not significant (Fig. 19).

Liver Glucose-6-phosphatase (G6Pase) activity varied greatly among the controls (Fig. 22). At 0.5 hour after injection, $PGF_{1\alpha}$, significantly increased liver G6Pase activity in snakeheads whereas PGB_1 intraperitoneal injection significantly decreased this enzyme activity. Moreover, no effect on liver G6Pase activity was observed with PGA_1, PGE_1 and arachidonic acid injections.

As well as G6Pase activity, liver Glucose-6-phosphate

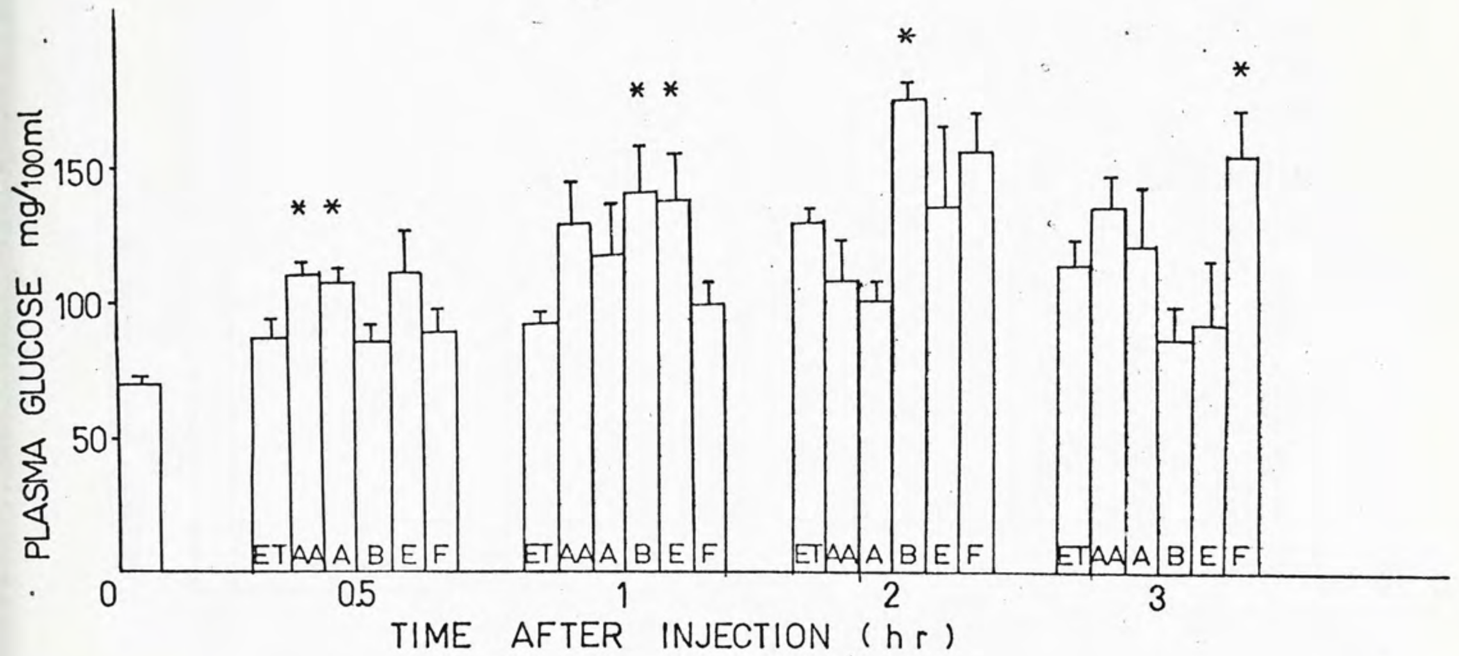


Fig. 20 Effect of intraperitoneal injection of ethanol (ET), arachidonic acid (AA), PGA_1 (A), PGB_1 (B), PGE_1 (E) and $PGF_{1\alpha}$ (F) in plasma glucose of six snakeheads. Vertical bar denotes SEM. * $P < 0.05$ when compared with controls.

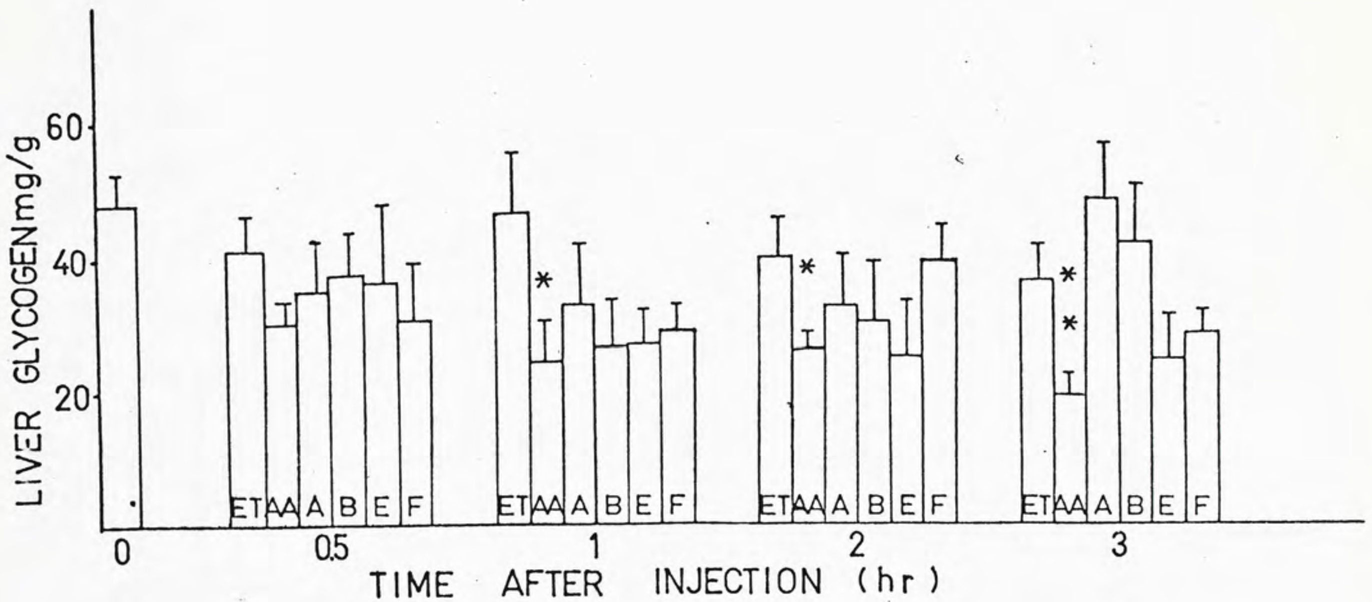


Fig. 21 Changes in liver glycogen content of six snakeheads after intraperitoneal injection of ethanol (ET), arachidonic acid (AA), PGA_1 (A), PGB_1 (B), PGE_1 (E) and $PGF_{1\alpha}$ (F). Vertical bar denotes SEM. * $P < 0.05$ and ** $P < 0.02$ when compared with ethanol controls

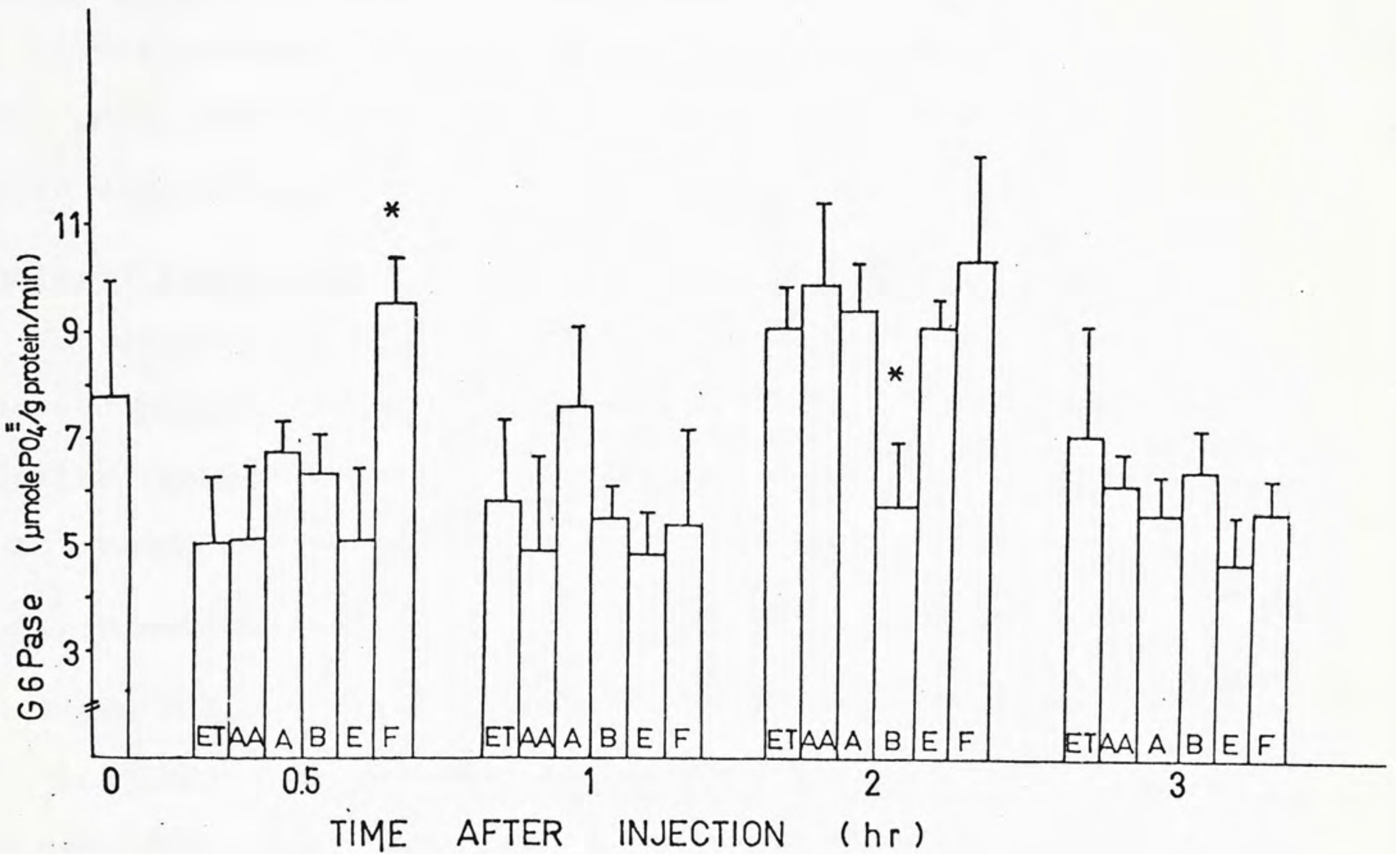


Fig. 22 Changes in liver glucose-6-phosphatase (G6Pase) activity of six snakeheads after injection of ethanol (ET), arachidonic acid (AA), PGA_1 (A), PGB_1 (B), PGE_1 (E) and $PGF_{1\alpha}$ (F). Vertical bar denotes SEM. * $P < 0.05$ when compared with controls.

dehydrogenase (G6PDH) activity also varied among the control fish (Fig. 23). No effect on this enzyme was observed after the injection of prostaglandins except $PGF_{1\alpha}$ significantly increased liver G6PDH activity 2 hours after injection.

Aspirin-treated Fish + Intraperitoneal Injection of PGE_1

Plasma glucose level was greatly elevated by PGE_1 (0.75 mg/kg) intraperitoneal injection in snakeheads with or without aspirin treatment (Fig. 19).

Cannulated Fish + Intravenous Injection of PGE_1

Intravenous injection of PGE_1 (30µg/kg) significantly increased plasma glucose concentration 30 and 60 min after injection whereas injection of ethanol saline (1:4 by vol.) did not alter plasma glucose level in snakehead (Fig. 24).

(c) Lipid metabolism

1 and 2 hours after intraperitoneal injection of PGE_1, a significant decrease in plasma lipid level was observed (Fig. 25). This result was repeatable in a separate experiment involving intravenous injection of PGE_1 into the gonadal vein via an indwelling cannula. However, there were no change in plasma lipid concentration after injection of PGA_1, PGB_1, $PGF_{1\alpha}$ and arachidonic acid in snakeheads. Also ethanol injection did not change plasma lipid level.

In aspirin treated fish, a decrease in plasma

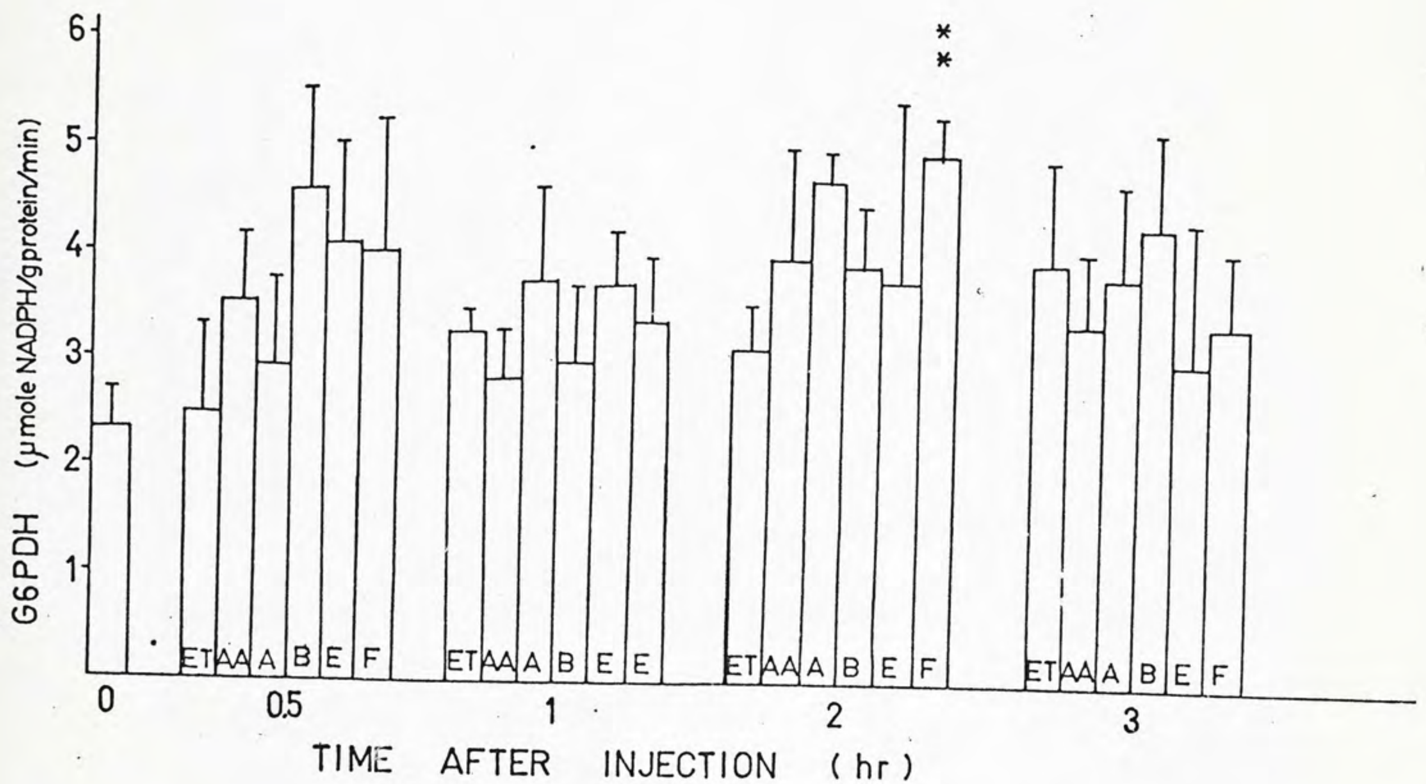


Fig. 23 Effect of intraperitoneal injection of ethanol (ET), arachidonic acid (AA), PGA_1 (A), PGB_1 (B), PGE_1 (E) and $PGF_{1\alpha}$ (F) in the liver glucose-6-phosphate dehydrogenase (G6PDH) activity of six snakeheads. Data were expressed in the Mean and SEM. ** $P < 0.02$ when compared with controls.

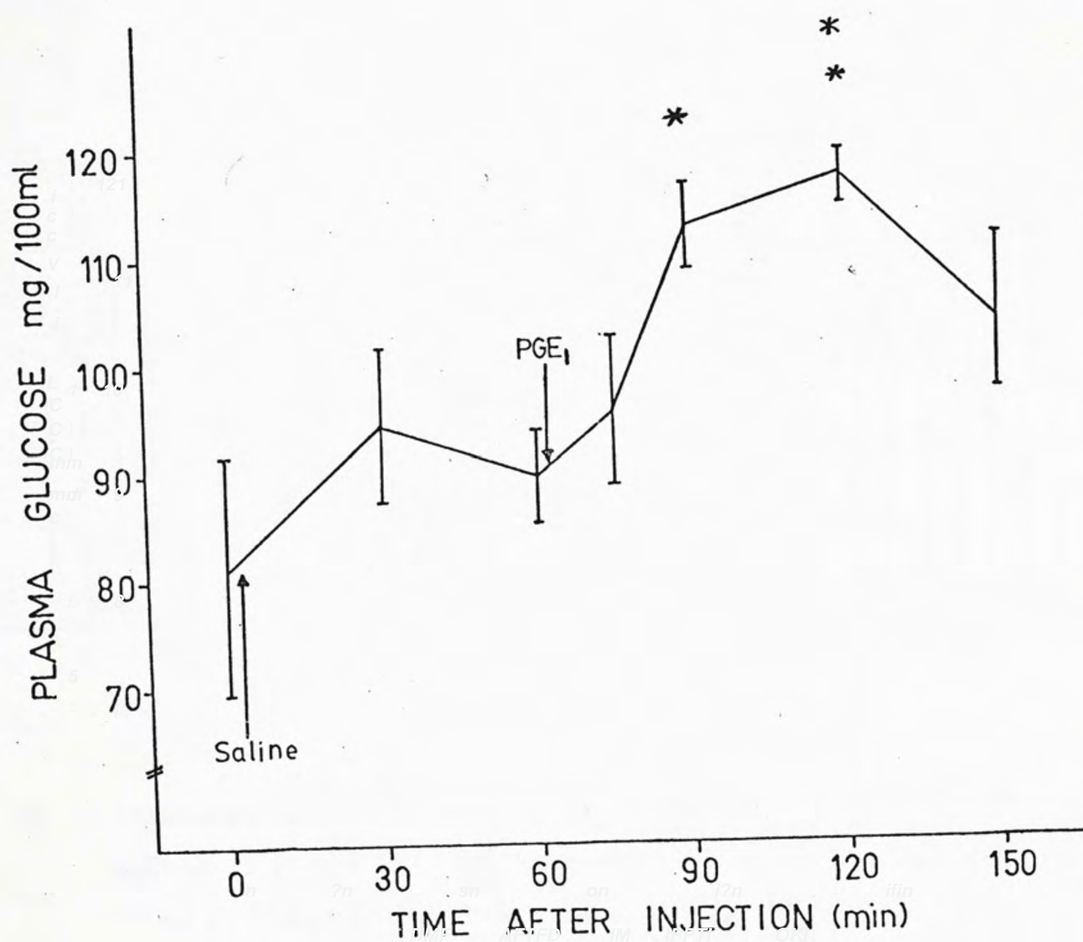


Fig. 24 Effect of intravenous injection of PGE_1 (30 µg/kg) in plasma glucose of five snakeheads. Data were presented in Mean ± SEM. * $P < 0.05$ and ** $P < 0.02$ when compared with controls.

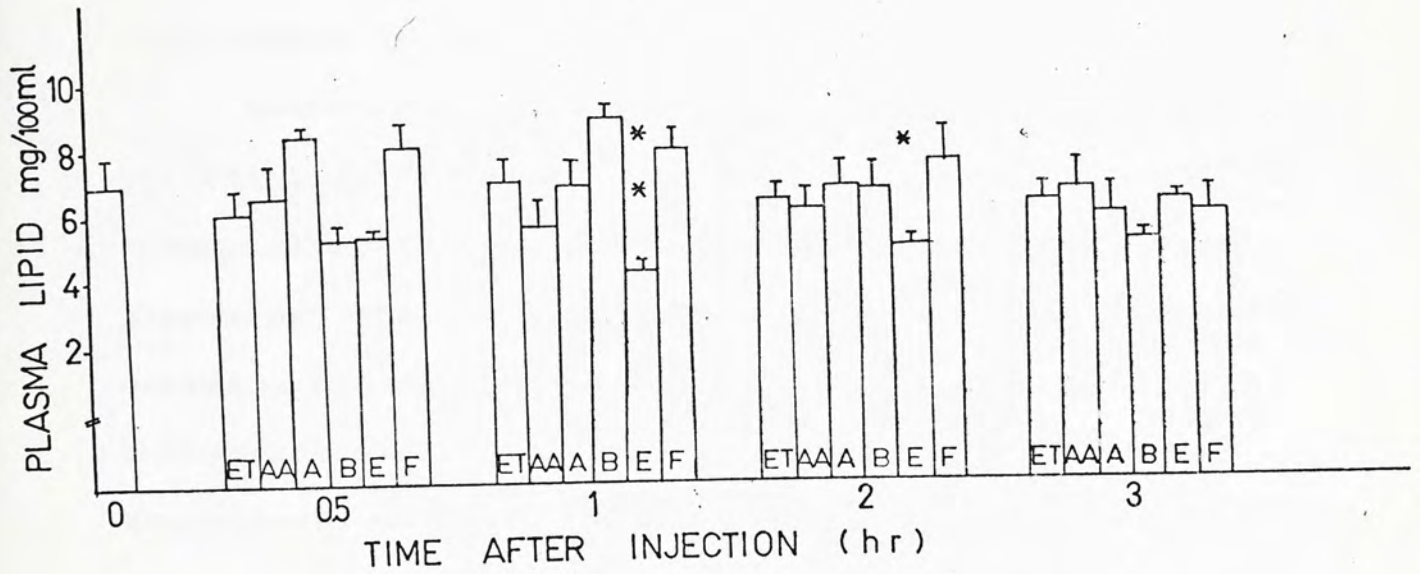


Fig. 25 Changes in plasma lipid level of six snakeheads after injection of ethanol (ET), arachidonic acid (AA), PGA_1 (A), PGB_1 (B), PGE_1 (E) and $PGF_{1\alpha}$ (F). Data were expressed in the Mean and SEM. * $P < 0.05$ and ** $P < 0.02$ when compared with controls.

lipid level was also observed (Fig. 26). However, injection of PGE_1 in the aspirin treated fish could not produce observable decrease in plasma lipid level when compared with aspirin treated controls.

