The effects of age, sex and a severe dietary stress upon plasma renin activity in the beagle

by

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INTRODUCTION

The renin-angiotensin system has been shown to be an important hormonal control system. The involvement of this system in the regulation of aldosterone secretion and the role it has in certain hypertensive diseases have been excellently reviewed by Davis (1971a) and Peart (1971) respectively. Johnson and Davis (1972) have also recently suggested that the system has an important role in the maintenance of arterial pressure in certain pathological states.

The activity of the renin-angiotensin system has been found to be elevated in humans suffering from kwashiorkor (Kritzinger, Kanengoni, and Jones, 1972). However, no mention was made of attempts to evaluate cardiovascular function and to correlate possible pressure changes with changes in plasma renin activity (PRA). Other workers dealing with malnutrition and starvation state that hypotension and bradycardia are consistent and significant findings (Keys et al., 1950).

Elevated PRA values have also been demonstrated in newborn animals. Granger et al. (1971) reported finding high levels in the dog during the first two weeks of life.

The primary goal of this project was to monitor the activity of the renin-angiotensin system in dogs undergoing a severe dietary stress. The stress was to be implemented by feeding a protein deficient diet to growing dogs. Attempts were to be made to correlate a predicted increase in PRA with possible changes in blood pressure, pulse rate, and serum electrolytes.

A secondary goal was to monitor PRA values in young, growing dogs being fed a control diet to investigate possible changes during maturation.

REVIEW OF LITERATURE

A physiological effect of the renin-angiotensin system was first noted by Tigersted and Bergman in 1898 (Helmer, 1971) when they reported that kidney extracts injected intravenously would produce an increase in blood pressure. The name renin was given to the unidentified agent responsible for this blood pressure rise. Kohlstaedt, Helmer, and Page (1938) demonstrated that a purified preparation of renin had no pressor activity itself, but appeared to react with a substance present in the protein fraction of plasma to give rise to a pressor agent.

Two groups of investigators, Page and Helmer (1940) and Braun-Menéndez et al. (1940) isolated the biologically active substance. It was later named angiotensin. Braun-Menéndez et al. (1940) were also the first to suggest that renin was an enzyme which acted upon a substrate (plasma renin substrate) in plasma.

In attempting to purify angiotensin Skeggs et al. (1954) found that it existed in two different forms. The first, angiotensin I, was the result of the action of renin but did not appear to be physiologically active. The second, angiotensin II, was the active agent. It resulted from the conversion of angiotensin I by an enzyme in the plasma. Ng and Vane (1967) have reported that the major site of this conversion appears to be the pulmonary circulation.

Anatomical Localization

Goormaghtigh (1939) first proposed that the location of renin in the kidney was the juxtaglomerular apparatus after noting that these cells

appeared to contain more secretory granules from subjects with arterial hypertension. This increase in granulation has since been noted in other circumstances which are known to produce increased plasma renin activity (PRA) such as low sodium intake (Hartroft and Hartroft, 1953) and adrenal insufficiency (Tobian et al., 1958).

The proposed area of renin location within the kidney received support from microdissection studies of the kidney. Cook and Pickering (1958) isolated an individual glomerulus, dissected it, and demonstrated that the renin which it contained was located within the area of the juxtaglomerular apparatus. In more recent work, Cook (1971) has isolated a sample of pure juxtaglomerular cell material and demonstrated that these cells contain renin.

Edelman and Hartroft (1961) and Warren, Johnson, and Hoobler (1966) also supported the proposed location with the use of a fluorescent antibody technique. They found that antirenin antibody appeared to localize in the area of the cells of the juxtaglomerular apparatus and the macula densa.

Barajas (1971) made an extensive study of the juxtaglomerular apparatus with the use of the electron microscope. He constructed a three-dimensional model of the area based on serial sections. With the use of this model he theorized that the distal tubule, the efferent arteriole, the afferent arteriole, and the mesangial region of the juxtaglomerular apparatus comprise a functional unit concerned with the synthesis, storage, and release of renin.

Control of Renin Release

The current literature offers three basic hypotheses to explain the regulation of renin release. These involve (i) a renal vascular receptor, (ii) renal sympathetic tone, and (iii) humoral or electrolyte receptors.

Renal vascular receptors

When renal ischemia created by constriction of the renal artery was demonstrated by Goldblatt et al. (1934) to produce persistent hypertension in the dog, this was assumed to be the stimulus for renin release.

Huidoboro and Braun-Menéndez (1942) later demonstrated that renal ischemia did not produce any acute increase in PRA if arterial pressure was maintained. They found that lowering blood pressure did apparently increase renin secretion. Therefore, they proposed that renin participated in maintaining blood pressure.

Divry (1951) found that progressive decreases in renal perfusion pressure would stimulate increases in the secretion of a "renal hypertensive substance". This could be demonstrated with either arterial or venous blood; thus eliminating the possible effect of ischemia. She suggested that the kidney contained a baro-sensitive device which regulated the release of this hypertensive substance.

Tobian, Tomboulian, and Janecek (1959) noted that renal perfusion pressure could affect the granulation of the juxtaglomerular cells which were believed to be the source of renin. They proposed that the renal afferent arterioles and the juxtaglomerular cells were "stretch receptors" regulating renin secretion based on changes in vascular volume and pressure.

Decreases in mean renal arterial pressure were found to increase renin secretion by Skinner et al. (1964). Changes in pulse pressure did not affect renin secretion if mean pressure was held constant.

Ayers et al. (1969) demonstrated that partial occlusion of the renal artery in dogs produced initial vasodilation followed by a gradual vasoconstriction. An increase in PRA was associated with the vasodilation and a decrease in PRA was associated with the vasoconstriction. Vasodilator drugs produced a small increase in PRA when given to normal dogs but produced a very marked increase in PRA in renovascular hypertensive dogs.

Blaine and Davis (1971) and Blaine et al. (1970 and 1971) provided strong evidence for the vascular receptor theory. These experiments were performed using a nonfiltering, denervated kidney model in adrenalectomized dogs. This procedure created a kidney in which there were no functioning nephrons. Thus, eliminating the possibility that humoral or electrolyte receptors at the macula densa, could control renin release. The adrenalectomy and the denervation eliminated the major influence of the sympathetic system on renin release. Under these conditions increases in PRA were found following hemorrhage, suprarenal aortic constriction, and other stimuli which could affect renal perfusion pressure.

Vander (1967) and Davis (1971b) outlined the possible causes of the changes in vascular tone as (i) changes in intravascular afferent arteriole pressure, (ii) changes in the pressure gradient between intravascular pressure and renal interstitial pressure, or (iii) changes in the tension of the wall of the afferent arteriole.

Renal sympathetic tone

The possibility that the sympathetic system could influence the control of renin synthesis or release was first presented by Taquini, Blaquier, and Taquini (1964). They reported that the renin content of a denervated kidney appeared to be reduced when compared to an intact kidney.

Vander (1965) reported that renin secretion could be increased by intravenous infusion of catecholamines or by stimulation of the renal nerves. Renal perfusion pressure was held constant during these experiments to eliminate the possible indirect influence of the baro-receptors. He suggested that the increased renin secretion could be due to a direct effect of the catecholamines or neural stimulation upon the renin secreting cells; or, an indirect effect due to the reduction in renal function which was noted. The effect of the renal nerve stimulation indicates that the adrenergic receptors which are responsible for the sympathetic effect on PRA are located within the kidney.

The possibility of a direct neural pathway was supported by Hartroft (1966) who observed nerve endings in the area of the macula densa and the juxtaglomerular cells.

Wathen et al. (1965) studied renin release after the infusion of either catecholamines or angiotensin II into the renal artery. Both produced renal vasoconstriction and both reduced renal function; however, only the catecholamine infusion increased renin release. Intravenous infusions of catecholamines did not increase renin secretion. After the intravenous infusion was stopped, systemic pressure decreased and renin was released. The author concluded that catecholamines did not directly promote renin

release, but may act indirectly by affecting the sensitivity of possible baro-receptors or by changing renal function.

Controlled hemorrhage was used by Bunag, Page, and McCubbin (1966a) to study the relationships between renin release and sympathetic activity. They found that slow hemorrhage would produce renin release without measurable changes in arterial pressure or total renal blood flow. This release was abolished by ganglion blockade or local anesthesia of renal nerves. During hemorrhage a "rough correlation" was found between compensatory tachycardia and release of renin.

The adrenergic receptors responsible for the sympathetic effect appear to be β-adrenergic receptors. The β-adrenergic agonist isoproterenol increases renin release when infused into the renal artery or intravenously at levels which would not significantly alter renal hemodynamics (Reid, Schrier, and Earley, 1972). However, this increase in renin release might be partially due to increased circulating catecholamines responding to the systemic effects of isoproterenol.

However, Winer et al. (1971) reported that both the β-adrenergic blocker propranolol and the α-adrenergic blocker phentolamine were capable of blocking the significant renin release due to renal artery constriction. These blocking effects were independent of significant changes in renal blood flow, glomerular filtration rate and urinary sodium excretion.

Electrolyte receptors

Vander and Miller (1964) first proposed that renin secretion was controlled by receptors within the macula densa monitoring intratubular fluid. They reported that the usual increase in renin secretion due to aortic

clamping could be prevented or reversed by induction of diuresis. These effects could not be explained by renal hemodynamics or plasma composition. They concluded that either the flow or the composition of the intratubular fluid was the controlling factor.

Brubacher and Vander (1968) reaffirmed that sodium restriction increased PRA levels. After noting that these increases were not abolished by renal denervation and adrenergic blockers, they concluded that the effects seen were not produced via the sympathetic nervous system. They also reported no significant changes in blood pressure, plasma electrolytes, or renal function during the period of sodium restriction.

Bunag, Page and McCubbin (1966b) reported that dogs maintained on a low-sodium diet released more renin and released it more readily than dogs fed a standard kennel diet. Aortic constrictions and infusions of norepinephrine were the stimuli used to elicit renin release. There was no correlation found between the hemodynamic effects of norepinephrine and renin release. They proposed that sodium concentrations in the distal tubule might modify the sensitivity of other receptors involved with the release of renin.

Vander and Carlson (1969) have proposed that sodium transport at the macula densa is important in the regulation of renin secretion. They administered furosemide to dogs which is believed to stop the transport of sodium at the macula densa. Sodium balance and volume balance were maintained by infusing isotonic saline. Under these conditions they found an increase in PRA, believed to represent renin release, without changes in plasma sodium concentration or glomerular filtration rate.

Ethacrynic acid, an inhibitor of sodium reabsorption which is believed to act in the ascending limb of the loop of Henle proximal to the macula densa, was used by Cooke et al. (1970) to study the effects of intratubular sodium concentration on renin release. They reported that infusion of ethacrynic acid effected a release of renin without volume depletion which was corrected for by reinfusion of urine. This release by ethacrynic acid was prevented by ureteral occlusion suggesting that it was independent of renal blood flow. They concluded that the stimulus for renin release was dependent on the sodium concentration of the distal tubular fluid.

Brunner et al. (1970) demonstrated that PRA was increased during potassium deprivation even though this was usually accompanied by sodium retention which would be expected to reduce PRA. Potassium administration was found to often suppress PRA independently of changes in diuresis and aldosterone secretion. Glomerular filtration rate and renal plasma flow were not evaluated but they felt that these functions were not affected. Therefore, they suggested that an intrarenal action of potassium ions either by direct action on renin release or indirectly by affecting sodium transport.

Regulation of Aldosterone Secretion

Denton, Goding and Wright (1959) were among the first to propose that a humoral agent other than corticotrophin (ACTH) was involved in the regulation of aldosterone secretion. Using conscious sheep with adrenal transplants they demonstrated that the blood of a sodium deprived donor would stimulate aldosterone in an adrenal of a sheep in normal sodium balance. The normal sheep had received exogenous hydroxycorticosteroid to suppress

ACTH production. The possible direct effect of electrolyte concentration did not appear to be the principal stimulant.

Davis et al. (1961) presented evidence that an aldosterone secreting hormone (ASH) was secreted by the kidney. They found that aldosterone secretion was increased in both decapitated and hypophysectomized dogs in response to acute hemorrhage. When the kidney was removed from hypophysectomized dogs, aldosterone levels declined and failed to respond to acute hemorrhage. Intravenous infusions of kidney extracts into nephrectomized-hypophysectomized dogs increased aldosterone secretion. They suggested that ASH was possibly renin or a renin-like compound which responded to cardiovascular stress by stimulating aldosterone release.

Laragh et al. (1960) demonstrated that intravenous infusions of angiotensin II appeared to increase aldosterone secretion in humans in both normal sodium balance and in a sodium depleted state. These effects were independent of changes in arterial pressure and pulse rate; thus indicating a possible direct effect of angiotensin II upon aldosterone secretion.

With the use of a radioimmunoassay for angiotensin II, Brown et al. (1972) monitored plasma aldosterone and plasma angiotensin II concurrently in normal humans during periods of sodium deprivation and sodium repletion. They found a significant positive correlation between aldosterone and angiotensin II with both increasing during deprivation and returning to control values following sodium repletion.

Luetscher et al. (1969) investigated the relationship between aldosterone and renin in humans with benign essential hypertension. They found increases in aldosterone secretion following sodium deprivation was

correlated with increases in PRA. However, during sodium loading aldosterone secretion remained high relative to the sodium load and level of PRA. They suggested that in certain pathologic conditions there was a failure in the normal feedback control of aldosterone production.

In an excellent review article, Blair-West et al. (1972) summarized the results and conclusions from several studies which they had done involving aldosterone secretion. By inhibiting the release of renin by slow renal arterial infusion of angiotensin II during the onset of sodium deficiency, they found that increases in aldosterone secretion could be demonstrated without increases in circulating levels of angiotensin II. They also found that rapid correction of sodium depletion would decrease aldosterone secretion while angiotensin II plasma levels remained elevated. They concluded that the renin-angiotensin system was important in the regulation of aldosterone secretion, but the degree of control was influenced by other factors.

Maintenance of Circulatory Homeostasis

The response of the renin-angiotensin system to circulatory stress has been well documented. It has served as the basis for (i) studies of the control of renin release (Goldblatt et al., 1934; Divry, 1951; Blaine et al., 1970, 1971; and Bunag et al., 1966a), (ii) studies of the control of aldosterone secretion (Davis et al., 1961), and (iii) development of bioassays for renin activity (Goldblatt, 1943; Helmer and Judson, 1963; and Pickens et al., 1965).

Scroop and Whelan (1966) demonstrated that the pressor activity of angiotensin II was mediated via two pathways. Intravenous infusions of

angiotensin II produced vasoconstriction in the hand of experimental subjects. This vasoconstriction was not demonstrated if the sympathetic vasoconstriction system to the hand was interrupted; thus indicating a central effect mediated via the sympathetic system. Angiotensin II in larger concentrations infused directly into the arteries of the hand produced vasoconstriction which was not dependent on the sympathetic system; thus indicating a direct effect on vascular smooth muscle.

Ueda, Katayama, and Kato (1972) injected microamounts of angiotensin II (0.1 to 1.0 μg) into different areas of the brain and brain stem of cats in attempts to locate the site of action of angiotensin II. They found that injections into the area postrema gave marked pressor responses. These responses were not found after injections of placebos.

Hall and Hodge (1971) monitored the circulating levels of catecholamines and angiotensin in the anesthetized dog during the cardiovascular stress of hemorrhage. They found that angiotensin levels increased before any significant decrease in arterial pressure. A decrease in arterial pressure preceded any increase in catecholamines. They suggested that the renin-angiotensin system was important in acute responses to cardiovascular stress as well as the possible longer term response via stimulation of aldosterone secretion.

Cowley, Miller, and Guyton (1971) evaluated the response of the renin-angiotensin system in dogs in which the cardiovascular control mechanisms of the central nervous system had been eliminated by decapitation and spinal cord destruction. Blood pressure was maintained at control levels by infusion of norepinephrine. Under these conditions they found that

reductions in renal perfusion pressures produced rapid elevations in mean arterial pressure and PRA. The elevations in pressure were inhibited by the injection of angiotensin II antiserum or infusion of angiotensin II before the reductions in renal perfusion pressure. Cardiac outputs were not significantly changed during the reductions in renal perfusion pressure. They concluded that the renin-angiotensin system was capable of having a significant involvement in the normal regulation of arterial pressure.

Johnson and Davis (1972) used an angiotensin II antagonist to study the role of angiotensin II in the maintenance of arterial pressure. In dogs with thoracic caval constriction they found that the administration of this analog reduced arterial pressure and aldosterone secretion while PRA was elevated. In normal dogs the analog failed to produce changes in arterial pressure or PRA. Citing earlier work by Davis and Howell in 1953 which stated that cardiac output was reduced in caval constriction and arterial pressure was maintained by increases in peripheral resistance, they suggested that angiotensin II was responsible for these increases in peripheral resistance.

Measurement of Plasma Renin Activity

Bioassay techniques

Goldblatt et al. (1943) were the first to attempt quantifications of renin potency. They used normal, trained, unanesthetized dogs to define the dog unit of renin as the amount needed "to raise blood pressure at least 30 and not more than 35 mmHg within 3 minutes in three dogs" weighing between 10 and 25 kilograms; serum was injected intravenously in most of their experiments.

Page (1940) was one of the first to attempt to use an isolated tissue preparation to observe the activity of the renin-angiotensin system. He noted that a segment of rabbit intestine would constrict after the addition of plasma and renin. Either substance added individually did not produce constriction.

Helmer and Judson (1963) attempted to quantitate renin in the plasma of patients with arterial hypertension with the use of an isolated segment of rabbit aorta and a nephrectomized, pithed cat. Their procedures involved the dialysis of the plasma to remove other vasoactive agents which would give false positive results. However, angiotensin could be destroyed before the assay as they neglected to inactivate angiotensinases in the plasma.

Feldberg and Lewis (1964) complicated the interpretation of bioassays using intact animals by demonstrating that angiotensin was a potent stimulant for secretion of catecholamines from the adrenals.

The rat colon was shown to be a highly sensitive and specific organ for angiotensin assays by Regoli and Vane (1964). Due to its reactions to various angiotensin analogs, they concluded that the receptors in the colon were the same type as those responding in the rat pressor assay.

