THE EFFECTS OF HYPOXIA AND HYPERCAPNIA ON PLASMA RENIN ACTIVITY IN THE RABBIT

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SUMMARY

The effects of hypoxia (7% and 10%) and hypercapnia (3% and 7%) on plasma renin activity (PRA) were studied in conscious male rabbits. Both stresses elevated PRA. However, statistically significant changes were produced during 7% O_2 and 7% CO_2. When animals breathed 7% O_2 for one hour, PRA rose from 3.799 $\pm$0.830 to 9.954$\pm$2.033 after 3o minutes, and to 11.186 ng/ml/hr after 60 minutes. That is a 162% and 194% increase, respectively. 7% CO_2 increased PRA to a statistically significant level after 60 minutes of exposure. PRA rose from 3.799$\pm$0.830 to 9.598$\pm$1.667 ng/ml/hr, which is a 152% increase.

Effects of hypoxia were also studied in sodium pentobarbital anaesthetized rabbits. Sixty minutes after induction of anaesthesia while animals were breathing room air, PRA increased by 47% but this was not statistically significant. When hypoxia (7% O_2) was substituted PRA rose very significantly 30 minutes ($p<0.002$) after starting hypoxia. The increases were from 2.691$\pm$0.308 to 9.738$\pm$1.821 ng/ml/hr after 30 minutes and to 15.997$\pm$2.209 ng/ml/hr after 60 minutes.

It can be concluded that severe hypoxia and hypercapnia elevate PRA in the rabbit; and pentobarbital anaesthesia exaggerates the increase in PRA to hypoxia.

CHAPTER ONE

INTRODUCTION

The renin-angiotensin system is an important humoral factor in the regulation of arterial blood pressure. In the operation of this system, renin is the most critical substance, since its secretion is believed to be the rate-limiting step. The secretion of renin is promoted by several stimuli. Hypoxia and hypercapnia, conditions that occur pathologically or because of extrinsic factors may also promote the secretion of renin.

Hypoxia and hypercapnia are known to be strong stimulators of the arterial chemoreceptors, resulting in reflex increase in pulmonary ventilation and in arterial blood pressure (Korner, 1971). This blood pressure response is mediated through the nervous system, and possibly by hormones (Braunwald, Ross, Kahler, Goffney, Goldblatt, and Masson, 1963; Hammill, Wagner, Latham, Frost, and Neil, 1979). Several studies have been made on the role of the renin-angiotensin system during hypoxic and hypercapnic stresses(Dusting and Staszewska-Barezak, 1976; Huidobro and Braun-Menendez, 1942; Liang and Gavras, 1978). The available experimental reports, reviewed below, are, however, inconsistent regarding the effects of hypoxia on renin secretion. The studies on the effects of hypercapnia are scanty and inadequate. Moreover,

previous studies have been made on dogs, (Skinner, McCubbin, and Page, 1964; Spath, Daugherty, Scott, and Haddy, 1971), rats (Gould and Goodman, 1970; Oliver and Brody, 1965), and humans (Hogan, Kotchen, Boyd and Hartley, 1973; Kotchen, Hogan, Boyd, Li, Sing, and Mason, 1973). There is no available study made with the rabbit, which is the most commonly-used laboratory animal in the study of experimental hypertension.

This study was, therefore, undertaken to investigate the effect of hypoxic (arterial) hypoxia and hypercapnia on renin secretion in the rabbit.

CHAPTER TWO

REVIEW OF LITERATURE

2.1. THE RENIN-ANGIOTENSIN SYSTEM

2.1.1. Components and Metabolism

The components of the renin-angiotensin system are renin, renin substrate (angiotensinogen), angiotensins I, II, III, and the angiotensin-I converting enzyme, also called kininase II.

Renin is a proteolytic enzyme synthesized and stored in the granules of the highly differentiated cells of the juxtaglomerular apparatus of the kidney (Figure 1). Earlier investigators with different technique have succeeded in localizing the specific site of renin production in the kidney. Pitcock, Hartroft, and Newmark (1959) and Tobian and associates (Tobian, Janecek, and Tomboulian, 1959) have reported that there is a correlation between the granule count of the juxtaglomerular (JG) cells and the renin content of the kidney. Hartroft (1963), using the fluorescent antibody technique, was able to localize renin by staining the JG cells. Robertson, Smeby, Bumpus, and Page (1966) noted that cultured JG cells *in vitro* produced granules. In 1963, Cook produced further evidence by actual sampling of the JG cells and assaying of the granules obtained from these JG cells for

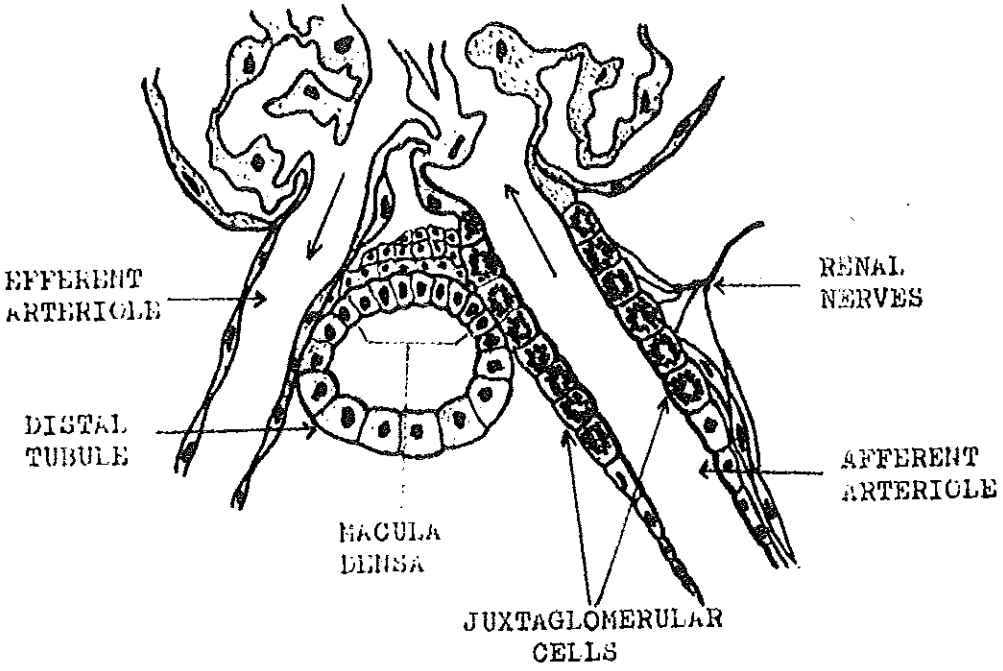

FIGURE 1

A Portion of the Glomerulus Showing the Juxtaglomerular Apparatus (From: Davis and Freeman, 1976)

renin activity. These studies have conclusively ascertained that the juxtaglomerular cells are the source of renin.

Though the kidney is known to be the primary source of renin, several studies indicate the presence of extra-renal sources of a renin-like pressor substance. These include the placenta (Symonds, Stanley, and Skinner, 1968), the uterus (Hodari, Carretero, and Hodgkinson, 1969), and the brain (Fischer-Ferraro, Nahmod, Goldstein, and Finkielman, 1971; Ganten, Minnich, Granger, Hayduk, Michael, Barbeau, Boucher, and Genest, 1971). However, the nature and the physiological significance of this renin-like pressor substance are not known, though it is believed to be identical with renin of renal origin.

Renal renin is released from the JG cells into the lumen of the renal afferent arterioles. It has a circulatory half-life of between 15 and 20 minutes, which was demonstrated in the anaesthetized dog by Hodge, Ng, and Vane (1967). In the circulation it acts on angiotensinogen, a plasma protein synthesized in the liver (Nasjletti and Masson, 1971), to produce the decapeptide angiotensin I (Figure 2). This decapeptide has a very weak pressor activity (Aiken and Vane, 1970; Ng and Vane, 1968). It is acted upon by the converting enzyme while passing through the lungs (Caldwell, Seegal,