Also plasma free fatty acid level was lowered 2 hours after PGE_1 injection while other PGs and arachidonic acid did not alter plasma free fatty acid concentration in snakeheads (Fig. 27).

(d) Plasma protein

Intraperitoneal injection of PGE_1 lowered plasma protein level 1 and 2 hours after injection (Fig. 28). 1 hour after intravenous injection of PGE_1 also decrease plasma protein level significantly (Fig. 29). $PGF_{1\alpha}$ also decrease plasma protein level 2 and 3 hours after intraperitoneal injection. However, PGA_1, PGB_1 and arachidonic acid and ethanol could not alter plasma protein at any time interval (Fig. 28).

Plasma albumin level significantly fell in snakeheads 3 hours after $PGF_{1\alpha}$ intraperitoneal injection (Fig. 30). 3 hours after intraperitoneal injection of PGE_1 and PGB_1 there was a drop in plasma albumin concentration. A fall in plasma albumin level was also observed in snakeheads 0.5 and 2 hours after PGA_1 injection and arachidonic acid also decreased albumin level 1 hour after injection.

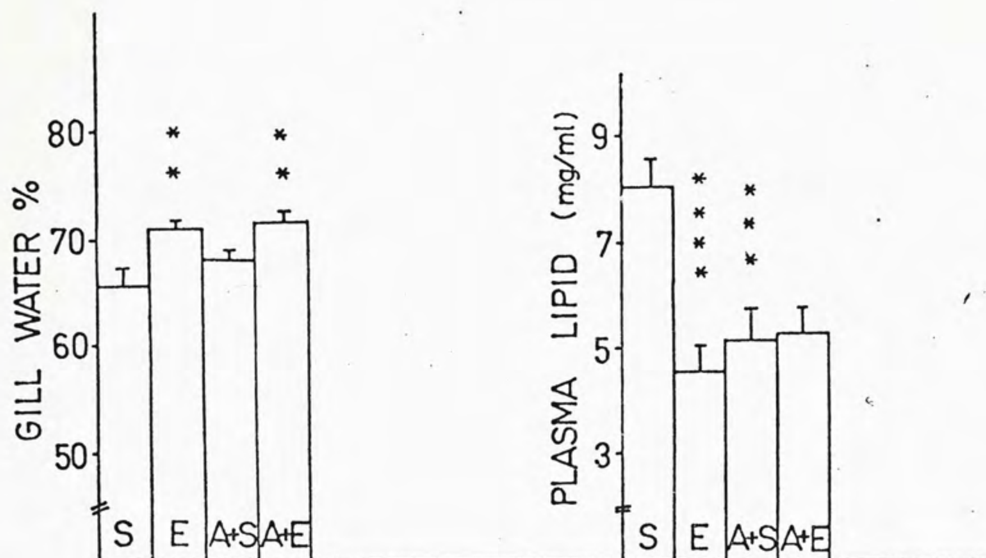


Fig. 26 Effect of intraperitoneal injection of PGE_1 in gill water and plasma lipid level of intact and aspirin treated fish. S=saline, E=PGE_1, A+S=saline injected into aspirin treated fish, A+E=PGE_1 injected into aspirin treated fish. Data (6 observation) were expressed in the Mean and SEM. ** $P < 0.02$, *** $P < 0.01$ **** $P < 0.001$ when compared with controls.

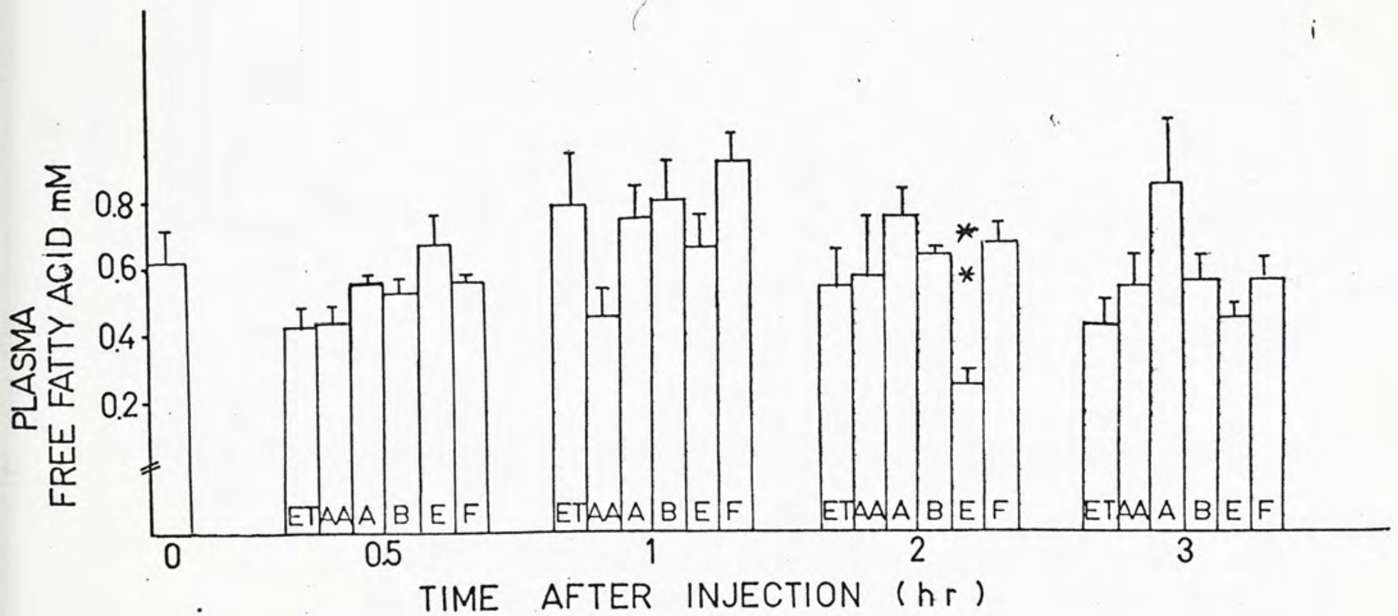


Fig. 27 Changes in plasma free fatty acid level after intraperitoneal injection of ethanol (ET), arachidonic acid (AA), PGA_1 (A), PGB_1 (B), PGE_1 (E) and $PGF_{1\alpha}$ (F). in six snakeheads. Data were expressed in the Mean and SEM. ** $P < 0.02$ when compared with controls.

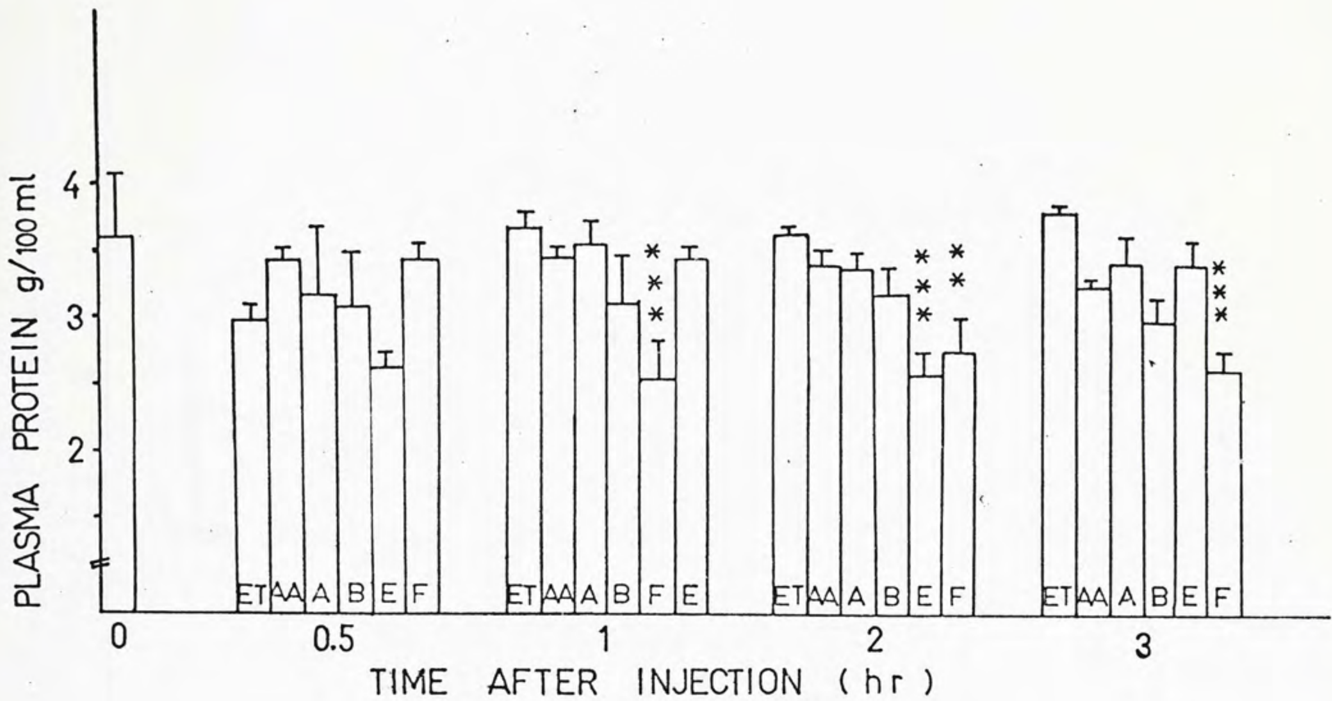


Fig. 28 Changes in plasma protein level of six snakeheads after injection of ethanol (ET), arachdonic acid (AA), PGA_1 (A), PGB_1 (B), PGE_1 (E) and $PGF_{1\alpha}$ (F). Data were expressed in the Mean and SEM. ** $P < 0.02$ and *** $P < 0.01$ when compared with corresponding controls.

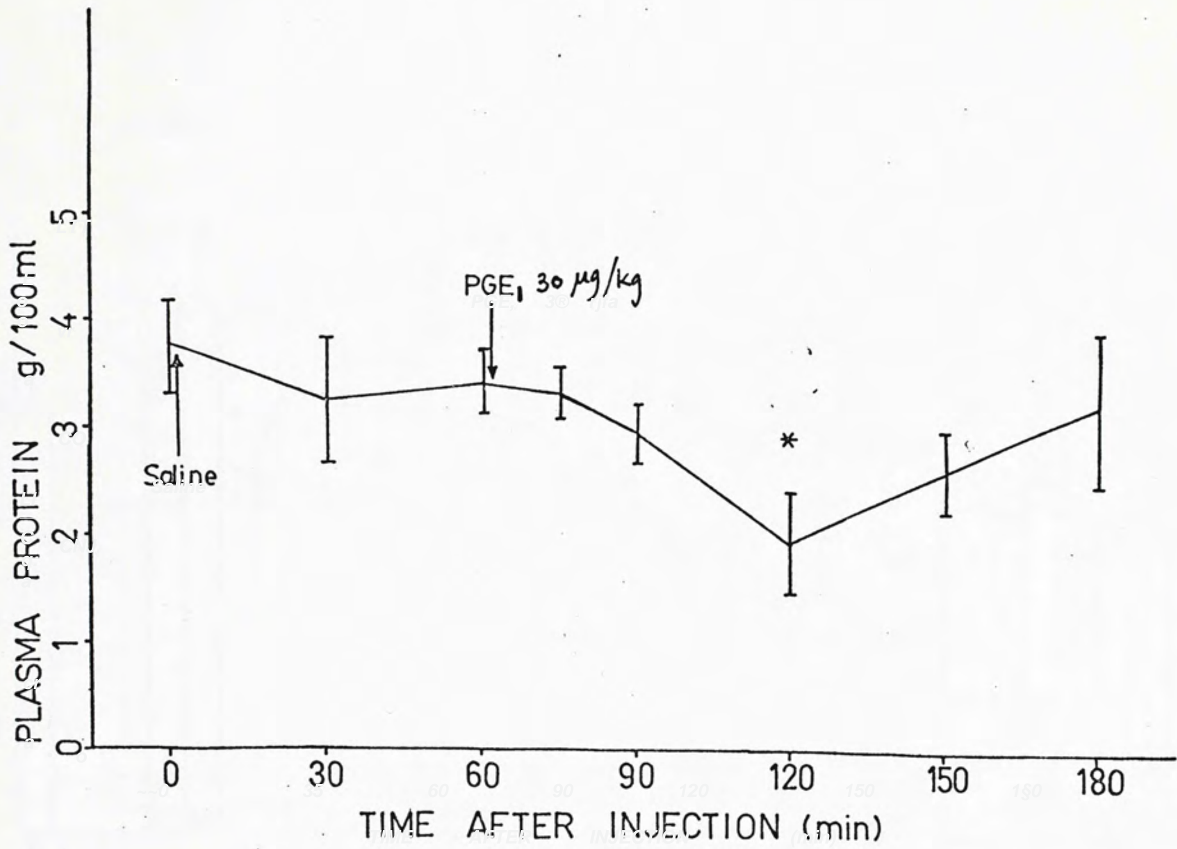


Fig. 29 **Effect of intravenous injection of PGE_1 in plasma protein level. Data were expressed in Mean ± SEM. *P < 0.05 when compared with controls.**

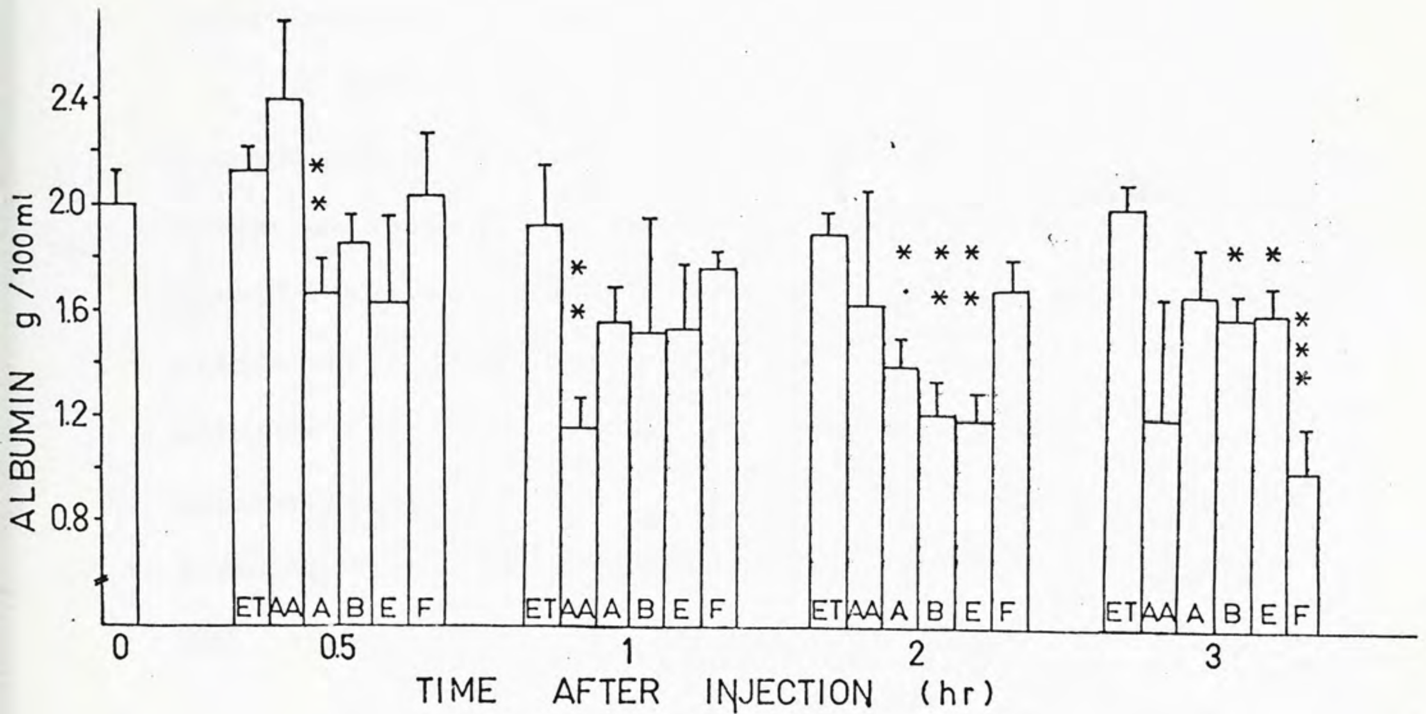


Fig. 30 Changes in plasma albumin level of six snakeheads after injection of ethanol (ET), arachidonic acid (AA), PGA_1 (A), PGB_1 (B), PGE_1 (E) and $PGF_{1\alpha}$ (F). Data were presented in the Mean and SEM. * $P < 0.05$, ** $P < 0.02$ and *** $P < 0.01$ when compared with corresponding controls.

A drop in plasma globulin was observed 3 hours after $PGF_{1\alpha}$ injection while there was no effect of arachidonic acid and other PGs injection (Fig. 31).

In aspirin experiment, a decrease in plasma protein was found in PGE_1 injected intact and aspirin treated fish (Fig. 19, p. 144).

(e) Plasma electrolytes

A decline in plasma Na^+ was observed in snakeheads 1 hour after intraperitoneal injection of $PGF_{1\alpha}$. 2 hours after arachidonic acid injection, plasma Na^+ level was lowered whereas PGA_1, PGE_1 and PGB_1 could not modify this electrolyte level in snakeheads (Fig. 32). However, aspirin treatment elicited a drop in plasma Na^+ concentration and PGE_1 injection in normal and aspirin treated fish did not alter plasma Na^+ level (Fig. 19, p. 144).

$PGF_{1\alpha}$ decreased plasma K^+ level 0.5 and 3 hours after intraperitoneal injection and PGE_1 also produced a decrease in K^+ level 1 hour after injection (Fig. 33). Aspirin treatment exhibited no effect on plasma K^+ in snakeheads. 2 hours after injection of PGE_1 in normal and aspirin treated fish, plasma K^+ level was unchanged. Plasma Cl^- was slightly but not significantly elevated by PGE_1 and aspirin treatment abolished this increase. Moreover, Mg^{++} and Ca^{++} levels were not altered by aspirin

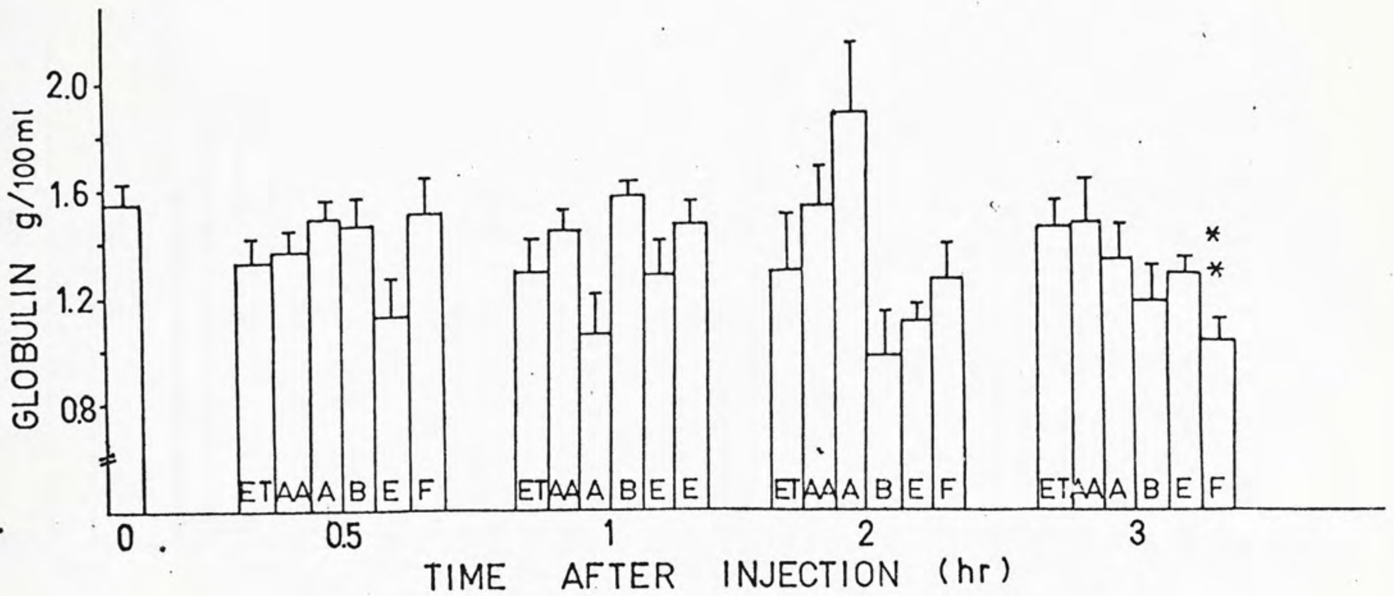


Fig. 31 Changes in plasma globulin level of six snakeheads after injection of ethanol (ET), arachidonic acid (AA), PGA_1 (A) PGB_1 (B), PGE_1 (E) and $PGF_{1\alpha}$ (F). Data were presented in the Mean and SEM. $**P < 0.02$ when compared with controls.