Pickens et al. (1965) reported the use of ethylenediaminetetraacetate (EDTA) and d-isopropylfluorophosphate as angiotensinase inhibitors. They also adjusted the pH of the plasma to 5.5 for incubation to allow the renin-renin substrate reaction to produce angiotensin I. This pH was considered optimal for the reaction. Based on the variability of reaction rates under various chemical conditions, they predicted that activators or

inhibitors of the renin-renin substrate reaction might be found in the plasma. EDTA also inhibits the conversion of angiotensin I to angiotensin II (Skeggs, Kahn, and Shumway, 1956). Therefore, the results of the assay of Pickens et al. (1965) could be affected by the converting enzyme capability of the assay animal.

Radioimmunoassay techniques

After Deodhar (1960) and Goodfriend et al. (1964) demonstrated that the octapeptide angiotensin II could be made antigenic by polymerizing it with a carrier protein, several laboratories developed radioimmunoassays to measure PRA.

The principle of the radioimmunoassay is based on the antigen-antibody reaction. The compound to be measured usually represents the antigenic compound in the reaction. A known amount of antibody against this antigen and a known amount of radiolabelled antigen are mixed with the unknown. The antibody being present in limited quantities requires the labelled antigen and the unknown antigen to compete for binding with the antibody. After this mixture has reached a state of equilibrium, the antibody bound antigen fraction and the free antigen fraction are separated. The ratio of isotope counts between these two fractions compared to a standard defines the amount of antigen present in the unknown.

Boyd, Landon, and Peart (1967) developed a radioimmunoassay using angiotensin II labelled with either ^{131}I or ^{125}I which was accurate to the level of 30 pg. It was very specific and not affected by angiotensin I. Dimercaprol and edetic acid were used as inhibitors of angiotensinases.

This procedure required a large amount of blood from which it was necessary to extract angiotensin II before measurement. This extraction was laborious and inefficient.

A double isotope radioimmunoassay developed by Catt, Cain, and Coghlan (1967) for angiotensin II also required an extraction procedure, but the loss of angiotensin II from the procedure could be calculated. A known amount of angiotensin II labelled with ^{125}I was added to the sample before the extraction and was used as an internal indicator to correct for loss during the procedure. The angiotensin II used for the radioimmunoassay was labelled with ^{131}I.

The use of a radioimmunoassay for angiotensin I for the measurement of PRA was reported by Haber et al. (1969). This assay did not require large amounts of blood for the measurement. ^{125}I was used as the label. A 3 hour incubation period was used to allow the renin-renin substrate reaction to produce measureable amounts of angiotensin I. The destruction of the angiotensin I produced and its conversion to angiotensin II was prevented by inhibitors present during the incubation. The assay was very specific and was found not to be significantly affected by the presence of angiotensin II.

A radioimmunoassay for angiotensin I using angiotensin I labelled with ^{125}I was also used by Cohen et al. (1971) for the measurement of plasma renin activity. The incubation time for this assay was only 15 to 30 minutes and was accomplished by adjusting the pH of the incubate to 5.5 which is optimal for the renin-renin substrate reaction. Shortening of the incubation time avoided renin substrate depletion and nonspecific plasma interference.

Kurtz (1971) developed a radioimmunoassay for plasma renin activity in which the antibody was labelled with ^{125}I. He adapted this assay to measure plasma renin concentration by making the assumption that during incubation endogenous and exogenous renin would produce angiotensin I at the same rate, when standard renin in varying amounts was added to plasma samples. Based on interpolation of the amounts of angiotensin I generated by these mixtures, the endogenous renin concentration could be calculated.

PART I. THE EFFECT OF AGE AND SEX UPON PLASMA RENIN ACTIVITY IN THE BEAGLE

LITERATURE REVIEW

The activity of the renin-angiotensin system was shown to be higher in newborn dogs than in adult dogs by Granger et al. (1971). They also demonstrated that the system was capable of increasing PRA in response to stress.

Using adrenal slices in isolated tissue studies, Kemény et al. (1964) found that the basal aldosterone production of infantile tissue was less than that of adult tissue. Slices of infantile adrenal tissue did not increase in aldosterone production in response to administration of progesterone; whereas, the adult tissue did show an increase in aldosterone production.

Cain, Williams, and Dluhy (1972) studied the renin-angiotensin-aldosterone system in hypertensive patients with acromegaly. They reported that in response to sodium restriction and volume depletion the rise in PRA appeared normal; however, the response of aldosterone secretion to this increase in PRA was inhibited. They suggested that this could be due to an altered metabolic clearance of aldosterone, a block in the action of renin-angiotensin on adrenal receptor sites, or some direct action of growth hormone on aldosterone production.

Helmer and Griffith (1952) reported that synthetic estrogens increased plasma renin substrate (PRS) in male rats. Testosterone propionate would inhibit this increase in PRS.

Weinberger et al. (1968) found that administration of oral contraceptives increased PRA in some women. They suggested that this was due to the increase in PRS. Katz and Romfh (1972) however were not able to demonstrate the increase in PRS during normal menstruation. At present the effects of oral contraceptives and estrogens upon PRS are unclear.

MATERIALS AND METHODS

Animal Techniques

Eleven beagle pups, six females and five males representing six different litters, were used as test animals. The dogs were housed in one room separated by sex into two groups. A commercial dog food[1] was fed ad libitum. The dogs received immunizations for canine distemper, canine infectious hepatitis, and leptospirosis[2] at 8, 14, and 24 weeks of age. Oral medication[3] was administered to control internal parasites when infestations were confirmed by fecal examinations.

Sampling and Sample Preparation

Blood plasma

Beginning at 9 weeks of age each dog was sampled every 2 weeks until the fifty-third week of age. Four ml of blood were drawn from the left external jugular vein while the dog was held in lateral recumbency on its right side. The blood was immediately transferred to a glass tube containing 7.5 mg disodium edetate (EDTA)[4]. The samples were held in an ice bath until they could be centrifuged at 200 X g for 15 minutes in a cold room (4 C) to recover the plasma.

The plasma was stored frozen (-20 C) until assayed for plasma renin activity (PRA). The sample preparation procedures were based upon the

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[1]Gaines Meal, General Foods Corporation, Battle Creek, Michigan.

[2]Enduracell d-h-l, Norden Laboratories, Lincoln, Nebraska.

[3]Vermiplex, Pitman-Moore, Inc., Fort Washington, Pennsylvania.

[4]Vacutainer #4770, Becton, Dickinson and Company, Rutherford, New Jersey.

recommendations given for the PRA radioimmunoassay technique used for the assays (Haber et al., 1969).

Blood serum

Beginning at 25 weeks of age each dog was sampled monthly until the fifty-third week of age. Four additional ml of blood were drawn at the same time that the blood for PRA determination was drawn. The blood was transferred to an empty glass tube[1] and placed in an ice bath. After clotting the samples were centrifuged at 200 X g for 15 minutes in a cold room (4 C) to recover the serum. The serum was stored frozen (-20 C) until assayed.

Electrocardiograms

Beginning at 23 weeks of age the electrocardiogram (EKG) of each dog was recorded each time a blood sample was taken. The bipolar lead II was recorded with the animal resting in right lateral recumbency. Subdermal platinum alloy electrodes and a Grass Model 7 Polygraph[2] were used for the recordings. The recordings were made on two channel Grass recording paper at a calibration setting of 1 mv/cm and a paper speed of 30 mm/sec.

Blood pressure

Beginning at 25 weeks of age the arterial blood pressure of each dog was recorded every 4 weeks through the fifty-third week of age. This procedure was done immediately after the blood sample for the PRA determination

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[1]Vacutainer, #4710, Becton, Dickinson and Company, Rutherford, New Jersey.

[2]Grass Instrument Company, Quincy, Massachusetts.

had been collected. EKG leads were attached to the animal and recordings of the standard lead II were made throughout the pressure recording procedure.

The dog was anesthetized with sodium methohexital[1] (9mg/kg) by intravenous injection into the cephalic vein of the left forelimb while resting in right lateral recumbency. The femoral artery was located in the femoral triangle by digital palpation. A 22 gauge 1 inch hypodermic needle attached to a vinyl tubing cannula (0.025 inch ID) filled with sterile saline was used to cannulate the artery. The cannula was attached to a Model 23Dc Statham pressure transducer[2]. Recordings from the transducer were made on a Grass Model 7 Polygraph[3]. After a recording period of 20 to 30 seconds the needle was withdrawn from the artery and digital pressure was applied to prevent hemorrhage. A blood sample for PRA determination was taken by the previously described procedure immediately after the removal of the cannula.

Temperature-humidity index

The temperature and humidity of the animal quarters were continuously recorded with the use of a hygro-thermograph[4].

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[1]Brevane, Elanco Products Company, Indianapolis, Indiana.

[2]Statham Laboratories, Inc., Hato Rey, Puerto Rico.

[3]Grass Instrument Company, Quincy, Massachusetts.

[4]Bendix Corporation, 1400 Taylor Avenue, Baltimore, Maryland.

Assay Procedures

Plasma renin activity

The PRA of the plasma samples was determined in random order with the use of a radioimmunoassay for angiotensin I. The protocol for these determinations was based upon the techniques described by Haber et al. (1969). Angiotensin I standard solution (10 ng/ml), angiotensin I labelled with ^{125}I, and angiotensin I antiserum were obtained from two commercial sources[1,2]. A detailed outline of the procedure is given in Appendix I.

Sodium and potassium

The serum samples previously described were randomized for assay at a later time. The assays were performed using a Baird-Atomic Flame Photometer Model KY-2[3].

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[1]Schwarz/Mann, Orangeburg, New York.

[2]New England Nuclear, 575 Albany Street, Boston, Massachusetts.

[3]Baird-Atomic, Inc., Cambridge, Massachusetts.

RESULTS

Data Analysis

An analysis of variance was used to test for between group differences. The experimental unit was considered to be the individual animal.

An analysis of variance was used to test for interactions.

A correlation matrix was used to display correlation coefficients and their significance. The degrees of freedom for the tests of significance of these coefficients were based on the number of observations.

Cardiovascular Functions

Arterial pressures

A significant difference between sexes was found with regard to both systolic ($p<0.025$) and diastolic ($p<0.005$) pressures. The females were higher in both cases. The mean values for the females were 157 mmHg for systolic and 109 mmHg for diastolic. The mean values for the males were 141 mmHg for systolic and 89 mmHg for diastolic.

There was no significant difference between sexes with regard to mean values for pulse pressure (49 mmHg for females and 52 mmHg for males).

Heart rate

A significant difference ($p<0.005$) was found between sexes with regard to heart rate after anesthesia. The mean for the females was 227 beats per minute while the mean for the males was 207 beats per minute.

There was no significant difference between sexes in mean heart rate before anesthesia. The mean values were 116 beats per minute for the females and 117 beats per minute for the males.

Plasma Renin Activity

Effect of estrus

During the course of the experiment five of the six females demonstrated physical signs of estrus. Four of these five subsequently demonstrated physical signs of pseudocyesis. The mean value for PRA in the unanesthetized state while physical signs of estrus or pseudocyesis were evident was higher (1.183 ng/ml/hr) than the mean value for females when no such physical signs were evident (0.434 ng/ml/hr). These mean values were adjusted for age.

The difference between these two periods was not highly significant ($0.050<p<0.100$). This low significance was probably due in part to the small number of observations (18 samples) taken when the signs were evident and the limited number of experimental subjects (5 dogs).

Effect of anesthesia

The mean value for PRA after anesthesia (1.417 ng/nl/hr) was significantly higher ($p<0.025$) than the mean value for PRA before anesthesia (0.389 ng/ml/hr). These means were adjusted for estrus and pseudocyesis

and were based on PRA determinations made on samples taken just before and just after anesthesia. This increase due to anesthesia has been reported by other investigators (Ganong, 1972).

The mean PRA values for the females demonstrating physical signs of estrus or pseudocyesis also significantly increased ($p<0.05$) due to anesthesia (1.183 ng/ml/hr before anesthesia and 3.063 ng/ml/hr after anesthesia). Statistical analysis found no interaction between the effects of estrus and anesthesia.

Effect of age and temperature-humidity index

The mean values for unanesthetized PRA of all dogs increased with increasing age. Female PRA values included in the means were from females not exhibiting physical signs of estrus or pseudocyesis. This increase due to age was shown statistically by both an analysis of variance ($p<0.05$) and by demonstrating a weak ($r = 0.19$) but significant ($p<0.005$) correlation between age and PRA. The effect of age on PRA was found to be independent of sex and body weight.

From a graph of mean PRA values (Figure 1) for all dogs in the unanesthetized state plotted versus age in weeks, it is evident that there is a large amount of variation between sampling periods. An attempt was made to correlate this variation with the temperature-humidity index of the animal quarters. However, no significant correlation was found.

Effect of sex and body weight

After eliminating values obtained for females when physical signs of estrus or pseudocyesis were evident, there was no significant difference

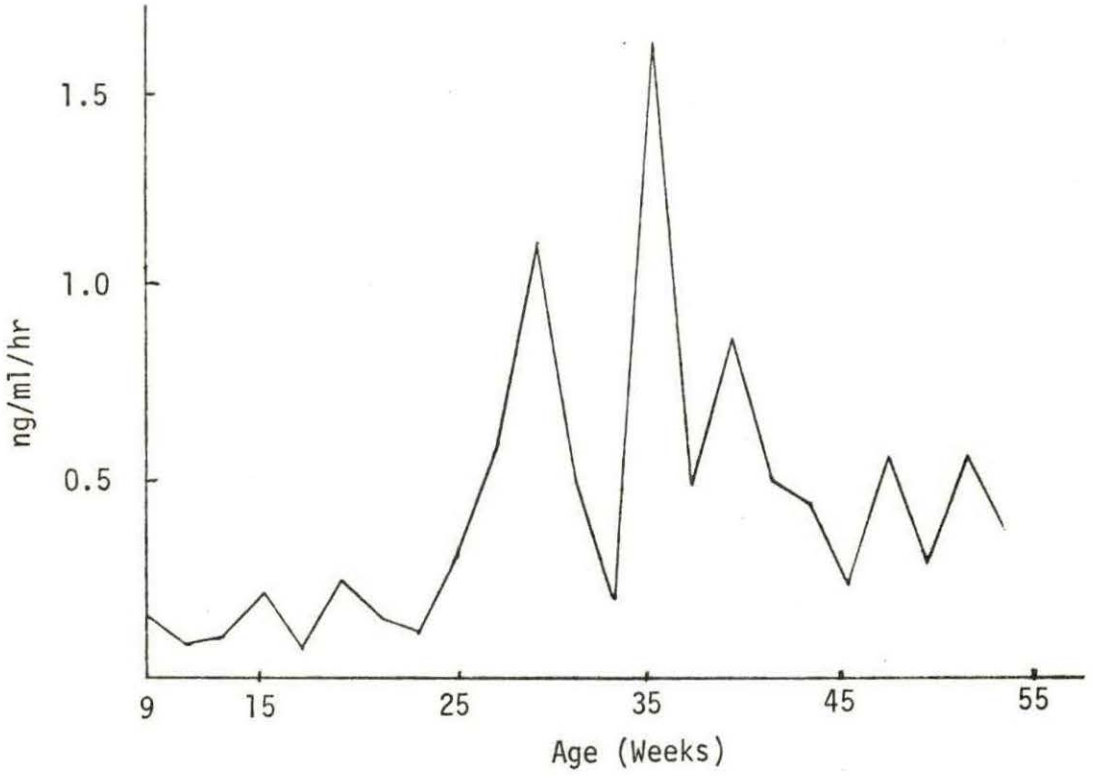

Figure 1. Mean PRA values in unanesthetized state

between sexes with regard to PRA in the unanesthetized state. The mean value for the females was 0.417 ng/ml/hr while the mean value for the males was 0.416 ng/ml/hr. In the anesthetized state under the same conditions, a difference was found between sexes with regard to mean PRA (1.975 ng/ml/hr for males and 1.152 ng/ml/hr for females). However, this difference was not highly significant ($0.10<p<0.20$).

Body weight demonstrated no significant effect upon PRA.

Correlation with cardiovascular functions

No significant correlation was found between PRA values obtained after anesthesia and systolic pressure, diastolic pressure or pulse pressure.

Heart rate demonstrated no significant correlation with PRA in either the unanesthetized or anesthetized state.

Correlation with serum electrolytes

The mean values for serum sodium concentration were 137.5 mEq/l for females. The mean values for serum potassium concentration were 4.40 mEq/l for males and 4.56 mEq/l for females. No significant sex difference was demonstrated by either electrolyte, and no significant correlation was demonstrated by either electrolyte with PRA values obtained from unanesthetized dogs.

DISCUSSION

Plasma renin activity in the dog from birth to 2 weeks of age was shown by Granger et al. (1971) to be significantly higher than PRA in the adult dog. These elevated levels would indicate that the mechanisms for renin synthesis and secretion are functional at this early age. Therefore, the increase due to age noted in the group of dogs studied in this project was not attributed to a maturation of secretory units.

If the aldosterone secretory apparatus in the newborn is not as responsive as in the adult, as suggested by Kemény et al. (1964), this could account for the elevated PRA in the newborn. The rapid reduction in PRA after birth would indicate that the apparatus matures rapidly.

New et al. (1966) showed that urinary aldosterone excretion in normal children was less than that in adults. However, there was no difference between children and adults when excretion rates were based on body surface area.

Minick and Conn (1964) found that urinary aldosterone excretion in children was greater than that in adults when evaluated on a per kilogram of body weight basis.

Plasma renin activity is estimated on the basis of angiotensin I generated per milliliter of plasma per hour. Therefore, the estimated effect of renin within the renin-angiotensin-aldosterone system should be independent of body size. Thus, the increase in PRA does not appear to be paralleled by an increase in aldosterone excretion. To draw more definite conclusions from what appears to be a changing relationship due to age, it would be necessary to monitor both PRA and plasma aldosterone concentrations concurrently.

The increase in PRA during estrus and pseudocyesis may be due to an increase in PRS; however, this explanation is somewhat illogical. Unless there was a need for an increase in the activity of the system, the mechanisms controlling the secretion of renin should reduce it so that PRA remained constant. Chesley (1963) reported that pregnant women were more resistant to pressor and renal effects of angiotensin II than nonpregnant women. If there was a decrease in the sensitivity of the system during periods of high circulatory levels of estrogens and progesterones, this would account for the increased PRA.