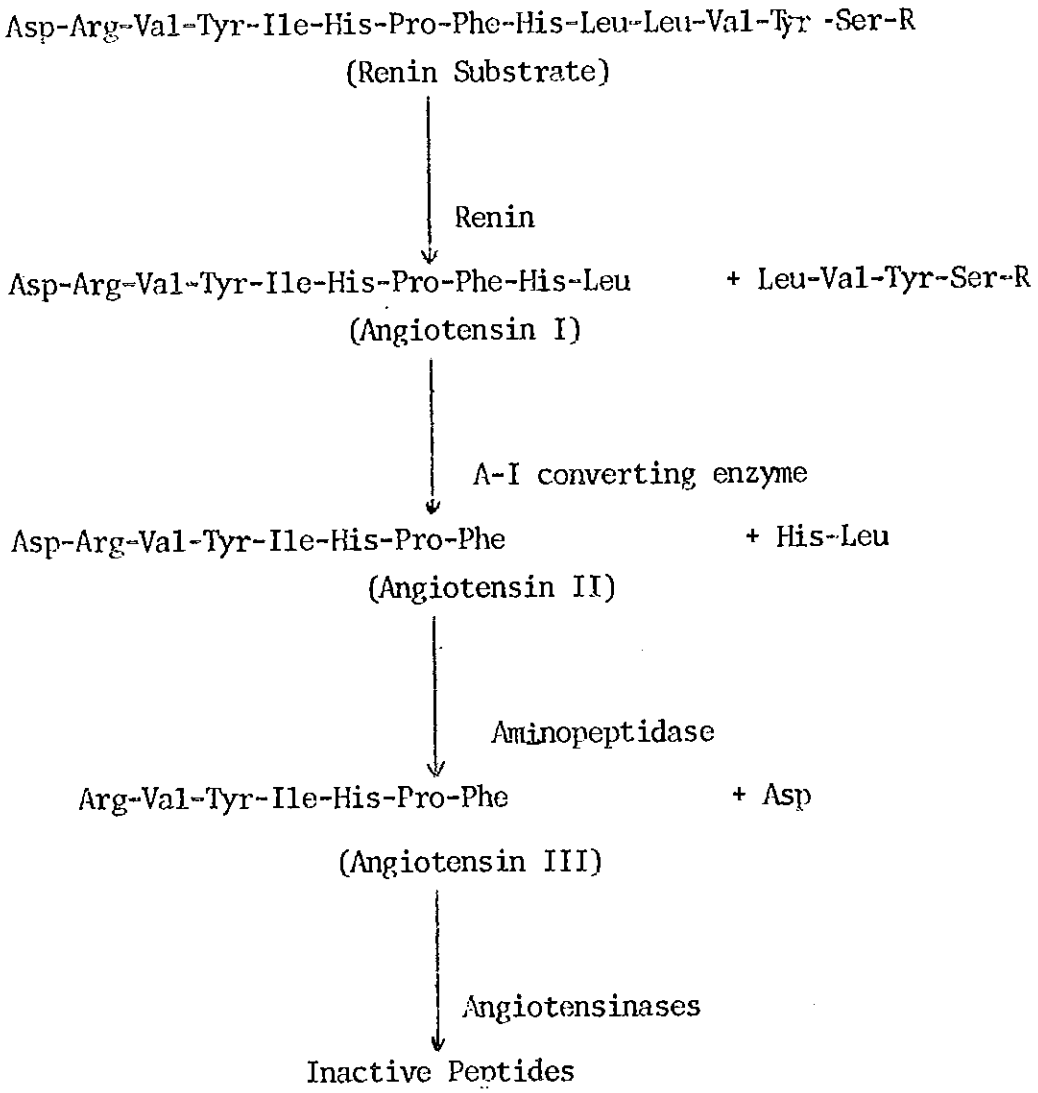

FIGURE 2

The Renin-Angiotensin System: Formation of Angiotensins I, II, and III from Angiotensinogen (Adapted From: Bakhle, Y.S. 1980 and Sofer, R.L., 1976).

and Hsu, 1976; Ng and Vane, 1967; Oparil, Sanders, and Haber, 1970a;Ryan, Stewart, Leary, and Ledingham, 1970; Yang, Erdos, and Levin, 1970). Two amino acids are split off to form the octapeptide angiotensin II, which is a biologically active component of the renin-angiotensin system. Though the lungs constitute a major site for the conversion of angiotensin I, other sites - the kidney, liver, pancreas, spleen - can convert angiotensin I to angiotensin II to some extent (Bakhle, Reynard, and Vane, 1969; Caldwell et al., 1976; Kreye and Gross, 1971; Oates and Stokes, 1974; Oparil et al., 1970a).

Angiotensin II has a short half-life in the circulation. In vitro determination, synthetic angiotensin II incubated with rat plasma showed a half-life of about 146 seconds (Hodge et al., 1967). But in vivo, in different vascular beds, it has a much lesser half-life of 15 to 20 seconds (Ferreira and Vane, 1967; Hodge et al., 1967; Oparil and Baillie, 1973). However, it has been reported (Hodge et al., 1967) that the half-lives of different angiotensin II varies. The horse angiotensin II has a half-life of 183 seconds, whereas that of the bovine is 282 seconds. Angiotensin II is rapidly metabolized in various vascular beds, notably the liver and kidney (Aiken and Vane, 1972;

Bakhle et al., 1969; Leary and Ledingham, 1969; Oparil and Bailie, 1973) to produce angiotensin III, a heptapeptide formed by the removal of a terminal amino aicd from angiotensin II. This heptapeptide, of the subsequent degradation products of angiotensin II, is the only fragment that has a significant biological activity. It is equipotent to angiotensin II in stimulating aldosterone secretion (Blair-West, Coghlan, Denton, Funder, Scoggins, and Wright, 1971; Bravo, Khosla, and Bumpus, 1976; Campbell, Brooks, and Pettinger, 1974), and its pressor action is nearly half that of angiotensin II (Freeman, Davis, Lohmeier, and Spielman, 1976; Steele, Neusy, and Lowenstein, 1976).

2.1.2. Physiological Significance

The renin-angiotensin system is known for its effect on arterial blood pressure. The magnitude of arterial blood pressure is determined by the cardiac output and the total peripheral resistance, and it is these two variables that are manipulated to regulate blood pressure. Thus, an increase in either arteriolar resistance or a rise in cardiac output results in increased blood pressure.

Blood pressure is regulated at a fairly constant level by several, intimately interrelated factors that

affect the cardiac output and/or the vascular resistance, either positively or negatively. The autonomic nervous system controls the cardiac output and the resistance of the vessels, and also activates other regulatory factors (Baum, 1977; Herd, 1970) depending on the input emanating from the various cardiorespiratory receptors (Korner, 1971). The endocrine system is also involved in the regulation of blood pressure. Epinephrine and norepinephrine, released from the adrenal medulla under the influence of the sympathetic system, do excite the heart and constrict most blood vessels, respectively increasing heart rate and vascular resistance, resulting in an elevated blood pressure (Baum, 1977). Cortisol, a glucocorticoid, appears to be essential for the effects of these catecholamines on blood pressure (Muriuki, Horrobin, and Burstyn, 1972).

Vasopressin (Antidiuretic hormone, ADH) with both antidiuretic and pressor properties (Johnston, Newman, and Woods, 1981) can elevate blood pressure by decreasing water loss from the kidneys and by its constrictor action or blood vessels. Another group of effector substances are the prostaglandins, released from various tissues of the body (Alpert and Hickler, 1971). These derivatives of essential fatty acids exhibit opposed functions: some are vasoconstrictors and others are

vasodilators (Herd, 1970). According to Alpert and Hickler, most of them increase cardiac output in dogs. Bradykinin causes a lowering of blood pressure because of its vasodilator activity (De Carvalho, 1971; Herd, 1970; Schachter, 1968).

Aldosterone, a mineralocorticoid hormone from the adrenal cortex, is considered (Guyton, Coleman, and Granger, 1972) as an important factor in long-term pressure regulation. This hormone increases the reabsorption of sodium by the renal tubules, causing an expansion of the extracellular fluid volume including the plasma portion of blood. This in turn elevates the cardiac output, thereby, raising arterial blood pressure. It also alters the sensitivity of the baroreceptors (Burstyn and Horrobin, 1970). The role of angiotensin II in pressure regulation is discussed below. All these factors work harmoniously to maintain the blood pressure at a fairly constant level in a resting subject.

The significance of angiotensin II in the maintenance of blood pressure has been shown by many investigators using different methods. Intravenous or intra-arterial administration of angiotensin II in doses between 0.2 to 0.4 µg in dogs (Bickerton and Buckley, 1961) and single intraventricular injection of 0.5 µg of angiotensin II in rats (Severs, Summy-

Long, Taylor, and Connor, 1970) elevate blood pressure markedly. Bean, Brown, Casals-Stenzel, Fraser, Lever, Morton, Petch, Riegger, Robertson, and Tree in 1979, showed that angiotensin II infused over a long period, can cause a progressive rise in arterial pressure which gradually dropped to normal in 48 hours when the infusion was stopped. In these studies synthetic angiotensin II was used. Johnson and Davis (1973) had approached the problem using an angiotensin II antagonist in dogs. An intravenous adminstration of the antagonist caused a marked fall in pressure which was accompanied by rise in plasma renin. Further evidence is also provided by the use of an angiotensin I converting enzyme inhibitors (CEI) given orally (McCaa, Hall, and McCaa, 1978) or intrarenally (Abe, Miura, Iamnishi, Yukimura, Komori, Okahara and Yamamoto, 1981) in dogs; and orally or intravenously in humans (MacGregor, Markandu, Roulston, Jones, and Morton, 1981; Niarchos, Pickering, Case, Sullivan, and Laragh 1979). In all these studies, administration of CEI produced a significant fall in systemic pressure, with a concomitant rise in plasma renin activity.

These studies support the view that the renin-angiotensin system contributes to the maintenance of blood pressure. However, it must be noted that excess

renin in the circulation may cause an abnormally high blood pressure-hypertension; and it is this that has attracted the interests of many researchers in the study of the renin-angiotensin system. Its determination in plasma has significant clinical value in the diagnosis of certain forms of hypertension.