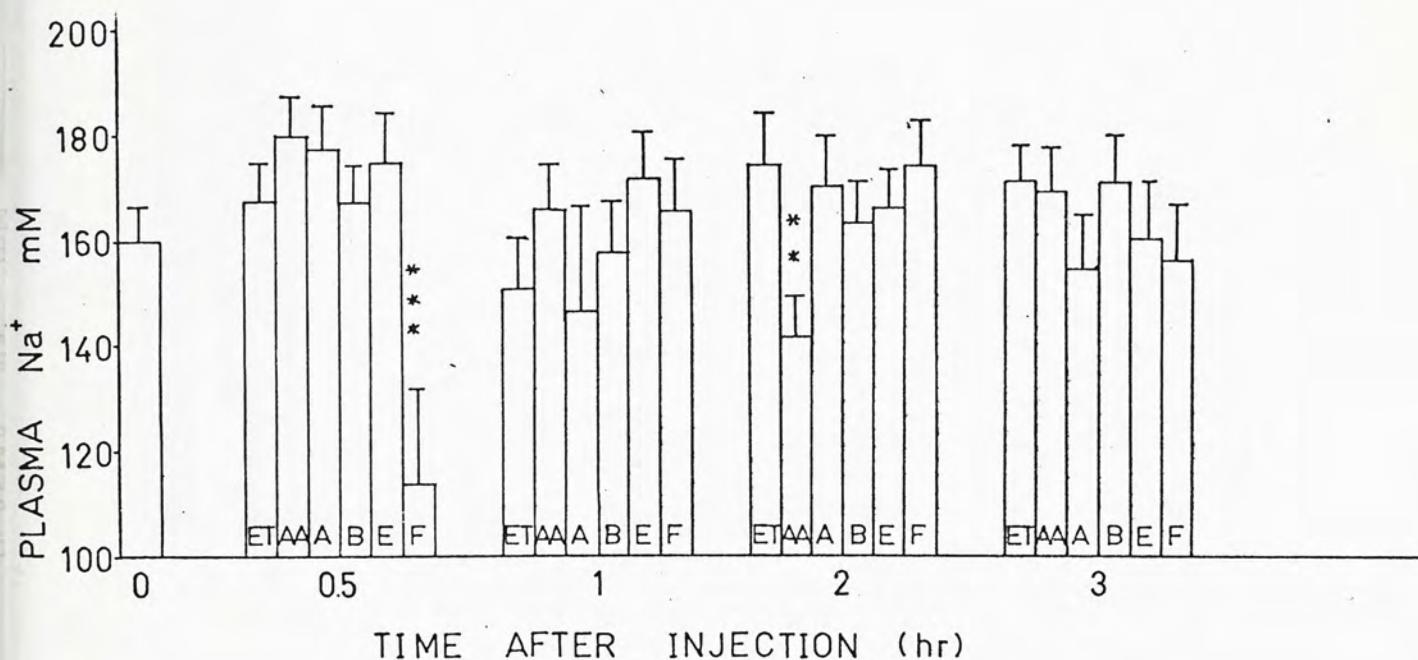


Fig. 32 Changes in plasma Na^+ level after injection of ethanol (ET), arachidonic acid (AA), PGA_1 (A), PGB_1 (B), PGE_1 (E) and $PGF_{1\alpha}$ (F) in six snakeheads. Data were expressed in the Mean and SEM. ** $P < 0.02$ and *** $P < 0.01$ when compared with controls.

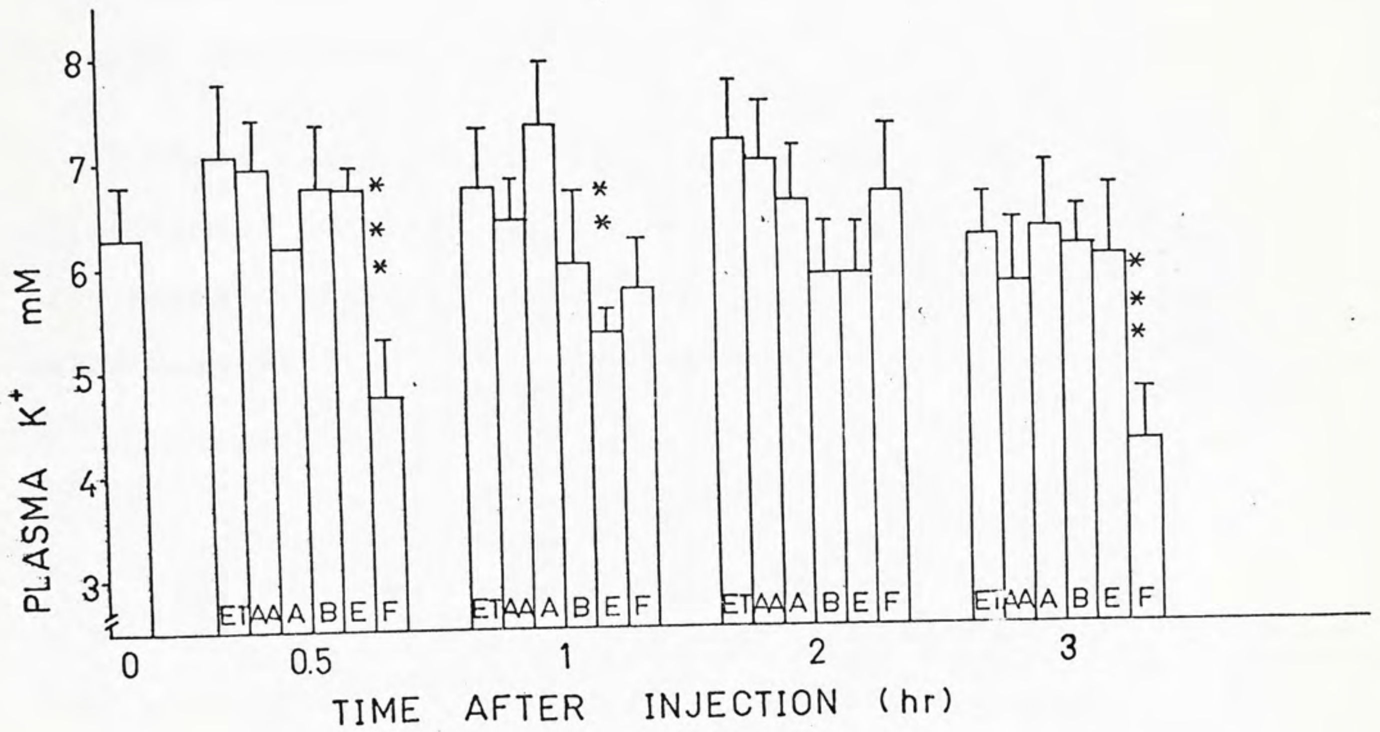


Fig. 33 Changes in plasma K^+ level after injection of ethanol (ET), arachidonic acid (AA), PGA_1 (A), PGB_1 (B), PGE_1 (E), $PGF_{1\alpha}$ (F) in six snakeheads. Data were expressed in the Mean and SEM. ** $P<0.02$ and *** $P<0.01$ when compared with controls.

treatment, PGE_1 injection in aspirin treated fish and single PGE_1 injection (Fig. 19, p. 144).

(f) Tissue water

Gill water content was elevated by intraperitoneal injection of PGE_1 in normal and aspirin treated fish (Fig. 26). However, aspirin treatment produced no effect on gill water content.

There was no change in muscle water content after injection of PGE_1 in normal and aspirin treated fish. Also aspirin treatment could not induce change in muscle water content (Fig. 19, p. 144).

(3) Discussion

(a) Carbohydrate metabolism

In the present experiments, intraperitoneal injection of ethanol (1.5 ml/kg) significantly increased plasma glucose level in snakehead 2 hours after administration (Fig. 20, pp. 146). Hellstedt et al. (1980) reported that intraperitoneal injection of ethanol in rat also increased plasma glucose level and the blood ethanol concentration fell to zero before 180 minutes. Moreover, chronic ethanol (5 weeks; liquid diet) consumption resulted in increase of oxidation of arachidonic acid to prostaglandin and suppression of prostaglandin E and F catabolism (PGE_2 to 15-keto-PGE_2 in lung) (Pennington et al., 1978). However, in the present experiments, only one low dose of ethanol was injected into the snakeheads. It is presumably that this single dose of ethanol may not result in increased PGs synthesis snakeheads.

Although intraperitoneal injection of ethanol increased plasma glucose in snakeheads, prostaglandins also produced hyperglycemia when compared with their corresponding ethanol controls. The hyperglycemic effect of prostaglandins in snakeheads is consistent and repeatable. In the aspirin experiments, PGE_1 (suspended in 0.9% NaCl) were injected in the normal and aspirin treated snakeheads, a marked increase in plasma glucose

was observed. This increase is about 200% of the basal glucose level. Moreover, intravenous injection of PGE_1 also significantly increased plasma glucose 30 and 60 min after administration. PGA_1, PGB_1 and $PGF_{1\alpha}$ also produced increase in glucose level in snakehead eventhough the PG precursor, arachidonic acid increased plasma glucose concentration. This suggests that these 4 types of prostaglandin can produce hyperglycemic effects in snakeheads.

The hyperglycemic effects of PGE_1, PGA_1 in mammals and birds have been published (Sanbar et al., 1967; Lemberg et al., 1971; Sacca et al., 1973; Berti et al., 1965; Grande and Prigge, 1972). The present results of PGE_1-and PGA_1-induced increase in plasma glucose in snakehead is in accordance with those reports on mammals and birds.

However, there are very few reports of PGB_1, $PGF_{1\alpha}$ and arachidonic acid effects on carbohydrate metabolism (Pento et al., 1970). The present investigation demonstrated that PGB_1, $PGF_{1\alpha}$ and arachidonic acid all exhibited hyperglycemic effect in snakehead although their effects occurred at different time interval after injection. PGA_1 and arachidonic acid induced hyperglycemia 0.5 hour after injection. PGB increased plasma glucose 1 and 2 hours after injection. 1 hour after PGE_1 injection and 3 hours after injection of $PGF_{1\alpha}$ induced a rise in plasma glucose.

The blood glucose level at any given time is determined by the balance between the amount of glucose entering the blood stream and the amount leaving it. The principal determinants are therefore the dietary intake, the rate of entry into the cells of muscle, adipose tissue and other organ, and the glucostatic activity of the liver (Gangong, 1977). In the present studies, the snakeheads were unfed for at least 2 weeks before experimentation, therefore, the increase in plasma glucose would not come from the dietary intake, PGE_1 has been shown to produce a sharp increase in the % of glucose transformed to fatty acid in adipose tissue (Crawford and Haessler, 1968; Rettberg, 1968). Hence this may eliminate the possibility that the rise in blood glucose is due to a decline in the rate of glucose into tissues. Therefore the increase in plasma glucose is most likely due to the alteration of glucostatic activity of the liver.

There are two possible ways of formation of glucose: gluconeogensis and glycogenolysis. Since liver contains a large amount of glycogen, an analysis of liver glycogen content will provide information of glycogenolytic activity. Intraperitoneal injection of arachidonic acid significantly depleted liver glycogen content 1, 2 and 3 hours after injection. PGA_1, PGB_1, PGE_1 and $PGF_{1\alpha}$ also slightly decreased liver glycogen level 1 and 2 hours after intraperitoneal injection. Hence, the source of plasma glucose seems to be the result of glycogenolysis.

However, a very slight but not significant decrease in liver glycogen occurred in PGE_1 injected normal or aspirin-treated fish. These results suggest that PGs may directly or indirectly affect the glycogenolytic activity in liver and promote glycogenolysis and result in increased plasma glucose level in snakeheads. May et al. (1969) published that intraperitoneal administration of PGE_1 to rat decreased glycogen content of the liver and induced hyperglycemia in mice. The present results in snakehead agrees with those results from mammals.

Moreover, the hydrolysis of G6P is a key reaction both in gluconeogenesis and in the conversion of liver glycogen to blood glucose and glucose-6-phosphatase (G6Pase) catalyses this reaction (Hochachka, 1969). $PGF_{1\alpha}$ significantly increased liver G6Pase activity ½ hour after injection whereas PGB_1 decreased G6Pase activity, 2 hours after administration. However, PGA_1, PGE_1 and arachidonic acid did not alter this enzyme activity. From the previous suggestion of PGs promoting glycogenolysis, an increase in G6Pase activity is expected. This anomalous findings cannot be explained in present experiment.

Moreover, it has been reported that in mammals, PGE_1 caused a significant decrease in glycogen synthetase activity regardless of nutritional status or presence or absence of adrenal glands (Curnow and Nuttall, 1972). In the present study on the snakehead, PGE_1 also increased

liver phosphophosphorylase activity. These changes in enzyme activities are compatible with the proposed increase in hepatic cAMP and suggested possible direct effects on hepatic glycogen level. On the other hand, Exton et al. (1971) and Levine (1974; 1979) showed that PGE_1 and PGF_2 perfusion in rat liver failed to alter cAMP levels or glucose production.

In Part II (pp. 117), a low O_2 consumption rate was observed 10-15 min after intravenous injection of PGE_1 and it was suggested that this decrease is due to the alteration of metabolism in snakeheads. However, Willebrands and Tasseron (1968) reported that PGE_1 and $PGF_{1\alpha}$ increased glucose oxidation in isolated rat heart. Moreover, PGE_1 and $PGF_{1\alpha}$ promoted oxidation of glucose to CO_2 in epididymal sperms. However, $PGF_{1\alpha}$ reduced O_2 consumption while PGE_1 had no effect in epididymal sperms. Besides the oxidation of glucose via glycolysis and Kreb's cycle, the other pathway is pentose phosphate pathway and glucose-6-phosphate dehydrogenase (G6PDH) is the key enzyme of this metabolic pathway. Only $PGF_{1\alpha}$ increased G6PDH activity 2 hours after injection whereas, PGA_1, PGB_1 and PGE_1 did not affect this enzyme activity. The exact importance of this finding is obscure. However, it must be pointed out that PGE_1 promoted glyceride-fatty acid formation from glucose in adipose tissue (Vaughan, 1966). In the present result, enhanced pentose phosphate pathway activity due to $PGF_{1\alpha}$ injection may

contribute to the promotion of fatty acid formation from glucose.

Although the present results cannot provide the general conclusion on the mechanisms of PGs actions in carbohydrate metabolism, PGs induced hyperglycemia is consistent. In addition, the subsequent experiments have been designed to examine the possible direct actions of PGs on liver glycogen metabolism.

(b) Lipid metabolism

In vivo effects of PGs on plasma lipid are dependent upon the dose of PGs and the kind of experimental mammals. PGA_1 infusion in anaesthetized dog produced an increase in plasma FFA level (Sacca et al., 1973) and PGE injection also elevated the plasma FFA and glycerol level in man (Bergström et al., 1965) and in goose (Grande and Prigge, 1972). However, Sanbar et al. (1967) reported a contradictory result and plasma FFA level was lowered by injection of PGE_1. From the present results, the PGE_1 effect on plasma free fatty acid and lipid in snakeheads was in conformity with the results of Sanbar et al. (1967). PGE_1 injection significantly decreased plasma free fatty acid and lipid level in the fish. However, these levels were not affected by other PGs, such as PGA_1, PGB_1, $PGF_{1\alpha}$ and arachidonic acid in snakeheads. From the results studied on humans, PGA_1 and PGE_1 infusion induced plasma FFA but F series of PG produced no significant change in

plasma FFA level (Carlson et al., 1970a).

In the other set of experiments done on snakeheads, a PGE_1-induced drop in plasma lipid was observed. On the other hand aspirin also lowered plasma lipid level in snakeheads. However, PGE_1 injection in aspirin treated fish did not decrease plasma lipid. The results are quite controversial and cannot be explained in the present investigation.

Moreover, PGE_1 produced a dose-dependent effect in plasma FFA level of anaesthetized dog (Bergström et al., 1966). A low dose of PGE_1 increased FFA level while high doses decreased plasma FFA. Bergström et al. as well as Somova and Dochev (1970) suggested that low dose of PGE_1 stimulated the sympathetic nervous activity (enhanced lipid mobilization) while high dose of PGE_1 has a direct inhibit effect on FFA mobilization from adipose tissue.

Due to the dose- and mammal-dependent response to PGE_1, however, intraperitoneal injection of PGE_1 (0.75 mg/kg) produced a fall in plasma lipid and free fatty acid in snakehead. In the succeeding experiments, an investigation of PGs actions on lipolysis in adipose tissue has been carried out.

(c)

(c) Protein metabolism

Most of the work on PGs and protein synthesis is related to collagen. In other experiments, local application of PGE_1 and PGE_2 seems to stimulate incorporation of labeled amino acids into collagen whereas $PGF_{2\alpha}$ inhibited this incorporation (Lupulescu, 1975) (for details, see Horrobin, 1978). However, the present studies seem to be far away from studies of PGs effects in collagen synthesis. Therefore, it is hard to relate the present results of PGs effects or plasma protein level in snakeheads in relation to collagen synthesis.

The present findings showed that 1 and 2 hours after PGE_1 intraperitoneal injection produced a significant drop in plasma total protein levels. $PGF_{1\alpha}$ also induced the same effect in snakehead 2 and 3 hours after injection. Regardless of the presence or absence of aspirin treatment, intraperitoneal injection of PGE_1 also lowered plasma protein. The same response occurred when the snakeheads received intravenous PGE_1 administration. From the above results, PGE_1-and $PGF_{1\alpha}$-induced decrease in plasma protein in snakehead is reliable. However, PGA_1, PGB_1 and arachidonic acid did not affect plasma total protein level in snakehead.

Since the main components of plasma protein are globulin and albumin, an examination of plasma globulin and albumin may indicate the cause of PGs-induced decreased

plasma protein.

Only $PGF_{1\alpha}$ was shown to lower plasma globulin level 3 hours after injection whereas no effects of other PGs were observed. Moreover, PGA_1, PGB_1, PGE_1, $PGF_{1\alpha}$ and arachidonic acid all could lower plasma albumin level in snakeheads although their effective time intervals were different. These results seem not to agree with the results on plasma protein (only PGE_1 and $PGF_{1\alpha}$ decreased plasma total protein). It has been well known that plasma protein is synthesized and released from the liver (Putman, 1975). Working on the killifish, Horseman and Meier (1979) demonstrated that prolactin stimulation of hepatic RNA synthesis was blocked by indomethacin. Furthermore, they showed that $PGF_{2\alpha}$ stimulated hepatic RNA synthesis. Thus, these results seem to be in contrast to our present findings.

Of course, the present examination cannot give any mechanism of PGs actions on protein metabolism in snakehead. Moreover, this new finding requires further investigation.

(d) Water and ion levels of plasma and tissues

$PGF_{1\alpha}$ significantly lowered plasma Na^+ and K^+ levels 0.5 hour after injection whereas no effect was observed in plasma osmolality and hematocrit value. This shows that $PGF_{1\alpha}$ -induced decrease in Na^+ and K^+ levels is not

due to hemodilution. Moreover, 3 hours after $PGF_{1\alpha}$ administration, decreased plasma osmolality and K^+ level were observed but there was no observable change in hematocrit and plasma Na^+. Although $PGF_{1\alpha}$ could not produce consistent results, it is certain that $PGF_{1\alpha}$ has an osmoregulatory role in snakeheads. Of course, it cannot be explained at the present time.

Furthermore, PGE_1 declined hematocrit value and plasma K^+ level 1 hour after intraperitoneal injection while plasma Na^+ and osmolality remained unchanged. In the other set of experiment, a remarkable drop in Plasma Na^+ level was found in aspirin treated fish. In the same experiment, PGE_1 injection could bring plasma Na^+ back to normal level in the aspirin treated fish. Plasma Cl^- inclined to increase but it was not statistically significant. Aspirin treatment seemed to abolish this PGE_1-induced increase in plasma Cl^-. However, aspirin treatment alone could not produce any effect on Cl^- level. From other reports, PGE_1 and $PGF_{2\alpha}$ were shown to inhibit Na^+ and Cl^- outflow in seawater acclimated mullet (Pic, 1975). Also PGE_1 injection elevated plasma Cl^- level in killifish (Horseman and Meier, 1978).

Besides, aspirin treatment also reduced plasma osmolality but PGE_1 could not modify this effect. Further, either PGE_1 injection or aspirin treatment did not alter plasma Mg^{++}, Ca^{++} levels and hematocrit value in snakeheads.

In addition, arachidonic acid decreased plasma osmolality 2 and 3 hours after injection and induced a drop in plasma Na^+. The other PGs, such as PGA_1 and PGB_1 did not change plasma Na^+, Cl^-, osmolality and hematocrit in this fish.

Besides estimating plasma ionic levels, muscle and gill water content of the aspirin-treated fish were also measured. PGE_1 declined gill water content of intact and aspirin-treated fish while muscle water content was elevated only in aspirin-treated fish. Other report demonstrated that an increase in PGE_2 synthesis of incubated gill from a marine bivalve was induced by hyposmotic stress (Freas and Grollman, 1980). Further, PGE_2 reduced Na influx in fresh water mussel (Graves and Dietz, 1979). In *in vitro* studies, PGE_1 reduced vasopressin and theophylline-induced osmotic flow across the toad urinary bladder and PGE_1 also increased short-circuit current in this preparation (Urakabe, 1975). All these results show that PGs are involved in osmoregulation in these animals.

The present experiments show that exogenous PGE_1 and $PGF_{1\alpha}$ do play a role in the regulation of plasma osmolality, ionic levels and tissue water content. At the same time, endogenous PGs are also shown to be involved in maintaining ionic levels in the snakeheads and this is in accordance with the report of Woo *et al*. (1980).

The present studies cannot reveal the mechansion of PGs actions in osmoregulation of this fish and further investigations are required.

(3) In vitro studies

1. Materials and Methods

(a) Removal of liver and fat tissue

Snakeheads were kept as previously described (pp. 83). The animals were sacrificed by cutting through the cervical vertebral column. Then the abdomen was cut open and the whole liver was amputated immediately. Fatty tissue distributed around the peripheral region of intestine was also removed. The isolation of liver and fat cells were undertaken immediately after removal.

(b) Liver cells preparation and incubation

This preparation followed the modified method of Seglen (1964).