Little information is available concerning estrogen levels in the dog during different stages of estrus. Thus, to outline the exact relationships it would be necessary to monitor estrogen levels, PRA, PRS, and plasma aldosterone concurrently in dogs during estrus.

The lack of an interaction between the effects of anesthesia and the effect of estrus and pseudocyesis would suggest that these effects are mediated through two separate mechanisms.

Although the exact mechanisms of the effects of age and sex upon PRA have not been defined, the conclusion that there are significant effects is important.

Hypertension is a current major medical problem. The renin-angiotensin-aldosterone system is most definitely involved with the maintenance of arterial pressure in both the normal and in certain hypertensive states. The effects of all variables, such as the effects of age and sex, involved with the control of this system must be considered when designing experiments to explore unknown aspects of the system.

PART II. THE EFFECT OF A SEVERE DIETARY STRESS UPON PLASMA RENIN ACTIVITY IN THE BEAGLE

LITERATURE REVIEW

Plasma renin activity has been shown to be elevated during periods of cardiovascular stress (Blaine et al., 1970; Hall and Hodge, 1971; and Johnson and Davis, 1972). It has also been demonstrated that the renin-angiotensin system is capable of significant involvement in the regulation of arterial pressure during cardiovascular stress (Cowley et al., 1971).

Elevated plasma renin activity has been found in children suffering from kwashiorkor (Kritizinger, Kanengoni, and Jones, 1972). In these cases the increase in activity was found not to be correlated with serum or urinary electrolytes. However, no mention was made of attempts to evaluate cardiovascular function and to correlate possible pressure changes with increases in plasma renin activity. Hypotension and bradycardia are common signs in humans suffering from dietary stresses such as starvation or severe malnutrition (Keys et al., 1950).

MATERIALS AND METHODS

Animal Techniques

Twenty-three beagle pups representing eight litters were randomly divided within litters into two groups at 7 weeks of age. The first group, six females and five males representing six of the eight litters, was designated as the control protein (CP) group. The second group, seven males and five females representing six of the eight litters, was designated as the low protein (LP) group. Members of each group were separated from their dams for a short time each day and fed their respective diets. At 9 weeks of age the dogs were weaned and feeding of the diets was begun ad libitum. The dogs received immunizations for canine distemper, canine infectious hepatitis and leptospirosis[1] at 8, 14, and 24 weeks of age. The dogs were checked for internal parasites by periodic fecal exams and oral medication[2] was administered when necessary. All dogs were housed in one room separated by sex and dietary group.

Sampling Intervals and Sample Preparation

Blood plasma

Beginning at 9 weeks of age each dog was sampled every 2 weeks until the fifty-third week of age. Four ml of blood were drawn from the left external jugular vein while the dog was held in lateral recumbency on its right side. The blood was immediately transferred to a chilled glass tube

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[1]Enduracell d-h-l, Norden Laboratories, Lincoln, Nebraska.

[2]Vermiplex, Pitman-Moore, Inc., Fort Washington, Pennsylvania.

containing 7.5 mg disodium edetate (EDTA)[1]. The samples were held in an ice bath until they could be centrifuged (200 X g for 15 minutes) in a cold room (4 C) to recover the plasma. The plasma was stored frozen at -20 C until assayed for PRA. The sample preparation procedures were based on the recommendations given for the PRA radioimmunoassay technique used for the assays.

Blood serum

Beginning at 25 weeks of age each dog was sampled monthly until the fifty-third week of age. Four additional ml of blood were drawn at the same time that the blood for PRA determination was taken. The blood was transferred to an empty glass tube[2] and placed in an ice bath. After clotting the samples were centrifuged (200 X g for 15 minutes) in a cold room (4 C) to recover the serum. The serum was stored frozen at -20 C until assayed.

Electrocardiograms

Beginning at 23 weeks of age the electrocardiogram (EKG) of each dog was recorded each time a blood sample was taken. The bipolar standard lead II was recorded with the animal resting in right lateral recumbency. Subdermal platinum alloy electrodes and a Grass Model 7 Polygraph[3] were used

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[1]Vacutainer #4770, Becton, Dickinson and Company, Rutherford, New Jersey.

[2]Vacutainer #4710, Becton, Dickinson and Company, Rutherford, New Jersey.

[3]Grass Instrument Company, Quincy, Massachusetts.

for the recordings. The recordings were made on two channel Grass recording paper at a calibration setting of 1 mv/cm and a paper speed of 30 mm/sec.

Blood pressure

Beginning at 25 weeks of age the arterial blood pressure of each dog was recorded monthly through the fifty-third week of age. This procedure was done immediately after the blood sample for the PRA determination had been collected. EKG leads were attached to the animal and recordings of the standard lead II were made throughout the procedure.

The dog was anesthetized with sodium methohexital[1] (9 mg/Kg) by intravenous injection into the cephalic vein of the left forelimb while resting in right lateral recumbency. The femoral artery was located in the femoral triangle of the right leg by digital palpation. A 22 gauge 1 inch hypodermic needle attached to a vinyl tubing cannula (0.025 inch ID) filled with sterile saline was used to cannulate the artery. The cannula was attached to a Model 23Dc Statham pressure transducer[2]. Recordings were made on a Grass Model 7 polygraph[3] at a paper speed of 60 mm/sec. After a recording period of 20 to 30 seconds the needle was withdrawn from the artery and digital pressure was applied to prevent hemorrhage. A blood sample for PRA determination was taken by the previously described procedure immediately after the removal of the cannula.

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[1]Brevane, Elanco Products Company, Indianapolis, Indiana.

[2]Statham Laboratories, Inc., Hato Rey, Puerto Rico.

[3]Bendix Corporation, 1400 Taylor Avenue, Baltimore, Maryland.

Temperature-humidity index

The temperature and humidity of the animal quarters were continuously recorded with the use of a hygro-thermograph[1].

Assay Procedures

Plasma renin activity

The PRA of the plasma samples was determined with the use of a radioimmunoassay for angiotensin I. The protocol for these determinations was based on the techniques described by Haber et al. (1969). Angiotensin I standard solution (10 ng/ml), angiotensin I labelled with ^{125}I and angiotensin I antiserum were obtained from two commercial sources[2,3]. A detailed outline of the procedure is given in Appendix I.

Sodium and potassium

The serum samples previously described were randomized for assay at a later time. The assays were performed using a Baird-Atomic Flame Photometer Model KY-2[4].

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[1]Bendix Corporation, 1400 Taylor Avenue, Baltimore, Maryland.

[2]Schwarz/Mann, Orangeburg, New York.

[3]New England Nuclear, 575 Albany Street, Boston, Massachusetts.

[4]Baird-Atomic, Inc., Cambridge, Massachusetts.

RESULTS

Data Analysis

An analysis of variance was used to test for between group differences. The experimental unit was considered to be the individual animal.

An analysis of variance was used to test for interactions.

A correlation matrix was used to display correlation coefficients and their significance. The degrees of freedom for the tests of significance of these coefficients were based on the number of observations.

Growth Patterns

The experimental plan which has been described was agreed upon by the investigator and a funding corporation. This corporation formulated and prepared the experimental low protein diet. A small number of pilot trials demonstrated that young, growing dogs could not survive on the low protein diet, and therefore, the experimental design was changed.

The low protein group was weaned using the low protein diet and was maintained on this diet until each dog experienced a 20% loss in body weight based on the maximum recorded weight. This was determined individually and at that point each dog was switched to the control diet. The average length of time on the low protein diet following weaning was 12 days.

At 25 weeks of age the low protein group was once again switched to the low protein diet. They were maintained on this feed throughout the remainder of the experiment.

During this second period of being fed the low protein diet, it again became evident that the LP group could not be maintained on the diet. The funding corporation furnished an analysis of the LP diet for study. It was decided to attempt to partially correct the deficiencies by amino acid supplementation. The amino acids selected and the levels of supplementation were methionine 36 mg/lb/day, histidine-d 20 mg/lb/day, and lipine-d 62 mg/lb/day. These levels of supplementation were twice the minimum daily requirements recommended for adult dogs (National Academy of Sciences, 1972).

The supplementation of the LP group was begun on an individual basis when each dog recorded a 20% weight loss based on its maximum recorded weight. The amino acids were administered orally each day in a #000 gelatin capsule[1]. The daily supplementation was continued throughout the remainder of the experiment.

This regime of supplementation arrested the losses of body weight. It also markedly increased the activity of the dogs of the LP group.

Cardiovascular Functions

Blood pressures

The mean values for systolic, diastolic, and pulse pressures (table 1) during the experimental period were significantly lower ($p<0.005$) for the LP group than for the CP group.

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[1]Eli Lilly and Company, Indianapolis, Indiana.

Table 1. Mean femoral pressures recorded in mmHg

	Control Protein			Low Protein		
	Group	Females	Males	Group	Females	Males
Systolic	148 ± 26[a]	157 ± 26[b]	141 ± 25[b]	119 ± 20[a]	117 ± 21	120 ± 19
Diastolic	97 ± 23[c]	109 ± 24[d]	87 ± 20[d]	76 ± 18[c]	74 ± 18	75 ± 18
Pulse	51 ± 11[e]	48 ± 11	54 ± 12	43 ± 11[e]	43 ± 10	45 ± 11

[a]Significant difference, $p<0.005$.

[b]Significant difference, $p<0.025$.

[c]Significant difference, $p<0.005$.

[d]Significant difference, $p<0.005$.

[e]Significant difference, $p<0.005$.

The systolic and diastolic values for the LP group at 25 weeks of age (126 mmHg systolic and 80 mmHg diastolic) were lower than the values for the CP group at the same age (138 mmHg systolic and 96 mmHg diastolic). The systolic and diastolic values for the LP group appeared to decrease even more during the second period of being fed the LP diet (figure 2).

Within the CP group, there was a significant difference between sexes in both systolic ($p<0.025$) and diastolic ($p<0.005$) pressures. The females had higher values for both. No significant sex difference was found in pulse pressure. There were no significant differences between sexes in the LP group with regard to systolic, diastolic or pulse pressure.

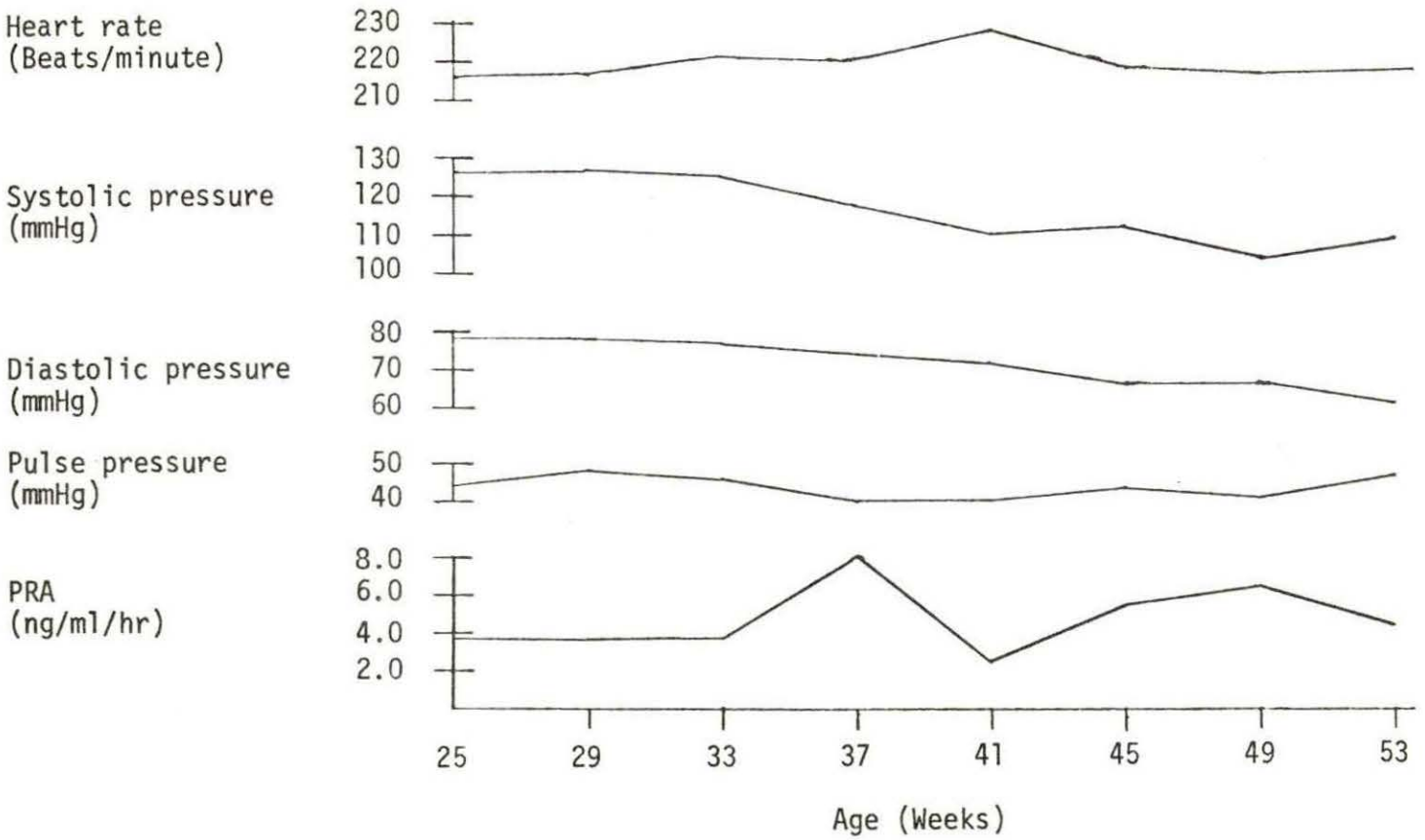

Figure 2. Progress of low protein group

Dietary supplementation of the LP group demonstrated no significant effect upon the arterial pressures of that group.

Heart rate

In the unanesthetized state no significant difference was found between the dietary groups with regard to heart rate (table 2).

Table 2. Mean heart rates recorded in beats per minute

	Control Protein			Low Protein		
	Group	Females	Males	Group	Females	Males
Unanesthetized	118 ± 24	116 ± 24	117 ± 23	112 ± 22	117 ± 26	109 ± 14
Anesthetized	217 ± 22	227 ± 21[a]	207 ± 28[a]	218 ± 28	226 ± 28[b]	209 ± 27[b]

[a]Significant difference, $p<0.005$.

[b]Significant difference, $p<0.05$.

Within the LP group, there was a significant sex difference ($p<0.050$) in heart rate in the unanesthetized state during the period that the dogs were fed the LP diet without supplementation. The males had the lower mean value (table 3). This sex difference was not found when the dogs received amino acid supplementation.

With regard to heart rate after anesthesia, there were significant sex differences in both the CP group ($p<0.005$) and the LP group ($p<0.050$). The females had higher values in both dietary groups (table 2). There was no

Table 3. Mean heart rates of low protein group

	Unanesthetized		Anesthetized	
	Males	Females	Males	Females
No supplementation	106 ± 13[a]	118 ± 28[a]	213 ± 28	223 ± 28
Amino acid supplementation	116 ± 16	117 ± 22	205 ± 26	228 ± 29

[a]Significant difference, $p<0.05$.

significant difference between dietary groups in heart rate after anesthesia. Within the LP group, dietary supplementation did not significantly affect heart rate in either sex.

Plasma Renin Activity

Effect of a dietary stress

Figure 3 illustrates the progress of the two dietary groups with regard to mean PRA recorded in the unanesthetized state. The LP group had a higher mean PRA at weaning, 9 weeks of age, than did the CP group. The growth curves, figure 4, indicated that the LP group was undergoing a dietary stress at this time.

During the period that both dietary groups were fed the CP diet, the difference in mean PRA between the two groups lessened. But, it was still significant ($p<0.005$).