Three different mechanisms are recognized by which angiotensin II participates in pressure regulation. First, angiotensin II, being a potent vasoconstrictor, primarily or arterioles (Vander, 1976), can cause a significant increase in vascular resistance and thus elevate blood pressure. Secondly, angiotensin II can activate the central nervous sytem resulting in increased sympthetic vasomotor discharge (Bickerton and Buckley, 1961; Ferrario, Gildenberg, and McCubbin, 1972; Severs, Daniels, Smookler, Kinnard, and Buckley, 1966; Smookler, Severs, Kinnard, and Buckley, 1966) and promotes the release of vasopressin (Severs et al., 1970; Suzuki, Kondo, Hondo, Kawabe, and Saruta, 1982) thus indirectly increasing arterial blood pressure. Finally, angiotensin II stimulates the secretion of aldosterone (Bravo et al.,1976; Campbell et al., 1974; Davis, Ayers, and Carpenter, 1961; Freeman et al., 1976; Laragh, Angers, Kelly, and Lieberman, 1960), which ultimately casues a rise in blood pressure.

2.1.3. Regulation of Renin Secretion

The stimulation of renin secretion can be effected by: 1) the renovascular baroreceptors sensing alterations in renal vascular pressure, 2) the macula densa receptor sensitive to the sodium load delivered to the distal tubule, and 3) the renal sympathetic nerves. The JG cells release renin when the distending (transmural) pressure in the afferent arterioles is lowered, the amount of sodium delivered to the macula densa is reduced, or when the activity in the renal sympathetic nerves is increased (Davis and Freeman, 1976).

Operating through the above mechanisms, several stimuli affect renin secretion by the kidneys. These include hemorrhage (McKenzie, Lee, and Cook, 1966; Weber, Thornell, and Stokes, 1973), anaesthesia (Pettinger, Tanaka Keeton, Campbell, and Brooks, 1975), use of oral contraceptives (Beckerhoff, Leitscher, Wilkinson, Gonzales, and Nokes, 1972; Dustan, Tarazi, and Frohlich, 1970), prolonged sodium deficiency (Brown, Fraser, Lever, Love, Morton, and Robertson, 1972) and postural changes (Cohen, Conn, and Rovner, 1967; Kala, Fyhrquist, and Eisola, 1974; Oparil, Vassaux, Sanders, and Haber, 1970b). Renin secretion is also increased in conditions which are associated with decreased oxygen and increased carbon dioxide tensions, such as asthma and heart failure.

2.2. HYPOXIA AND HYPERCANIA

Hypoxia, literally meaning low oxygen is of four types (Folkow and Neil, 1971). These are:

1. Hypoxic or arterial hypoxia: a situation where the arterial blood oxygen tension (PaO_2) is low. This type of hypoxia is encountered at high altitudes and during severe excercise (Korner, 1959). It may also be the result of impaired ventilation in chronic lung diseases such as asthma, pneumonia, pulmonary oedema, and destruction of lung tissue in tuberculosis. Hypoventilation due to decreased pulmonary compliance, or neuromuscular disorder, can produce arterial hypoxia (Guyton, 1976).

2. Stagnant hypoxia: is where the amount of oxygen delivered to tissues is insufficient due to poor circulation, as in tissue ischemia and reduced cardiac output.

3. Anaemic hypoxia: caused by a reduction in haemoglobin available to transport oxygen.

4. Histotoxic hypoxia: where the ability of cells to utilize oxygen is reduced due to paralysis of cellular enzymes. A common case is cyanide poisoning.

Hypercapnia, meaning excess carbon dioxide, is a condition where the arterial blood carbon dioxide tension

($PaCO_2$) is increased above the normal value. It usually occurs in association with hypoxia, when the latter is caused by hypoventilation due to impaired neuromuscular capability. It is also caused by circulatory deficiencey, where diminished flow of blood decreases removal of carbon dioxide, and in chronic emphysaema (Guyton, 1976); or it may occur in a person who is confined to a small space for a long time.

2.2.1. Circulatory Effects

Hypoxia and hypercapnia entail various circulatory and respiratory adjustments, but the responses observed in different experiments are not in complete agreement. During hypoxia, elevation in blood pressure has been observed in the cat (Weissman, Rubinstein, and Sonnenschein, 1976) and in dogs (Borgia and Horvath, 1977; Hammill et al., 1979; Kontos, Vetrovec, and Richardson, 1970; Sylvester, Scharf, Gilbert, Fitzgerld, and Traystman, 1979). In these studies the gas mixtures used were 7 to 10% oxygen in nitrogen. On the other hand, Daly and Scott (1963) in dogs artificially respiring 5% oxygen, found a small fall in blood pressure in ninteen tests, with a rise in only two tests. Working with the unanaesthetized rabbit, Korner (1963) reported that blood pressure did not change when animals respired 10.4% and 9.6% oxygen in

nitrogen. And according to Macleod and Scott (1964), in cats the observed blood pressure change was variable though the predominant response was a rise in pressure.

This variability in observations is also true for other circulatory parameters. Cardiac output and the vascular resistance, which are determinants of blood pressure, either are elevated or reduced. But in most of the research, results favour an elevation of cardiac output (Daly and Scott, 1963; Downing et al., 1962) and a fall in the vascular resistance (Baugh, Cornett, and Hatcher, 1959; Daly and Scott, 1963; Hammill et al., 1979)

The responses observed during hypoxia are also manifested when an animal is subjected to hypercapnia. But little work has been done to see the cardiovascular effect of hypercapnia. Richardson and collaborators (Richardson, Wasserman, and Patterson, 1961) studied the effect of this stress in humans exposed to 5 to 7% carbon dioxide in air for periods of 5 to 15 minutes. They were able to demonstrate that the responses to hypercapnia include increases in heart rate, cardiac output, and blood pressure, but a decrease in the total vascular resistance. However, Weissman et al., (1976) have noted a significant increase in vascular resistance and blood pressure in cats respiring 10% carbon dioxide. The variability here

could be due to difference in the degree of hypercapnia. Dusting and Staszewska-Barczak (1976) and Staszewska-Barczak (1978) also have demonstrated an elevation in arterial pressure in dogs breathing 10% carbon dioxide in air. They did not measure other circulatory parameters.

The special chemosensitive cells of the peripheral chemoreceptors are important for the various circulatory responses to hypoxia and hypercapnia.

2.2.2. The Peripheral Chemoreceptors

The peripheral chemoreceptors consist of the aortic and carotid chemoreceptor bodies, respectively located at the aortic arch and at the bifurcation of the common carotid artery (Biscoe, 1971). These chemoreceptor bodies are innervated by the sinus nerve and by the superior cervical ganglion. These receptors are concerned with the control of respiration and adjustments of the cardiovascular activity in response to changes in oxygen and carbon dioxide tensions of the arterial blood. These chemoreceptors send signals to the medullary center (Shephered and Vanhoutte, 1979) concerning decreases in oxygen tension and pH, or increases in carbon dioxide tension of the arterial blood. Their impulse activity is increased in circumstances of hypoxia and hypercapnia

(Heymans and Neil, 1958; Korner, 1959; Sampson and Hainsworth, 1972), their discharge rising exponentially with reduction in oxygen, or rise in carbon dioxide tensions. The peripheral chemoreceptors, as stated by Korner (1971), account for virtually the entire cardiorespiratory responses at all levels of hypoxia, but during changes in blood carbon dioxide tension, the central chemoreceptors located in the ventral medulla are also involved (Braunwald et al., 1963; Sorensen, 1971).

Experiments show that stimulation of these receptors with hypoxia or hypercapnia elicit reflex increase in respiration and in arterial blood pressure, and that these receptors are responsible for these reflex responses. Korner, in 1965, showed that the respiratory and cardiovascular responses to 10.5% oxygen were abolished when the aortic and carotid nerves were sectioned. This finding has been confirmed in the works of Chalmers, Korner, and White, (1967) and Crocker, Johnson, Korner, Uther, and White (1968) in the rabbit.

The activity of the receptors during arterial hypoxia has also been studied by Kontos et al., (1970). In their experiments dogs breathing 7.5% oxygen exhibited elevated arterial blood pressure, but after chemoreceptor-inactivation by injection of acetic acid, blood pressure failed

to change from the control values, indicating the importance of the chemoreceptor bodies. Pelletier (1972) also demonstrated that independent changes in PaO_2 and $PaCO_2$ at the carotid bodies causes circulatory responses through the chemoreflex mechanism, and that their magnitude is proportional to the intensity of the stimulation. At the same time, Sampson and Hainsworth (1972) found that the activity of the aortic body chemoreceptors increased when PaO_2 was lowered and when $PaCO_2$ was elevated in cats.

From the foregoing it is obvious that chemoreceptor activation with hypoxia and hypercapnia produce a reflex increase in blood pressure, caused mainly by increased cardiac output during hypoxia, and increased cardiac output and total vascular resistance during hypercapnia. The circulatory response under the two stresses are mediated by the sympathetic nervous system (Braunwald et al., 1963; Downing et al., 1962; Hammill et al., 1979) and the adrenal catecholamines (Baugh et al., 1959; Korner and White, 1966; Sechzer, Egvert, Linde, Cooper, Dripps, and Price, 1960; Tenney, 1969), and probably by the renin-angiotensin system also. Investigating the role of the renin-angiotensin system as a factor in this response has been the subject of interest for some time.