(i) Preparation of liver cells

a. Composition of buffers used for collagenase perfusion

	Ca^{++}-free perfusion buffer	Collagenase buffer	Washing buffer	Suspension buffer
NaCl	8,300	3,900	8,300	4,000
KCl	500	500	500	400
$CaCl_2 2H_2O$	--	700	180	180
$MgCl_2$ $6H_2O$	--	--	--	130
KH_2PO_4	--	--	--	150
Na_2SO_4	--	--	--	100
HEPES	2,400	24,000	2,400	7,200
TES	--	--	--	6,900
Tricine	--	--	--	6,500
1M NaOH	5.5	66.0	5.5	52.5
Collagenase	--	500	--	--
pH	7.4	7.6	7.4	7.6

Salt concentrations were given in milligrams per 1000 ml of final solution, and the concentration of NaOH (1M) as milliliters per 1000 ml of final solution. The two strongly buffered solution (collagenase buffer and suspension buffer) were designed to withstand continous acidification by the liver cells, and therefore had a high initial pH. All buffer solutions were prepared with well aerated distilled water.

b. After removal from the fish, the liver was immediately placed in a petri dish containing 20 ml of Ca^{++}-free perfusion buffer. The hepatic vein was catheterized with a polyethylene tube (PE-60). This cannula was connected with another polyethylene tube (diameter about 0.3 cm). The perfusion buffer was aerated and pumped by a varistatic pump into the liver via the polyethylene tube at a rate of 0.05 ml/sec. Then the perfusate was allowed to leak freely from the hepatic portal vein and hepatic artery. The liver was continously perfused with recirculating buffer for 20 min.

c. After 20 min perfusion, the Ca^{++}-free perfusion buffer was replaced by collagenase buffer (15 ml). Then the perfusion was continued for 1 hour and the buffer was aerated throughout the perfusion.

d. After all of the above treatments, the liver became swollen to more than twice its original size. The liver was then transferred to a small petri dish containing 10 ml washing buffer. While the liver was held in the

portal region with forceps and gently shaken, the cells were liberated from the connective vascular tissue by careful raking with another pair of forceps.

e. The cell suspension was filtered through a coarse nylon filter to remove connective tissue debris and clumps of infarctious tissue. The cell suspension was centrifuged and the washing buffer was pipetted out. Then the content was rewashed again. Then the washing buffer was discarded and replaced by approiate amount of suspension buffer. The liver cell suspension was subjected to experimentation.

(ii) Incubation of liver cell suspension

The liver cell suspension (containing 10-15 mg of liver wet weight) was incubated with PGA_1, PGB_1, PGE_1 and $PGF_{1\alpha}$ (dose 25 µg/ml) (all PGs were initially prepared in 0.5 mg/ml ethanol). Ethanol (50 µl) was added into the cell suspension as control. In another set of experiment, insulin (4 unit/ml) and epinephrine (50 µg/ml) was added into the cell suspension and an appropriate amount of 0.9% NaCl was added in the cell suspension as control. The cell suspensions were incubated at 20°C and were continously shaken. At zero, 1, 2 and 3 hours after incubation, 100 µl of cell suspension was taken out. Then the content was centrifuged and supernatant was transferred into another tube. Then the glucose content of the supernatant was estimated by glucose oxidase-peroxidase

method (see p. 134). The precipitate was saved and the excess supernatant was poured out. Then the precipitate was weighed and 200 µl distilled water was used to break down the liver cells. Then the content was frozen for later analysis of enzymatic activities.

Another 100 µl of cell suspension was also taken out at zero, 1, 2 and 3 hours after incubation. Then the cell suspension was centrifuged and the supernatant was discarded. The precipitate was saved for glycogen measurement (see p 137).

(iii) Enzymatic activities of liver cells

The soluble protein content of liver cells was estimated (see p. 134). G6Pase, GOT and GPT activities of liver cells were also determined. The measurement of G6Pase activitiy was same as previously described (p. 139).

Activities of the enzymes, glutamate oxaloacetate transaminase (GOT; L-aspartate : 2-oxoglutarate aminotransferase; E.C.2.6.1.1) and glutamate pyruvate transaminase, in the liver were assayed by the method described in Sigma Technical Bulletin No. 505-Transaminase (GOT and GPT). A micromodification was employed in which 0.5 ml substrate, 0.05 ml liver homogenate (20 fold dilution), 0.5 ml color reagent and 5 ml 0.4N NaOH solution were used. The assay was carried out at 20°C in a water bath. The enzymatic activities

were expressed as S-F units mg protein^{-1} (one theoretical Sigma-Frankel (S-F) unit of GOT or GPT will form 4.82 X 10^{-4} µM of glutamate min^{-1} at pH 7.5 and 25°C).

(c) Liver slice preparation and incubation

Liver was removed from fish and then was immediately frozen. A cooled slicer (Stadie-Riggs) was used for slicing the liver. The liver slices were about 0.5-1 mm thick. About 100 mg of liver slice was weighed and then was suspended in 1 ml suspension medium (the same suspension medium as in liver cell incubation). 25 µg of dry PGA_1, PGB_1, PGE_1, $PGF_{1\alpha}$ arachidonic acid and 25, 50 µg of epinephrine were added into the suspension medium. The content was incubated at 20°C water bath and was continously shaken for 2 hours. After incubation, the liver slice solution was centrifuge and the precipitate was homogenized using an Ultra-Turrax homogenizer. The homogenate was stored in freezer for later GOT, GPT and glycogen phosphorylase A activities estimation.

GPT and GOT activities measurement were understaken as previous described (pp. 179). Glycogen phosphorylase A activity was determined by method of Bergmeyer (1974). Glycogen phosphorylase A activity was expressed in PO_4^{3-} mM • g protein^{-1} • hr^{-1}.

(d) Fat cell preparation and incubation

A modification method of Rodbell (1964) was employed to prepare the fat cells. All containers used in fat cell preparation and incubation were made of plastic.

(i) Composition of Krebs Ringer + Collagenase

	Concentration (g/100ml)	Volume (ml)
NaCl	0.9	100
KCl	1.15	8
$CaCl_2$	1.22	6
KH_2PO_4	2.11	2
$MgSO_4 \cdot 7H_2O$	3.82	2

19 ml $NaHCO_3$ (1.3%) was added in 100 ml of above mixture. 2 g albumin (Sigma) and 108 mg glucose (Sigma) were added into the second mixture. Then 10 µg collagenase per ml of final Ringer solution was prepared.

(ii) Isolation of fat cells

The fat tissue (2 g) removed from the fish was immediately transferred into a plastic beaker with 3 ml of Kreb's Ringer + Collagenase solution. The fat tissue was cut into small pieces and was digested for 1 or 1.5 hour at room temperature. Then the cell suspension was filtered through a nylon filter. The filtrate containing free fat cells was washed with Ringer solution and then was centrifuged. After low speed centrifugation, the fat cells floated on the top

and the lower fraction was discarded. The cell suspension was rewashed again and the cell suspension was prepared for incubation.

(iii) Incubation of fat cells

PGA_1, PGB_1, PGE_1 and $PGF_{1\alpha}$ were gifts from Upjohn Company. These PGs were dissolved in 0.5 mg/ml ethanol. Appropriate amount of PGs (20 µg/1.5 ml) was added into fat cell suspension (0.1-0.2 g of fat weigh). 20 µl of ethanol was added in the cell suspension and was used as control.

The cell suspension was incubated at 20°C and was continuously shaken. At zero, 1,2, 3,4 and 5 hours, 200 µl cell suspension was taken out(before taking out the cell suspension, full mixing of fat cells and suspension medium was ensured). The samples were immediately frozen and saved for glycerol determination.

(iv) Glycerol determination

Glycerol content was determined by enzymatic method (glycerol dehydrogenase, Sigma). The formation of NADH after the enzymatic reaction was detected. The reagents were prepared as follow :

0.1 M Ammonium Sulfate
β-NAD Solution (7 mg β-NAD (Sigma), grade III, per ml H_2O, adjust pH to 7.0-7.5 with solid $NaHCO_3$)
0.5 M Carbonate Buffer pH 10.0
1.0 M Glycerol (For standard)
5 unit/ml Glycerol Dehydrogenase Solution (0.05 potassium phosphate pH 7.5)

To a cuvet of 1 cm lightpath, 0.05 ml Ammonium sulfate, 0.05 ml β-NAD solution, 0.3 ml carbonate buffer and 0.7 ml distilled water were added. Then 200 µl sample and 20 µl glycerol dehydrogenase were mixed with the above solution. Then the mixture was allowed to incubate at room temperature for 3 hours and then was read at 340 nm.

(e) Statistical analysis

All data were expressed as Mean ± Standard Error of the Mean. Statistical analysis was determined by Student's t test.

(a) Liver cells

A gradual release of glucose from incubated liver cells was observed (Fig. 35). However, PGA_1, $PGF_{1\alpha}$, PGB_1, PGE_1, ethanol could not produce any change in the rate of glucose release from liver cells . Although epinephrine declined glucose release, a statistical significance was only observed at 120 min after incubation.

In the case of liver cell glycogen, a moderate decrease of glycogen content was shown (Fig. 36). The glycogen content was significantly higher in PGE_1 and $PGF_{1\alpha}$ treated group when compared with their corresponding controls (ethanol group) 2 hours after incubation. A higher glycogen content was found in insulin-treated liver cell 1 hour after incubation. Other treatments such as epinephrine, PGA_1 and PGB_1 did not alter the glycogen content of liver cells at any time interval.

Since glycogen level of liver cell was significantly elevated 2 hours after PGE_1 and $PGF_{1\alpha}$ treatment, the enzyme activities of liver cells at this interval were measured. GOT and GPT activities were not altered by PGE_1, $PGF_{1\alpha}$ and epinephrine when compared with their corresponding controls (Fig. 37). Also no change was found in G6Pase activity in all these treatment (Fig. 38).

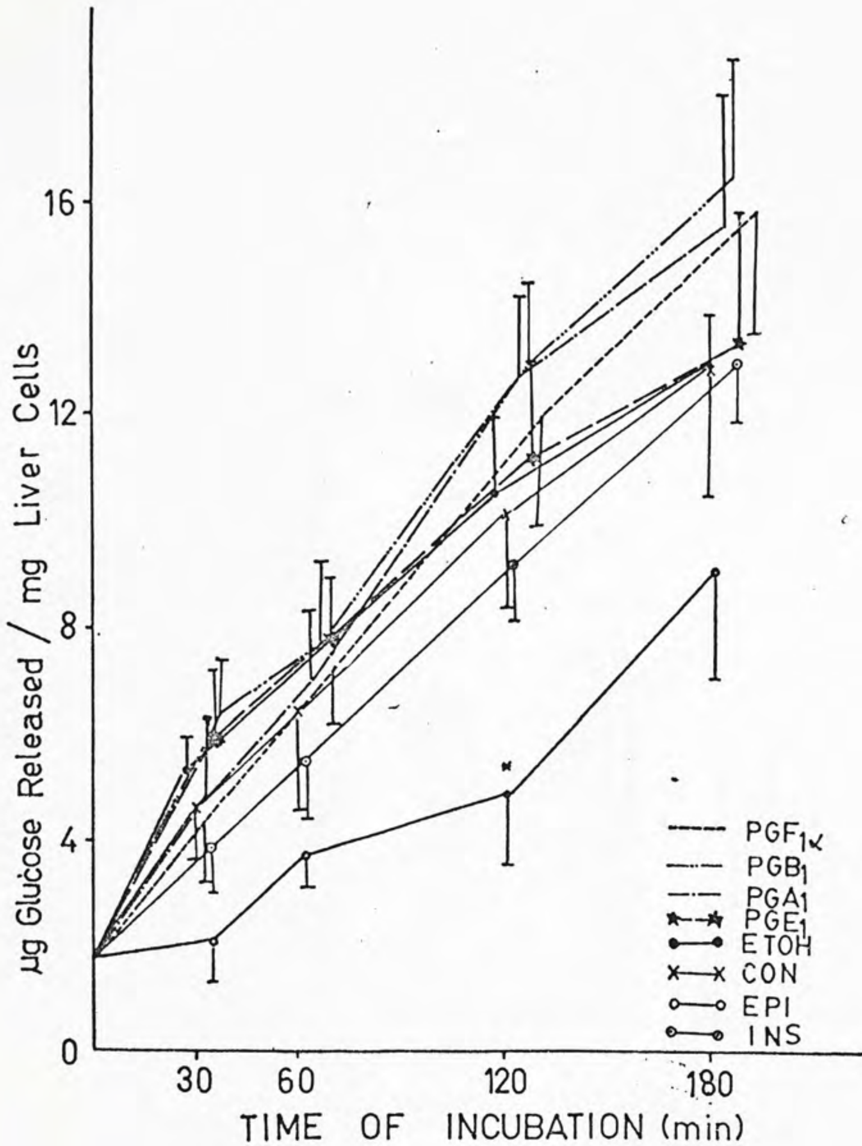


Fig. 35 Effect of PGs, ethanol (ETOH), epinephrine (EPI), insulin (INS), saline (CON) in the glucose release of isolated liver cells. Data were expressed in the Mean and SEM.
* $P < 0.05$ when compared with controls.

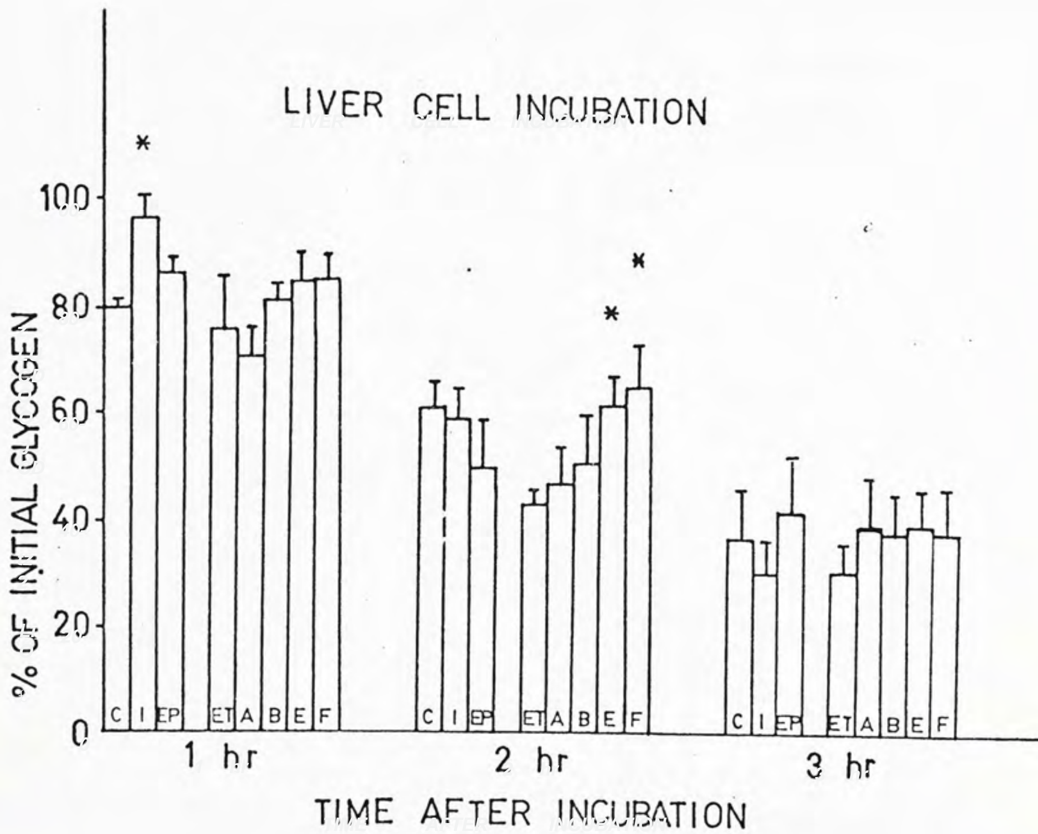


Fig. 36 Effect of PGs (A=PGA_1, B=PGB_1, E=PGE_1 F=$PGF_{1\alpha}$), ethanol (ET), epinephrine (EP), and insulin (I) (C=control) in glycogen content of isolated liver cells. Data were presented in the Mean and SEM. * $P < 0.05$ when compared with controls.

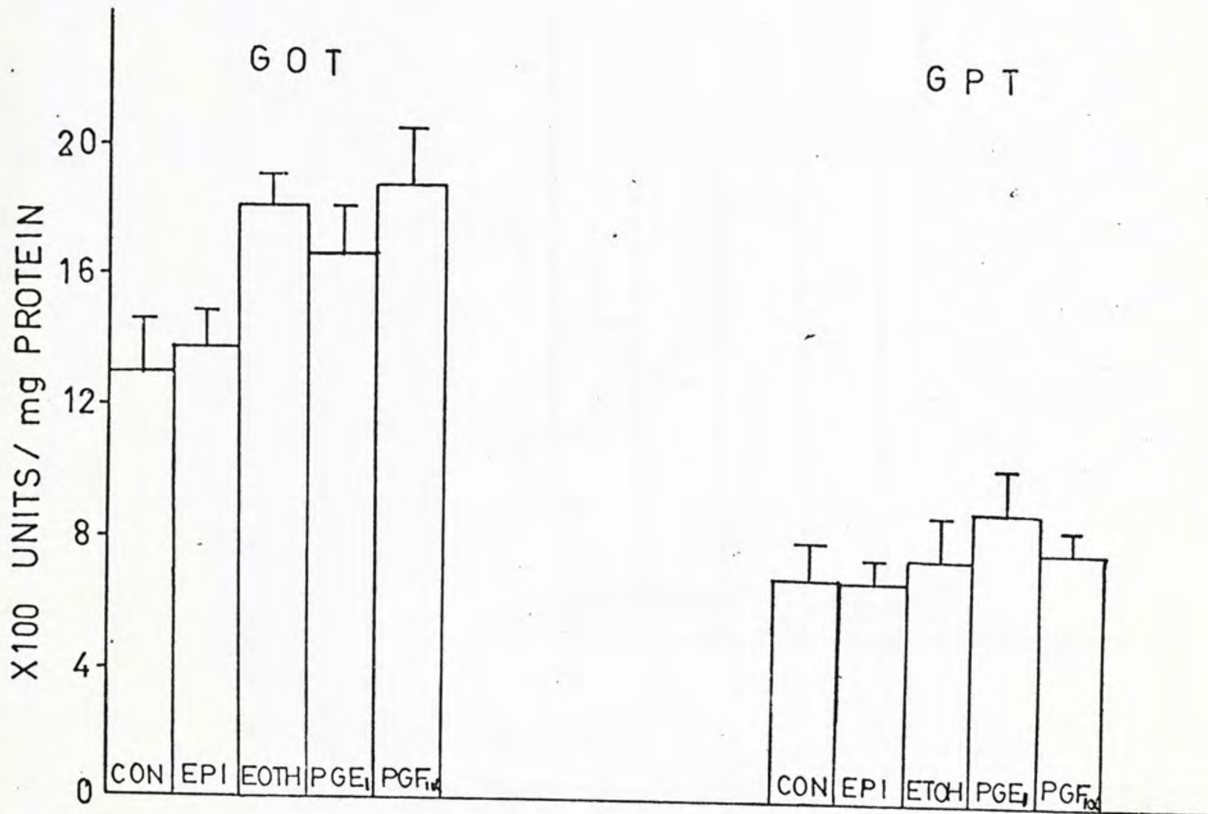


Fig. 37 Effect of epinephrine (EPI), ethanol (ETOH) PGE_1, $PGF_{1\alpha}$ (CON=control) in glutamate oxal-acetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) activities of isolated liver cells at 2 hours after incubation. Data (six observation) were presented in the Mean and SEM. No significant changes were observed.

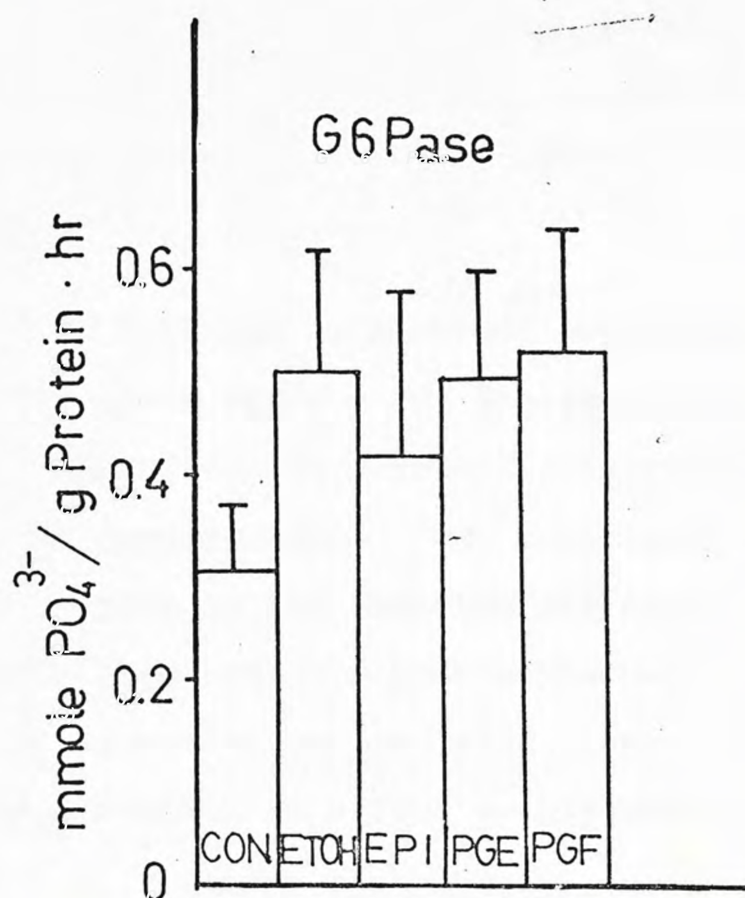


Fig. 38 Effect of PGE_1 (PGE), $PGF_{1\alpha}$ (PGF), epinephrine (EPI) and ethanol (ETOH) (CON=saline control) in glucose-6-phosphatase (G6Pase) activity of isolated liver cells at 2 hours after incubation. Data (6 observation) were expressed in the Mean and SEM.

(b) Liver slice

The GOT and GPT activities of liver slice in controls were 1844.4 ± 214 and 1058.8±127 unit/mg protein respectively while glycogen phosphorylase A activity was 92 ± 9.2 $PO_4^{\ 3-}$ mM/g protein/hr. No effect was observed in GPT, GOT and glycogen phosphorylase A activities in any treatment (Table 8).

(c) Fat cell

A plot of % change in glycerol production over buffer control after various PGs treatment was presented in Fig. 39. A decrease in glycerol production caused by ethanol was demonstrated. PGB_1 remarkably promoted glycerol production in the isolated fat cells. An $PGF_{1\alpha}$-induced increase in glycerol production was also shown but the elevation was not significant. Further, PGE_1 and PGA_1 produced no effect on glycerol production.