At 25 weeks of age, the LP group was again fed the LP diet. The growth curve for the LP group shows a decrease in mean weight from this point indicating a dietary stress. The unanesthetized PRA of the LP group

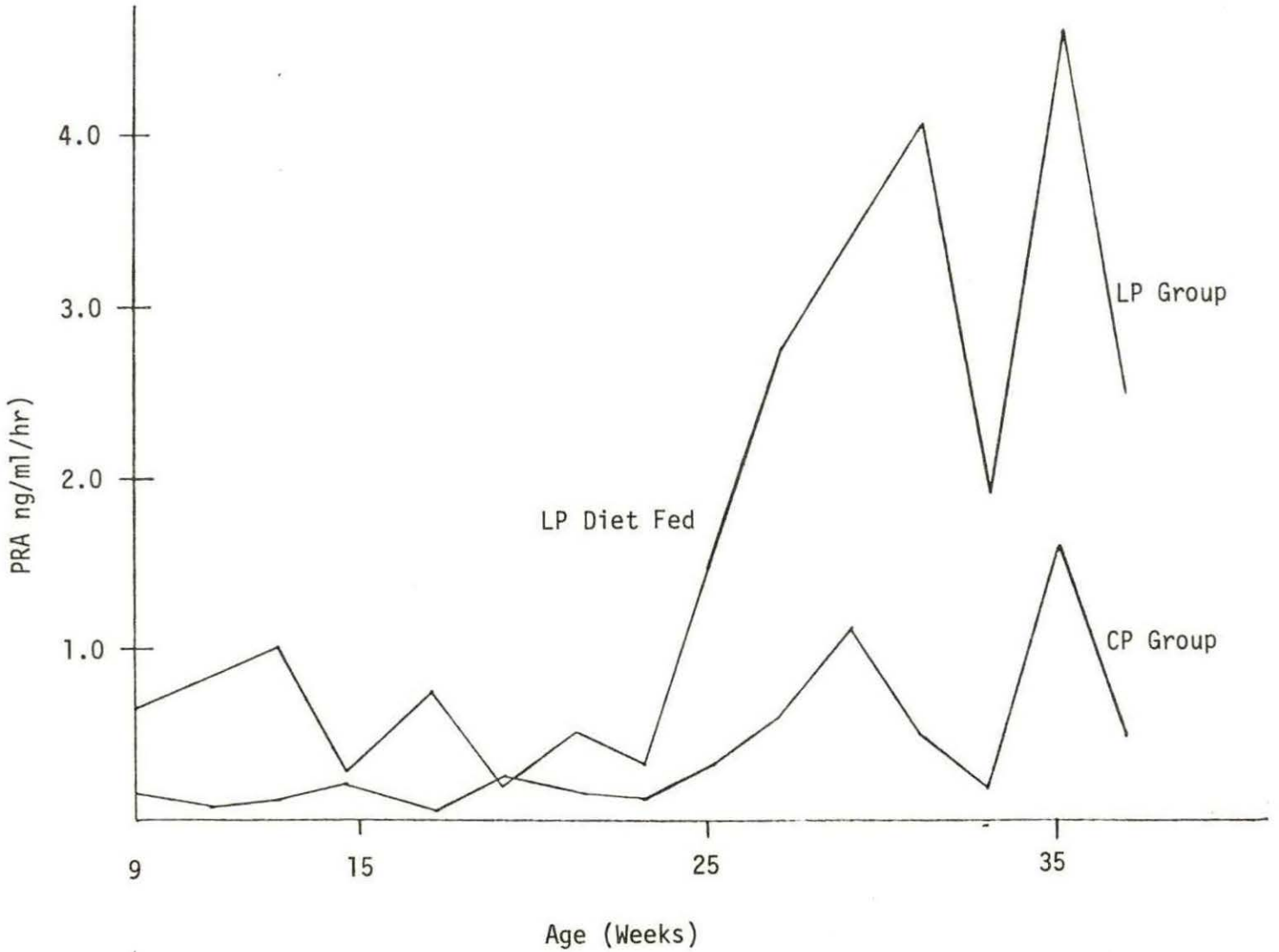

Figure 3. Mean PRA of CP and LP groups in unanesthetized state

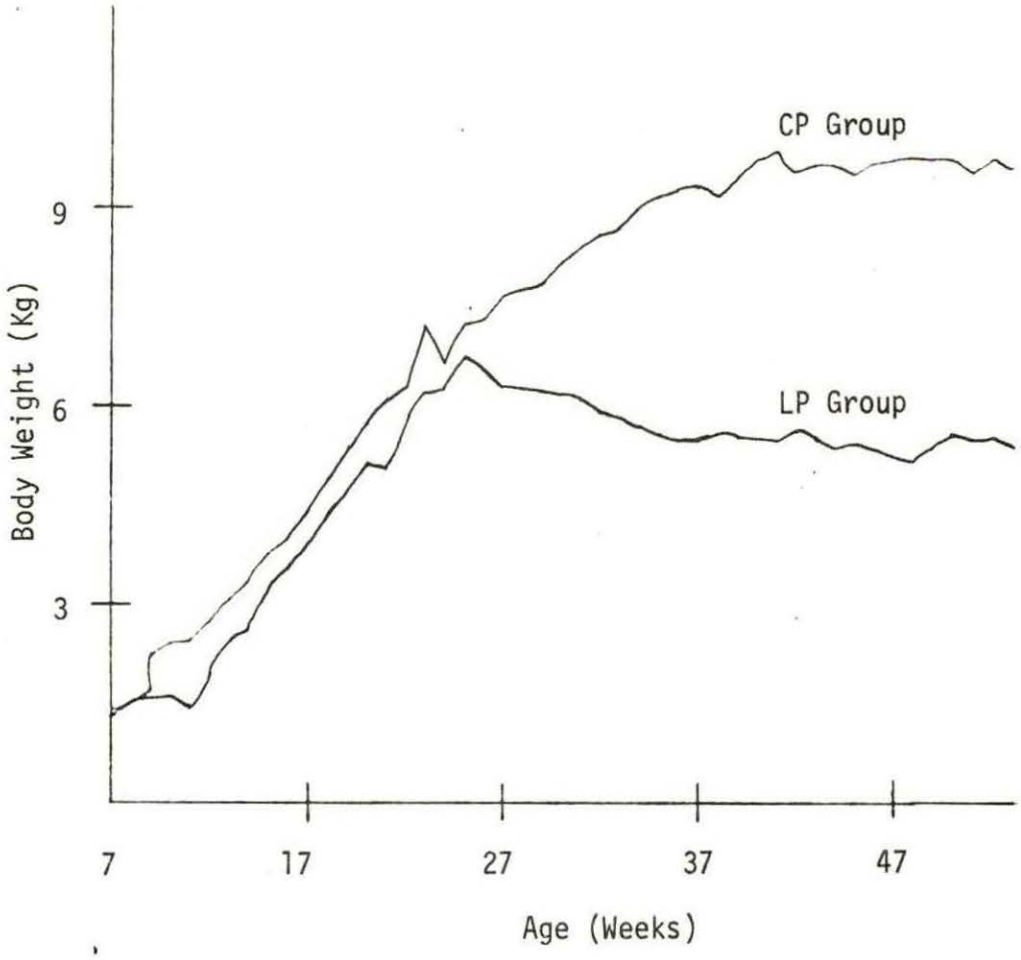

Figure 4. Growth curves

rose rapidly and remained elevated during the dietary stress. The difference between the two groups during this second dietary stress, based on mean values adjusted for age, estrus and pseudocyesis, was highly significant ($p<0.005$).

Dietary supplementation of the LP group demonstrated no significant effect upon PRA of the LP group.

Correlations with cardiovascular functions

PRA demonstrated no statistically significant correlation with systolic pressure, diastolic pressure, pulse pressure, or heart rate in either dietary group.

However, within the LP group it did appear that systolic and diastolic pressures were decreasing while PRA values from anesthetized animals were increasing (figure 2). The CP group showed no decrease in pressures.

No significant correlation was found between PRA values from unanesthetized animals and serum sodium concentration or serum potassium concentration in either dietary group. No significant difference was found between dietary groups or sexes with respect to either electrolyte (table 4).

Effect of anesthesia

PRA was significantly higher in the anesthetized state than in the unanesthetized state (figure 5) for both the CP group ($p<0.005$) and the LP group ($p<0.050$). An analysis of variance demonstrated no significant interaction between dietary group and state of anesthesia. This increase in PRA after barbiturate anesthesia has been observed by other authors (Ganong, 1972).

Table 4. Serum sodium and serum potassium concentrations recorded in mEq/l

	Control Protein			Low Protein		
	Group	Females	Males	Group	Females	Males
Sodium	137.3±7.3	137.2±7.4	137.5±7.2	137.5±8.0	137.2±9.0	137.7±6.9
Potassium	4.48±0.46	4.56±0.46	4.40±0.42	4.42±0.51	4.37±0.41	4.64±0.56

Within both dietary groups, the males showed a greater increase in PRA after anesthesia than the females. However, this difference was not significant.

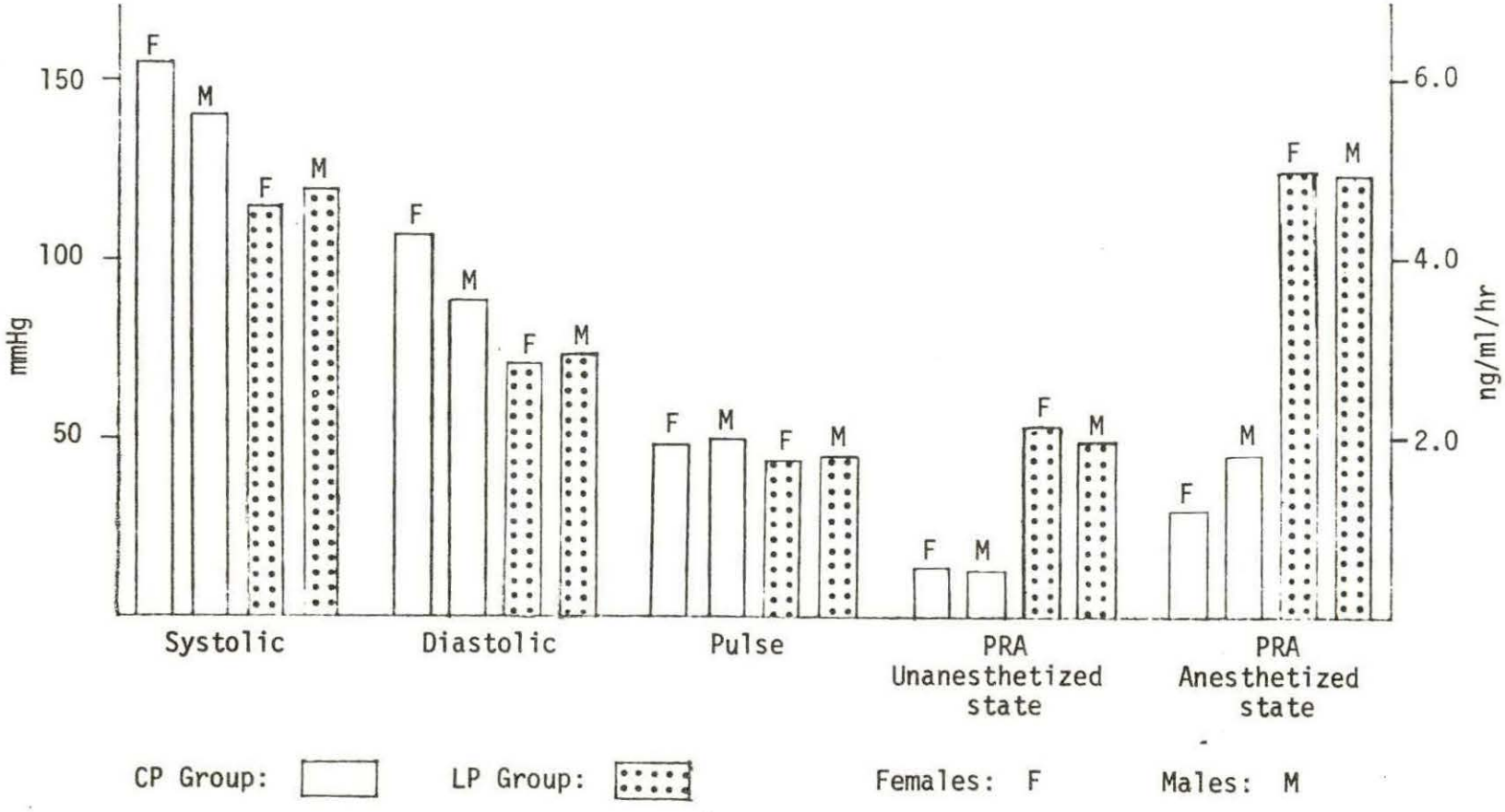

Figure 5. Arterial pressure and PRA mean values for age period 25-53 weeks

DISCUSSION

The dietary stress endured by the LP group of dogs was extremely severe as was shown by the rapid loss of body weight when the animals were fed the LP diet.

The rapid rise in PRA (unanesthetized state) after the LP group was fed the LP diet at 25 weeks of age indicates that the renin-angiotensin system was responding to the stress. The only other variable which demonstrated a change at this time was the decline in the heart rate of the unanesthetized males of the LP group.

After being fed the LP diet for 8 weeks, the mean PRA in the unanesthetized of the LP group began to increase. At the same time systolic pressure, diastolic pressure, and pulse pressure began to decline. These relationships between PRA and femoral pressures suggested that the stimuli responsible for the increases in PRA during the dietary stress were decreases in arterial pressure.

The sex difference in heart rate during the dietary stress and the lack of a sex difference in PRA during the same period suggested that the increases in PRA were not mediated through the sympathetic system. This was supported by the significant effect of amino acid supplementation upon the heart rate and physical activity of the males of the LP group in the absence of an effect on PRA.

The sex differences in the CP group with regard to arterial pressures, heart rates before and after anesthesia, and increases in PRA due to anesthesia suggested a possible mechanism for the increase in PRA due to anesthesia. The females appeared to be more capable than the males of respond-

ing to the effect of barbiturate anesthesia by mechanisms other than the renin-angiotensin system. This was indicated by the higher heart rates and higher arterial pressures. These other physiological mechanisms for maintaining blood pressure were apparently not as efficient in the males and thus the renin-angiotensin system received greater stimulation.

The dietary stress appeared to decrease the ability of the females to maintain arterial pressures by physiological mechanisms other than the renin-angiotensin system. This was indicated by the lack of a sex difference in arterial pressures and PRA after anesthesia in the LP group. The sex difference in the LP group with regard to heart rates after anesthesia is important for it indicates that the females were still more capable of responding to barbiturate anesthesia than were the males.

SUMMARY OF CONCLUSIONS

It was concluded that plasma renin activity increases in the normal, growing beagle from weaning to 1 year of age. This increase was independent of body weight and sex when the effects of estrus and pseudocyesis in the female are removed. Interpretation of this increase pointed to a maturation of control systems rather than maturation of secretory units.

Plasma renin activity increases in the female beagle during estrus or pseudocyesis. This increase was independent of the increase in PRA due to anesthesia thus indicating two separate pathways. Consideration must be given to these effects of estrus and pseudocyesis in experimental designs involving measurement of PRA in the female beagle.

An increase in PRA of beagles undergoing a severe dietary stress was found and this increase was apparently not mediated through the sympathetic system. The stimuli eliciting the increases appeared to be decreases in arterial pressure which, in turn, would increase renin secretion.

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APPENDIX I.

THE DETERMINATION OF PLASMA RENIN ACTIVITY WITH THE USE OF A RADIOIMMUNOASSAY FOR ANGIOTENSIN I

The PRA of the samples was determined with the use of a radioimmunoassay for angiotensin I. The protocol for these determinations was based on the technique described by Haber et al. (1969).

The frozen plasma samples, containing EDTA as previously described, were thawed carefully at 4 C. To 1 ml of each cold plasma sample 10 µl of 8-hydroxyquinoline (0.340 M) and 2 µl of dimercaprol (0.806 M) were added. These acted as enzyme inhibitors to prevent the conversion of angiotensin I to angiotensin II and to prevent the enzymatic destruction of the angiotensin I which will be generated. A 0.5 ml aliquot of this mixture was removed and refrigerated at 4 C. The remainder was placed in a 37 C waterbath and incubated for 3 hours to allow for the renin-renin substrate reaction.

During the incubation period 0.9 ml of 0.1 M Tris buffer (pH 7.4), containing 1 mg lysozyme per ml, was added to a 12 x 75 mm polystyrene culture tube[1] being chilled in an ice bath. Six such tubes were prepared for each plasma sample and 30 such tubes, numbered 1 through 30, were prepared for the calculation of a standard curve. A standard curve was prepared and calculated daily with each group of 15 to 30 samples.

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[1]Falcon Plastics, Oxnard, California.

Angiotensin I standard solution[1,2] (10 ng/ml) was added to 18 tubes to locate points on a standard curve. Beginning with tube number seven, triplicate points were located using the following volumes: 0.005 ml, 0.010 ml, 0.020 ml, 0.030 ml, 0.050 ml, and 0.075 ml.

At the end of the 3 hour incubation period, 0.05 ml of the plasma aliquot incubated at 4 C was added to each of three tubes prepared for that sample. The same volume of the aliquot incubated at 37 C was added to the other three tubes prepared for that sample.

Angiotensin I[1,2] labelled with ^{125}I was added to each tube with the exception of tubes 25, 26, 29, and 30 of the standard curve. Tube numbers 29 and 30 were procedural checks to register the counts per minute (cpm) of the background. When Schwartz-Mann reagents were used 0.05 ml of the labelled angiotensin I was added, and when New England Nuclear reagents were used 0.10 ml of the labelled angiotensin I was added. These amounts were sufficient to give the recommended level of 4500 to 5000 cpm per tube.

Twice the routine amount and three times the routine amount of labelled angiotensin I were added to tubes 25 and 26 respectively. These were procedural checks demonstrating that these amounts of labelled angiotensin I would not significantly affect the assay.

Angiotensin I antiserum[1,2] was then added to each tube with the exception of tubes 1, 2, 3, 28, 29, and 30 of the standard curve. When Schwarz-Mann reagents were used 0.05 ml of the antiserum was used, and

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[1]Schwarz-Mann, Orangeburg, New York.

[2]New England Nuclear, 575 Albany Street, Boston, Massachusetts.

when New England Nuclear reagents were used 0.10 ml of the antiserum was added. These amounts were sufficient to bind approximately 50% of the labelled angiotensin I. It was at this percentage of binding that the precision of the assay was maximized.

Following the addition of the antiserum, the tubes were mixed on a Vortex Genie Mixer[1], covered with parafilm, and incubated at 4 C for 20 to 24 hours.

Following the incubation 1 ml of a dextran-coated charcoal suspension was added to each tube with the exception of tubes 27, 28, 29, and 30 of the standard curve. The charcoal suspension containing 2.5 g Norit A charcoal and 0.25 Dextran, radioimmunoassay grade, suspended in 400 ml of barbital buffer. The buffer was prepared by dissolving 3.825 g sodium chloride, 0.7357 g sodium barbital, and 0.4857 g sodium acetate trihydrate in 450 ml distilled water. The pH of this solution was adjusted to 7.4 with 0.10 N hydrochloric acid and distilled water was added to give a total volume of 500 ml.

The charcoal suspension and the incubate were mixed on a Vortex Genie Mixer and then centrifuged at 1750 X g at 4 C for 20 minutes.

The clear supernatant and the charcoal residue were separated by decanting the clear supernatant into an identical and similarly numbered tube. Each tube was counted in sequence, with the supernatant being first and then its residue, in a well-type solid crystal scintillation gamma counter[2] for 5 minutes.

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[1]American Hospital Supply Corporation, Evanston, Illinois.

[2]Picker Nuclear, North Haven, Connecticut.

Calculations

All calculations were done using programs designed for a Wang Model 500 Programable Calculator[1]. A total of four such programs were used.

Program titled Renin I calculated the ratio F/Bc for each tube where F was the average cpm of free labelled angiotensin I in the supernatant and Bc was the average corrected cpm of labelled angiotensin I in the charcoal residue bound to the antibody.

The number of counts in tubes 1, 2, and 3 of the standard curve were first used by Renin I to calculate a correction factor to be applied to all other tubes. This was done by dividing the cpm of the residue of each of the three tubes by the sum of the cpm in the residue and its corresponding supernatant. Labelled angiotensin I, Tris buffer, and the charcoal suspension were the only reagents added to these tubes. Therefore, theoretically the charcoal should have absorbed the total quantity of the labelled angiotensin. However, there is some mechanical loss of counts into the supernatant. The correction factor accounts for this loss.

The total number of cpm for each tube was obtained by summing the cpm of the supernatant and the residue. This total (T) was multiplied by the average correction factor obtained from the first three tubes to give the corrected total (Tc). The cpm of the residue was then subtracted from the corrected total (Tc) leaving as a remainder the cpm of the antibody bound fraction corrected (Bc).