2.2.3. Hypoxia, Hypercapnia and Renin Secretion

The role of the renin-angiotensin system in blood pressure rise during hypoxia and hypercapnia has not been established with certainity. However, various studies have been made since the pioneering work of Huidobro and Braun-Menendez in 1942, but the results obtained so far are not in complete agreement.

Huidobro and Braun-Menendez (1942) did some experiments to see if hypoxia and hypercapnia have any effect on renin secretion. In dogs respiring hypoxic (7-8% O_2) and hypercapnic (2-5% CO_2) gas mixtures, they found no demonstrable change in renin secretion. A decade latter, Divey (1951) also reported that there is no change in arterial pressor activity when dog kidney was subjected to local hypoxia produced by perfusing it with venous blood from a donor dog. The same result was obtained by Skinner et al., (1964) working with dogs. In these experiments renin secretion was determined by bioassay.

Since increased granularity of the JG cells indicates increased renin secretion, other investigators approached the problem using the JG cells granularity as a measure of renin secretion. Thus Goldfarb and Tobian (1962) using this technique observed no change in the granule count of the JG cells in rats rendered hypoxic by being

kept in a chamber at one-half atmospheric pressure for 12 hours. However, Demopoulos, Highman, Altland, Gerving, and Kaley in 1965, during intermittent exposures of rats to simulated high altitudes for a period of six weeks, and Oliver and Brody (1965), in rats kept for two weeks in an hypoxic atmosphere, have demonstrated an increase in the granularity of the JG cells.

Slater and co-workers (Slater, Tuffley, Williams, Bersford, Sonksen, Edwards, Eking, and McLaughlin) exposed men to mild hypoxia produced by an ascent to an altitude of 3500 m (11,438 ft.). In all the subjects plasma renin activity rose slightly on ascent and fell on descent. Using the radioimmunoassay technique for determining plasma renin, Gould and Goodman (1970) studied the effect of hypoxia an plasma renin activity on rats kept at a reduced air pressure of 0.48 atmosphers from five to twenty five days. During the first 24 hours of hypoxia, while renin substrate was elevated there was no effect on plasma renin activity. But a significant increase in plasma renin was noted during the long-term hypoxia of 25 days. The increase was first noted after three days of exposure followed by a greater increase in kidney renin on the fifth day of hypoxia. Spath et al., (1971) experimented with dogs by subjecting the

kidney to local hypoxia in situ. Eventhough the renal hypoxia was severe (6 mm Hg), renin activity was not significantly different from that of the controls. However, it must be noted that their work by-passes the stimulation of the chemoreceptors from which responses to hypoxia and hypercapnia are known to originate. In unanaesthetized dogs exposed to hypoxic gas mixture for 20 minutes, Liang and Gavras (1978) have found a significant increase in PRA when oxygen is lowered from 8% to 5%. At 5% oxygen plasma renin activity rose from 2.8 ± 0.4 to 8.4 ± 1.8 ng/ml/hr, while at 8% oxygen it increased from 2.3 ± 0.4 to 4.9 ± 0.8 ng/ml/hr.

Other studies have produced results which contradict the above. Braverman and Rostorfer (1970) have reported that hypoxia inhibit the secretion of renin. This observation has also been reported by Hogan et al., (1973) and Kotchen and his group (1973). In their studies men were exposed to a simulated altitude of 12, 000 ft. - comparable to that employed by Slater et al., 1969 - for 72 hours. The results show that plasma renin was decreased during all three days of exposrue to hypoxia.

Studies concerning the effects of hypercapnia demonstrate that the rise of arterial blood pressure during this stress is associated with increased plasma renin (Dusting and Staszewska-Barszak, 1976; Stas ewska-Barczak, 1978).

CHAPTER THREE

MATERIALS AND METHODS

Animals

Seven male albino rabbits (weight range 1.75-2.32 kg, mean weight 2.08 kg) obtained from the Central Laboratory and Research Institute (CLRI) and the Ethiopian Meat Enterprises (EME), were used for the experiments with hypoxia and hypercapnia. Another group of five rabbits (mean weight 1.65 kg) were used to determine the time interval at which supplementary anaesthesia is to be given during the experiments in anaesthetized animals. The rabbits were kept in separate compartments, water and feed ('coarse wheat meal' supplemented with cabbages) given ad libitum.

Gas Mixtures

Hypoxic (10% and 7% O_2) and hypercapnic (3% and 7% CO_2) gas mixtures were prepared from cylinders containing oxygen, carbondioxide, or nitrogen gasses, using a gas flow meter (wet-type Gallenkamp, England). Hypoxic gas mixtures of 10% and 7% oxygen were respectvively prepared by mixing 10 liters and 7 liters of oxygen with nitrogen to give 100 liters of gas mixture. Hypercapnic gas mixtures of 7% or 3% carbon dioxide were prepared

by mixing 7 or 3 liters of carbon dioxide with 20 liters of oxygen and the balance of nitrogen to give a total volume of 100 liters. All gas mixtures were collected into a Douglas bag fresh before an experiment. For a certain number of these gas mixtures, the actual percentages of O_2 and CO_2 were analyzed using a Lloyd Gas Analyser (Gallenkamp, England). The results, presented in appendix 1, indicate that the gas mixtures prepared correspond to the intended percentage with less than 0.5% deviation.

Determination of Time Interval for Supplementing Anaesthesia

In experiments with anaesthetized rabbits, 40 mg/kg body weight of sodium pentobarbital (40 mg/ml in 0.9% saline) was given initially, supplemented with 4 mg/kg, administered through the marginal ear vein. The dose used was from the literature, but it was necessary to determine the interval when a supplementary dose of anaesthesia had to be given in order to maintain the level of anaesthesia. Therefore, the time interval at which supplementary anaesthesia was to be given was determined in five rabbits. Each rabbit received an initial dose of pentobarbital (40 mg/kg) over a period of 5 minutes. Fifteen minutes after administering of anaesthesia, rabbits were checked for the level of anaesthesia by

tail-pinching every five minutes. The supplementary dose (4 mg/kg) was given whenever there was a response to the tail-pinching. This observation was continued for 90 to 120 minutes. The cummulative dose (mg/kg) at every positive response was plotted against time, and a regression line drawn (Appendix 2). From this, the average rate at which the supplementary anaesthesia should be infused after the initial dose, was determined from the graph. The average rate thus found was 0.21 ml per minute, or 4 mg per 20 minutes.

Experimental Protocol

All experiments were done between 9:00 and 12:30 a.m. During an experiment, a rabbit was kept in a restraint box in the prone position with the head protruding out and breathing spontaneously either room air or one of the gas mixtures through a funnel fitted around the mouth and nose (Figure 3). The funnel was connected to a Palmer ideal pump (Bioscience, England) pumping at the rate of 54 strokes per minute and 14 ml per stroke.

In all rabbits blood samples to determine the basal PRA value, were taken earlier, before starting the experiments, without using the respirating funnel and the ideal pump. In experiments with hypoxia and hypercapnia,

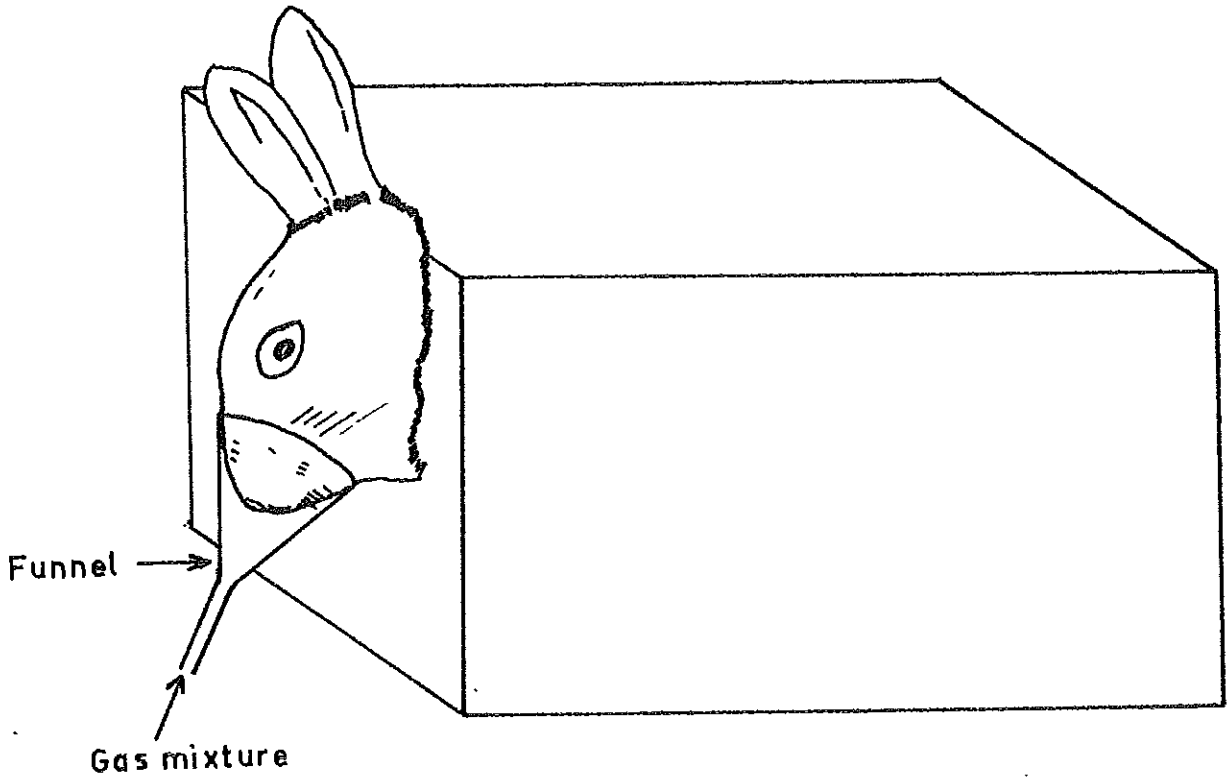

FIGURE 3

Position of rabbit during an experiment. The funnel is connected to a Palmer ideal pump which, in turn, is connected to a douglas bag containing gas mixture.

blood samples for PRA were collected at 30 and 60 minutes after starting breathing a gas mixture.