Table 8 : Enzymatic Activities of Liver Slice[+]

µg/ml	G O T unit/mg protein	G P T unit/mg protein	Glycogen Phosphorylase A PO_4^{-3} mM/g protein/hr
Saline[‡]	44 ± 214	1058 ± 127	92 ± 9.2
PGA_1 25	2052 ± 259	1076 ± 120	102 ± 12.2
PGB_1 25	1825 ± 187	1028 ± 130	109 ± 12.7
PGE_1 25	1878 ± 189	951 ± 118	112 ± 13.3
PGF_1 25	2054 ± 273	1072 ± 142	120 ± 12.3
Arachidonic acid 25	2255 ± 182	1372 ± 244	108 ± 11
Epinephrine 25	2148 ± 217	1098 ± 141	123 ± 13.7
Epinephrine 50	3137 ± 189	1372 ± 244	109 ± 9.7

+ Liver slice was incubated for 2 hours and then GOT, GPT and glycogen phosphorylase A activities were determined.

‡ 0.9% NaCl (20 µl/ml) was added as control in the liver slice incubation medium.

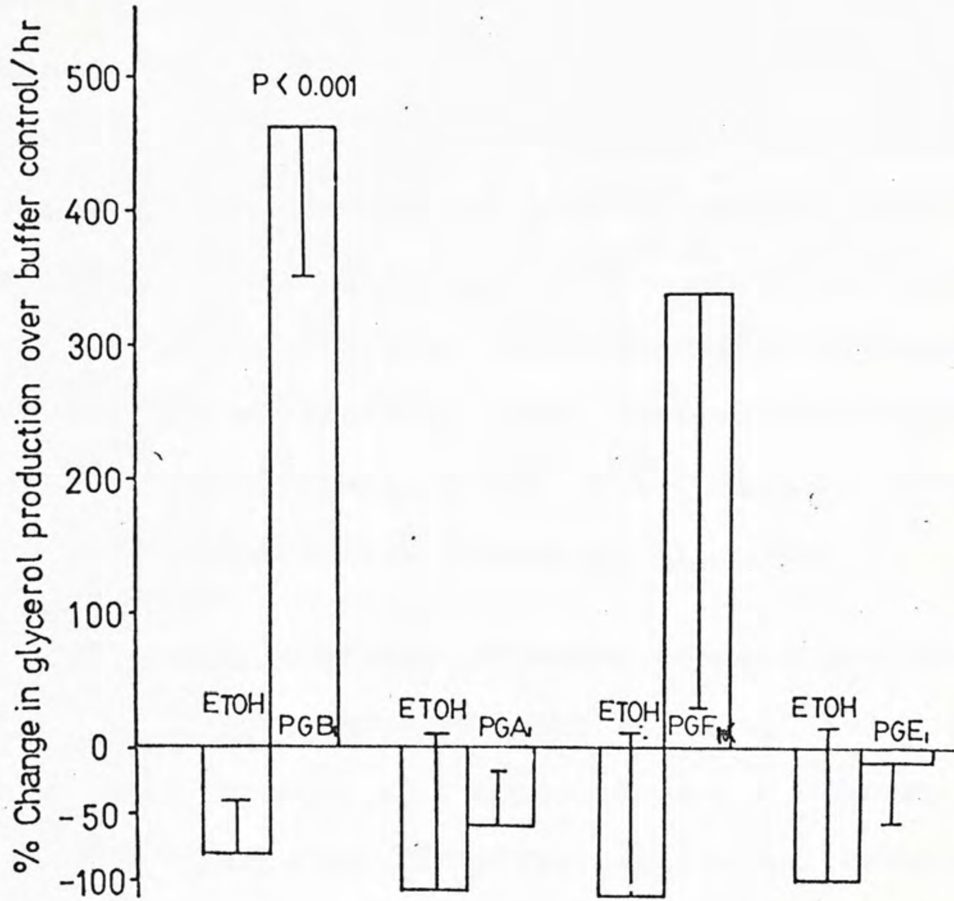


Fig. 39 Effect of ethanol (ETOH) and PGs in glycerol production of isolated fat cells. Data (6 observation) were expressed in the Mean and SEM. PGB_1 significantly increased glycerol production ($P < 0.001$).

3 Discussion

A gradual decrease in glycogen content of liver cells and a moderate increase in glucose level of suspension medium indicated that a degradation of glycogen into glucose and released out was proceeding in the liver cells during incubation. The data showed that PGs did not affect the rate of glucose release from liver cells. Only epinephrine depressed glucose release from liver cells. This result is in contrast to the result reported on other fishes : epinephrine produced hyperglycemia in the eel (Larsson, 1973; Woo, 1976) and goldfish (Young et al., 1965).

Moreover, a higher glycogen content was observed in the liver cells treated with insulin. However, this increase in glycogen did not accompany a decrease in glucose release. Furthermore, insulin has been shown to promote glycogen synthesis and glycogen is deposited in the muscle of lamprey (Murat et al., 1981).

PGE_1 and $PGF_{1\alpha}$ also elevated liver glycogen content in liver cells whereas PGA_1 and PGB_1 produced no effect. This is also a suprise to find that PGE_1 and $PGF_{1\alpha}$ induced increase glycogen content in liver cells since many reports have been published that PGE_1 infusion induced a significant increment in the average glucose levels in the perfusate of the isolated perfused rat liver (Lemberg et al., 1971). PGE_1 also activated

glycogen synthetase activity and increased glycogen phosphorylase A activity in rat liver (Curnow and Nuttall, 1972). However, in present studies, liver phosphorylase A activity in snakehead was not altered by any PGs. Also G6Pase activity in the isolated liver cell was not modified by PGE_1 or $PGF_{1\alpha}$ or epinephrine treatment. Levine (1979) also found that PGE_1 did not promote hyperglycemia, glycogenolysis, lipolysis, or prevent epinephrine-induced hyperglycemia in the isolated perfused rat liver. Further, this author and his coworker (Levine and Schwertzel, 1980) reported that PGE_2 alone could not depress gluconeogensis while PGE_2 inhibited glucagon induced gluconeogensis in rat liver. Therefore, the inability of PGs in activation of gluconeogensis or glycogenolysis in isolated snakehead liver slice or liver cells indicates that hyperglycemic effect of PGs may be mediated through the action of other hormone(s) (e.g. glucagon).

In the previous results, PGE_1 and $PGF_{1\alpha}$ significantly declined plasma protein level in snakeheads. Further GPT and GOT are the enzymes involved in the transamination from an amino acid to a keto acid. A change in activity of these enzymes may show an alternation of amino acid metabolism. However, PGA_1, PGB_1, PGE_1, $PGF_{1\alpha}$, arachidonic acid and epinephrine did not change these two enzymatic activities in the liver slice.

In the present studies, only PGB_1 was shown to stimulate lipolysis in the isolated fat cells since a marked increase in glycerol production was demonstrated. $PGF_{1\alpha}$ also elicited a lipolytic effect on fat cell although the effect was not statistically significant. Other PGs (PGA_1 and PGE_1) did not exhibit action on lipolysis in fat cells. Ethanol could not exert a significant effect on lipolysis in fat cell. This result agreed with the report of Nilsson and Belfrage (1978) on effects of ethanol on lipolysis in isolated rat adipocytes.

Since no report on PGB_1 effect on lipolysis has been published, the present result of PGB_1-induced lipolysis in snakehead fat cells cannot be compared with other report. It has published that $PGF_{1\alpha}$ inhibited epinephrine-induced lipolysis in mammal (Handler *et al.*, 1965). However, in the snakehead fat cells, $PGF_{1\alpha}$ seems to promote rather than inhibit lipolysis.

The present data showed that PGE_1 could not inhibit lipolysis *in vitro* fat cells. This result is in contrast to the previous result of PGE_1 in *in vivo* studies. In *in vivo* studies, PGE_1 significantly lowered plasma FFA and lipid level 2 hours after injection. This decrease in plasma FFA is presumbably due to the

antilipotic effect of PGE_1 (for details, see also Literature Review, pp. 53-58). Many reports have demonstrated that PGE_1 has antilipolytic action in both the *in vitro* and *in vivo* experimental conditions (see Literature Review, pp. 53-58). One of the possibility causing the disaccordance of the results from *in vitro* and *in vivo* studies in snakeheads is that PGE_1-induced lipolysis is mediated by other hormones (such as insulin), therefore in *in vitro* studies, this mediation is blocked and no lipolytic effect can be observed. The other possible factor may be due to the absence of GTP in the fat cell incubation medium. Other reports showed that GTP is necessary on the action of PGs and other α-adrenergic agonists in reducing hamster fat cell adenylate cyclase activity (Aktories, *et al.*, 1979; Watanabe *et al.*, 1979; Kather and Simon, 1979). However, the present investigation cannot provide support for these two possibilities.

CHAPTER IV CONCLUSION

CHAPTER IV CONCLUSION

It has been well known that prostaglandins have a wide spectrum of biological and pharmacological functions. However, most of these studies were performed on mammalian systems. Therefore, the present studies only focused on the effects of PGs in a lower vertebrate-snakehead fish. Since PGs possess versatile actions, only the studies of certain effects of PGs were carried out in the present investigation. The present results showed that PGs do exert cardiovascular, metabolic and osmoregulatory actions in this fresh water fish.

The doses of PGs employed in the fish were high and it may be in pharmacological rather than physiological range. However, a minimal dose of 2 µg/kg of PGs was required to produce cardiovascular response in snakeheads. Form the other reports on mammals, a very low dose of PGE_1 (0.02 µg/kg) could elicit these cardiovascular responses (Jones, 1972) while 9 µg/kg of PGE_2 was required to produce the same effect in bullfrog (Leffler <u>et al</u>., 1980). Also the duration of response to PGE are different among mammals, amphibians and fish and a longer recovery time is found in lower vertebrates (in human : 3 min (Karim <u>et al</u>., 1971) ; in bullfrog : 10 min (Leffler <u>et al</u>., 1980) ; in snakehead : 20 min). Moreover, PGE_1 intravenous injection produced a hyperglycemic effect in snakehead 1 and 2 hours after administration whereas the same effect occurred 10 min after PGE_1 infusion in non-anaesthetized dog (Bergström <u>et al</u> ., 1966). Similarly, other metabolic effects of PGE_1, such as those on plasma

lipid and free fatty acid occurred 1 and 2 hours after injection. Other reports have shown that the effect of insulin in fish requires several hours to occur while only a few minutes is required to elicit the same effect in mammals. This phenomenon is not only simply explained by the temperature-related Arrhenius plot and is an intrinsic property of lower vertebrate biology. It seems that hormonal regulation of metabolism is less critical in the lower vertebrates which consequently allows them to better withstand long-lasting perturbation of the 'milieu intérieur' (Murat _et al._, 1981). The assumption of Murat _et al._ may also explain the present delayed effects of PGs in snakeheads.

Besides, PGB_1 has been shown to have hypotensive action in cat (Jones, 1972) but its action in snakehead was hypertension instead of hypotension. Further, $PGF_{1\alpha}$ produced species-dependant cardiovascular effect in mammals (either hypertension or hypotension). In leopard frog (_Rana pipien_) $PGF_{2\alpha}$ infusion increased arterial pressure whereas $PGF_{2\alpha}$ decreased arterial pressure in bullfrog (_Rana catesbiana_) (Leffler _et al._, 1980). It is a surprise that $PGF_{1\alpha}$ in doses ranging from 5 µg/kg to 120 µg/kg could not produce any cardiovascular effect in snakehead. From the above data, the different responses of PGs in different classes of animal may have an evolutionary significance. There is another example that different response to the same molecule exists in different groups of animal . Urotensin I (a hormone secreted from the caudal neurosecretory system of fish) exerts a uniquely long-lasting depression of systemic blood

pressure in mammals (see Chan and Bern, 1976 ; Woo and Bern, 1979). In some amphibian and reptilian species, urotensin I elicits either hyper- or hypotensive actions while it produces hypertension in eel (see Chan and Bern, 1976 ; Woo and Bern, 1979). Therefore, it seems that there is an evolution of vascular response to urotensins in vertebrates : a hypertensive response in lower vertebrates is gradually succeeded by hypotensive effects in higher vertebrates. In view of the present findings, it is likely that a similar evolution of vascular response to PGs may exist. As comparative studies are extended, many more deviation from the conventional (mammalian) functional patterns will possibly be revealed. However, there is no information on the effect of PGs on cardiovascular system in reptiles. Also there are very scarce reports on the other lower vertebrates such as amphibians and fish. Thus it seems to be premature at the present time to construct an evolutionary trend of action of PGs in different vertebrate groups. Nevertheless, these new findings of PGB_1 and $PGF_{1\alpha}$ effects on snakeheads and this will provide more information on the comparative aspects of physiology.

Although the doses used in present studies were high, it was expected to be a pharmacological dose rather than a physiological dose. However, only high doses of PGs would produce observable effects in snakeheads. There was no other alternative except using a high dose for the present investigation. Furthermore, the distribution of PGE was demonstrated in the various tissues of snakehead and its quantity varied

considerably among different tissues. The ubiquitous presence of PGE in the snakehead indicates that PGE has a role in the physiology of this fish. Besides, PG-blockers (aspirin and indomethacin) were employed in the fish to verify the possible participation of endogenous PGs in the physiology of snakehead. Indomethacin treatment significantly lowered the basal arterial blood pressure and this indicates the actual involvement of endogenous PGs in normal blood pressure regulation. Also aspirin treatment lowered plasma Na^+ level. It is presumably that PGs participated in Na^+ regulation in snakehead. From the above evidence, it undoubtedly suggests that PGs do have physiological functions in snakehead.

According to the original use of the word, hormones are chemical agents which are synthesized by circumscribed parts of the body generally specialized ductless glands and are carried by the circulatory blood to another part of the body where they evoke systemic adjustments by acting on specific tissues and organs (Turner and Bagnara, 1976). From this definition of hormone, PGs seem not to fulfil the above criteria as a hormone. Since PGEs and PGFs do not survive a single passage through the lung so that a role for PGs as circulating hormones seems excluded. Notwithstanding, there is general acceptance of the fact that PGE is produced in the medulla of the kidney to regulate the haemodynamics of the cortex (Herbaczynska-Cedro and Vane, 1973). $PGF_{2\alpha}$ has been shown to be the agent, formed in the uterus to shut off progesterone production in the corpus luteum (luteolysin=$PGF_{2\alpha}$) (McCracken et al., 1973). Thus it is clear that PGEs and $PGF\alpha$s function

as local hormonal regulators and this may be their primary function. Since PGs are produced in all tissues so far examined which in turn have the ability to respond to PG action, it seems that these mediators may function as intracellular regulators in many instances.

In addition, there is ample evidence to demonstrate the relationship between PGs and cAMP. Numerous reports showed that many hormonal effects are mediated by a sequence of synthesis of two mediators, PG and cAMP. For example, urotensin I-induced vasodilatory action is mediated via cAMP and cGMP formation. An antagonistic interaction between PGs of the B, E and F series and urotensin I is indicated in experiments using such PGs and the PG synthetase inhibitor. The latter substance potentiated the effects of urotensin I on the isolated rat tail artery (Lederis and Bern, 1976). Other hormones, such as LH, stimulate cAMP formation in isolated mouse ovaries. A PG antagonist blocks the actions of both PGE and LH in stimulating cAMP formation and it was suggested that PGs play an essential role in the action of LH (Kuehl et al., 1970). Similar observations were made for the action of other hormones on their target tissues leading to the same conclusion. Finally, in any event, it is reasonable to predict that PGs will prove to be related to cyclic nucleotide in some manner. Therefore the PG effects in snakehead is also expected to mediate via cAMP formation.

In addition, PGE_1 has higher metabolic potency than PGA_1, PGB_1 and $PGF_{1\alpha}$ in snakeheads. These four types of PG uniformly

elevated plasma glucose in this fish and this effect is generally true in mammals. PGE_1 was also shown to decrease plasma protein, lipid, free fatty acid and oxygen consumption. However, the exact mechanism of these effects remains unknown further investigations are required.

Further studies along the lines set out in this thesis on sub-mammalian vertebrates may prove to be interesting for those involved in the comparative and evolutionary aspects of prostaglandin actions.

To recapitulate the major findings in this thesis, the presence of prostaglandins in the various tissues was clearly established and endogenous PGs were shown to exert physiological functions in this fish. Thus, PGs undoubtedly play a role in physiology of snakehead.

REFERENCES

REFERENCES

Aiken, J.W. (1972). Aspirin and indomethacin prolong parturition in rats : Evidence that prostaglandins contribute to expulsion of fetus. Nature (London) 240 : 21-22.

Aiken, J.W. and Vane, J.R. (1971). Blockade of angiotensin release from dog kidney by indomethacin. Pharmacologist 13 : 15.

Aktories, K., Schultz, G. and Jakobs, K.H. (1979). Inhibition of hamster fat cell adenylate cyclase by prostaglandin E_1 and epinephrine : Requirement for GTP and sodium ion. FEBS Let. 107 (1) : 100-104.

Albro, P.W. and Fishbein, L. (1969). Determination of prostaglandins by gas-liquid chromatography. J. Chromato. 44 : 443-451.

Ambache, N., Brummer, H.C., Rose, J.G. and Whiting, J. (1966). Thin-layer chromatography of spasmaogenic unsaturated hydroxy-acids from various tissues. J. Physiol. 185 : 77-78.

Andersen, N.H. (1969). Preparative thin-layer and column chromatography of prostaglandins. J. Lipid. Res. 10 : 316-319.

Andersson, B. and Keskell, L.G. (1975). Effects on fluid balance of intraventricular infusions of prostaglandin E_1. Acta Physiol. Scand. 93 : 286-288.

Änggård, E. (1964). The identification of prostaglandins $F_{2\alpha}$ in lungs from man, monkey and guinea-pig. Abstracts 6th Congr. Biochem. N.Y. VII, pp.562.

Änggård, E. and Samuelsson, B. (1964). Smooth-muscle stimulating lipids in sheep iris. The identification of prostaglandin $F_{2\alpha}$. Biochem. Pharmaccol. 13 : 281-283.

Änggård, E. and Samuelsson, B. (1966). Purification and properties of a 15-hydroxyprostaglandin dehydrogenase from swing lung. Ark. Kemi. 25 : 293.

Änggård, E., Larsson, C. and Samuelsson, B. (1971). The distribution of 15-hydroxyprostaglandin dehydrogenase and prostaglandin-Δ^{13}-reductase in tissues of the swine. Acta Physiol. Scand. 81 : 396-404.

Attallah, A. and Stahl, R. (1980). Inhibition of renal prostaglandin E_2 biosythesis in vivo : Renal differences. Prostaglandins 19 (5) : 649-650.

Attallah, A, Payakkapan, W., Lee, J., Carr, A. and Brazelton, E. (1974). PGA : Fact, not artifact. Prostaglandins 5 : 69-72.

Bartholomew, G.A. (1977). Body temperature and energy metabolism. In 'Animal physiology', ed. by Gordon, M.S., Bartholomew, G. A., Grinnell, A.D., Jørgensen, C.B. and White, F. N., third edition, pp.372. MacMillan, Lond.

Bartholomew, G.A. (1977). Energy metabolism. In 'Animal physiology', ed. by Gordon, M.S., Bartholomew, G.A., Grinnell, A.D., Jørgensen, C.B. and White, F.N., third edition, pp.65. MacMillan, Lond.

Beerthuis, R.K., Nugterenc, D.H., Pabon, H.J.J. and von Dorp, D.A. (1968). Biologically active prostaglandins from some new odd-numbered essential fatty acids. Rec. Trav. Chim. 87 : 461.

Bennett, A., Friedmann, C.A. and Vane, J.R. (1967). Release of prostaglandin E_1 from the rat stomach. Nature (Lond) 216 : 873-876.

Bennett, A., Murray, J.G. and Wyllie, J.H. (1968). Occurrence of prostaglandin E_2 in the human isolated gastric muscle. Bri. J. Pharmac. Chemother. 32 : 339-349.

Bergmeyer, H.U. (1974). Glycogen phosphorylase A. In 'Methods of enzymatic analysis', ed. by Bergmeyer, H.U. Verlag Chemie, Academic Press, New York-San Francisco-London.

Bergström, S. (1966). Isolation, structure and action of the prostaglandins. In 'Nobel symbosium 2, prostaglandins', ed. by Bergström, S. and Samuelsson, B., pp.21-30. Almqvist and Wiksell, Stockholm.

Bergström, S. (1966). The prostaglandins. Recent. Prog. Horm. Res. 22 : 153-175.

Bergström, S. and Euler, U.S. von (1963). The biological activity of prostaglandin E_1, E_2 and E_3. Acta Physiol. Scand. 59 : 493.

Bergström, S. and Samuelsson, R. (1962). Isolation of prostaglandin E_1 from seminal plasma. J. Biol. Chem. 237 : 3005-3006.

Bergström, S. and Sjövall, J. (1957). The isolation of prostaglandin. Acta Chem. Scand. 11 : 1086.

Bergström, S. and Sjövall, J. (1960). The isolation of prostaglandin F from sheep prostate glands. Acta Chem Scand. 14 : 1693-1700.

Bergström, S., Carlson, L.A. and Orö, L. (1964). Effect of prostaglandins on catecholamin-induced changes in the free fatty acids of plasma and in blood pressure in the dog. Acta Physiol. Scand. 60 : 170-180.

Bergström, S., Carlson, L. A. and Orö, L. (1966). Effect of different doses of prostaglandin E_1 on free fatty acids of plasma bolld glucose and heart rate in the nonanesthetized dog. Acta Physiol. Scand. 67 : 183-195.

Bergström, S., Carlson, L.A. and Weeks, J.R. (1968). The prostaglandins : A family of biologically active lipids. Pharmacol. Rev. 20 : 1-40.

Bergström, S., Carlson, L.A., Ekelund, L.G. and Orö, L. (1965). Effect of prostaglandin E_1 on blood pressure, heart rate and concentration of free fatty acids of plasma in man. Proc. Soc. Exptl. Biol. Med. 118 : 110.

Bergström, S., Danielsson, H. and Samuelsson, B. (1964). The enzymatic formation of prostaglandin E_2 from arachidonic acid . Biochim. Biophys. Acta 90 : 207-210.

Bergström, S., Danielsson, H., Klenberg, D. and Samuelsson. B. (1964). The enzymatic conversion of essential fatty acids into prostaglandins. J. Biol. Chem. 239 : 4006-4008PC.