Each point on the standard curve and each sample fraction was performed in triplicate. The program titled Renin II was a rejection criteria

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[1]Wang Laboratories, Inc., Tewksbury, Massachusetts.

applied to each group of triplicate results. This criteria compared each individual value to the mean of the three values and each individual value to the mean of the other two values. The limits which were acceptable were based upon prior experience with the PRA assay. The values which this criteria used for comparison were the F/Bc ratios.

The next program, Renin III, was used to calculate the standard curve and to arrive at unknown values from the standard curve. This program fit the best linear line for the plot of the F/Bc ratios of the tubes versus the standard added to those tubes. The program would then calculate unknown angiotensin amounts by interpolation from this curve with the use of the F/Bc ratios of the unknowns.

The last program, Renin IV, converted the amount of angiotensin obtained by the program Renin III to the units of ng/ml/hr. This was done by multiplying the amount by the appropriate dilution factor, and dividing this product by three, the incubation time.

APPENDIX II. TABLES

Table 5. Analysis of variance to test for differences in systolic pressure between CP group and LP group while LP group being fed LP diet

Source	s.s.	m.s.	F value	P
Between dietary groups	26488	26488	53	<0.005
Residual	66486	504		

Table 6. Analysis of variance to test for differences in diastolic pressure between CP group and LP group while LP group being fed the LP diet

Source	s.s.	m.s.	F value	P
Between dietary groups	14439	14439	36	<0.005
Residual	52350	397		

Table 7. Analysis of variance to test for differences in pulse pressure between CP group and LP group while LP group being fed the LP diet

Source	s.s.	m.s.	F value	P
Between dietary groups	1814	1814	15	<0.005
Residual	15559	118		

Table 8. Analysis of variance to test for effect of age on PRA (unanesthetized state) in CP group

Source	s.s.	m.s.	F value	P
Age	7.3	7.3	9.5	<0.025
Residual	199.1	0.8		

Table 9. Analysis of variance to test for effects of dietary group, sex and age on PRA (unanesthetized state)

Source	s.s.	m.s.	F value	P
Between dietary groups	432.6	432.6	45.5	<0.005
Between sexes	0.2	0.2	0.0	N.S.
Increasing age	575.1	18.6	2.0	<0.05
Residual	5860.8	9.5		

Table 10. Analysis of variance to test for differences in PRA (unanesthetized state) between CP group and LP group while LP group fed LP diet, corrected for age

Source	s.s.	m.s.	F value	P
Between dietary groups	417	417	66	<0.005
Residual	1983	6		

Table 11. Analysis of variance to test for sex difference in systolic pressure in CP group

Source	s.s.	m.s.	F value	P
Between sexes	5410	5410	9	<0.025
Residual	46630	614		

Table 12. Analysis of variance to test for sex difference in diastolic pressure in CP group

Source	s.s.	m.s.	F value	P
Between sexes	7559	7559	17	<0.005
Residual	33951	447		

Table 13. Analysis of variance to test for effect of anesthesia on PRA of CP group; based on sampling periods where PRA was recorded both before and after anesthesia; values for female demonstrating signs of estrus or pseudocyesis were not used

Source	s.s.	m.s.	F value	P
Before and after anesthesia	54	54	14	<0.005
Residual	649	4		

Table 14. Analysis of variance to test for a sex difference in heart rate after anesthesia in the LP group while being fed the LP diet

Source	s.s.	m.s.	F value	P
Between sexes	4513	4513	6	<0.05
Residual	51380	816		

Table 15. Analysis of variance to test for a sex difference in heart rate after anesthesia in the CP group; values for females demonstrating signs of estrus or pseudocyesis were not used

Source	s.s.	m.s.	F value	P
Between sexes	6752	6752	14	<0.005
Residual	32373	498		

Table 16. Analysis of variance to test for litter differences in PRA (unanesthetized state) within CP group

Source	s.s.	m.s.	F value	P
Between litters	9.6	1.9	0.76	N.S.
Residual	848.8	2.5		

Table 17. Analysis of variance to test for differences in PRA (unanesthetized state) between CP group and LP group while both fed CP diet

Source	s.s.	m.s.	F value	P
Between dietary groups	6.1	6.1	12.9	<0.005
Residual	80.0	0.5		

Table 18. Analysis of variance to test for effect of estrus on PRA (unanesthetized state) of females of CP group

Source	s.s.	m.s.	F value	P
Age	6.6	6.6	6.6	<0.05
Estrus	7.5	7.5	7.5	<0.05
Residual	136.6	1.0		

Table 19. Analysis of variance to test for effects of anesthesia and sex on the PRA of the LP group

Source	s.s.	m.s.	F value	P
Before and after anesthesia	410	410	25	<0.005
Between sexes	1	1	0	N.S.
Residual	5107	17		

APPENDIX III. DATA SETS

Legend for Data Sets

Each of the following data sets are preceded by a description of the set of which the included observations are members. Each line of the sets is a group of different observations all recorded from the same dog at the same time.

The columns of each set contain the following:

Column 1: dog identification number
Column 2: sex of dog; coded 1 for female and 2 for male
Column 3: litter identification number
Column 4: age of dog; recorded in weeks
Column 5: weight of dog; recorded in pounds
Column 6: heart rate of dog; recorded in beats per minute
Column 7: systolic blood pressure; recorded in mmHg
Column 8: diastolic blood pressure; recorded in mmHg
Column 9: state of anesthesia; coded 1 for unanesthetized and 2 for anesthetized
Column 10: dietary supplementation; coded 1 for no supplementation and 2 when dietary supplements were being administered
Column 11: plasma renin activity; recorded in ng/ml/hr
Column 12: assay identification number
Column 13: estrus or pseudocyesis; coded 1 when no physical signs of estrus or pseudocyesis were seen and 2 when such signs were evident
Column 14: diet being fed; coded 1 for control diet and 2 for low protein diet
Column 15: temperature-humidity index of 4 hour period just prior to sampling
Column 16: temperature-humidity index of 72 hour period just prior to sampling
Column 17: serum sodium concentration; recorded in mEq/l
Column 18: serum potassium concentration; recorded in mEq/l
Column 19: arterial pulse pressure; recorded in mmHg

Data Set I. Observations from control protein group

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
22	2	5	09	4.6	-	-	-	1	1	0.480	29	1	1	68.1	62.7	-	-	-
22	2	5	13	8.0	-	-	-	1	1	0.062	48	1	1	68.4	68.6	-	-	-
22	2	5	15	10.1	-	-	-	1	1	0.198	33	1	1	72.4	72.6	-	-	-
22	2	5	19	14.4	160	-	-	1	1	0.125	38	1	1	66.9	67.8	-	-	-
22	2	5	21	16.0	180	-	-	1	1	0.011	48	1	1	67.6	68.9	-	-	-
22	2	5	23	16.6	120	-	-	1	1	0.012	46	1	1	78.3	74.6	-	-	-
22	2	5	25	18.6	150	-	-	1	1	0.437	12	1	1	72.6	75.0	-	-	-
22	2	5	26	-	-	-	-	1	1	1.093	20	1	1	-	-	142	5.2	-
22	2	5	27	19.3	120	-	-	1	1	0.302	20	1	1	78.5	81.1	-	-	-
22	2	5	29	20.5	120	-	-	1	1	5.434	43	1	1	72.2	76.2	-	-	-
22	2	5	29	20.5	210	115	95	2	1	-	-	1	1	72.2	76.2	-	-	20
22	2	5	31	22.0	120	-	-	1	1	0.244	48	1	1	71.0	74.2	-	-	-
22	2	5	33	22.8	110	-	-	1	1	0.128	37	1	1	65.9	69.0	-	-	-
22	2	5	35	21.4	100	-	-	1	1	1.079	17	1	1	69.3	69.0	132	4.0	-
22	2	5	35	21.4	215	142	92	2	1	3.430	11	1	1	69.3	69.0	-	-	50
22	2	5	37	24.1	100	-	-	1	1	0.667	36	1	1	65.5	62.5	-	-	-
22	2	5	39	26.3	125	-	-	1	1	2.020	16	1	1	61.5	61.2	129	4.2	-
22	2	5	39	26.3	195	117	60	2	1	1.344	45	1	1	61.5	61.2	-	-	57
22	2	5	41	24.0	90	-	-	1	1	1.217	48	1	1	57.3	56.5	-	-	-
22	2	5	43	25.7	100	-	-	1	1	0.144	43	1	1	48.4	48.5	-	-	-
22	2	5	45	25.0	90	-	-	1	1	0.073	17	1	1	53.8	54.4	132	3.6	-
22	2	5	45	25.0	180	140	85	2	1	2.076	12	1	1	53.8	54.4	-	-	55
22	2	5	47	30.1	130	-	-	1	1	1.024	45	1	1	57.3	45.7	-	-	-
22	2	5	49	25.8	100	-	-	1	1	0.254	27	1	1	61.8	58.8	137	4.6	-
22	2	5	49	25.8	195	140	90	2	1	0.543	30	1	1	61.8	58.8	-	-	50
22	2	5	51	26.9	100	-	-	1	1	0.220	-	1	1	63.4	62.1	-	-	-
22	2	5	53	24.8	90	-	-	1	1	0.799	39	1	1	52.4	57.3	137	4.6	-
22	2	5	53	24.8	180	102	55	2	1	2.938	27	1	1	52.4	57.3	-	-	47
16	2	4	09	3.5	-	-	-	1	1	0.039	35	1	1	-	-	-	-	-
16	2	4	11	4.2	-	-	-	1	1	0.040	13	1	1	63.5	65.0	-	-	-
16	2	4	13	5.8	-	-	-	1	1	0.034	36	1	1	69.3	69.0	-	-	-
16	2	4	15	7.5	-	-	-	1	1	0.223	10	1	1	69.4	70.0	-	-	-

Data Set I. (continued)

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
16	2	4	17	9.0	-	-	-	1	1	0.056	23	1	1	68.8	66.5	-	-	-
16	2	4	19	10.6	144	-	-	1	1	0.107	25	1	1	72.2	74.7	-	-	-
16	2	4	21	13.0	160	-	-	1	1	0.033	10	1	1	72.8	71.6	-	-	-
16	2	4	23	14.3	180	-	-	1	1	0.326	46	1	1	75.4	76.3	-	-	-
16	2	4	25	15.0	150	-	-	1	1	0.026	42	1	1	73.6	77.1	-	-	-
16	2	4	25	15.2	160	-	-	1	1	0.024	43	1	1	74.0	75.8	-	-	-
16	2	4	25	15.2	200	147	100	2	1	0.011	13	1	1	74.0	75.8	-	-	47
16	2	4	27	17.0	140	-	-	1	1	1.716	27	1	1	-	-	-	-	-
16	2	4	29	17.6	130	-	-	1	1	3.256	38	1	1	72.6	76.9	-	-	-
16	2	4	31	19.0	140	-	-	1	1	0.073	40	1	1	70.0	73.3	128	4.5	-
16	2	4	31	19.0	216	157	105	2	1	0.018	40	1	1	70.0	73.3	-	-	52
16	2	4	33	19.9	130	-	-	1	1	-	-	1	1	67.3	73.9	141	4.9	-
16	2	4	33	19.9	180	-	-	1	1	3.769	46	1	1	67.3	73.9	-	-	-
16	2	4	35	20.7	144	-	-	1	1	0.792	48	1	1	69.6	71.5	-	-	-
16	2	4	37	21.8	130	-	-	1	1	1.474	37	1	1	59.5	60.2	133	4.3	-
16	2	4	37	21.8	250	195	130	2	1	4.455	37	1	1	59.5	60.2	-	-	65
16	2	4	39	22.1	120	-	-	1	1	0.535	36	1	1	64.0	64.4	-	-	-
16	2	4	41	22.4	110	-	-	1	1	0.142	16	1	1	54.8	55.6	136	4.2	-
16	2	4	41	22.4	210	110	65	2	1	2.320	19	1	1	54.8	55.6	-	-	45
16	2	4	43	22.5	130	-	-	1	1	0.222	16	1	1	60.1	58.0	-	-	-
16	2	4	45	22.2	90	-	-	1	1	0.330	35	1	1	46.9	47.6	135	4.0	-
16	2	4	45	22.2	170	145	99	2	1	0.374	43	1	1	46.9	47.6	-	-	46
16	2	4	47	21.6	130	-	-	1	1	0.583	12	1	1	55.0	55.1	-	-	-
16	2	4	49	21.4	125	-	-	1	1	0.777	39	1	1	47.0	45.7	135	4.7	-
16	2	4	49	21.4	190	157	102	2	1	2.700	21	1	1	47.0	45.7	-	-	55
16	2	4	51	19.4	100	-	-	1	1	1.046	48	1	1	61.4	60.0	-	-	-
16	2	4	53	21.8	80	-	-	1	1	0.459	46	1	1	55.4	58.6	154	4.9	-
16	2	4	53	21.8	230	210	140	2	1	0.191	40	1	1	55.4	58.6	-	-	70
16	2	4	55	21.5	110	-	-	1	1	0.147	50	1	1	62.0	62.0	-	-	-
14	1	4	09	4.5	-	-	-	1	1	0.086	50	1	1	-	-	-	-	-
14	1	4	11	5.3	-	-	-	1	1	0.037	43	1	1	63.5	65.0	-	-	-
14	1	4	13	7.0	-	-	-	1	1	0.249	35	1	1	69.3	69.0	-	-	-

Data Set I. (continued)

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
14	1	4	15	8.3	-	-	-	1	1	0.767	23	1	1	69.4	70.0	-	-	-
14	1	4	17	11.0	-	-	-	1	1	0.140	32	1	1	68.8	66.5	-	-	-
14	1	4	19	11.4	140	-	-	1	1	0.714	45	1	1	72.2	74.7	-	-	-
14	1	4	21	14.6	150	-	-	1	1	0.186	45	1	1	72.8	71.6	-	-	-
14	1	4	23	17.1	180	-	-	1	1	0.513	32	1	1	75.4	76.3	-	-	-
14	1	4	25	18.2	140	-	-	1	1	1.110	9	1	1	73.6	77.1	-	-	-
14	1	4	25	17.7	120	-	-	1	1	0.098	20	1	1	74.0	75.8	-	-	-
14	1	4	25	17.7	200	150	97	2	1	0.039	42	1	1	74.0	75.8	-	-	53
14	1	4	27	19.8	130	-	-	1	1	1.068	31	1	1	-	-	-	-	-
14	1	4	29	20.8	130	-	-	1	1	0.066	42	1	1	72.6	76.9	-	-	-
14	1	4	31	21.7	120	-	-	1	1	0.530	46	1	1	70.0	73.3	133	4.2	-
14	1	4	31	21.7	230	147	102	2	1	0.194	42	1	1	70.0	73.3	-	-	45
14	1	4	33	24.7	130	-	-	1	1	0.015	32	1	1	67.3	73.9	142	4.7	-
14	1	4	33	24.7	200	145	105	2	1	0.007	32	1	1	67.3	73.9	-	-	40
14	1	4	35	24.5	120	-	-	1	1	0.167	28	1	1	69.6	71.5	-	-	-
14	1	4	37	24.6	90	-	-	1	1	0.704	10	1	1	59.5	60.2	148	4.9	-
14	1	4	37	24.6	230	165	110	2	1	1.308	36	1	1	59.5	60.2	-	-	55
14	1	4	39	25.4	120	-	-	1	1	0.304	39	2	1	64.0	64.4	-	-	-
14	1	4	41	26.9	100	-	-	1	1	0.521	31	2	1	54.8	55.6	145	4.9	-
14	1	4	41	26.9	230	150	95	2	1	0.377	48	2	1	54.8	55.6	-	-	55
14	1	4	43	26.6	110	-	-	1	1	0.262	39	1	1	60.1	58.0	-	-	-
14	1	4	45	24.9	80	-	-	1	1	0.114	39	1	1	46.9	47.6	126	4.4	-
14	1	4	45	24.9	200	144	98	2	1	0.403	32	1	1	46.9	47.6	-	-	46
14	1	4	47	25.8	120	-	-	1	1	1.333	14	2	1	55.0	55.1	-	-	-
14	1	4	49	26.3	120	-	-	1	1	0.213	43	1	1	47.0	45.7	139	5.1	-
14	1	4	49	26.3	210	150	90	2	1	0.837	19	1	1	47.0	45.7	-	-	60
14	1	4	51	27.0	130	-	-	1	1	0.983	48	2	1	61.4	60.0	-	-	-
14	1	4	53	26.5	120	-	-	1	1	0.352	22	1	1	55.4	58.6	135	4.6	-
14	1	4	53	26.5	210	140	100	2	1	1.560	14	1	1	55.4	58.6	-	-	40
14	1	4	55	27.8	120	-	-	1	1	0.150	17	1	1	62.0	62.0	-	-	-
13	1	4	09	4.5	-	-	-	1	1	0.115	44	1	1	-	-	-	-	-
13	1	4	11	5.4	-	-	-	1	1	0.022	35	1	1	63.5	65.0	-	-	-

Data Set I. (continued)