Four experiments were carried out on each of the seven unanaesthetized rabbits to see the effects of hypoxia and hypercapnia on renin release. To investigate the effects of pentobarbital anaesthesia on the renin secretion during hypoxia, a total of nine experiments were made on four anaesthetized rabbits. In these experiments, blood samples for PRA were taken one hour after anaesthesia, with the animals breathing room air, and at 30 and 6o minutes with the animals breathing 7% oxygen.

Collection of Plasma

Each blood sample, of about 2.5 ml., was collected from the ear artery into a pre-chilled glass tube containing 3.2 mg of disodium ethylenediaminetetra-acetic acid (Na_2EDTA). The blood was immediately centrifuged at 2500 rpm for 20 minutes, the plasma separated and stored at -20°C until the day of the assay.

Determination of Plasma Renin Activity (PRA)

Plasma renin activity was determined by radioimmunoassay (RIA) using 'renin activity radioimmunoassay' kits (Becton Dickinson Immunodiagnostics, Mountain View Avenue, Orangeburg, New York 10962-1294). The protocol followed was that provided with the RIA

kit, which is based on the method of Haber, Koerner, Page, Kliman, and Pernode (1969). This is as follows:

"To 1 ml of plasma buffered at pH 7.4 with tris Chloride buffer (100 µl) is added 10 µl of each of dimercaprol and 8-hydroxy-quinoline, which are angiotensin-I-converting enzyme inhibitors. Half the amount is incubated at 37°C for 3 hours in a water bath to generate angiotensin I, and the other half (blank) is simultaneously kept at 4°C for the same period of time. After incubation 50 µl aliquots are assayed for angiotensin I. The radioimmuno-assay of these plasma is made together with the angiotensin standards. 100 µl of the angiotensin I tracer (labelled with ^{125}I on the tyrosine residue), and 100 µl of the antibody, are added to known amounts of angiotensin I, covering the range of 0.02 ng to 0.50 ng per tube, and the plasma samples in tris acetate buffer, pH 7.4. The tubes are then incubated at 4°C to equilibriate. At the end of equilibriation, antibody-bound and free tracer peptide are separated by dextran-coated charcoal. That is, 1 ml of the charcoal suspension added to each tube, is mixed and then centrifuged at 2700 rpm for 15 minutes at 4°C. Radioactivity in the clear supernatant is counted in a gamma counter for 1 minute or more.

The concentration of angiotensin I per tube in plasma is read from a standard curve, constructed using known quantities of synthetic angiotensin I. The amount of angiotensin I per ml of plasma is then computed for the incubated (37°C) and the blank (4°C) samples. Subtracting the blank value from the incubated sample yields the amount of angiotensin I generated in the 3 hours of incubation.

This value divided by 3 (hr of incubation) gives the angiotensin I generation rate or the plasma renin activity, expressed as ng AI/ml/hr, that is, ng of AI generated per ml of plasma per hour of incubation".

A Wilj 2001 gamma counter was used during this investigation, and the counting time was 100 seconds. Worked example to show the radioimmunoassay determination of PRA is presented in Appendix 3-A, B and C.

Statistical Analysis

The data obtained were analyzed by computing the means and standard errors of the means, and paired t-tests for differences were considered statistically significant for $p \leq 0.05$.

CHAPTER FOUR

RESULTS

Hypoxia

Changes in plasma renin activity produced by ventilation with 10% and 7% oxygen in unanaesthetized animals are summarized in Table 1. Breathing 10% hypoxic gas mixture did not induce significant renin release in a one hour duration. PRA rose from a basal value of 3.799 $\pm$0.830ng/ml/hr to 7.684$\pm$2.130 and 7.775$\pm$1.808ng/ml/hr after 30 and 60 minutes of hypoxia, respectively.

On the other hand, 7% oxygen, which is a stronger stimulus, had a striking effect on renin release. At this level of hypoxia, PRA rose from a baseline value of 3.799$\pm$0.830 to 9.954 $\pm$2.033 in 30 minutes and to 11.186 $\pm$2.958ng/ml/hr in 60 minutes after hypoxia was started. These increases in PRA were statistically significant ($p<0.05$). Even though significant rises in PRA occurred at both 30 and 60 minutes, as compared with control values, the increase during the first 30 minutes was greater than that which occurred during the second 30 minutes (Figure 4). While the total increase in PRA at 60 minutes was 194%, the increase at 30 minutes was 162%.

Hypercapnia

Table 2 shows the results of the experiments in unanaesthetized rabbits during hypercapnic stimulation.

Breathing gas mixture containing 3% carbon dioxide for one hour had only a small effect on renin release. The percent increase at 30 minutes of hypercapnia was 25.4% and remained unchanged (25.2%) during the following 30 minutes period (Figure 5).

TABLE I

Plasma Renin Activity in 7 unanaesthetized rabbits before and during hypoxia

RABBIT		BASAL VALUE	PLASMA RENIN ACTIVITY 9ng AI/ml/hr)			
			10% O_2		7% O_2	
No.	WEIGHT (kg.)		30 min	60 min	30 min	60 min
1	2.32	1.672	1.753	4.495	9.533	4.371
2	2.00	3.720	10.083	12.628	6.351	10.268
3	2.12	2.486	18.795	15.525	20.995	24.258
4	1.75	2.987	3.733	3.806	11.449	7.431
5	2.20	8.059	8.353	9.102	7.832	-
6	2.25	2.444	6.204	5.676	9.152	14.212
7	1.95	5.229	4.869	3.190	4.363	6.578
	Mean	3.799	7.684	7.775	9.954	11.186
	±SE	0.830	2.130	1.808	2.033	2.958

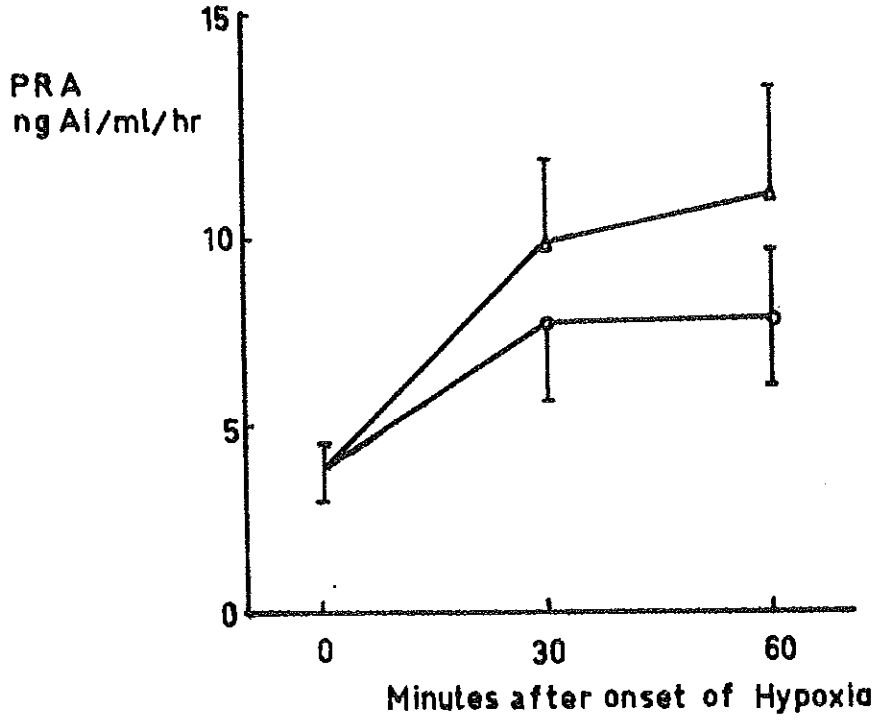

FIGURE 4

Plasma renin activity in unanaesthetized rabbits during respiration of 10% (o—o) and 7% (Δ—Δ) oxygen

TABLE 2

Plasma renin activity in 7 unanaesthetized rabbits before and during Hypercapnia

RABBIT No.	WEIGHT (Kg.)	BASAL VALUE	PLASMA RENIN ACTIVITY (ng Al/ml/hr)			
			10% O_2		7% O_2	
			30 min	60 min	30 min	60 min
1	2.32	1.672	1.269	1.599	9.145	16.082
2	2.00	3.720	11.491	3.718	9.592	-
3	2.12	2.486	8.599	-	10.707	11.853
4	1.75	2.987	1.124	5.378	7.138	9.944
5	2.20	8.059	5.317	11.752	1.621	8.737
6	2.25	2.444	4.107	4.473	4.583	4.693
7	1.95	5.229	1.445	1.635	4.664	6.227
	Mean	3.799	4.765	4.759	6.779	9.589
	±SE	0.830	1.521	1.530	1.243	1.667