Bergström, S., Duner, H., Euler, U.S. von, Pernow, B. and Sjövall, J. (1959). Observations on the effects of infusions of prostaglandin E_1 in man. Acta Physiol. Scand. 45 : 145.

Berti, F., Lentati, R. and Grafnetter, D. (1967). Effecti della sommistrazione di prostaglandina E_1 sulla lipasi lipoproteica cariaca. Bol. Soc. Ital. Sper. 43 : 515-518.

Berti, F., Lentati, R. and Usardi, M.M. (1965). La prostaglandina E_1 (PGE_1) spiega attivita iperglicemizzante. Soc. Ital. Bio. Sper. 41 : 1327-1329.

Berti, F., Kabir, M., Lentati, R., Usardi, M.M., Mantegazza, P. and Paoletti, R. (1967). Relations between some in vitro in vivo effects of prostagalandin E_1. Progr. Biochem. Pharmaco. 3 : 110-121.

Bhattercherjee, P. and Eakins, K. (1973). Inhibition of prostaglandin synthetase systems in ocular tissues by indomethacin. Pharmacologist 15 : 201-215.

Bohle, E. and May, B. (1967). Metabolic effects of PGE_1 upon lipid and carbohydrate metabolism. In 'Prostaglandin symposium of the Worcester Foundation for experimental biology', ed. by Ramwell, P.W. and Shaw, J.E., pp.115-129. Interscience, New York-London-Sydney-Toronto.

Bohle, E., Ditschuneit, H.H., Dober, R. and Ditschuneit, H. (1967). Animal experimentation on the influence of prostagalandin E_1 on fat and carbohydrate metabolism. Verh. Deut. Ges. Inn. Med. 73 : 793.

Bressler, R., Vargas-Gordon, M. and Lebovitz, H.E. (1968). Tranylcypromine : a potent insulin secretagogue and hypoglycemic agent. Diabetes 17 : 617-624.

Butcher, R.W. and Sutherland, E.W. (1967). The effect of catecholamines adrenergic blocking agents, prostaglandin E_1 and insulin on cyclic AMP levels in the rate epididymal fat pad in vitro. Ann. N.Y. Acad. Sci. 139 : 849.

Butcher, R.W., Pike, J.E. and Sutherland, E.W. (1967). The effect of prostaglandin E_1 on adenosine 3',5'-monophosphate levels in adipose tissue. In 'Nobel symposium 2 : prostaglandins', ed. by Bergström, S. and Samuelsson, B., pp.133-138. Interscience, London.

Butcher, R.W., Robison, G.A., Hardman, J.G. and Sutherland, E.W. (1968). The role of cyclic AMP in hormone actions. Advan. Enzyme Reg. 6 : 357.

Bygdeman, M., Hamberg, M. and Samuelsson, B. (1966). The content of different prostaglandins in human seminal fluid and their threshold doses on the human myometrium. Mem. Soc. Endocrinol. 14 : 49.

Caldwell, B.V., Burstein, S., Brock, W.A. and Speroff, L. (1971). Radioimmunoassay of the F prostaglandins. J. Clin. Endocrinol. Metab. 33 : 171-175.

Carlson, L.A. (1965). Inhibition of the moblilization of free fatty acids from adipose tissue. Ann. N.Y. Acad. Sci. 113 : 119.

Carlson, L.A. (1967). Metabolic and cardiovascular effects in vivo of prostaglandins. In 'Nobel symposium 2 : Prostaglandins', ed. by Bergström, S. and Samuelsson, B., pp. 123-132. Almqvist and Wiksell, Stockholm.

Carlson, L.A. and Orö, L. (1966). Effect of prostaglandin on blood pressure and heart rate in thedog. Acta Physiol. Scand. 67 : 89.

Carlson, L.A. Ekelund, L.G. and Orö, L. (1969). Circulatory and respiratory effects of different doses of prostaglandin E_1 in man. Acta Physiolo. 75 : 161.

Carlson. L.A., Ekelund, L.G. and Orö, L. (1970a). Effect of intravenous prostaglandin E_1 on noradrenaline-stimulated mobilization of plasma free fatty acids in man. Acta Med. Scand. 188 : 379-383.

Carlson, L.A., Ekelund, L.G. and Orö, L. (1970b). Clinical, metabolic and cardiovascular effects of different prostaglandins in man. Acta Med. Scand. 188 : 553.

Cavanaugh, A.H., Farnsworth, W.F., Greizerstein, H.B. and Wojtowicz, C. (1980). A novel effect of indomethacin on prostaglandin $F_{2\alpha}$ synthesis and metabolism by human prostate. Life Sci. 26 : 19-28.

Chan, D.K.O. and Bern, H.A. (1976). The caudal neurosecretory system : A critical evaluation of the two hormone hypothesis. Cell. Tiss. Res. 174 : 339-354.

Chan, D.K.O. and Woo, N.Y.S. (1978). The respiratory metabolism of the Japanese eel, Anguilla japonica : Effects of ambient oxygen, temperature, season, body weight and hypophysectomy. Gen. Comp. Endocrinol. 35 : 160-168.

Chapple, D.J., Dusting, G.J., Hughes, R. and Vane, J.R. (1980). Some direct and reflex cardiovascular actions of prostaglandin E_2 in anaesthetized dogs. Bri. J. Pharmacol. 68 : 437-447.

Chow, T.W., Cheng, D.I. and Cheng, M.D. (1962). A prelimiary study on the reproduction ecology of the snake-head fish, Ophiocephalus argus Cantor. J. Shanton Univ. 4 : 61-73.

Christ, E.J. and Dorp, D.A. van (1972). Comparative aspects of prostaglandin biosynthesis in animal tissues. Biochim. Biophys. Acta 270 : 537-545.

Christ, E.J. and Nugteren, D.H. (1970). The biosynthesis and possible function of prostaglandins in adipose tissue. Biochim. Biophys. Acta 218 : 296-307.

Colin, D. and William, C.H. (1974). Cyclic AMP regulation of prostaglandin biosynthesis in fat cells. Prostaglandins 6 (3) : 227-235.

Collier, J.G. and Flower, R.J. (1971). Effect of aspirin on human seminal prostaglandins. Lancet 11 : 852-853.

Collier, J.G., Herman, A.G. and Vane, J.R. (1973). Appearance of prostaglandins in the renal venous blood of dogs in response to acute systemic hypotension produced by bleeding or endotoxin. J. Physiol. (Lond) 230 : 19-20P.

Collier, J.G., Karim, S.M.M., Robinson, B. and Somers, K. (1972). Action of prostaglandins A_2, B_1, E_2 and $F_{2\alpha}$ on superficial hand veins of man. Bri. J. Pharmacol. 3 :347P.

Conn, E.E, and Stumpf, P.K. (1976). The metabolism of ammonia and nitrogen-containing monomers. In 'Outlines of biochemistry', ed. by Conn, E.E. and Stumpf. P.K., fourth edition, pp.462-463. Wiley, New York-London-Sydney-Toronto.

Conte, F.P. (1969). Salt secretion, In 'Fish physiology', ed. by Hoar, W.S. and Randall, D.J., pp.241-283. Academic Press, New York-London.

Corey, E.J., Albonico, S.M., Koelliker, U., Schaaf, T.K. and Varma, R.J. (1971). New reagents for stereoselective carbonyl reduction. Improved synthetic route to the primary prostaglandins. J. Am. Chem. Soc. 93 : 1491-1493.

Corker, C.S., Norymberski, J.K. and Thow, R. (1962). Some aspects of the Zimmermann reaction. Biochem. J. 83 : 583-588.

Crawford, J.D. and Haessler, H.A. (1968). Insulin-like activities of PGE_1. In 'Prostaglandin symposium of the Worcester Foundation for experimental biology', ed. by Ramwell, P.W. and Shaw, J.E., pp.103-113. Interscience, New York.

Curnow, H.T. and Nuttall, F.Q. (1972). Effect of prostaglandin E_1 administration on the liver glycogen synthetase and phosphorylase systems. J. Biol. Chem. 247 : 1892-1898.

Curtis-Prior, P.B. (1976a). Agents affecting prostaglandin metabolism. In 'Prostaglandins : An introduction to their biochemistry, physiology, and pharmacology', ed. by Curtis-Prior, P.B., pp.41-44. North-Holland Biomedical Press, Amsterdam.

Curtis-Prior, P.B. (1976b). Characterization of prostaglandin dehydrogenase from rabbit kidney. Biochem. Pharmacol. 25 (4) : 409-411.

Curtis-Prior, P.B. (1976c). Effects of age and nutritional status on prostaglandin dehydrogenase activity of a high speed supernatant from rat kidney. Prostaglandins 11 : 645-646.

Curtis-Prior, P.B. (1976d). History and nomenclature. In 'Prostaglandins : An introduction to their biochemistry, physiology and pharmacology', pp.1-7. North-Holland, Biomedical Press, Amsterdam.

Curtis-Prior, P.B. (1976e). Biosynthesis of prostaglandins. In 'Prostaglandins : An introduction to their biochemistry, physiology and pharmacology', pp.13. North-Holland Biomedical Press, Amsterdam.

Curtis-Prior, P.B. (1976f). Catabolism of prostaglandins. In 'Prostaglandins : An introduction to their biochemistry, physiology and pharmacology', pp. 21-40. North-Holland Biomedical Press, Amsterdam.

Curtis-Prior, P.B., Jenner, M., Smethurst, M. and Woodward, J.W. (1978). Plasma prostaglandin levels in fed and starved lean, normal and obese women. Experientia 35 : 911-912.

Damas, J. (1978). Action hypotensive de l'acide arachidonique substance à action lente de l'anaphylaxie (SRS-A) et thromboxane A_2, chez le Rat. Comp. Rend. Sẽa. Soc. Biol. 172 (5) : 1025-1028.

Damas, J. and Troquet, J. (1978). L'action hypotensive de l' acide arachidonique chez le Rat et la lapin. Arch. Inter. Physiol. Biochim. 86 : 1147-1151.

Damas, J. and Volon, G. (1978). L'activation de l'acide arachidonique dans le système cardio-vasculaire du Rat. Comp. Ren. Sèa. Soc. Biol. 172 (6) : 1250-1253.

DeRubertis, F. R., Zenser, T. V. and Curnow, R.T. (1974). Inhibition of glucagon-mediated increases in hepatic cyclic adenosine 3',5'-monophosphate by prostaglandin E_1 and E_2. Endocrinology 94 : 93-101.

Donker, A.J., Arisz, L., Brentjen, J.R.H., Hem, G.K. van der, and Hollemans, H.J.G. (1976). The effect of indomethacin on kidney function and plasma renin activity. Nephron 17 : 288-296.

Dorp, D.A. van, Berthuis, R.K., Nugteren, D.H. and Vonkeman, H. (1964). Enzymatic conversion of all-cis-polyunsaturated fatty acids into prostaglandins. Nature 203 : 839-841.

Du, K.S. (1962). On the biology of the Chinese snakehead Ophiocephalus argus, in Liang-Tse Lake. Acta Hydrobid. sinica 2 : 54-66.

DuCharme, D.W. and Weeks. J.R. (1967). Cardiovascular pharmacology of prostaglandin $F_{2\alpha}$, a unique pressor agent. In'Nobel symposium 2 : Prostaglandins', ed. by Bergström, S. and Samuelsson, B., pp. 173-181. Almqvist and Wiksell, Stockholm.

DuCharme, D.W., Weels, J.R. and Montgomery, R.G. (1968). Studies on the mechanism of the hypertensive effect of prostaglandin $F_{2\alpha}$. J. Pharmacol. Expt. Therap. 160 : 1.

Eakins, K.E., Whitelocke, R.I.F., Perkins, E.S., Bennett, A. and Ungar, W.G. (1972). Release of prostaglandins in ocular inflammation in the rabbit. Nature New Biol. 239 : 248.

Eastman, A.R. and Dowsett, M. (1976). The simultaneous separation of individual prostaglandins by thin-layer chromatography on an unmodified support. J. Chromat. 444 : 33-35.

Eglinton, G., Raphael, R.A., Smith, G.N., Hall, W.J. and Pickles, V.R. (1963). The isolation andidentification of two smooth muscle stimulants from menstrual fluid. Nature (Lond) 200 : 993-995.

Emerson, T.E. Jr., Jelks, G.W. Daugherty, R.M. Jr. and Hodgmen, R.E. (1971). Effects of prostaglandin E_1 and $F_{2\alpha}$ on venous return and other parameters in the dog. Am. J. Physiol. 220 : 243.

Euler, U.S. von (1935a). Uber die spezifische Blutdrucksenkende Substanz des menschlichen prostataund Samen-blasensekrates. Klin. Wschr. 14 : 1182-1183.

Euler, U.S. von (1935b). A depressor substance in the vesicular gland. J. Physiol. Lond. 84 : 21P.

Euler, U.S. von (1934). Zur Kenntnis der pharmakologischen Wirkungen von natirsekreten und Extrackten mannlicher accessorischer Geshlechtsdrüsen. Arch. Exp. Path. Pharmak. 175 : 78-84.

Exton, J.H., Robinson, G.S., Sutherland. E.W. (1971). Studies on the role of adenosine 3',5'-monophosphate in the hepatic action of glucagon and catecholamines. J. Biol. Chem. 246 : 6166-6177.

Ferreira, S.H. and Moncada, S. (1971). Inhibition of prostaglandin synthesis augments the effects of sympathetic nerve stimulation the the cat spleen. Bri. J. Pharmacol. 43 : 491P.

Ferreira, S.H. and Vane,J.R. (1967). Prostaglandins : their disappearance from and release into the circulation. Nature 216 : 868-873.

Ferreira, S.H. and Vane, J.R. (1974). Aspirin and prostaglandins. In 'The prostaglandins', ed. by Ramwell, P.W., vol.2, pp. 1-39. Plenum Press, New York.

Ferreira, S.H., Herman, A. and Vane, J.R. (1972). Prostaglandin generation maintains the smooth muscle tone of the rabbit isolated jejunum. Bri. J. Pharmacol. 44 : 328P.

Ferreira, S.H., Moncada, S. and Vane, J.R. (1971). Indomethacin and aspirin abolish prostaglandin release from the spleen. Nature New Biol. 231 : 237-239.

Fiske, C.H. and Subbarow, Y. (1925). The colorimetric determination of phosphorus. J. Biol. Chem. 66 : 375-400.

Flower, R.J. (1974). Drugs which inhibit prostaglandin biosynthesis. Pharmacol. Rev. 26 : 33-67.

Flower, R.J. and Vane, J.R. (1972). Inhibition of prostaglandin synthetase in brain explains the anti-pyretic activity of paracetamol (4-acetamidophenol). Nature Lond. 240 : 410-411.

Flower, R.J., Cheung, H.S. and Cushman, D.W. (1973). Quantitative determination of prostaglandins and malondialdehyde formed by the arachidonate oxygenase system of bovine seminal fluid. Prostaglandins 4 : 325-341.

Flower, R.J., Gryglewski, R., Herbaczynska-Cedro, K. and Vane, J.R. (1972). The effects of anti-inflammatory drugs on prostaglandin biosynthesis. Nature New Biol. 238 : 104-106.

Frame, M.H. and Main, H.M. (1980). Effects of arachidonic acid on rat gastric acid secretion in response to different secretagogues ; inhibition of these effects by indomethacin. Bri. J. Pharmacol. 69 : 171-178.

Freas, W. and Grollman, S. (1980). Ionic and osmotic influences on prostaglandin release from the gill tissue of a marine bivalve, Modiolus demissus. J. Exp. Biol. 84 : 169-185.

Frölich, J.C. (1973). Gas chromatography-mass spectrophotometry of prostaglandins. In 'Prostaglandin', ed. by Ramwell, P.W., pp.180. Plenum Press, New York.

Frölich, J.C. (1980). Prostaglandins : Role in renin regulation and mediation of anti-phpertensive drug effects. Arch. Inter. Pharmacody. Ther. Suppl. 1980 pp. 213-224.

Frölich, J.C. Hollifield, J.W., Dormois, J.C., Frölich, B.L., Seyberth, H.J., Michelakis, A.M. and Oates, J.A. (1976). Circ. Res. 39 : 447-452.

Frölich. J.C., Sweatman, B.J., Carr, K., Splawinski, J., Watson, J.T., Änggård, E. and Oates, J.A. (1973). Occurrence of prostaglandin in urine. Adv. Biosciences 9 : 321-330.

Gangong, W.F. (1977). Energy balance, metabolism and nutrition. In 'Review of medical physiology', ed. by Gangong, W.F., 8th edition, pp.213. Lange Medical Publications, California.

Gerencser, G.A., Tyler, T. and Cassin, S. (1977). Sodium transport by isolated bullfrog small intestine effect of prostaglandin E_1. Biochim. Biophys. Acta 488 : 159-169.

Giles, T.D., Quiroz, A.C. and Burch, G.E. (1969). The effects of prostaglandin E_1 on the systemic and pulmonary circulations of intact dogs. The influences of urethane and pentobarbital anesthesia. Experentia 25 : 1056.

Glaviano, V. and Master, T. (1968). Inhibition action of prostaglandin E_1 on myocardial lipolysis. Circulation 38 : 83 (Suppl. VI).

Godard, C., Lambert, B. and Jacquemin, C. (1976). Integrated procedure for the determination of free fatty acid, glycerol, triacylglycerol and prostaglandins in rat epididymal fat pads. Biochimie 58 : 1413-1415.

Goldblatt, M.W. (1933). A depressor substance in seminal plasma. J. Sco. Chem. Ind. Lond. 52 : 1056-1057.

Goldblatt, M.W. (1935). Properties of human seminal plasma. J. Physiol. Lond. 84 : 208-218.

Gordon, M. S. (1977). Chemical correlation and control. In 'Animal physiolofy', ed. by Gordon, M.S., Bartholomew, G.A., Grinnell, A.D., Jørgensen, C.A. and White, F.D., 3rd edition, pp.614. Collier MacMillan, London.

Grande, F. and Prigge, W.F. (1972). Influence of prostaglandin E_1 on the adipokinetic effect of glucagon in birds. Proc. Soc. Exp. Biol. Med. 140 : 999-1004.

Graves, S.Y. and Dietz, T.H. (1979). Prostaglandin E_2 inhibition of sodium transport in the freshwater mussel. J. Exptl. Zool. 210 : 195-201.

Gray, D.E. (1971). Statistics for medical students : a practical guide, pp.17-23. Hong Kong University Press, Hong Kong.

Greaves, M.W. and McDonald-Gibson, W. (1972). Inhibition of prostaglandin biosynthesis by corticosteroids. Bri. Med. J. 2 : 83.

Gréen, K. (1971). The metabolism of prostaglandin $F_{2\alpha}$ in the rat. Biochim. Biophys. Acta 231 : 419.

Gréen, K. and Samuelsson, B. (1964). Prostaglandins and related factors : XIX. Thin layer chromatography of prostaglandins. J. Lipid Res. 5 : 117-120.

Gréen, K., Granström, E., Samuelsson, B. and Axen, U. (1973). Methods for quantitative analysis of $PGF_{2\alpha}$, $PGE_{2\alpha}$, 9α,11α 15-keto-prost-5-enoic acid and 9α,11α,15-trihydroxy-prot-5-enoic acid from body fluids using deuterated carriers and gas chromatography-mass spectrometry. Anal. Biochem. 54 : 434-440.

Greenberg, S., Engelbrecht, J.A. and Wilson, W. R. (1973). Cardiovascular pharmacolgy of prostaglandin B_1 and B_2 in the intact dog. Proc. Soc. Exp. Biol. Med. 143 : 1008.

Greenberg, S., Howard, L. and Wilson, W.R. (1974). Comparative effects of prostaglandins A_2 and B_2 on vascular and airway resistances and adrenergic neurotransmission. Can J. Physiol. Pharmacol. 52 : 699.

Gryglewski, R. and Vane, J.R. (1972). The release of prostglandins and rabbit aorta contracting substance (RCS) from rabbit spleen and its antagonism by anti-inflammatory drugs. Bri. J. Pharmacol. 45 : 37-47.

Güllner, H-G., Lake, C.R., Bartter, F.C. and Kafka, M.S. (1979). Effect of inhibition of prostaglandin synthesis on sympathetic nervous system function in man. J. Clin. Endocrinol. Meta. 49 (4) : 552-556.

Haessler, H.A. and Crawford, J.D. (1967). Insulin-like inhibition of lipolysis and stimulation of lipogensis by prostaglandin E_1 (PGE_1). J. Clin. Invest. 46 : 1065.

Halushka, P.V., Levanho., A. and Auber, M. (1980). Arachidonic acid stimulates short-circuit current in the isolated toad urinary bladder. J. Pharmacol. Exp. Ther. 213 (3) : 462-467.

Ham, E.A., Cirillo, K.J., Zanetti, M., Shen, T.Y. and Kuehl, F.A. (1972). Studies on the mode of action of non-steroidal, anti-inflammatory agents. In 'Prostaglandins in cellular biology', ed. by Ramwell, P.W. and Pharriss, B.B., pp. 345-352. Plenum Press. New York.

Hamberg, M. (1972). Inhibition of prostaglandin synthesis in man. Biochem. Biophys. Res. Commun. 49 : 720-726.

Hamberg, M. and Samuelsson,,B. (1971). On the metabolism of prostaglandin E_1 and E_2 in man. J. Biol. Chem. 246 : 6713-6721.

Hamberg, M. and Samuelsson, B. (1972). On the metabolism of prostaglandin E_1 and E_2 in the guinea-pig. J. Biol. Chem. 247 : 3495.

Hamberg, M., Svennon, J., Wakabayasi, T. and Samuelsson, B. (1974). Isolation and structure of two prostaglandin endoperoxides that cause platelet aggregation. Proc. Nalt. Acad. Sci. U.S.A. 71 : 345.

Handler, J.S., Butcher, R.W., Sutherland, E.W. and Oroloff, J.(1965). The effect of vasopressin and of theophylline on the concentration of adenosine 3',5'-monophosphate in the intact urinary bladder of the toad. J. Biol. Chem. 240 : 4524.

Harper, A.E. (1963). Glucose-6-phosphate. In 'Methods of enzymatic analysis', ed. by Bergmeyer, H.U. Academic Press, New York.