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
13	1	4	13	6.4	-	-	-	1	1	0.072	45	1	1	69.3	69.0	-	-	-
13	1	4	15	7.9	-	-	-	1	1	0.452	41	1	1	69.4	70.0	-	-	-
13	1	4	17	9.5	-	-	-	1	1	-	-	1	1	68.8	66.5	-	-	-
13	1	4	19	10.4	134	-	-	1	1	0.339	50	1	1	72.2	74.7	-	-	-
13	1	4	21	12.8	-	-	-	1	1	0.171	46	1	1	72.8	71.6	-	-	-
13	1	4	23	14.1	165	-	-	1	1	0.053	50	1	1	75.4	76.3	-	-	-
13	1	4	25	14.3	150	-	-	1	1	0.552	-3	1	1	73.6	77.1	-	-	-
13	1	4	25	15.4	140	-	-	1	1	0.042	36	1	1	74.0	75.8	-	-	-
13	1	4	25	15.4	220	157	107	2	1	0.022	30	1	1	74.0	75.8	-	-	50
13	1	4	27	16.3	110	-	-	1	1	0.110	40	1	1	-	-	-	-	-
13	1	4	29	17.5	110	-	-	1	1	0.217	10	1	1	72.6	76.9	-	-	-
13	1	4	31	18.3	170	-	-	1	1	1.147	16	1	1	70.0	73.3	129	4.8	-
13	1	4	31	18.3	204	155	110	2	1	0.095	29	1	1	70.0	73.3	-	-	45
13	1	4	33	18.3	90	-	-	1	1	0.564	19	1	1	67.3	73.9	134	4.7	-
13	1	4	33	18.3	210	165	120	2	1	-	-	1	1	67.3	73.9	-	-	45
13	1	4	35	19.3	110	-	-	1	1	0.567	23	1	1	69.6	71.5	-	-	-
13	1	4	37	19.0	100	-	-	1	1	0.302	23	1	1	59.5	60.2	134	4.1	-
13	1	4	37	19.0	210	165	110	2	1	0.530	29	1	1	59.5	60.2	-	-	55
13	1	4	39	19.3	160	-	-	1	1	2.983	12	1	1	64.0	64.4	-	-	-
13	1	4	41	21.2	100	-	-	1	1	0.140	23	2	1	54.8	55.6	146	4.3	-
13	1	4	41	21.2	240	175	130	2	1	0.520	19	2	1	54.8	55.6	-	-	45
13	1	4	43	21.8	150	-	-	1	1	0.131	18	1	1	60.1	58.0	-	-	-
13	1	4	45	20.7	90	-	-	1	1	2.140	29	2	1	46.9	47.6	134	4.6	-
13	1	4	45	20.7	180	168	120	2	1	4.589	17	2	1	46.9	47.6	-	-	48
13	1	4	47	20.0	120	-	-	1	1	0.225	45	1	1	55.0	55.1	-	-	-
13	1	4	49	20.4	110	-	-	1	1	0.396	44	1	1	47.0	45.7	138	4.5	-
13	1	4	49	20.4	200	160	110	2	1	0.227	46	1	1	47.0	45.7	-	-	50
13	1	4	51	20.4	120	-	-	1	1	0.337	12	2	1	61.4	60.0	-	-	-
13	1	4	53	20.5	-	-	-	1	1	0.242	31	1	1	55.4	58.6	150	5.4	-
13	1	4	53	20.5	-	-	-	2	1	0.354	45	1	1	55.4	58.6	-	-	-
13	1	4	55	20.5	90	-	-	1	1	0.264	26	1	1	62.0	62.0	-	-	-
11	1	4	09	4.1	-	-	-	1	1	0.000	43	1	1	-	-	-	-	-

Data Set I. (continued)

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
11	1	4	11	5.2	-	-	-	1	1	0.020	34	1	1	63.5	65.0	-	-	-
11	1	4	13	6.4	-	-	-	1	1	0.063	44	1	1	69.3	69.0	-	-	-
11	1	4	15	8.4	-	-	-	1	1	0.110	32	1	1	69.4	70.0	-	-	-
11	1	4	17	9.6	-	-	-	1	1	0.029	35	1	1	68.8	66.5	-	-	-
11	1	4	19	11.2	-	-	-	1	1	1.024	16	1	1	72.2	74.7	-	-	-
11	1	4	21	12.2	120	-	-	1	1	0.423	29	1	1	72.8	71.6	-	-	-
11	1	4	23	13.7	120	-	-	1	1	0.073	41	1	1	75.4	76.3	-	-	-
11	1	4	25	13.6	110	-	-	1	1	0.117	44	1	1	73.6	77.1	-	-	-
11	1	4	25	13.9	120	-	-	1	1	0.092	37	1	1	74.0	75.8	-	-	-
11	1	4	25	13.9	230	170	110	2	1	0.267	11	1	1	74.0	75.8	-	-	60
11	1	4	27	15.1	80	-	-	1	1	0.367	43	1	1	-	-	-	-	-
11	1	4	29	16.0	80	-	-	1	1	0.464	43	1	1	72.6	76.9	-	-	-
11	1	4	31	17.0	100	-	-	1	1	1.560	50	1	1	70.0	73.3	130	4.0	-
11	1	4	31	17.0	252	195	140	2	1	4.455	41	1	1	70.0	73.3	-	-	55
11	1	4	33	17.3	90	-	-	1	1	0.073	42	1	1	67.3	73.9	140	4.6	-
11	1	4	33	17.3	230	172	127	2	1	2.658	21	1	1	67.3	73.9	-	-	45
11	1	4	35	14.9	80	-	-	1	1	0.096	13	1	1	69.6	71.5	-	-	-
11	1	4	37	17.2	80	-	-	1	1	1.173	12	1	1	59.5	60.2	135	3.7	-
11	1	4	37	17.2	260	185	125	2	1	5.790	47	1	1	59.5	60.2	-	-	60
11	1	4	39	18.7	80	-	-	1	1	3.553	39	2	1	64.0	64.4	-	-	-
11	1	4	41	19.1	90	-	-	1	1	1.839	18	2	1	54.8	55.6	135	4.3	-
11	1	4	41	19.1	240	165	125	2	1	2.044	22	2	1	54.8	55.6	-	-	40
11	1	4	43	19.6	90	-	-	1	1	0.112	45	2	1	60.1	58.0	-	-	-
11	1	4	45	18.5	70	-	-	1	1	7.451	45	2	1	46.9	47.6	129	4.6	-
11	1	4	45	18.5	210	220	165	2	1	10.933	29	2	1	46.9	47.6	-	-	55
11	1	4	47	18.8	105	-	-	1	1	2.089	15	1	1	55.0	55.1	-	-	-
11	1	4	49	18.5	90	-	-	1	1	2.442	48	2	1	47.0	45.7	134	4.9	-
11	1	4	49	18.5	240	210	160	2	1	2.464	43	2	1	47.0	45.7	-	-	50
11	1	4	51	18.4	80	-	-	1	1	1.280	13	1	1	61.4	60.0	-	-	-
11	1	4	53	18.2	80	-	-	1	1	0.547	18	1	1	55.4	58.6	143	5.3	-
11	1	4	53	18.2	260	207	142	2	1	1.100	48	1	1	55.4	58.6	-	-	65
11	1	4	55	18.7	100	-	-	1	1	1.022	17	1	1	62.0	62.0	-	-	-

Data Set I. (continued)

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
20	2	3	09	6.0	-	-	-	1	1	0.044	35	1	1	-	-	-	-	-
20	2	3	11	7.0	-	-	-	1	1	0.110	36	1	1	63.5	67.2	-	-	-
20	2	3	13	10.4	-	-	-	1	1	0.095	36	1	1	68.4	68.6	-	-	-
20	2	3	15	12.6	-	-	-	1	1	0.004	46	1	1	72.4	72.6	-	-	-
20	2	3	17	15.9	-	-	-	1	1	0.044	48	1	1	75.0	73.5	-	-	-
20	2	3	19	17.6	150	-	-	1	1	0.020	35	1	1	66.9	67.8	-	-	-
20	2	3	21	18.2	150	-	-	1	1	0.482	20	1	1	67.6	68.9	-	-	-
20	2	3	23	19.0	120	-	-	1	1	0.077	46	1	1	78.3	74.6	-	-	-
20	2	3	23	18.8	140	-	-	1	1	0.159	11	1	1	78.5	74.6	142	3.8	-
20	2	3	23	18.8	230	115	90	2	1	0.032	35	1	1	78.5	74.6	-	-	25
20	2	3	25	20.4	130	-	-	1	1	0.727	22	1	1	72.6	75.0	-	-	-
20	2	3	27	20.6	100	-	-	1	1	0.073	13	1	1	78.5	81.1	127	3.7	-
20	2	3	29	21.8	108	-	-	1	1	0.055	32	1	1	72.2	76.2	-	-	-
20	2	3	29	21.8	210	115	80	2	1	0.130	40	1	1	72.2	76.2	-	-	35
20	2	3	31	23.0	100	-	-	1	1	0.584	23	1	1	71.0	74.2	145	4.4	-
20	2	3	33	24.4	130	-	-	1	1	0.737	21	1	1	65.9	74.2	133	4.4	-
20	2	3	33	24.4	230	170	107	2	1	0.367	25	1	1	65.9	74.2	-	-	63
20	2	3	35	22.4	80	-	-	1	1	1.376	17	1	1	69.3	69.0	-	-	-
20	2	3	37	25.1	100	-	-	1	1	0.324	27	1	1	65.5	62.5	134	4.1	-
20	2	3	37	25.1	220	172	107	2	1	1.900	19	1	1	65.5	62.5	-	-	65
20	2	3	39	25.8	110	-	-	1	1	0.489	47	1	1	61.5	61.2	-	-	-
20	2	3	41	24.6	95	-	-	1	1	0.279	40	1	1	57.3	56.5	143	3.8	-
20	2	3	41	24.6	220	150	85	2	1	1.903	32	1	1	57.3	56.5	-	-	65
20	2	3	43	26.4	110	-	-	1	1	1.190	35	1	1	48.4	48.5	140	4.5	-
20	2	3	43	26.4	190	110	65	2	1	1.763	27	1	1	48.4	48.5	-	-	45
20	2	3	45	25.8	110	-	-	1	1	0.029	30	1	1	53.8	54.4	-	-	-
20	2	3	47	25.9	90	-	-	1	1	0.341	43	1	1	57.3	57.3	146	4.7	-
20	2	3	47	25.9	210	165	100	2	1	1.164	18	1	1	57.3	57.3	-	-	65
20	2	3	49	26.2	100	-	-	1	1	0.051	32	1	1	61.8	58.8	-	-	-
20	2	3	51	26.3	110	-	-	1	1	0.264	48	1	1	63.4	62.1	145	5.2	-
20	2	3	51	26.3	200	-	-	2	1	0.721	50	1	1	63.4	62.1	-	-	-
20	2	3	53	24.8	95	-	-	1	1	0.484	33	1	1	52.4	57.3	-	-	-
19	1	3	09	3.5	-	-	-	1	1	0.071	41	1	1	-	-	-	-	-

Data Set I. (continued)

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
19	1	3	11	4.1	-	-	-	1	1	0.064	26	1	1	63.5	67.2	-	-	-
19	1	3	13	4.6	-	-	-	1	1	0.073	36	1	1	68.4	68.6	-	-	-
19	1	3	15	6.4	-	-	-	1	1	0.096	21	1	1	72.4	72.6	-	-	-
19	1	3	17	7.9	-	-	-	1	1	0.027	25	1	1	75.0	73.5	-	-	-
19	1	3	19	9.0	130	-	-	1	1	0.026	40	1	1	66.9	67.8	-	-	-
19	1	3	21	9.4	150	-	-	1	1	0.070	32	1	1	67.6	68.9	-	-	-
19	1	3	23	9.7	110	-	-	1	1	0.032	41	1	1	78.3	74.6	118	4.1	-
19	1	3	23	10.2	140	-	-	1	1	0.038	24	1	1	78.5	74.6	-	-	-
19	1	3	23	10.2	240	157	110	2	1	0.051	14	1	1	78.5	74.6	-	-	47
19	1	3	25	10.5	140	-	-	1	1	0.343	9	1	1	72.6	75.0	-	-	-
19	1	3	27	11.9	130	-	-	1	1	0.005	43	1	1	78.5	81.1	129	4.7	-
19	1	3	29	11.6	130	-	-	1	1	0.380	20	1	1	72.2	76.2	-	-	-
19	1	3	29	11.6	230	125	105	2	1	0.086	28	1	1	72.2	76.2	-	-	20
19	1	3	31	12.2	110	-	-	1	1	0.011	50	1	1	71.0	74.2	131	3.7	-
19	1	3	33	13.1	120	-	-	1	1	0.077	37	1	1	65.9	69.0	139	4.8	-
19	1	3	33	13.1	240	185	150	2	1	0.389	45	1	1	65.9	69.0	-	-	35
19	1	3	35	13.5	110	-	-	1	1	1.344	19	1	1	69.3	69.0	-	-	-
19	1	3	37	13.8	120	-	-	1	1	0.068	36	1	1	65.5	62.5	136	4.5	-
19	1	3	37	13.8	250	182	137	2	1	2.136	47	1	1	65.5	62.5	-	-	45
19	1	3	39	14.3	110	-	-	1	1	0.077	38	1	1	61.5	61.2	-	-	-
19	1	3	41	13.7	100	-	-	1	1	0.413	29	1	1	57.3	56.5	130	3.9	-
19	1	3	41	13.7	235	-	-	2	1	8.191	16	1	1	57.3	56.5	-	-	-
19	1	3	43	14.1	110	-	-	1	1	0.046	14	1	1	48.4	48.5	141	3.7	-
19	1	3	43	14.1	245	160	100	2	1	0.064	41	1	1	48.4	48.5	-	-	60
19	1	3	45	13.8	120	-	-	1	1	0.019	20	1	1	53.8	54.4	139	4.0	-
19	1	3	47	14.4	110	-	-	1	1	0.089	21	1	1	57.3	57.3	141	5.2	-
19	1	3	47	14.4	230	165	110	2	1	0.073	30	1	1	57.3	57.3	-	-	55
19	1	3	49	14.4	120	-	-	1	1	-	-	1	1	61.8	58.8	-	-	-
19	1	3	51	14.2	100	-	-	1	1	1.053	16	1	1	63.4	62.1	143	5.3	-
19	1	3	51	14.2	220	155	-	2	1	-	-	1	1	63.4	62.1	-	-	-
19	1	3	53	14.5	105	-	-	1	1	0.102	12	1	1	52.4	57.3	-	-	-
18	1	2	09	3.3	-	-	-	1	1	0.193	34	1	1	-	-	-	-	-
18	1	2	11	4.5	-	-	-	1	1	0.022	35	1	1	-	-	-	-	-

Data Set I. (continued)

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
18	1	2	13	6.0	-	-	-	1	1	0.337	47	1	1	63.5	65.0	-	-	-
18	1	2	15	6.9	-	-	-	1	1	0.020	26	1	1	69.3	69.0	-	-	-
18	1	2	17	7.8	-	-	-	1	1	0.210	48	1	1	69.4	70.0	-	-	-
18	1	2	19	9.0	-	-	-	1	1	0.015	30	1	1	68.8	66.5	-	-	-
18	1	2	21	10.0	-	-	-	1	1	0.051	43	1	1	72.2	74.7	-	-	-
18	1	2	23	10.5	120	-	-	1	1	0.071	31	1	1	72.8	71.6	-	-	-
18	1	2	24	-	144	-	-	1	1	0.102	11	1	1	69.3	68.9	-	-	-
18	1	2	24	-	228	132	100	2	1	0.760	10	1	1	69.3	68.9	134	4.0	32
18	1	2	25	11.9	160	-	-	1	1	0.020	26	1	1	75.4	76.3	-	-	-
18	1	2	27	11.7	-	-	-	1	1	2.387	25	1	1	73.6	77.1	-	-	-
18	1	2	27	12.5	132	-	-	1	1	0.213	18	1	1	74.0	75.8	-	-	-
18	1	2	27	12.5	220	133	80	2	1	0.220	34	1	1	74.0	75.8	-	-	53
18	1	2	29	14.7	140	-	-	1	1	2.090	46	1	1	-	-	-	-	-
18	1	2	31	14.0	130	-	-	1	1	0.364	42	1	1	72.6	76.9	-	-	-
18	1	2	33	14.1	120	-	-	1	1	0.218	23	1	1	70.0	73.3	146	5.1	-
18	1	2	33	14.1	220	145	80	2	1	0.200	23	1	1	70.0	73.3	-	-	65
18	1	2	37	14.7	120	-	-	1	1	0.125	31	1	1	67.3	73.9	121	3.7	-
18	1	2	37	14.7	240	160	90	2	1	2.147	15	1	1	67.3	73.9	-	-	70
18	1	2	39	15.6	120	-	-	1	1	0.037	13	1	1	69.6	71.5	144	4.7	-
18	1	2	39	15.6	250	-	-	2	1	0.513	43	1	1	69.6	71.5	-	-	-
18	1	2	41	15.3	100	-	-	1	1	0.027	20	1	1	59.5	60.2	-	-	-
18	1	2	43	16.2	110	-	-	1	1	1.357	50	1	1	64.0	64.4	-	-	-
18	1	2	45	16.9	95	-	-	1	1	0.108	30	2	1	54.8	55.6	-	-	-
18	1	2	45	16.9	230	160	110	2	1	3.104	47	2	1	54.8	55.6	141	4.9	50
18	1	2	47	17.3	-	-	-	1	1	0.102	14	2	1	60.1	58.0	-	-	-
18	1	2	49	16.9	70	-	-	1	1	0.027	45	2	1	46.9	47.6	-	-	-
18	1	2	49	16.9	180	140	115	2	1	0.474	41	2	1	46.9	47.6	140	4.8	25
18	1	2	51	16.2	90	-	-	1	1	0.853	15	1	1	55.0	55.1	-	-	-
18	1	2	51	16.2	200	125	87	2	1	-	-	1	1	55.0	55.1	-	-	38
18	1	2	53	16.2	-	-	-	1	1	0.719	35	2	1	47.0	45.7	-	-	-
18	1	2	55	16.2	90	-	-	1	1	1.085	48	2	1	61.4	60.0	-	-	-
18	1	2	57	16.7	100	-	-	1	1	0.051	44	1	1	55.4	58.6	-	-	-
18	1	2	57	16.7	170	117	72	2	1	0.073	18	1	1	55.4	58.6	142	5.0	45

Data Set I. (continued)