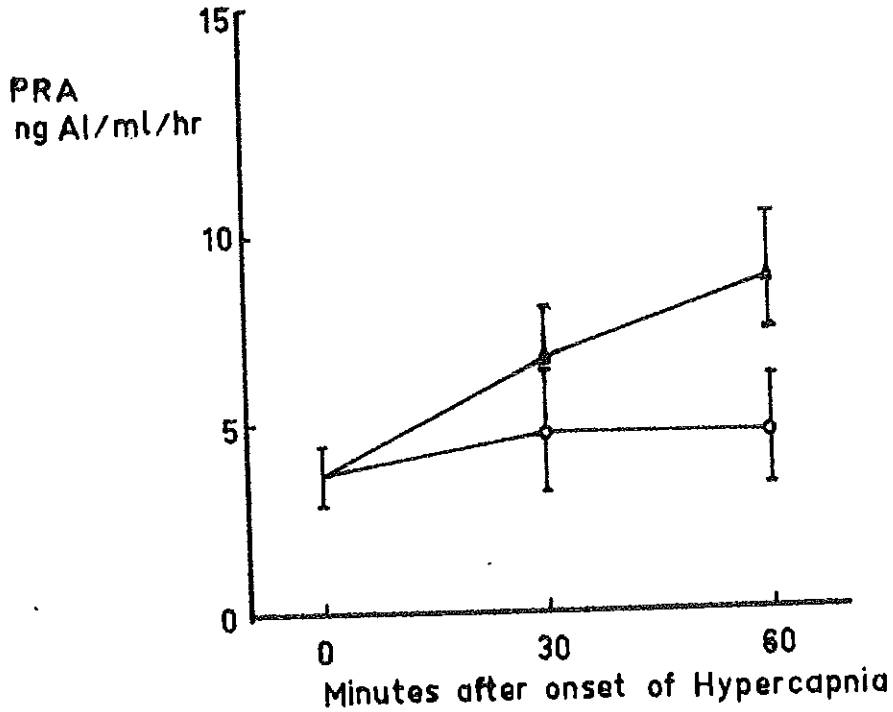

FIGURE 5

Plasma renin activity in unanaesthetized rabbits during respiration of 3% (o—o) and 7% (Δ—Δ) carbon dioxide

A more severe hypercapnia (7% CO_2), was found to elevate renin release markedly (Fig. 5). This increase in renin release was statistically significant ($p<0.05$) only after 60 minutes of breathing the gas mixture. At this time there was a 152% increase from the basal value, however, the increase in the first 30 minutes was not statistically sgnificant. But, as is the case with hypoxia, the change in PRA was greater in the first half hour (78%) than in the second half-hour (74%), though the difference was small (Fig.5).

Hypoxia during Anaesthesia

Sodium pentobarbital is one of the commonly-used anaesthetic agents in experiments with animals. Studies were undertaken in four rabbits to see if this anaesthetic agent modified the response in renin release to hypoxia. This was done with 7% oxygen only. (Experiments with 10% oxygen, and hypercapnia were not done due to limitation of time). The results are presented in Table 3. Plasma renin activity after 60 minutes of anaesthesia while breathing room air was compared with that of the basal value before the anaesthesia. Though PRA was elevated from 2.691 ± 0.308 to 3.950 ± 0.603ng/ml/hr, which is a 47% increase, this difference was not statistically significant ($p>0.1$).

TABLE 3

Plasma renin activity before anaesthesia, 60 minutes after anaesthesia, and during anaesthesia with hypoxia (7% O_2). '___' indicates blood sample was not taken

RABBIT		BASAL VALUE	Plasma renin activity (ng AI/ml/hr		
No.	Weight (Kg.)		60 min after anaesthesia (room air)	7% O_2	
				30 min	60 min
1	2.32	1.357	-	3.124	-
		1.987	-	4.840	-
2	2.00	2.956	4.146	15.143	18.773
		4.484	4.591	16.067	19.507
3	2.12	2.673	3.945	12.562	15.439
		2.539	3.913	17.065	25.630
		2.248	1.298	7.811	13.134
4	1.75	3.664	5.807	4.956	8.074
		2.310	-	6.073	11.425
	Mean	2.691	3.950	9.738	15.997
	±SE	0.308	0.603	1.821	2.209

When animals were breathing 7% oxygen during anaesthesia, the induction of renin release is rapid in onset (Figure 6) and is highly significant both at 30 minutes ($p<0.01$) and 60 minutes ($p<0.002$). The highly significant increase at 60 minutes is not only because of the large increase at 30 minutes, but is also due to a continuous rise in renin release. The elevation in PRA from the 30 minute value to the 60 minute value is very significant, ($p<0.001$), with a 158% increase. When these values are compared with that observed for the unanaesthetized animals (Fig. 7),it indicates that pentobarbital anaesthesia considerably modifies the effect of hypoxia on renin release. In the anaesthetized animals the mean difference between the 30 minute PRA and the control value is 7.047 (an increase of 262%), whereas in the conscious rabbit the difference for the same period is 6.155ng/ml/hr,which is a 162% increase.

In both groups the initial increase in PRA is strikingly large in the first half hour of hypoxia, while further increment is relatively lower in the conscious rabbit than in the anaesthetized ones.

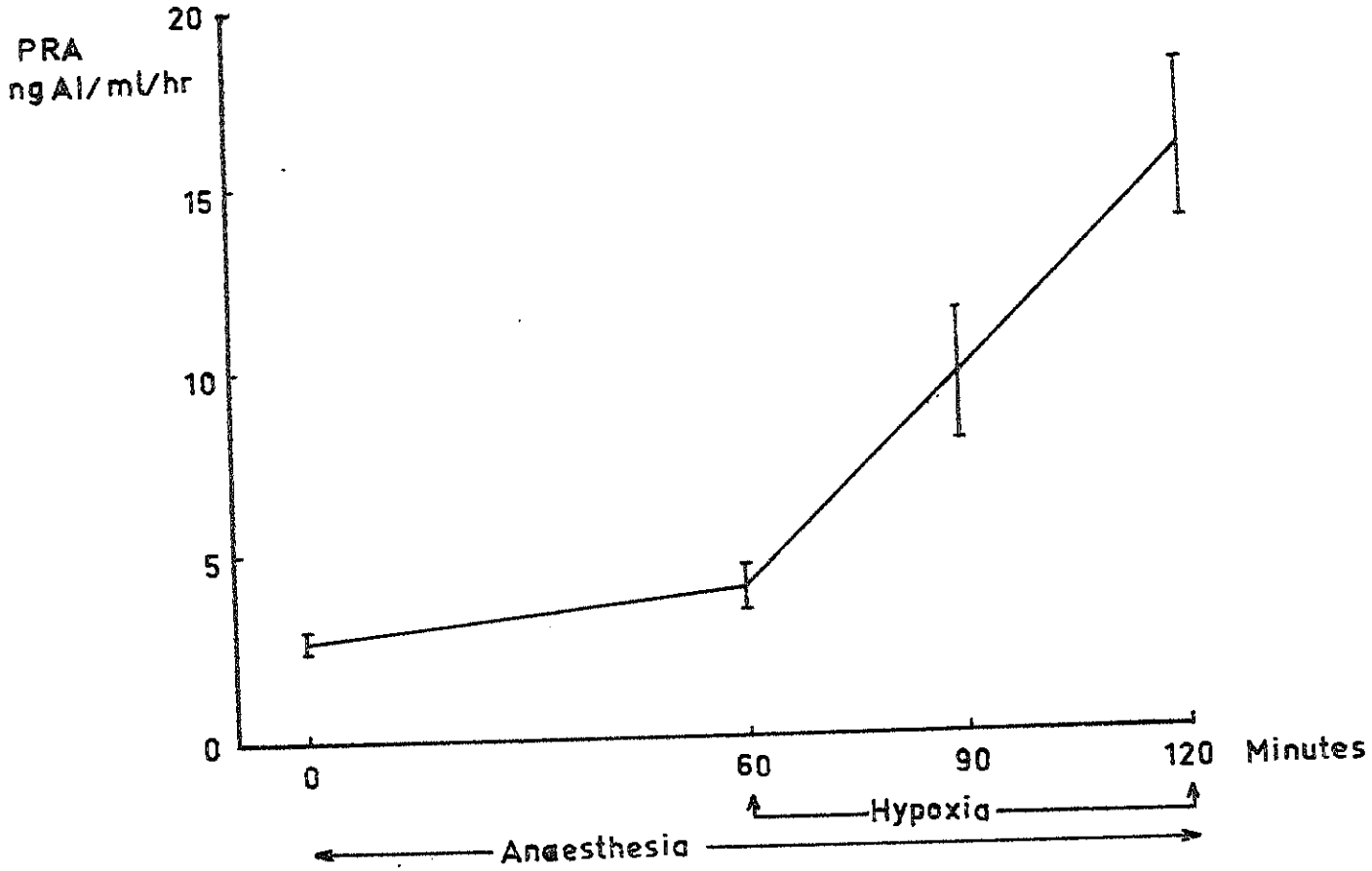

FIGURE 6

Plasma renin activity in anaesthetized rabbits during hypoxia (7% Oxygen)