Hartree, F.F. (1972). Determination of protein : a modification of the Lowry method that gives a linear photometric response. Anal. Biochem. 48 : 422-427.

Heftmann, E. (1975). Chromatography, Van Nostrand-Reinhold, New York.

Hensby, C. (1977). Physical methods in prostaglandin research. In ' Monograph of organic chemistry : Prostaglandin research', ed. by Crabbe, P., vol.36, pp.89-120. Academic Press New York.

Herbaczynska-Cedro, K. and Vane, J.R. (1973). Contribution of intrarenal generation of prostaglandin to autoregulation of renal blood flow in the dog. Cir. Res. 33 : 428-436.

Herman, A.G., Claeys, M., Moncada, S. and Vane, J.R. (1978). Prostacyclin production by rabbit aorta, pericardium, pleura, peritoneum and dura mater. Arch. Inter. Pharmacodyn. Ther. 236 (2) : 302-304.

Hornych, A., London, G., Safar, M., Weiss, Y., Simon, A., Guyene, T.T., Bariéty, J. and Milliez, P. (1979). L'hémodynamique de l'hypertension artérielle labile et les prostaglandins. Arch. Mal. Caeur. 73 : 32-38.

Higgins, C.B. and Braunwald, E. (1972). The prostaglandins. Am. J. Med. 53 : 92-112.

Higgins, C.B., Vatner, S.F., Franklin, D. and Branuwald, E. (1970). Augmentation of coronary blood flow and cardiac output in conscious dogs by intravenous prostaglandin A_1. Circulation 42 : 123 (Suppl III).

Higgins, C.B., Vatner, S.F., Franklin, D., Patrick, T. and Braunwald, E. (1971). Effects of prostaglandin A_1 on the systemic and coronary circulations in the conscious dog. Cir. Res. 28 : 638.

Hochachka, P.W. (1969). Intermediary metabolism in fishes. In 'Fish physiology', ed. by Hoar, W.S. and Randall, D.J., vol.1, pp.359. Academic Press, New York-London.

Hockel, G.M. and Cowley, A.W. (1980). Role of the renin-angiotensin system in prostaglandin E_2-induced hypertension. Hypertension 2 (4) : 529-537.

Hoffman, W.E. and Schmid, P.G. (1979). Cardiovascular and antidiuretic effects of central prostaglandin E_2. J. Physiol. 288 : 159-169.

Hollstedt, C., Rydberg, U., Olsson, O. and Buijten, J. (1980). Effects of ethanol on the developing rat. I. Ethanol metabolism and effects on lactate, pyruvate, and glucose concentration. Med. Biol. 58 : 158-163.

Holmes, S.W., Horton, E.W. and Main, I.H.M. (1963). The effect of prostaglandin E_1 on responses of smooth muscle to catecholamine angiotensin and vasopressin. Bri. J. Pharmacol. 21 : 538.

Holmes, S.W., Horton, E.W. and Main, I.H.M. (1963). The effect of prostaglandin E_1 on responses of smooth muscle to catecholamines, angiotensin and vasopress. Bri. J. Pharmacol. 21 : 538.

Horrobin, D.F. (1978). Metabolism. In 'Prostaglandins : physiology, pharmacology and clinical significance', ed. by Horrobin, D.F., pp.149-150. Churchill livingstone, Vermont.

Horrobin, D.F. (1978). Chemistry, biosynthesis and metabolism. In 'Prostaglandins : physiology, pharmacolgy and clinical significance', ed. by Horrobin, D.F., pp.9. Churchill livingstone, Vermont.

Horseman, N.D. and Meier, A.H. (1978). Prostaglandin and the osmoregulatory role of prolactin in a teleost. Life Sci. 22 : 1485-1490.

Horseman, N.D. and Meier, A.H. (1979). Circadian-dependent prolactin effects : hepatic RNA metabolism and prostaglandin mediation. Gen. Comp. Endocrinol. 38 : 269-274.

Horton, E.W. (1972). Prostaglandins : monograph in endocrinology, pp.11-46. Spring-Verlag, Berlin-New York.

Horton, E.W. and Jones, R.L. (1969). Prostaglandins A_1, A_2 and 19-hydroxy A_1 : their actions on smooth muscle and their inactivity on passing through the pulmonary and hepatic portal vascular beds. Bri. J. Pharmacol. 37 : 705-722.

Horton, E.W. and Main, I.H.M. (1963). Acomparison of the biological activities of four prostaglandins. Bri. J. Pharmacol. 21 : 182-189.

Horton, E.W. and Main, I.H.M. (1967). Identification of prostaglandins in central nervous tissues of the cat and chicken. Bri. J. Pharmac. Chemother. 30 : 582-602.

Horton, E.W., Jones, R.L. and Marr, G.G. (1973). Effects of aspirin on prostaglandin and fructose levels in human semen. J. Reprod. Fert. 33 : 385-382.

Horton, E.W., Jones, R.L., Thompson, C.L. and Poyser, N.L. (1971). Release of prostaglandins. Ann. N.Y. Acad. Sci. 180 : 351-361.

Houvenaghel, A., Schrauwen, E. and Wechsung, L. (1979). Influence of primary prostaglandins, prostacyclin and arachidonic on mesenteric hemodynamics in the pig. Prost. Med. 2 : 83-95.

Hu, H.H. and Chen, S.L. (1964). The reproduction behaviour and embryonic development of the snakehead. Biol. Commu. 2 : 17-19. (in Chinese)

Iino, Y. and Imai, M. (1978). Effects of prostaglandins on Na transport in isolated collecting tubules. Pflugers Arch. 373 : 125-132.

Illiano, G. and Cuatrecasas, P. (1971). Endogenous prostaglandins modulate lipolytic processes in adipose tissue. Nature New Biol. 234 : 72.

Jaffe, B.M. and Behrman, H.R. (1974). Prostaglandins E, A and F. In 'Methods of hormone radioimmunoassay', ed. by Jaffe, B.M. and Behrman, H.R., pp. 19-34. Academic Press, New York.

Jaffe, B.M., Behrman, H.R. and Parker, C.W. (1973). Radioimmunoassay measurement of prostaglandin E, A and F in human plasma. J. Clin. Invest. 52 : 398-405.

Jarabak, J. (1972). Human placental 15-hydroxyprostaglandin dehydrogenase. Proc. Nat. Acad. Sci. 69 : 533-534.

Jones, R.L. (1970). A prostaglandin isomerase in cat plasma. Biochem. J. 119 : 64P.

Jones, R.L. (1972). Properties of a new prostaglandin. Proc. Bri. Pharmacol. Soc. 28-29th March, pp.144-155.

Jones, R.L. (1976). In 'Advances in prostaglandin and thromboxane research' ed. by Samuelsson, B. and Paoletti, R., vol.1, pp.19-27.

Jones, R.L. and Cammock, S. (1973). Purification, properties and biological significance of prostaglandin A isomerase. Adv. Biosci. 9 : 61-63.

Jones, R.L., Cammock, S. and Horton, E.W. (1972). Partial purification and properties of a cat plasma prostaglandin A isomerase. Biochim. Biophys. Acta. 280 : 588-601.

Jones, R.L., Kane, K.A. and Ungar, A. (1974). Cardiovascular actions of prostaglandin C in the cat and dog. Bri. J. Pharmacol. 51 : 157.

Jubliz, W., Frailey, J., Child, C. and Bartholomew. K. (1972). Physiological role of prostaglandins of the E (PGE), F (PGF) and A (PGA) and B (PGB) groups. Estimation by radioimmunoassay in unextracted human plasma. Prostaglandins 2 : 471-483.

Kannegiesser, H. and Lee, J.B. (1971). Difference in response to prostaglandin A and E. Nature (Lond). 229 : 498.

Kaplan, L., Lee, S-C. and Levine, L. (1975). Partial purification and some properties of human erythrocyte prostaglandin 9-keto reductase and 15-hydroxyprostaglandin dehydrogenase. Arch. Biochem. Biophys. 167 : 287-293.

Karim, S.M.M. (1968). Appearance of prostaglandin $F_{2\alpha}$ in human blood during labour. Bri. Med. J. 4 : 618-621.

Karim, S.M.M., Hillar, K. and Devlin, J. (1968). Distribution of prostaglandins E_1, E_2, $F_{1\alpha}$ and $F_{2\alpha}$ in some animals tissue. J. Pharm. Pharmacol. 20 : 749-753.

Karim, S.M.M., Sandler, M. and Williams, E.D. (1967). Distribution of prostaglandins in human tissues. Bri. J. Pharmacol. Chemother. 31 : 340-344.

Karim, S.M.M., Somers, K. and Hillier, K. (1969). Cardiovascular actions of prostaglandins $F_{2\alpha}$ infusion in man. Eur. J. Pharmacol. 5 : 117-120.

Karim, S.M.M., Somers, K. and Hillier, K. (1971). Cardiovascular and other effects of prostaglandins E_2 and $F_{2\alpha}$ in man. Cardiovasc. Res. 5 : 255.

Kather, H. and Simon, B. (1979). Biophysical effects of prostaglandin E_2 on the human fat cell adenylate cyclase. J. Clin. Invest. 64 : 609-612.

Keirse, M.J.N.C. and Turnbull, A.C. (1973). Gas chromatographic determination of E prostaglandins in human amniotic fluid. Prostaglandins 4 : 263-267.

Kirschenbaum, M.A. and Serros, E.R. (1980). Are prostaglandins natriuretic? Mineral Electrolyte Metab. 3 : 113-121.

Kondo, K., Okuno, T., Saruta, T. and Kato, E. (1979). Effects of intracerebroventricular administration of prostaglandins I_2, E_2, $F_{2\alpha}$ and indomethacin on blood pressure in the rat. Prostaglandins 17 (5) : 769-774.

Kondo, K., Okuno, T., Suzuki, H., Nakamura, R., Eguchi, T., Yasui, T., Nagahama, S. and Saruta, T. (1980). Effects of prostaglandins E_2 and I_2, arachidonic acid and indomethacin on vascular reactivity to norepinephrine in the rat. Jap. Cir. J. 44 (5) : 384-387.

Krupp, M.A., Sweet, N.J., Jawetz, E., Biglieri, E.G., Roe, R.L. and Camargo, C.A. (1979). Physician's handbook, 9th edition, pp.238. Lange Medical Publication, Singapore.

Kuehl, F.A.Jr., Cirillo, V.J., Ham, E.A. and Humes, J.L. (1973). The regulatory role of the prostaglandins on the cyclic 3',5'-AMP system. In 'Advances in the biosciences', ed. by Bergstöm, S. and Bernhard, S., vol.9, pp.155-172. Pergamon Press, Oxford.

Kuehl, F.A. Jr., Humes, J.L., Tarnoff, J., Cirillo, V.J. and Ham, E.A. (1970). Prostaglandin receptor site : evidence for an essential role in the action of luteinizing hormone. Science 169 : 883-886.

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

Kushiku, K. and Furukawa, T. (1979). Effects of prostaglandins E_1, E_2 and $F_{2\alpha}$ on the cardiac chronotropism by affecting the cardiac ganglionic transmission in spinal dogs. Life Sci. 26 : 41-47.

Lands, W.E.M., Le Tellier, P.R., Rome, L.H. and Vanderhoek, J.Y. (1973). Inhibition of prostaglandin biosynthesis. In 'Advances in the biosciences' ed. by Bergström, S. and Bernhand, S., vol.9, pp.15-28. Pergamon Press, Vieweg-Braunschweig.

Larsson, A.L. (1973). Metabolic effects of epinephrine and norepinephrine in the eel Anguilla anguilla L. Gen. Comp. Endocrinol. 20 : 155-167.

Larsson, C. and Änggard, E. (1973). Regional differences in the formation and metabolism of prostaglandins in the rabbit kidney. Eur. J. Pharmacol. 21 : 30.

Larsson, C. and Änggard, E. (1974). Increased juxta medullary blood flow on stimulation of intrarenal prostaglandin bio-synthesis. Europ. J. Pharmacol. 28 : 391.

Lederis, K. and Bern, H.A. (1976). Caudal neurosecretory system of fishes in 1976. In 'Neurosecretion and neuro-endocrine activity, evolution, structure and function', ed. by Bargmann, W., Oksche, A., Polerov, A. and Scharrer, B. Springer-Verlag, Berlin-New York.

Lee, S-C. and Levine, L. (1974). Prostaglandin metabolism I. Cytoplasmic reduced nicotinamide adenine dinucleotide phosphate-dependent and microsomal reduced nicotinamide adenine dinucleotide-dependent prostaglandin E 9-keto reductase activities in monkey and pigeon tissue. J. Biol. Chem. 249 : 1369-1375.

Lee, J.B., Covino, B.F., Takman, B.H. and Smith, E.T. (1965). Renomedullary vasodepressor substance, medullin : isolation, chemical characterization and physiological properties. Cir. Res. 17:57-77.

Lefebvre, P.J. and Luyckx, A.S. (1973). Stimulation of insulin secretion after prostaglandin E_1 in the anaesthetized dog. Biochem. Pharmacol. 22 : 1773-1779.

Leffler, C.W., Hanson, R.C. and Schneider, E.G. (1980). Effects of prostaglandins and prostaglandin precursors on the artial pressure of the bullfrog, Rana catesbiana. Comp. Biochem. Physiol. 66 : 199-202.

Lemberg, A., Wikinski, R., Izurieta, E.M., Halperin, H., Paglione, A.M., De Neuman, P. and Jauregui, H. (1971). Effects of prostaglandin E_1 and norephinephrine on glucose and lipid metabolism in isolated perfused rat liver. Biochim. Biophys. Acta 248 : 198-204.

Leskell, L.G. (1976). Influence of prostaglandin E_1 on cerebral mechanisms involved in the control of fluid balance. Acta Physiol. Scand. 98 : 85-93.

Levine, R.A. (1974). Effect of prostaglandin E_1 on hepatic cAMP activity, carbohydrate and lipid metabolism. Prostaglandins 6 : 509-521.

Levine, R.A. (1979). Effect of prostaglandins on hepatic cyclic nucleotide concentration, carbohydrate and lipid metabolism. Yale J. Bio. Med. 52 : 107-116.

Levine, R.A., Schwartzel, E.H. Jr. (1980). Prostaglandin E_2 inhibition of glucagon-induced hepatic gluconeogenesis and cyclic adenosine 3',5'-monophosphate accumulation. Biochem. Pharmacol. 29 : 681-684.

Lijnen, P.J., Amery, A.K. and Huysecom, J, (1979). Radioimmunoassay of plasma prostaglandins A,E and $F\alpha$. Arch. Inter. Physiol. Biochim. 87 (3) : 629.

Lipson, L., Hynie, S. and Sharp, G. (1971). Effect of prostaglandin E_1 on osmotic water flow and sodium transport in the toad bladder. Ann. N.Y. Acad. Sci. 180 : 261-277.

Lupulescu, A.P. (1975). Effects of prostaglandins on protein, RNA, DNA and cllagen synthesis in experimental wounds. Prostaglandins 10 : 573-579.

Malik, K.U. and McGiff, J.C. (1976). Cardiovascular actions of prostaglandins. In 'Prostaglandins : physiological, pharmacological and pathological aspects', ed. by Karim, S.M.M., pp.103-200. University Park Press, Baltimore.

Marrazzi, M.A. and Andersen, N.H. (1974). Prostaglandin dehydrogenase. In 'The prostaglandins', ed. by Ramwell, P.W. vol.2, pp.99-155. Plenum Press, New York.

Mathews, D.W., Muir, G.G. and Baron, D.N. (1964). Estimation alpha-amine nitrogen in plasma and urine by the colorimetric ninhydrin reaction. J. Clin. Path. 17 : 150-153.

Maxwell, G.M. (1967). The effect of prostaglandin E_1 upon the general and coronary haemodynamics and metabolism of the intact dog. Bri. J. Pharmacol. Chemother. 31 :162.

May, B.D., Helmstaedt, J.K. and Bohle, E. (1969). Uber metabolische wirkungen verschiedener prostaglandine tierversuch. Verh. Deut. Gesell. Inn. Med. 75 : 806-808.

McCracken, J.A., Barcikowski, B., Carlson, J.C. Green, K. and Samuelsson, B. (1973). The physiology role of prostaglandin F_2 in corpus luteum regression. In 'Advances in the biosciences', ed. by Bergström, S. and Bernhard, S., vol. 9, 599-624. Pergamon Press, Oxford.

McDonald-Gibson, R.G., Flack, J.D. and Ramwell, P.W. (1973). Inhibition of prostaglandin biosynthesis by 7-oxa- and 5-oxa-prostaglandin analogues. Biochem. J. 132 :117-120

McGill, J.C., Terrango, N.A., Strand, J.C., Lee, J.B., Lonigro, A.J. and Ng, K.K.F. (1969). Selective passage of prostaglandins across the lung. Nature (Lond.) 223 : 742.

Meyer, F.H., Jawetz,E. and Goldfien, A. (1972). In 'Review of medical pharmacology', pp. 96-98. Maruzen, Japan.

Milton, A.S. (1973). Prostaglandin release in the central nervous system during endotoxin-induced fever. In 'Supplementum to advances in the biosciences', ed. by Bergström, S. and Bernhard, S., vol.9, pp.79. Pergamon Press, Viewag-Braunschweig.

Milton, A.S. and Wendlandt, S. (1971). Effect on body temperature of prostaglandins of the A, E, and F series on injection into the third ventricle of unanaesthetized cats and rabbits. J. Physiol. (Lond.) 218 : 325.

Mullane, K.M., Dusting, G.J., Salmon, J.A., Moncada, S. and Vane, J.R. (1979). Biotransformation and cardiovascular effects of arachidonic acid in the dog. Eur. J. Pharmacol. 54 : 217-228.

Murat, J.C. and Serfaty, A. (1974). Simple enzymatic determination of polysaccharide (glycogen) content of animal tissues. Clin. Chem. 20 : 1576-1577.

Murat, J.C., Plisetskaya, E.M. and Woo, N.Y.S. (1981). Endocrine control of nutrition in cyclostomes and fish. Comp. Biochem. Physiol. 68A : 149-158.

Nakano, J. (1971). Effects of the metabolites of prostaglandin E_1 on the systemic and peripheral circulations in the dogs. Proc. Soc. Exptl. Biol. Med. 136 : 1265.

Nakano, J. (1973). Cardiovascular actions. In 'The prostaglandins', ed. by Ramwell, P.W., vol.1, pp.239-307. Plenum Press, New York-London.

Nakano, J. and Cole, B. (1969). Effects of prostaglandins E_1 and $F_{2\alpha}$ on systemic, pulmonary and splanchnic circulations in dogs. Am. J. Physiol. 217 : 222.

Nakano, J. and McCurdy, J.R. (1967). Cardiovascular effects of prostaglandin E_1. J. Pharmacol. Exptl. Therp. 156 : 538.

Ng, H.L., Kam, K.P. and Ai, Y. (1977). The Chinese poisionous and medical used fishes. Shanghai Sci. Techn. Pub. Shanghai pp. 267-269. (in Chinese).

Nilsson, N.Ö. and Belfrage, P. (1978). Effects of acetate, acetaldehyde, and ethanol on lipolysis in isolated rat adipocytes. J. Lipid Res. 19 (6) : 737-741.

Nomura, T. and Ogata, H. (1976). Distribution of prostaglandins in the animal kindom. Biochim. Biophys. Acta 431 : 127-131.

Nomura, T., Ogata, H. and Ito, M. (1973). Occurrence of prostaglandins in fish testis. Toh. J. Agric. Res. 24 : 138-143.

Nugteren, D. H. and Hazelhof, E. (1973). Isolation and properties of intermediates in prostaglandin biosynthesis. Biochim. Biophys. Acta. 326 : 448-461.

Nugteren, D.H., Beerthuis, R.K. and Dorp, D.A. van (1966). The enzymic conversion of all-cis 8,11,14-eicosatrienoic acid into prostaglandin E_1. Rec. Trav. Chim. 85 : 405.

Oates, J.A., Whorton, A.R., Gerkens, J.F., Branch, R.A., Hollifield, J.W. and Frölich, J.C. (1979). The participation of prostaglandins in the control of renin release. Fed. Proc. 38 (1) : 72-74.

Ogata, H. and Nomura, T. (1975). Isolation and identification of PGE_2 from the gastrointestinal tract of shark Triakis scyllia. Biochim. Biophys. Acta 388 : 84-91.

Ogata, H., Nomura, T. and Hata, M. (1978). Prostaglandin biosynthesis in the tissue homogenates of marine animals. Bull. Jap. Soc. Fish. 44 (12) : 1367-1370.

Okahara, T., Abe, Y., Imanishi, M., Miura, K. and Yamamoto, K. (1980). Effects of calcium ionophore, A23187, on prostaglandin E_2 and renin release in dogs. Jap. Cir. J. 44 (5) : 394-399.

Okuno, T., Kondo, K., Suzuki, H. and Saruta, T. (1980). Effect of prostaglandins E_2, I_2 and $F_{2\alpha}$, arachidonic and indomethacin on pressor responses to norepinephrine in conscious rats. Prostaglandins 19 (6) : 855-863.

Oliw, E. (1979). Prostaglandins and kidney function : an experimental study in the rabbit. Acta Physiol. Scand. (suppl.) 461.

Ono, T, Motoyama, Y., Sakai, S., Yoneda, J. and Kumada, S. (1979). Interaction between aspirin and prostaglandins in the isolated guinea-pig tracheal muscle. Jan. J. Pharmacol. 29 : 865-875.

Orczyk, P.G. and Behrman, H.R. (1972). Ovulation blockade by aspirin and indomethacin-in vivo evidence for a role of prostaglandin in gonadotrophin secretion 1 : 3.

Orloff, J., Handler, J.S. and Bergström, S. (1965). Effect of prostaglandin (PGE_1) on the permeability response on the toad bladder to vasopressin, theophylline and adenosine 3',5'-monophosphate. Nature 205 : 397.

Pace-Aciak, C.R. and Carrara, M.C. (1979). Age-dependent increase in the formation of prostaglandin I_2 by intact and homogenated aortae from the developing spontaneously hypertension rat. Biochim. Biophys. Acta 574 : 177-181.