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
18	1	2	59	16.5	90	-	-	1	1	0.122	35	1	1	62.0	62.0	-	-	-
6	2	1	09	3.2	-	-	-	1	1	0.142	48	1	1	-	-	-	-	-
6	2	1	11	4.5	-	-	-	1	1	0.029	32	1	1	-	-	-	-	-
6	2	1	13	5.9	-	-	-	1	1	0.051	39	1	1	-	-	-	-	-
6	2	1	15	7.4	-	-	-	1	1	-	-	1	1	63.5	65.0	-	-	-
6	2	1	17	8.2	-	-	-	1	1	0.038	27	1	1	69.3	69.0	-	-	-
6	2	1	19	9.8	-	-	-	1	1	0.118	15	1	1	69.4	70.0	-	-	-
6	2	1	21	11.6	-	-	-	1	1	0.033	32	1	1	68.8	66.5	-	-	-
6	2	1	23	12.4	136	-	-	1	1	0.078	44	1	1	72.2	74.7	-	-	-
6	2	1	25	13.1	-	-	-	1	1	0.451	38	1	1	71.4	71.6	-	-	45
6	2	1	25	13.1	168	-	-	2	1	1.427	48	1	1	71.4	71.6	-	-	-
6	2	1	27	15.1	150	-	-	1	1	0.024	46	1	1	75.4	76.3	135	4.2	-
6	2	1	29	15.8	140	-	-	1	1	0.200	48	1	1	73.6	77.1	-	-	-
6	2	1	29	15.0	130	-	-	1	1	0.023	25	1	1	74.0	75.8	-	-	-
6	2	1	29	15.0	180	122	55	2	1	0.007	24	1	1	74.0	75.8	-	-	67
6	2	1	31	18.6	110	-	-	1	1	0.042	38	1	1	-	-	-	-	-
6	2	1	33	18.0	120	-	-	1	1	0.007	34	1	1	72.6	76.9	128	4.5	-
6	2	1	33	18.0	-	-	-	1	1	0.105	44	1	1	74.9	75.4	-	-	-
6	2	1	34	18.2	110	-	-	1	1	0.093	35	1	1	72.2	76.2	-	-	-
6	2	1	34	18.2	210	140	100	2	1	0.194	35	1	1	72.2	76.2	-	-	40
6	2	1	37	19.2	100	-	-	1	1	0.088	50	1	1	67.3	73.9	128	4.0	-
6	2	1	37	19.2	200	130	90	2	1	0.121	39	1	1	67.3	73.9	-	-	40
6	2	1	39	21.0	105	-	-	1	1	0.160	26	1	1	69.6	71.5	-	-	-
6	2	1	41	20.3	90	-	-	1	1	0.109	15	1	1	59.5	60.2	134	3.5	
6	2	1	41	20.3	230	185	115	2	1	0.778	26	1	1	59.5	60.2	-	-	70
6	2	1	43	21.3	110	-	-	1	1	0.315	42	1	1	64.0	64.4	-	-	-
6	2	1	45	20.6	120	-	-	1	1	1.098	24	1	1	54.8	55.6	139	4.5	-
6	2	1	45	20.6	200	140	75	2	1	17.052	46	1	1	54.8	55.6	-	-	65
6	2	1	47	19.4	110	-	-	1	1	0.064	31	1	1	60.1	58.0	-	-	-
6	2	1	49	18.7	80	-	-	1	1	0.334	46	1	1	46.9	47.6	129	4.5	-
6	2	1	49	18.7	150	128	70	2	1	0.856	25	1	1	46.9	47.6	-	-	58
6	2	1	51	19.2	100	-	-	1	1	0.064	41	1	1	55.0	55.1	-	-	-

Data Set I. (continued)

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
6	2	1	53	18.6	90	-	-	1	1	0.056	20	1	1	47.0	45.7	143	4.8	-
6	2	1	53	18.6	180	142	80	2	1	-	-	1	1	47.0	45.7	-	-	62
6	2	1	55	19.4	90	-	-	1	1	1.022	48	1	1	61.4	60.0	-	-	-
6	2	1	57	20.0	100	-	-	1	1	0.682	36	1	1	55.4	58.6	131	4.8	-
6	2	1	57	20.0	-	117	60	2	1	1.983	50	1	1	55.4	58.6	-	-	57
6	2	1	59	19.2	100	-	-	1	1	0.032	32	1	1	62.0	62.0	-	-	-
23	1	6	09	5.6	-	-	-	1	1	0.222	9	1	1	66.0	67.0	-	-	-
23	1	6	11	6.9	-	-	-	1	1	0.347	33	1	1	69.5	68.0	-	-	-
23	1	6	13	8.5	-	-	-	1	1	-	-	1	1	69.4	70.0	-	-	-
23	1	6	15	8.6	-	-	-	1	1	0.289	26	1	1	65.4	65.4	-	-	-
23	1	6	17	11.0	140	-	-	1	1	0.006	12	1	1	72.2	74.7	-	-	-
23	1	6	19	12.6	180	-	-	1	1	0.024	44	1	1	72.8	71.6	-	-	-
23	1	6	21	13.5	210	-	-	1	1	0.026	47	1	1	75.4	76.3	-	-	-
23	1	6	23	14.6	170	-	-	1	1	0.037	11	1	1	73.6	77.1	-	-	-
23	1	6	25	16.6	80	-	-	1	1	0.380	26	1	1	-	-	149	5.0	-
23	1	6	27	17.3	120	-	-	1	1	0.336	28	1	1	72.6	76.9	-	-	-
23	1	6	29	17.2	150	-	-	1	1	0.942	17	1	1	72.2	76.2	-	-	-
23	1	6	29	17.2	250	122	85	2	1	1.166	37	1	1	72.2	76.2	-	-	37
23	1	6	33	19.9	110	-	-	1	1	0.122	41	1	1	65.9	69.0	150	4.5	-
23	1	6	33	19.9	250	167	95	2	1	0.366	37	1	1	65.9	69.0	-	-	72
23	1	6	35	19.8	115	-	-	1	1	7.421	41	1	1	69.3	69.0	-	-	-
23	1	6	37	21.0	120	-	-	1	1	0.328	48	1	1	65.5	62.5	141	4.6	-
23	1	6	37	21.0	250	132	95	2	1	1.293	21	1	1	65.5	62.5	-	-	37
23	1	6	39	20.3	90	-	-	1	1	0.117	37	1	1	61.5	61.2	-	-	-
23	1	6	41	21.2	110	-	-	1	1	0.616	45	1	1	57.3	56.5	155	4.3	-
23	1	6	41	21.2	220	120	70	2	1	3.109	43	1	1	57.3	56.5	-	-	50
23	1	6	43	21.1	110	-	-	1	1	0.711	46	1	1	48.4	48.5	-	-	-
23	1	6	45	21.0	100	-	-	1	1	0.037	30	1	1	53.8	54.4	-	-	-
23	1	6	45	21.0	225	152	95	2	1	0.198	50	1	1	53.8	54.4	-	-	57
23	1	6	47	21.3	90	-	-	1	1	0.293	50	2	1	57.3	57.3	-	-	-
23	1	6	49	21.8	130	-	-	1	1	0.036	13	1	1	61.8	58.8	139	4.4	-
23	1	6	49	21.8	230	120	70	2	1	-	-	1	1	61.8	58.8	-	-	50
23	1	6	51	22.0	110	-	-	1	1	0.098	50	1	1	63.4	62.1	-	-	-

Data Set I. (continued)

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
23	1	6	53	22.3	120	-	-	1	1	0.345	29	1	1	52.4	57.3	136	4.2	-
23	1	6	53	22.3	250	162	120	2	1	0.596	41	1	1	52.4	57.3	-	-	42
25	2	6	09	6.9	-	-	-	1	1	0.238	36	1	1	66.0	67.0	-	-	-
25	2	6	11	8.5	-	-	-	1	1	0.016	10	1	1	69.5	68.0	-	-	-
25	2	6	13	11.0	-	-	-	1	1	0.034	44	1	1	69.4	70.0	-	-	-
25	2	6	15	11.8	-	-	-	1	1	0.026	46	1	1	65.4	65.4	-	-	-
25	2	6	17	14.9	122	-	-	1	1	0.018	48	1	1	72.2	74.7	-	-	-
25	2	6	19	17.9	180	-	-	1	1	0.423	46	1	1	72.8	71.6	-	-	-
25	2	6	21	19.8	180	-	-	1	1	0.088	30	1	1	75.4	76.3	-	-	-
25	2	6	23	21.0	140	-	-	1	1	0.193	27	1	1	73.6	77.1	-	-	-
25	2	6	25	22.5	140	-	-	1	1	0.369	46	1	1	-	-	145	4.8	-
25	2	6	27	24.1	130	-	-	1	1	0.799	45	1	1	72.6	76.9	-	-	-
25	2	6	29	24.2	130	-	-	1	1	0.193	40	1	1	72.2	76.2	-	-	-
25	2	6	29	24.2	240	125	80	2	1	2.691	48	1	1	72.2	76.2	-	-	45
25	2	6	33	26.6	150	-	-	1	1	0.048	37	1	1	65.9	69.0	130	4.4	-
25	2	6	33	26.6	240	-	-	2	1	6.937	44	1	1	65.9	69.0	-	-	-
25	2	6	35	24.7	90	-	-	1	1	1.660	30	1	1	69.3	69.0	-	-	-
25	2	6	37	28.2	130	-	-	1	1	0.007	32	1	1	65.5	62.5	148	4.6	-
25	2	6	37	28.2	210	130	80	2	1	0.013	13	1	1	65.5	62.5	-	-	50
25	2	6	39	29.8	120	-	-	1	1	1.300	39	1	1	61.5	61.2	-	-	-
25	2	6	41	27.6	110	-	-	1	1	1.234	43	1	1	57.3	56.5	153	4.6	-
25	2	6	41	27.6	230	105	67	2	1	5.744	35	1	1	57.3	56.5	-	-	38
25	2	6	43	29.0	125	-	-	1	1	0.205	38	1	1	48.4	48.5	-	-	-
25	2	6	45	28.1	90	-	-	1	1	0.108	40	1	1	53.8	54.4	132	3.6	-
25	2	6	45	28.1	240	147	90	2	1	0.622	24	1	1	53.8	54.4	-	-	57
25	2	6	47	30.1	110	-	-	1	1	0.134	42	1	1	57.3	57.3	-	-	-
25	2	6	49	28.5	110	-	-	1	1	0.099	20	1	1	61.8	58.8	143	4.4	-
25	2	6	49	28.5	-	140	90	2	1	0.924	43	1	1	61.8	58.8	-	-	50
25	2	6	51	29.5	120	-	-	1	1	0.033	13	1	1	63.4	62.1	-	-	-
25	2	6	53	28.2	115	-	-	1	1	0.324	10	1	1	52.4	57.3	-	-	-
25	2	6	53	28.2	230	150	95	2	1	1.383	44	1	1	52.4	57.3	-	-	55

Data Set II. Observations from low protein group

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
10	1	4	09	3.0	-	-	-	1	1	0.308	48	1	2	-	-	-	-	-
10	1	4	11	3.6	-	-	-	1	1	0.037	48	1	1	63.5	65.0	-	-	-
10	1	4	13	6.0	-	-	-	1	1	0.048	48	1	1	69.3	69.0	-	-	-
10	1	4	15	7.8	-	-	-	1	1	0.029	25	1	1	69.4	70.0	-	-	-
10	1	4	17	10.0	-	-	-	1	1	0.040	22	1	1	68.8	66.5	-	-	-
10	1	4	19	11.3	136	-	-	1	1	0.013	28	1	1	72.2	74.7	-	-	-
10	1	4	21	12.3	140	-	-	1	1	0.684	9	1	1	72.8	71.6	-	-	-
10	1	4	23	13.8	150	-	-	1	1	0.134	45	1	1	75.4	76.3	-	-	-
10	1	4	25	14.8	150	-	-	1	1	0.341	46	1	1	73.6	77.1	-	-	-
10	1	4	25	13.8	135	-	-	1	1	2.382	17	1	1	74.0	75.8	-	-	-
10	1	4	25	13.8	216	147	107	2	1	-	-	1	1	74.0	75.8	-	-	40
10	1	4	27	15.3	120	-	-	1	1	5.273	48	1	2	-	-	-	-	-
10	1	4	29	15.0	144	-	-	1	1	10.462	9	1	2	72.6	76.9	-	-	-
10	1	4	31	14.4	130	-	-	1	1	10.787	21	1	2	70.0	73.3	132	4.2	-
10	1	4	31	14.4	270	162	110	2	1	2.064	36	1	2	70.0	73.3	-	-	52
10	1	4	33	14.9	130	-	-	1	1	0.227	40	1	2	67.3	73.9	136	4.3	-
10	1	4	33	14.9	200	135	80	2	1	6.564	19	1	2	67.3	73.9	-	-	55
10	1	4	35	14.7	100	-	-	1	1	6.107	12	1	2	69.6	71.5	-	-	-
10	1	4	37	13.7	130	-	-	1	1	-	-	1	2	59.5	60.2	132	4.0	-
10	1	4	37	13.7	220	135	100	2	1	2.946	47	1	2	59.5	60.2	-	-	35
10	1	4	39	13.6	140	-	-	1	1	2.473	20	1	2	64.0	64.4	-	-	-
10	1	4	41	13.1	120	-	-	1	1	7.787	44	1	2	54.8	55.6	154	4.6	-
10	1	4	41	13.1	230	130	95	2	1	4.027	24	1	2	54.8	55.6	-	-	35
10	1	4	43	13.1	120	-	-	1	1	1.599	39	1	2	60.1	58.0	-	-	-
10	1	4	45	12.0	80	-	-	1	1	0.227	43	1	2	46.9	47.6	-	-	-
10	1	4	45	12.0	200	124	90	2	1	18.480	47	1	2	46.9	47.6	-	-	34
10	1	4	47	12.7	150	-	-	1	2	5.383	43	1	2	55.0	55.1	-	-	-
10	1	4	49	12.4	140	-	-	1	2	1.397	25	1	2	47.0	45.7	138	4.9	-
10	1	4	49	12.4	240	115	65	2	2	25.575	47	1	2	47.0	45.7	-	-	50
10	1	4	51	12.8	130	-	-	1	2	3.060	48	1	2	61.4	60.0	-	-	-
10	1	4	53	13.5	120	-	-	1	2	17.507	47	1	2	55.4	58.6	134	5.0	-
10	1	4	53	13.5	220	115	65	2	2	0.572	30	1	2	55.4	58.6	-	-	50
10	1	4	55	12.5	100	-	-	1	2	6.013	48	1	2	62.0	62.0	-	-	-

Data Set II. (continued)

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
17	2	4	09	3.2	-	-	-	1	1	3.037	26	1	2	-	-	-	-	-
17	2	4	11	3.3	-	-	-	1	1	0.092	34	1	1	63.5	65.0	-	-	-
17	2	4	13	5.7	-	-	-	1	1	0.208	48	1	1	69.3	69.0	-	-	-
17	2	4	15	7.5	-	-	-	1	1	0.860	15	1	1	69.4	70.0	-	-	-
17	2	4	17	10.1	-	-	-	1	1	0.203	33	1	1	68.8	66.5	-	-	-
17	2	4	19	11.7	138	-	-	1	1	0.154	32	1	1	72.2	74.7	-	-	-
17	2	4	21	13.9	144	-	-	1	1	0.053	10	1	1	72.8	71.6	-	-	-
17	2	4	23	15.1	150	-	-	1	1	0.440	36	1	1	75.4	76.3	-	-	-
17	2	4	25	15.1	140	-	-	1	1	0.523	10	1	1	73.6	77.1	-	-	-
17	2	4	25	15.0	140	-	-	1	1	0.662	33	1	1	74.0	75.8	-	-	-
17	2	4	25	15.0	216	155	100	2	1	0.117	40	1	1	74.0	75.8	-	-	55
17	2	4	27	16.0	110	-	-	1	1	0.167	50	1	2	-	-	-	-	-
17	2	4	29	15.4	100	-	-	1	1	6.440	24	1	2	72.6	76.9	-	-	-
17	2	4	31	15.0	108	-	-	1	1	7.914	21	1	2	70.0	73.3	140	4.5	-
17	2	4	31	15.0	240	155	110	2	1	2.419	48	1	2	70.0	73.3	-	-	45
17	2	4	33	14.7	130	-	-	1	1	2.151	17	1	2	67.3	73.9	132	4.0	-
17	2	4	33	14.7	210	125	90	2	1	-	-	1	2	67.3	73.9	-	-	35
17	2	4	35	14.9	90	-	-	1	1	10.213	12	1	2	69.6	71.5	-	-	-
17	2	4	37	13.6	100	-	-	1	1	7.980	15	1	2	59.5	60.2	135	4.4	-
17	2	4	37	13.6	230	145	100	2	1	2.947	17	1	2	59.5	60.2	-	-	45
17	2	4	39	14.3	120	-	-	1	1	1.073	22	1	2	64.0	64.4	-	-	-
17	2	4	41	16.3	130	-	-	1	1	2.260	9	1	2	54.8	55.6	135	4.0	-
17	2	4	41	16.3	260	110	65	2	1	2.533	9	1	2	54.8	55.6	-	-	45
17	2	4	43	17.2	120	-	-	1	1	9.797	47	1	2	60.1	58.0	-	-	-
17	2	4	45	14.7	120	-	-	1	1	2.409	34	1	2	46.9	47.6	126	4.4	-
17	2	4	45	14.7	190	125	75	2	1	3.053	18	1	2	46.9	47.6	-	-	50
17	2	4	47	14.2	120	-	-	1	2	3.938	29	1	2	55.0	55.1	-	-	-
17	2	4	49	14.6	130	-	-	1	2	0.311	40	1	2	47.0	45.7	140	6.0	-
17	2	4	49	14.6	200	135	90	2	2	3.073	18	1	2	47.0	45.7	-	-	45
17	2	4	51	14.8	130	-	-	1	2	3.975	50	1	2	61.4	60.0	-	-	-
17	2	4	53	15.0	120	-	-	1	2	2.204	44	1	2	55.4	58.6	148	5.6	-
17	2	4	53	15.0	240	127	65	2	2	4.840	47	1	2	55.4	58.6	-	-	62

Data Set II. (continued)