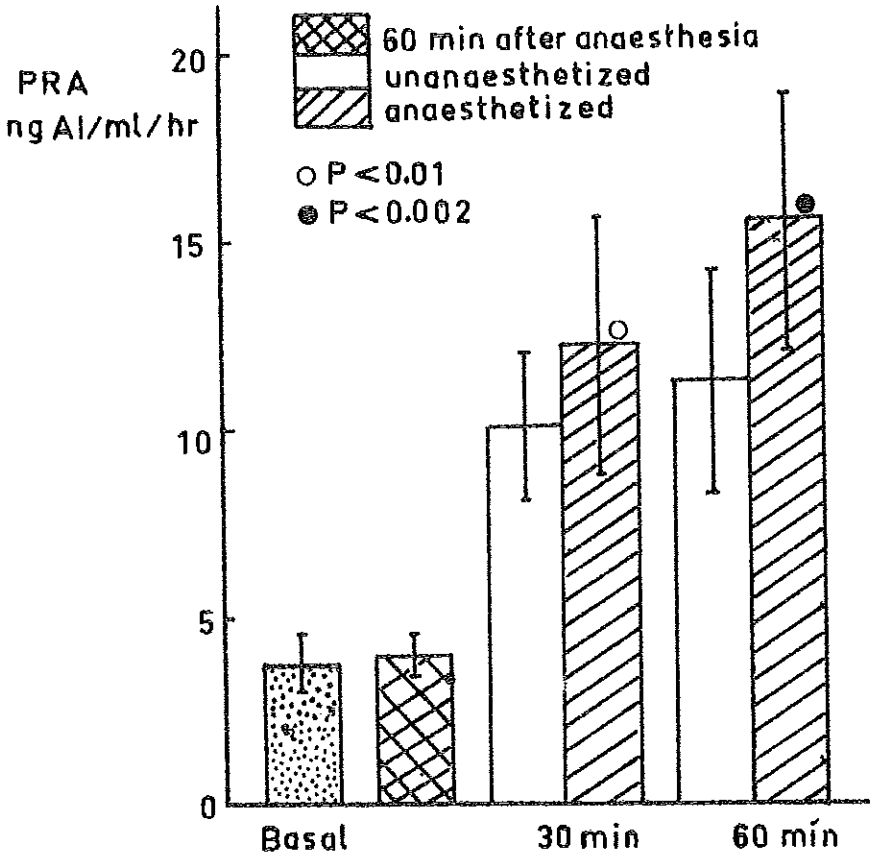

FIGURE 7

Plasma renin activity during Hypoxia ($7\%\ O_2$) in four anaesthetized and seven unanaesthetized rabbits

DISCUSSION AND CONCLUSION

Hypoxia and hypercapnia produce several changes in an animal. Their effects on the secretion of renin was studied in this investigation. The results of the present investigation indicate that hypoxia and hypercapnia stimulate the release of renin. The effects of hypoxic stimulation is more pronounced than that caused by hypercapnia .

Although renin release is increased during stimulation with 7% and 10% oxygen, the increase is statistically significant only with the 7% oxygen. This suggests that a more severe hypoxic stimulation is required to produce a significant increase in the plasma level of renin. This result is in agreement with the results obtained in dogs by Liang and Gavras (1978). They demonstrated that a more significant elevation in PRA is seen at 5% oxygen than at 8% oxygen.

Hypercapnia, like hypoxia, is another condition that induces varied physiological changes. However, there are only few studies to see its effect on renin release. The report of Dusting and Staszewska-Barczak (1976) show that 10% carbon dioxide elevates plasma renin level. The present investigation shows that hypercapnia of a lesser degree (3% and 7% CO_2) can, to some extent, produce a

rise in plasma renin. But it is only the 7% CO_2 that produced a statistically significant elevation. Yet, it was a weak stimulator, since there was not a statistically significant rise in PRA after 30 minutes of hypercapnic stimulation, as is the case with hypoxia.

In all the four gas mixture studied it is seen that the increase in PRA is more during the first 30 minutes after starting breathing the gas mixtures. The increase during the subsequent half hour of exposure is always less. This may indicate that the effects of hypoxia and hypercapnia on renin release is shorrt-term, but this could not be conclusive from the present study, since the data are only for a one hour period. Therefore, further investigation for a prolonged period of time is needed to see the long-term effects of these stresses on renin release in the rabbit. Indeed, Gould and Goodman (1970) have studied the long-term effects of hypoxia in rats. There was a delayed response in PRA which was significant only after the third day of exposing rats to hypoxia.

In the present study the effects of hypoxia was also investigated in anaesthetized animals. Pentobarbital anaesthesia alone is known (Pettinger et al.,1975) to cause a rise in PRA in rats. However, its effect is significant initially within 15 minutes of induction of anaesthesia,

and gradually the PRA value falls to normal (Johnson and Malvin, 1975). From the results of the present study it is evident that pentobarbital significantly modifies the response to hypoxic stimulation (Figure 7). During anaesthesia, when animals are respiring room air, the level of PRA was near the basal value after 60 minutes. When 7% hypoxia was substituted, PRA rose very significantly ($P<0.01$) after 30 minutes of exposure. Further increase in PRA is also more significant than it is with unanaesthetized animals. The results indicate that experiments with anaesthetized animals will yield an exaggerated PRA values.

From the results of the present investigation it can be concluded that plasma renin level is increased during hypoxia and hypercapnia. Thus, the results of this study give further support that hypoxia and hypercapnia stimulate the secretion of renin, and also shows that different levels of hypoxia and hypercapnia influence renin relase to different degrees.

From the experiments with anaesthetized rabbits, it could be concluded that pentobarbital anaesthesia considerably modifies renin release in response to hypoxia, and probably to hypercapnia too. In addition, the present study may indicate that the response of the

rabbit to hypoxia and hypercapnia, in relation to renin secretion, is similar to dogs (Ling and Gavras, 1978); Staszewska-Barczak, 1978), and rats (Demopoulos, et al., 1965; Oliver and Brody, 1965).

It is proposed to further investigate the long-term effects of hypoxia and hypercapnia on renin release in the rabbit.

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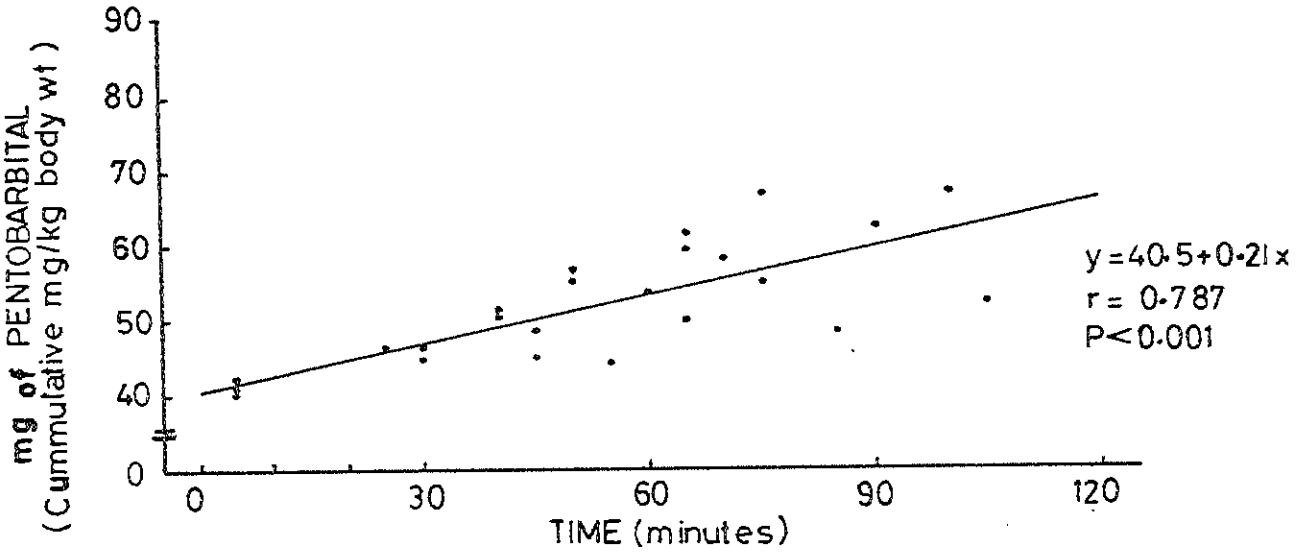
APPENDIX 1

Results of gas analysis made with Lloyd Gas Analyser of four different gas mxitures

Gas Mixture Prepared	Volume (L)	Contents (L)			Gas Mixture %	% of Gas Mixture Determined With Gas Analyser				Mean ± SE	
		O_2	N_2	CO_2		1	2	3	4		
Hypercapnic	100	20	77	3	3 (CO_2)	3.2	3.4	2.6	2.4(CO_2)	2.9	0.48
					20 (O_2)	20.08	20.4	20.5	19.8 (O_2)		
Hypoxic	100	7	93	-	7	7.5	7.6	6.78	-	7.29	0.45
Hypoxic	50	3.5	46.5	-	7	6.6	6.9	7.2	6.1	6.7	0.47
Hypoxic	50	5	45	-	10	9.6	9.78	9.8	-	9.7	0.11