Pace-Asciak, C.R. and Rangaraj. G. (1977). Distribution of prostaglandin biosynthetic pathways in organs and tissues of the fetal lamb. Biochim. Biophys. Acta 528 : 512-514.

Pace-Asciak, C.R. and Rangaraj, G. (1977). Distribution of prostaglsndin biosynthesis pathways in several rat tissues. Formation of 6-ketoprostaglandin F_1 . Biochim. Biophys. Acta 486 : 579-582.

Pace-Asciak, C.R. and Rangaraj, G. (1977). The 6-ketoprostaglandin $F_{1\alpha}$ pathway in the lamb ductus arteriosus. Biochim. Biophys. Acta 486 : 583-585.

Pace-Asciak, C.R. and Wolfe, L.S. (1971). N-butylboronate derivative of the F prostaglandins. Resolution of prostaglandins of the E and F series by gas-liquid chromatography. J. Chroma. 56 : 129-133.

Pace-Ascial, C.R., Carrara, M.C. and Nicolaou, K.C. (1978). Prostaglandin I_2 has more potent hypotensive properties than prostaglandin E_2 in the normal and spontaneously hypertensive rat. Prostaglandins 15 : 99-102.

Pace-Asciak, C.R., Rosenthal, A. and Dimazet, Z. (1979). Comparison between the in vivo rate of metabolism of prostaglandin I_2 and its blood pressure lowering response after intravenous administration in the rat. Biochim. Biophys. Acta 574 : 182-186.

Palmer, M.A., Piper, P.J. and Vane, J.R. (1973). Release of rabbit aorta contracting substance (RCS) and prostaglandins induced by chemical or mechanical stimulation of guinea-pig lungs. Bri. J. Pharmacol. 49 : 226.

Papanicolaou, N., Lefkos, N., Safar, M., Paris, M., Bariety, J. and Milliez, M. (1976). Direct relationship between urinary prostaglandin E and sodium excretion in essential hypertensive patient. Experientia 32 : 1280-1281.

Pennington, S.N., Smith, C.P. Jr. and Strider, J.B.Jr. (1979). Alternations in PG catabolism in rats chronically dose with ethanol. Biochem. Med. 21 : 246-252.

Pento, J.T., Cenedella, R.J. and E.K. Inskeep. (1970). Effects of prostaglandins E_1 and F_1 upon carbohydrate metabolism of ejaculated and epididymal ram spermatozoa in vitro. J. Am. Sci. 30 : 409-411.

Peyraud-Waitzenegger, M., Nomura, T., Peyraud, T. and Bras, Y. (1974). Effects cariovasculaire et ventilatoire de la prostaglandine (PGE_2) chez la Carpe (Cyprinus carpio L.). J. Physiol. 69 : 286A.

Peyraud-Waitzenegger, M., Nomura, T. and Peyraud, C. (1975). Cardiovascular and ventilatory effects of PGE_2 in the carp (Cyprinus carpio L.). International conference on prostaglandins, Florence, May 26-30, pp.126.

Pic, P. (1975). Effets des prostaglandines (PG) E_1 et $F_{2\alpha}$ sur les échanges branchiaux d'eau, de Na^+ et Cl^- chez Mugil capito adapte a l'eau de mer. J. Physiol. (Paris) 71 :146A.

Pickles, V.R., Hall, W.J., Best, F.A. and Smith, G.N. (1965). Prostaglandin in endometrium and menstrual fluid from normal and dysmenorrhoeic subjects. J. Obstet. Gynaec. Br. Commonw. 72 : 185-192.

Pinelli, A. (1973). A new colorimetric method for plasma fatty acid analysis. Clin. Chim. Acta 44 : 385-390.

Piper, P.J., Vane, J.R. and Wyllie, J.H. (1970). Inactivation of prostaglandins by the lungs. Nature 255 : 600.

Pletka, P. and Hikler, R.B. (1974). Blood prostaglandin A (PGA) levels in normal human subjects. Prostaglandins 7 : 107-115.

Polet, H. and Levine, L. (1975). Metabolism of prostaglandins E. A and C in serum. J. Biol. Chem. 250-351.

Pong, S.S. and Levine, L. (1977). Prostaglandin biosynthesis and metabolism as measured by radioimmunoassay. In 'The prostaglandins', ed. by Ramwell, P.W., vol.3, pp.41-76. Plenum Press, New York.

Powell, W.S. (1980). Distribution of prostaglandin ω-hydroxylase in different tissues. Prostaglandins 19 (5) : 701-710.

Powell, W.S., Hammarström, D. and Samulsson, B. (1974). PGF_2 receptor in ovine corpra lutea. Eur. J. Biochem. 41 : 103-107.

Poyser, N.L. (1972). Production of prostaglandins by guinea-pig uterus. J. Endocrinol. 54 : 147-159.

Putnam, F.W. (1975). Hepatoglobin in 'Plasma protein', ed. by Putman, F.W., vol.2, pp.2. Academic Press, New York-San Francisco-London.

Ramwell, P.W. (1973). The prostaglandins, vol.1. Plenum Press, New York-London.

Ramwell, P.W. (1974). The prostaglandins, vol.2. Plenum Press, New York-London.

Ramwell, P.W. (1977). The prostaglandins, vol.3. Plenum Press, New York-London.

Ramwell, P.W. and Shaw, J.E. (1970). Biological significance of the prostaglandins. Recent Progr. Hormone Res. 26 : 139.

Rettberg, H. (1968). Untersuchungen uber die wirkung von PGE_1 und insulin auf das epididymale fettgewebe und diaphragma bei ratten nach intraperitonealer injektion von $U-C^{14}$-glukose. Dissertation, Medizinischen Universitatsklinik, Johann Wolfgang Goethe Universitat, Frankfurt am Main 51P.

Rizack, M.A. (1964). Activation of an epinephrine-sensitive lipolytic activity from adipose tissue by adenosine 3',5'-monophosphate. J. Biol. Chem. 239 :392-395.

Robertson, R.P. (1973). Inhibition of insulin scretion by prostaglandin E_1 independent of hypotensive or alpha adrenergic effects. Diabetes. (Suppl I) 22 : 305.

Rodbell, M. (1964). Metabolism of isolated fat cells. I Effects of hormones on glucose metabolism and lipolysis. J. Biol. Chem. 239 : 375-380.

Romero, J.C., Dunlap, C.L. and Strong, C.G. (1976). The effect of indomethacin and other ant-inflammatory drugs on the renin-angiotensin system. J. Clin. Invest. 58 :282.

Rose, J.C. and Kot, P.A. (1977). Cardiovascular responses to the prostaglandin precursors, In 'The prostaglandins', ed. by Ramwell, P.W., vol.3, pp.135-144. Plenum Press, New York.

Rose, J.C., Johnson, M., Ramwell, P.W. and Kot, P.A. (1974). Effects of arachidonic acid on systemic arterial pressure, myocardial contractility and platelets in thedog. Proc. Soc. Exp. Biol. Med. 147 : 652.

Rossini, A.A., Lee, J.B. and Frawley, F.T. (1971). An unpredictable lack of effect of prostaglandins on insulin release in isolated rat islets. Diabetes. 20 : 374.

Sacca, L., Rengo, F., Chairiello, M. and Condorelli, M. (1973). Glucose intolerance and impaired insulin secretion by PGA_1 in fasting anesthetized dogs. Endocrinology.92 (1) : 31-34.

Salmon, J.A. and Karim, S.M.M. (1976). Methods for analysis of prostaglandins. In 'Prostaglandins : chemical and biological aspects', ed. by Karim, S.M.M., pp.25-86. Universisity Park Press, Baltimore.

Samuelsson, B. (1963a). Prostaglandins of human seminal plasma. Biochem. J. 89 : 34P.

Samuelsson, B. (1963b). Isolation and identification of prostaglandins from human seminal plasma. J. Biol. Chem. 238 : 3229-3234.

Samuelsson, B. (1964). Synthesis of tritium-labelled prostaglandin E_1 and studies on its distribution and excretion in the rat. J. Biol. Chem. 239 : 4091-4096.

Samuelsson, B. (1965). On the incorporation of oxygen in the conversion of 8,11,4,-eicosatrienoic acid into prostaglandin E_1. J. Am. Chem. Soc. 87 : 3011.

Samuelsson, B. (1971). Metabolism of prostaglandin. Ann. N.Y. Acad. Sci. 180 : 138-163.

Samuelsson, B. (1972). Biosynthesis of prostaglandins. Fed. Proc. 31 : 1442-1450.

Samuelsson, B. (1973). Quantitative aspects on prostaglandin synthesis in man. In 'Advances in the biosciences' ed. by Bergström, S. and Bernhard, S., vol.9, pp7-14. Pergamon, New York.

Samuelsson, B. (1978). Prostaglandins and Thromboxanes. In 'Recent progress in hormone research', ed. by Greep, R.O. vol.34, pp.239-258.

Samuelsson, B., Granström, E., Green, K. and Hamberg, M. (1971). Metabolism of prostaglandins. In 'Prostaglandins', ed. by Ramwell, P. and Shaw, J.E., pp. 138. Academic Press, New York.

Sanbar, S.S., Mitchell, S.A., Lockey, R.F., Vlcek, E.A. and Green,J.J.Jr. (1967). Clin. Res. pp.410.

Saunders, R.N. and Moser, C. A. (1972). Changes in vascular resistance induced by PGE_2 and $PGF_{2\alpha}$ in the isolated rat pancreas. Arch. Int. Pharmacodyn. 197 : 86-92.

Sato, M., Abe, K., Yasujima, M., Otsuka, Y., Chiba, S., Harugama, Sato, K., Imai, Y., Sakurai, Y., Ito, T., Omata, K. and Yoshinag, K. (1980). Effect of propranolol on the urinary excretion of prostaglandin E and plasma renin activity in hypertensive patient. PGs Med. 5 : 1-9.

Scherer, B, and Weber, D.C. (1979). Time dependent change in prostaglandin excretion in responses to frusemide in man. Clin. Sci. 56 : 77-81.

Scherer, B., Siess, W., and Weber, P.C. (1977). Radioimmunological decreases of PGE_2 excretion at high NaCl intake. Prostaglandins 13 : 1127-1139.

Scherer, B., Friedmann, B., Dumbs, A., Holzmann, K. and Weber, P.C. (1980). Urinary prostaglandins in human neonates : relationship to kidney function and blood pressure. Klin. Wochenschr. 58 : 449-455.

Schneider, W.P. (1976). The chemistry of prostaglandins. In 'Prostaglandins : chemical and biochemical aspects', ed. by Karim, S.M.M., pp.1-23. University Park Press, Baltimore.

Schrauwen, E. and Houvenaghel, A. (1978). Influence of arachidonic acid and its metabolites on mesenteric vascular resistance in the pig. Arch. Inter. Pharmacol. Ther. 236 (2) : 320-322.

Schrauwen, E., Beetens, J. and Korteweg, M. (1979). Low doses of prostaglandin $F_{2\alpha}$ stimulate prostacyclin (PGI_2) production in the mesenteric circulation of the pig. PGs Med. 3 : 95-96.

Schölkens, B.A. (1978). Antihypertensive effect of prostacyclin (PGI_2) in experimental hypertension and its influence on plasma renin activity in rats. PGs Med. 1 :358-372.

Seglen, P.O. (1964). Preparation of isolated rat liver cells, In 'Methods in biology', ed. by Prescott, D.M., vol.13. pp.29-83. Academic Press, New York.

Shaw, J.E. and Ramwell, P.W. (1966). Prostaglandin release from the adrenal gland. In 'Nobel symposium 2 : prostaglandins', ed. by Bergström, S. and Samuelsson, B., pp. 293-299. Almqvist and Wiksell, Stockholm.

Shaw, J.E. and Ramwell, P.W. (1968). Release of prostaglandin from rat epididymal fat pad on nervous and hormonal stimulation. J. Bio. Chem. 243 : 1498-1503.

Shaw, J.E. and Ramwell, P.W. (1969). Separation, identification and estimation of prostaglandins. Methods Biochem. Anal. 17 : 325-371.

Shehadeh, Z., Price, W.E. and Jacobson, E. D. (1969). Effects of vasoactive agents on intestinal blood flow and motility in the dog. Am. J. Physiol. 216 : 386-392.

Smith, W.L. and Lands, W.E.M. (1971). Stimulation and blockade of prostaglandin biosynthesis. J. Biol. Chem. 21 : 6700-6702.

Smith, J.B. and Willis, A.L. (1971). Aspirin selectively inhibits prostaglandin production in human platelets. Nature New Biol. 231 : 237.

Smith, J.B. and Willis, A.L. (1971). Formation and release of prostaglandins by platelets in response to thrombin. Bri. J. Pharmacol. 40 : 545P.

Somova, L. (1973). Inhibition of prostaglandin synthsis in the kidneys by aspirin-like drugs. In 'Advances in the biosciences', ed. by Bergström, S. and Bernhard, S., vol. 9, Pergamon Press, Vieweig-Braunschweig.

Somova, L. and Dochev, D. (1970). Tracing the metabolic effect of different PGE_1 and PGE_2 doses in rats with experimental hypertension. Comp. Rend. Acad. Bul. Sci. 23 : 1461-1464.

Steinberg, D., Vaughan, M., Nestle, P.J. and Bergström, S. (1963). Effects of prostaglandin E opposing those of catecholamine on blood pressure and on triglyceride breakdown in adipose tissue. Biochem. Pharmacol. 12 : 764-766.

Steinberg, D., Vaughan, M., Nestle, P.J., Strand, O. and Bergström, S. (1964). Effects of prostaglandins on hormone-induced mobilization of free fatty acids. J. Clin. Invest. 43 : 1533-1554.

Sun, F.F. and Tayler, B.M. (1978). Metabolism of prostacyclin in rat. Biochemistry 17 : 4096-4104.

Tai, H.H. and Hollander, C.S. (1973). Regulation of prostaglandin synthetase activity in rabbit kidney medulla : a possible mechanism of hormonal and drug action. In 'Supplementation to advances in the biosciences', ed. by Bergström, S. and Bernhard, vol.9, pp.5. Pergamon Press, Vieweg-Braunschweig.

Ten Berg, R.G.M., De Jong, W. and Nijkamp, F.P. (1980). Attenuated depressor response to arachidonic acid and prostaglandins in unclipped renal hypertensive rats. Bri. J. Pharmacol. 68 : 519-524.

Tietz, N.W. (1973). Electrolytes. In 'Fundamentals of clinical chemistry', ed. by Tietz, N.W., pp.673-697.

Tong, W.C.M. (1980). Studies on the osmoregulatory and metabolic effects of the urophysis in the snakehead, Ophiocephalus maculatus Lacépède. Master's Thesis. The Chinese University of Hong Kong.

Turner, C.D. and Bagnara, J.T. (1976). Introduction. In 'General Endocrinology', 6th edition, pp.3. Saunders, Philadelphia-London.

Urakabe, S., Takamitsu, Y., Shirai, D., Yuasa, S., Kimura, G., Orita, Y. and Abe, H. (1975). Effect of different prostaglandins on the permeability of the toad urinary bladder. Comp. Biochem. Physiol. 52C : 1-4.

Unger, W.G., Stamford, I.F. and Bennett, A. (1971). Extraction of prostaglandins from human blood. Nature (Lond.) 233 : 336-337.

Vance, V.K. (1973). Inhibition of prostaglandin biosynthesis as the mechanism of action of aspirin-like drugs. Adv. Biosci. 9 : 391-411.

Vane, J.R. (1969). The release and fate of vaso-active hormones in the circulation. Bri. J. Pharmacol. 35 : 209-242.

Vane, J.R. (1971). Inhibition of prostaglandlin synthesis as a mechanism of action for aspirin-like drugs. Nature New Biol. 231 : 232-235.

Vane, J.R. (1972). Prostagladins and the aspirin-like drugs. Hosp. Pract. 7 : 61-71.

Vaughan, M. (1966). An effect of prostaglandin E_1 on glucose metabolism in rat adipose tissues. In 'Nobel symposium 2 : Prostaglandins', ed. by Bergström, S. and Samuelsson, B., pp.139-142. Almqvist and Wilsell, Stockholm.

Vergroesen, A.J., de Boer, J. and Gottenbos. J.J. (1966). Effects of prostaglandins on perfused isolated hearts. In 'Nobel symposium 2 : Prostaglandins', ed. by Bergström, S. and Samuelsson, B., pp.211-218. Almqvist and Wiksell, Stockholm.

Vogt, W., Suzuki, I and Distrlkötter, B. (1966). Release of prostaglandin from frog intestine. In 'Nobel symposium 2 Prostaglandins', ed. by Bergström, S. and Samuelsson, B., pp.237-240. Almqvist and Wiksell, Stockholm.

Watanabe, A.M., McConnanghy, M.M., Strawbridge, R.A. Flemine, J.W., Jones, L.R. and Besch, K.H. Jr. (1979). J. Biol. Chem. 360 : 223.

Weber, P.C., Scherer, B., Siess, W., Held, E. and Schnermann, J. (1979). Formation and action of prostaglandins in the kidney. Klin. Wochenschr. 57 : 1021-1029.

Weeks, J.R. (1969). The prostaglandins : Biological active lipids with implications in circulatory physiogy. Cir. Res. 24 : 1.

Weeks, J.R. and Wingerson, F. (1964). Cardiovascular action of prostaglandin E_1 evaluated using unanaesthetized relatively unrestrained rats. Fedn. Proc. Fed. Am. Socs. Exp. Biol. 23 : 327.

Weeks, J.R., Schultz, J.R. and Brown, W.E. (1968). Evaluation of smooth muscle bioassays. for prostaglandins E_1 and $F_{1\alpha}$. J. Appl. Physiol. 25 : 783-785.

Weeks, J.R., Sekhar, N.C. and Ducharme, D.W. (1969). Relative activity of prostaglandins E_1, A_1, E_2 and A_2 on lipolysis, platelet, aggregation, smooth muscle and the cardiovascular system. J. Pharm. Pharmacol. 21 : 103.

Weiner, R. and Kaley, R. (1969). Influence of PGE_1 on the terminal vascular bed. Am. J. Physiol. 217 : 563-565.

Weinheimer, A.J. and Spraggins, R.L. (1969). The occurrence of two new prostaglandin derivatives (15-epi-PGA_2 and its acetate, methyl ester) in the gorgonian Plexaura homomalla chemistry of Coelenterates. XV. Tetra. Lett. 59 : 5185-5188.

Westura, E.E., Kannegiesser, H., O'Toole, J.D. and Lee, J.B. (1970). Antihypertensive effects of prostaglandin A_1 in essential hypertenion, Cir. Res. 27 : 1 (suppl. I).

Wheeler, G.E. and Epand, R.M. (1975). Prostaglandin E_1 : anommalous effects on glucose production in rat liver. Mole. Pharmacol. 11 : 335-339.

Wickramasinghe, J.A.F. and Shaw, S.R. (1973). Separation of prostaglandins A, B and C by thin layer chromatography on ferric chloride impregnated silic gel, Prostaglandins 4(6) : 903.

Willebrands, A.F. and Tasseron, S.J.A. (1968). Effect of hromones on substrate preference in isolated rat heart. Am. J. Physiol. 215 : 1089-1098.

William, E.A. (1971). The extraction of prostaglandin E_1 from human plasma. Life Sci. 10 (1) : 1181-1191.

Wilson, D.E. and Handewych, M. (1973). Prostaglandin E_1 effects on hepatic glycogenolysis. Gastroenterology 63 (3) :576.

Wilson, D.E. and Levine, R.A. (1970). Inhibition of hepatic glucose ultilization by prostaglandin E_1 (PGE_1). Clin. Res. 18 : 468.

Wolfe, L., Mamer, O. and Rostworowski, K. (1972). Prostaglandin levels in human body fluids. Clin. Res. 20 : 925.

Wong, P.Y.P., Bedwani, J.R. and Cuthbert, A.W. (1972). Hormone action and the levels of cyclic AMP and prostaglandins in the toad bladder. Nature New Biol. 238 : 27-31.

Woo, N.Y.S. (1976). The effects of salinity and hormonal factors on the intermediary metabolism of the Japanese eel, Anguilla japonica Temminck and Schlegel. Ph. D. thesis, University of Hong Kong, pp.479.

Woo, N.Y.S. and Bern, H.A. (1979). Vasoactive properties of urotensins I and II in vertebrates, with special reference to birds. Gunna Sympo. Endocrinol. 16 29-37.

Woo, N.Y.S. and Cheung, S.Y. (1980). Metabolic effects of starvation on snakehead, Ophiocephalus maculatus. Comp. Biochem. Physiol. 67A : 623-627.

Woo, N.Y.S. and Tong, W.C.M. (1981). Salinity adaptation in the snakehead, Ophiocephalus maculatus Lacépède : Changes in oxygen consumption, branchial Na^+-K^+-ATPase and body chemical composition. J. Fish. Biol. (in press).

Woo, N.Y.S., Tong, W.C.M. and Chan, E.P.L. (1980). Effects of urophysial extracts on plasma electrolyte and metabolite levels in Ophiocephalus maculatus. Gen. Comp. Endocrinol. 41 : 458-467.

Woodman, D.D. and Price, C.P. (1972). Estimation of serum total lipids. Clin. Chim. Acta 38 : 39-43.

Yamamoto, S., Ogino, N., Ohki, S., Yoshimoto, T., Bhat, S.G., Oka, J. and Hayaishi, O. (1977). Enzymological studies of prostaglandin biosynthesis. In 'Biochemical aspects of prostaglandins and thromboxanes', ed. by Kharasch, N. and Fried, J., pp.1-14. Academic Press, New York.

Young, J.E. and Chavin, W. (1965). Effects of glucose, epinephrine or glucagon upon serum glucose levels in the goldfish (Carassius auratus L.). Am. Zool. 5 : 688-689.

Ziboh, V.A., McElligot, T. and Hsia, S.L. (1973). Prostaglandin E_2 biosynthesis in human skin : subcellular localization and inhibition by unsaturated fatty acids and anti-inflammatory agents. In 'Supplementum to advances in the biosciences', ed. by Bergström, S. and Bernhard, S., vol.9, pp.71. Pergamon Press, Vieweg-Braunschweig.

Zuman, R.M. (1972). Quantitative conversion of PGA or PGE to PGB. Prostaglandins 1 : 167-168.

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