APPENDIX 2

REGRESSION OF CUMMULATIVE PENTOBARBITAL (mg/kg body wt) ON TIME IN 5 RABBITS



APPENDIX 3A

A) Radioimmunoassay determination of PRA for a batch of four plasma samples

Tubes 1-18 are for the standard curve, 19-42 for plasma samples (For each plasma sample 3 duplicated tubes are used: one for the 4°C sample, and the other two for the 37°C sample. Average PRA values are in parenthesis)

	Tube	c/s	Total counts	cpm	Corrected counts	Average Counts	% Bound	% of Trace Binding	AI ng/tube	Calculated PRA ng AI/ml/hr
Total	1	142.8	14280	8568	8412	8218		-	-	
Counts	2	136.32	13632	8179	8023					
Blank	3	2.61	261	156.6	-	155.7				
	4	2.58	258	154.8	-					
Trace	5	79.69	7969	4781	4625	4697	57.2	-	0	
	6	82.06	8206	4924	4768					
AI	7	59.46	5946	3568	3412			72.6	0.020	
Stan-	8	59.54	5954	3572	3416			72.7	0.020	
dard	9	46.83	4683	2810	2654			56.5	0.040	
(7-18)	10	47.96	4796	2878	2722			57.9	0.040	
	11	32.34	3234	1940	1784			38.0	0.080	
	12	30.15	3015	1809	1653			35.2	0.080	

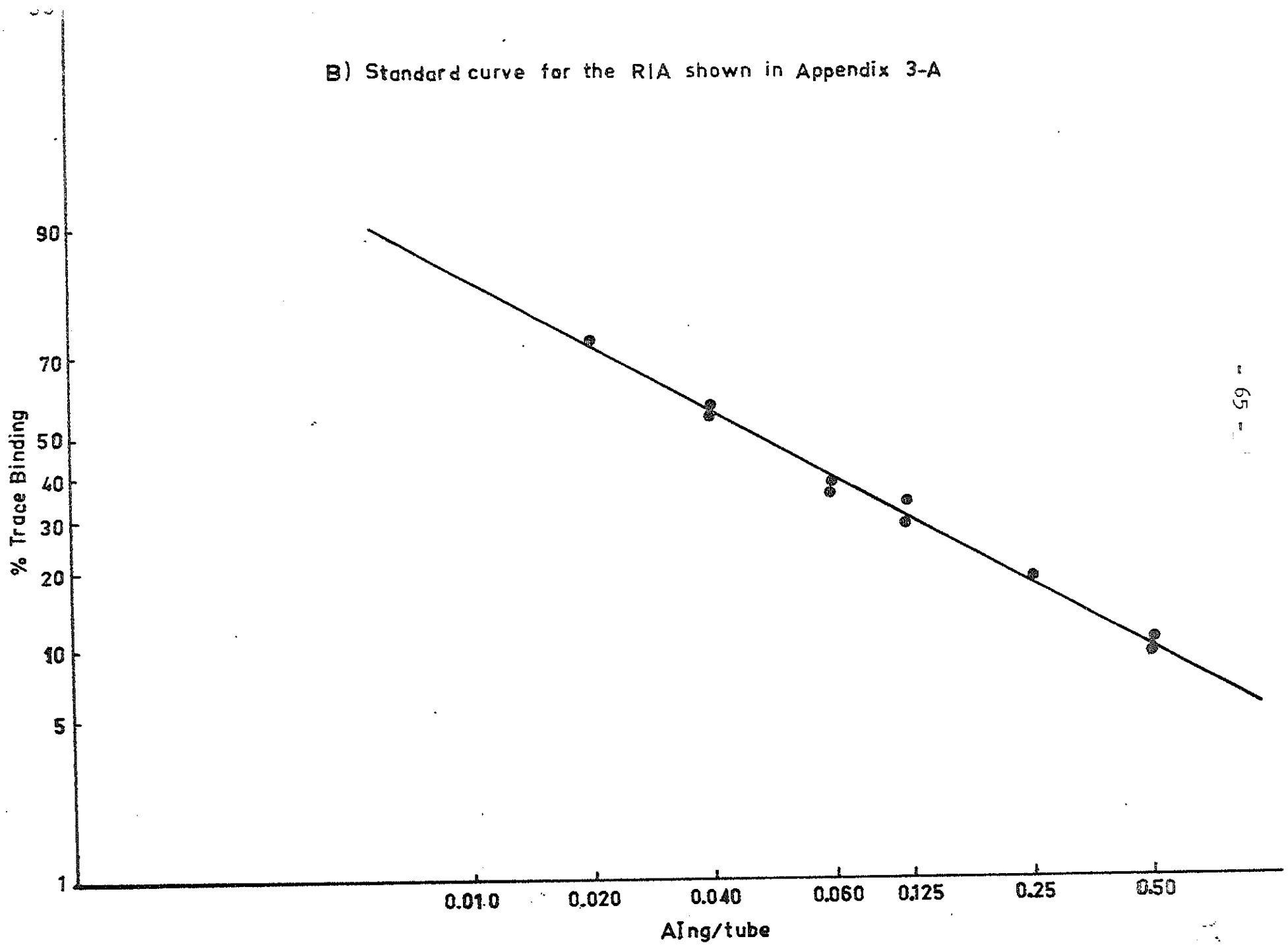
APPENDIX 3A (Cont'd)

Tube	c/s	Total counts	cpm	Corrected counts	Average counts	% Bound	% of Trace Binding	AI ng/tube		Calculated PRA ng AI/ml/hr
13	28.65	2865	1719	1563			33.3	0.125		
14	25.39	2539	1523	1367			29.1	0.125		
15	16.77	1677	1006	850			18.1	0.250		
16	16.53	1653	992	834			17.8	0.250		
17	10.60	1060	636	480			10.2	0.500		
18	9.98	998	599	443			9.0	0.500		
19	48.13	4813	2888	2734			58.2	0.038	(0.036)	
20	50.45	5045	3027	2869			61.1	0.034		
21	15.35	1535	921	765			16.3	0.274	(0.275)	1.753
22	15.50	1550	930	774			16.5	0.275		
23	44.17	4417	2650	2494			53.1	0.047	(0.049)	
24	42.34	4234	2540	2384			50.8	0.050		
25	52.23	5223	3134	2978			63.4	0.030	(0.032)	
26	50.62	5062	3037	2881			61.3	0.033		
27	9.18	918	551	395			8.4	0.590	(0.605)	4.495
28	8.86	886	532	376			8.0	0.620		

APPENDIX 3A (Cont'd)

Tube	c/s	Total counts	cpm	Corrected counts	Average counts	% Bound	% of Trace Binding	AI ng/tube		Calculated PRA ng AI/ml/hr
29	26.62	2662	1597	1441			30.7	0.122	(0.129)	
30	24.88	2488	1493	1337			28.5	0.135		
31	37.79	3779	2267	2111			45.0	0.064	(0.065)	
32	37.27	3727	2236	2080			44.3	0.066		
33	5.97	597	358	202			4.3	-	(-)	9.533
34	5.68	568	341	185			4.0	-		
35	16.84	1684	1010	854			18.2	0.240	(0.273)	
36	14.33	1433	860	704			15.0	0.305		
37	44.53	4453	2672	2516			53.6	0.045	(0.044)	
38	46.19	4619	2771	2615			55.7	0.042		
39	9.67	967	580	424			9.0	0.550	(0.575)	4.371
40	9.08	908	545	389			8.3	0.600		
41	24.57	2457	1474	1318			28.1	0.136	(0.128)	
42	26.71	2671	1603	1447			30.8	0.120		

B) Standard curve for the RIA shown in Appendix 3-A



C) Calculations

i) Corrected count for each tube = counts per minute for each tube-average counts for tubes 1 & 2

ii) % Bound = $\frac{\text{average corrected counts for tubes 5 \& 6}}{\text{corrected total counts for tubes 1 \& 2}}$ X 100

iii) % of trace binding for each tube

$$= \frac{\text{corrected counts for each tube}}{\text{average corrected counts for tubes 5 \& 6}} \times 100$$

iv) AI ng/tube for the plasma is determined by interpolation from the standard curve

v) Worked example for sample '1.7' (tubes 19-24)

average ng AI for tubes 19 & 20 (ng^{4^oC}) = 0.036

" " " " " 21 & 22 (ng^{37^oC}) = 0.275

PRA, ng AI/ml/hr = ($ng^{37^oC} - ng^{4^oC}$) X 20 X 1.1/3

where, 20 is the factor used since only 50 µl of plasma is used

1.1 used to compensate for thedilution of plasma with 100 µl of buffer

3 is the hour of incubation

PRA = (0.275 - 0.036) X 20 X 1.1/3

= 1.753 ng AI/ml/hr

If the 10 µl sample (tubes 23 & 24) is used to determine ng $^{37^oC}$, the ng/tube value is multiplied by 5 before calculating PRA.