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
17	2	4	55	14.6	120	-	-	1	2	13.244	23	1	2	62.0	62.0	-	-	-
26	2	6	09	4.3	-	-	-	1	1	0.452	48	1	2	66.0	67.0	-	-	-
26	2	6	11	3.7	-	-	-	1	1	0.898	28	1	2	-	-	-	-	-
26	2	6	13	5.4	-	-	-	1	1	0.469	41	1	1	69.4	70.0	-	-	-
26	2	6	15	7.0	-	-	-	1	1	0.066	38	1	1	65.4	65.4	-	-	-
26	2	6	17	9.0	-	-	-	1	1	0.826	48	1	1	72.2	74.7	-	-	-
26	2	6	19	10.5	132	-	-	1	1	0.093	18	1	1	72.8	71.6	-	-	-
26	2	6	21	11.6	150	-	-	1	1	0.070	23	1	1	75.4	76.3	-	-	-
26	2	6	23	13.7	160	-	-	1	1	0.010	27	1	1	73.6	76.6	-	-	-
26	2	6	25	15.0	130	-	-	1	1	5.784	38	1	1	-	-	139	4.7	-
26	2	6	27	13.8	120	-	-	1	1	2.112	32	1	2	72.6	76.9	-	-	-
26	2	6	29	13.8	90	-	-	1	1	1.551	16	1	2	72.6	76.2	-	-	-
26	2	6	29	13.8	200	110	62	2	1	7.963	19	1	2	72.6	76.2	-	-	48
26	2	6	33	13.1	100	-	-	1	1	0.086	42	1	2	65.9	69.0	140	4.8	-
26	2	6	33	13.1	265	140	70	2	1	6.482	15	1	2	65.9	69.0	-	-	70
26	2	6	35	12.4	90	-	-	1	1	5.775	29	1	2	69.3	69.0	-	-	-
26	2	6	37	11.7	115	-	-	1	1	1.160	18	1	2	65.5	62.5	149	4.4	-
26	2	6	37	11.7	235	100	75	2	1	4.840	47	1	2	65.5	62.5	-	-	25
26	2	6	39	10.9	110	-	-	1	1	21.831	47	1	2	61.5	61.2	-	-	-
26	2	6	41	10.6	115	-	-	1	2	0.601	42	1	2	57.3	56.5	137	4.4	-
26	2	6	41	10.6	220	-	-	2	2	2.639	47	1	2	57.3	56.5	-	-	-
26	2	6	43	10.1	115	-	-	1	2	3.425	31	1	2	48.4	48.5	-	-	-
21	2	5	09	4.3	-	-	-	1	1	0.968	36	1	2	68.1	62.7	-	-	-
21	2	5	13	5.4	-	-	-	1	1	0.027	28	1	1	68.4	68.6	-	-	-
21	2	5	15	7.6	-	-	-	1	1	0.196	48	1	1	72.4	72.6	-	-	-
21	2	5	19	11.7	170	-	-	1	1	0.436	38	1	1	66.9	67.8	-	-	-
21	2	5	21	13.3	160	-	-	1	1	0.051	47	1	1	67.6	68.9	-	-	-
21	2	5	23	14.4	150	-	-	1	1	0.550	15	1	1	78.3	74.6	-	-	-
21	2	5	25	15.8	130	-	-	1	1	0.696	18	1	1	72.6	75.0	-	-	-
21	2	5	27	14.1	80	-	-	1	1	0.341	40	1	2	78.5	81.1	127	4.5	-
21	2	5	29	13.2	110	-	-	1	1	3.133	19	1	3	72.2	76.2	-	-	-
21	2	5	29	13.2	210	140	105	2	1	2.420	34	1	2	72.2	76.2	-	-	35

Data Set II. (continued)

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
21	2	5	31	14.0	100	-	-	1	1	3.058	34	1	2	71.0	74.2	-	-	-
21	2	5	33	13.8	110	-	-	1	1	0.095	45	1	2	65.9	69.0	-	-	-
21	2	5	35	13.0	90	-	-	1	1	0.829	48	1	2	57.3	56.5	129	4.6	-
21	2	5	35	13.0	160	130	95	2	1	3.843	32	1	2	57.3	56.5	-	-	35
21	2	5	37	13.2	110	-	-	1	1	2.156	35	1	2	65.5	62.5	-	-	-
21	2	5	39	12.6	90	-	-	1	1	8.936	39	1	2	61.5	61.2	133	4.5	-
21	2	5	39	12.6	190	130	90	2	1	-	-	1	2	61.5	61.2	-	-	40
21	2	5	41	13.0	100	-	-	1	2	4.740	18	1	2	57.3	56.5	-	-	-
21	2	5	43	12.6	110	-	-	1	2	1.184	34	1	2	48.4	48.5	-	-	-
21	2	5	45	12.6	160	-	-	1	2	3.005	44	1	2	53.8	54.4	-	-	-
21	2	5	45	12.6	210	110	65	2	2	12.166	44	1	2	53.8	54.4	-	-	45
21	2	5	47	12.0	120	-	-	1	2	3.409	15	1	2	57.3	57.3	-	-	-
21	2	5	49	11.1	90	-	-	1	2	0.165	42	1	2	61.8	58.8	129	4.8	-
21	2	5	49	11.1	160	120	87	2	2	5.247	15	1	2	61.8	58.8	-	-	33
21	2	5	51	11.0	80	-	-	1	2	0.773	28	1	2	-	-	-	-	-
21	2	5	53	11.2	100	-	-	1	2	1.110	24	1	2	52.4	57.3	139	6.0	-
21	2	5	53	11.2	170	100	65	2	2	1.354	44	1	2	52.4	57.3	-	-	35
21	2	5	56	11.8	-	-	-	1	1	0.638	16	1	2	-	-	-	-	-
27	1	7	09	2.8	-	-	-	1	1	0.513	36	1	2	70.2	69.5	-	-	-
27	1	7	11	3.4	-	-	-	1	1	0.077	26	1	1	68.4	68.6	-	-	-
27	1	7	13	4.6	-	-	-	1	1	3.351	48	1	1	72.4	72.6	-	-	-
27	1	7	15	5.2	-	-	-	1	1	0.350	38	1	1	75.0	73.5	-	-	-
27	1	7	17	7.2	160	-	-	1	1	0.022	37	1	1	-	-	-	-	-
27	1	7	19	7.5	150	-	-	1	1	0.070	30	1	1	67.6	68.9	-	-	-
27	1	7	21	9.0	120	-	-	1	1	0.183	47	1	1	78.3	74.6	-	-	-
27	1	7	23	11.3	130	-	-	1	1	0.269	33	1	1	72.6	75.0	-	-	-
27	1	7	25	11.0	120	-	-	1	1	0.195	29	1	1	78.5	81.1	134	4.2	-
27	1	7	27	10.6	90	-	-	1	1	0.920	23	1	2	72.2	76.2	-	-	-
27	1	7	27	10.6	210	125	90	2	1	1.380	26	1	2	72.2	76.2	-	-	35
27	1	7	29	10.1	-	-	-	1	1	0.460	40	1	2	71.0	74.2	131	4.0	-
27	1	7	31	9.8	75	-	-	1	1	1.820	14	1	2	65.9	69.0	-	-	-
27	1	7	33	10.9	90	-	-	1	1	0.553	22	1	2	69.3	69.0	134	4.2	-
27	1	7	33	10.9	260	110	80	2	1	2.818	39	1	2	69.3	69.0	-	-	30

Data Set II. (continued)

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
27	1	7	35	10.1	70	-	-	1	1	2.102	47	1	2	65.5	62.5	-	-	-
27	1	7	37	10.4	80	-	-	1	1	1.213	28	1	2	61.5	51.2	151	4.8	-
27	1	7	37	10.4	260	100	60	2	1	2.004	46	1	2	61.5	51.2	-	-	40
27	1	7	39	10.0	100	-	-	1	2	0.022	39	1	2	57.3	56.5	-	-	-
27	1	7	41	10.3	100	-	-	1	2	0.146	48	1	2	48.4	48.5	-	-	-
27	1	7	41	10.3	240	92	65	2	2	0.797	19	1	2	48.4	48.5	-	-	27
27	1	7	43	10.2	100	-	-	1	2	0.103	30	1	2	53.8	54.4	-	-	-
27	1	7	45	9.9	100	-	-	1	2	5.104	47	1	2	57.3	57.3	139	4.8	-
27	1	7	45	9.9	250	97	52	2	2	0.946	31	1	2	57.3	57.3	-	-	45
27	1	7	47	10.2	100	-	-	1	2	1.606	48	1	2	61.8	58.8	-	-	-
27	1	7	49	10.3	80	-	-	1	2	0.364	14	1	2	-	-	132	3.8	-
27	1	7	49	10.3	230	100	-	2	2	-	-	1	2	-	-	-	-	-
27	1	7	51	10.7	80	-	-	1	2	1.569	43	1	2	52.4	57.3	-	-	-
27	1	7	53	10.5	-	-	-	1	1	0.709	25	1	2	-	-	-	-	-
29	1	8	09	4.4	-	-	-	1	1	0.103	34	1	2	-	-	-	-	-
29	1	8	11	4.2	-	-	-	1	1	0.913	48	1	1	-	-	-	-	-
29	1	8	15	9.5	180	-	-	1	1	0.120	26	1	1	-	-	-	-	-
29	1	8	17	10.3	240	-	-	1	1	2.000	14	1	1	67.6	68.9	-	-	-
29	1	8	19	14.0	165	-	-	1	1	0.103	29	1	1	78.3	74.6	-	-	-
29	1	8	21	15.1	200	-	-	1	1	4.058	24	1	1	72.6	75.0	-	-	-
29	1	8	23	16.1	170	-	-	1	1	0.270	21	1	1	78.5	81.1	-	-	-
29	1	8	24	16.8	-	-	-	1	1	0.403	47	1	1	74.9	75.4	123	3.7	-
29	1	8	25	16.6	130	-	-	1	1	0.966	45	1	2	72.2	76.2	-	-	-
29	1	8	25	16.6	210	120	65	2	1	2.667	48	1	2	72.2	76.2	-	-	55
29	1	8	27	15.6	120	-	-	1	1	3.500	14	1	2	71.0	74.2	-	-	-
29	1	8	29	15.3	110	-	-	1	1	5.915	29	1	2	65.9	69.0	118	3.7	-
29	1	8	29	15.3	210	125	75	2	1	-	-	1	2	65.9	69.0	-	-	50
29	1	8	31	14.7	110	-	-	1	1	1.265	46	1	2	69.3	69.0	-	-	-
29	1	8	33	14.2	110	-	-	1	1	3.664	20	1	2	65.5	62.5	142	4.2	-
29	1	8	33	14.2	220	95	55	2	1	3.110	22	1	2	65.5	62.5	-	-	40
29	1	8	35	13.9	110	-	-	1	1	2.750	48	1	2	61.5	61.2	-	-	-
29	1	8	37	13.3	120	-	-	1	2	3.020	18	1	2	57.3	56.5	151	4.3	-
29	1	8	37	13.3	235	115	65	2	2	12.173	29	1	2	57.3	56.5	-	-	50

Data Set II. (continued)

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
29	1	8	39	13.9	160	-	-	1	2	3.623	48	1	2	55.0	55.1	-	-	-
29	1	8	41	13.8	130	-	-	1	2	0.371	50	1	2	53.8	54.4	155	4.1	-
29	1	8	41	13.8	230	115	65	2	2	1.151	42	1	2	53.8	54.4	-	-	50
29	1	8	43	13.8	150	-	-	1	2	2.256	46	1	2	57.3	57.3	-	-	-
29	1	8	45	13.7	110	-	-	1	2	1.082	39	1	2	61.8	58.8	135	4.3	-
29	1	8	45	13.7	235	120	72	2	2	1.118	48	1	2	61.8	58.8	-	-	48
29	1	8	47	13.7	100	-	-	1	2	0.523	26	1	2	-	-	-	-	-
29	1	8	49	13.8	140	-	-	1	2	0.530	31	1	2	52.4	57.3	152	5.0	-
29	1	8	49	13.8	230	90	55	2	2	1.322	13	1	2	52.4	57.3	-	-	35
29	1	8	53	12.5	-	-	-	1	1	0.942	50	1	2	-	-	-	-	-
30	2	8	09	3.7	-	-	-	1	1	0.136	36	1	2	-	-	-	-	-
30	2	8	11	2.9	-	-	-	1	1	1.331	24	1	2	-	-	-	-	-
30	2	8	15	7.6	180	-	-	1	1	0.154	47	1	1	-	-	-	-	-
30	2	8	17	8.5	180	-	-	1	1	2.250	48	1	1	67.6	68.9	-	-	-
30	2	8	19	12.2	150	-	-	1	1	0.007	42	1	1	78.3	74.6	-	-	-
30	2	8	21	13.8	140	-	-	1	1	0.230	29	1	1	72.6	75.0	-	-	-
30	2	8	23	16.1	150	-	-	1	1	0.706	43	1	1	78.5	81.1	143	4.5	-
30	2	8	25	16.0	105	-	-	1	1	2.114	44	1	2	72.2	76.2	-	-	-
30	2	8	25	16.0	180	87	50	2	1	15.287	21	1	2	72.2	76.2	-	-	37
30	2	8	27	15.3	108	-	-	1	1	1.743	27	1	2	71.0	74.2	-	-	-
30	2	8	29	14.5	110	-	-	1	1	1.797	45	1	2	65.9	69.0	129	4.7	-
30	2	8	29	14.5	200	110	45	2	1	4.180	48	1	2	65.9	69.0	-	-	65
30	2	8	31	12.9	100	-	-	1	1	4.133	14	1	2	69.3	69.0	-	-	-
30	2	8	33	11.6	100	-	-	1	1	0.183	16	1	2	65.5	62.5	143	4.9	-
30	2	8	33	11.6	192	-	-	2	1	1.793	22	1	2	65.5	62.5	-	-	-
30	2	8	35	10.3	100	-	-	1	1	0.400	39	1	2	61.5	61.2	-	-	-
30	2	8	36	9.8	-	-	-	1	2	2.383	45	1	2	-	-	-	-	-
31	2	8	09	3.5	-	-	-	1	1	0.064	34	1	2	-	-	-	-	-
31	2	8	11	3.1	-	-	-	1	1	0.530	24	1	2	-	-	-	-	-
31	2	8	15	6.9	150	-	-	1	1	1.395	29	1	1	-	-	-	-	-
31	2	8	17	6.8	180	-	-	1	1	0.589	25	1	1	67.6	68.9	-	-	-
31	2	8	19	10.0	144	-	-	1	1	0.168	36	1	1	78.3	74.6	-	-	-
31	2	8	21	12.2	140	-	-	1	1	0.147	35	1	1	72.6	75.6	-	-	-

Data Set II. (continued)

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
31	2	8	23	13.8	150	-	-	1	1	0.557	36	1	1	78.5	81.1	141	4.4	-
31	2	8	25	14.0	120	-	-	1	1	2.083	30	1	2	72.2	76.2	-	-	-
31	2	8	25	14.0	230	140	87	2	1	2.506	48	1	2	72.2	76.2	-	-	53
31	2	8	27	13.6	108	-	-	1	1	6.457	43	1	2	71.0	74.2	-	-	-
31	2	8	29	12.7	110	-	-	1	1	0.308	45	1	2	65.9	69.0	140	5.1	-
31	2	8	29	12.7	210	102	75	2	1	1.215	32	1	2	65.9	69.0	-	-	27
31	2	8	31	11.9	110	-	-	1	1	-	-	1	2	69.3	69.0	-	-	-
31	2	8	33	10.9	140	-	-	1	1	1.391	24	1	2	65.5	62.5	128	4.4	-
31	2	8	33	10.9	240	127	70	2	1	-	-	1	2	65.5	62.5	-	-	57
31	2	8	35	10.2	110	-	-	1	1	0.198	35	1	2	61.5	61.2	-	-	-
31	2	8	37	-	120	-	-	1	2	1.943	42	1	2	57.3	56.5	137	5.1	-
31	2	8	37	-	210	-	-	2	2	40.623	37	1	2	57.3	56.5	-	-	-
31	2	8	39	10.2	114	-	-	1	2	0.691	28	1	2	48.4	48.5	-	-	-
31	2	8	41	9.6	130	-	-	1	2	0.973	15	1	2	53.8	54.4	129	4.0	-
31	2	8	41	9.6	190	100	45	2	2	1.281	32	1	2	53.8	54.4	-	-	55
32	2	8	09	4.2	-	-	-	1	1	0.103	12	1	2	-	-	-	-	-
32	2	8	11	3.3	-	-	-	1	1	3.604	15	1	2	-	-	-	-	-
32	2	8	15	9.2	170	-	-	1	1	0.086	14	1	1	-	-	-	-	-
32	2	8	17	10.8	180	-	-	1	1	1.485	42	1	1	67.6	68.9	-	-	-
32	2	8	19	13.7	140	-	-	1	1	0.337	44	1	1	78.3	74.6	-	-	-
32	2	8	21	15.0	140	-	-	1	1	0.048	42	1	1	72.6	75.0	-	-	-
32	2	8	23	16.8	130	-	-	1	1	0.054	42	1	1	78.5	81.1	-	-	-
32	2	8	24	-	-	-	-	1	1	1.693	17	1	1	-	-	133	3.9	-
32	2	8	25	16.4	120	-	-	1	1	5.491	18	1	2	72.2	76.2	-	-	-
32	2	8	25	16.4	240	100	60	2	1	2.141	40	1	2	72.2	76.2	-	-	40
32	2	8	27	15.8	100	-	-	1	1	1.433	20	1	2	71.0	74.2	-	-	-
32	2	8	29	15.2	100	-	-	1	1	0.561	40	1	2	65.9	69.0	146	4.8	-
32	2	8	29	15.2	240	160	100	2	1	3.986	23	1	2	65.9	69.0	-	-	60
32	2	8	31	14.4	90	-	-	1	1	0.179	36	1	2	69.3	69.0	-	-	-
32	2	8	33	13.7	90	-	-	1	1	5.923	50	1	2	48.4	48.5	144	3.9	-
32	2	8	33	13.7	230	140	92	2	1	4.312	32	1	2	48.4	48.5	-	-	48
32	2	8	35	13.2	105	-	-	1	1	4.114	38	1	2	61.5	61.2	-	-	-

Data Set II. (continued)

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
32	2	8	37	13.4	100	-	-	1	2	3.984	47	1	2	57.3	56.5	152	4.2	-
32	2	8	37	13.4	180	102	55	2	2	2.768	47	1	2	57.3	56.5	-	-	47
32	2	8	39	13.7	110	-	-	1	2	2.134	36	1	2	48.4	48.5	-	-	-
32	2	8	41	13.4	130	-	-	1	2	1.325	29	1	2	53.8	54.4	-	-	-
32	2	8	41	13.4	230	110	55	2	2	1.398	36	1	2	53.8	54.4	-	-	55
32	2	8	43	13.4	120	-	-	1	2	0.963	9	1	2	57.3	57.3	-	-	-
32	2	8	45	13.4	120	-	-	1	2	2.968	38	1	2	61.8	58.8	139	5.5	-
32	2	8	45	13.4	220	110	65	2	2	2.383	48	1	2	61.8	58.8	-	-	45
32	2	8	47	13.7	110	-	-	1	2	1.087	19	1	2	-	-	-	-	-
32	2	8	49	13.8	120	-	-	1	2	0.323	40	1	2	52.4	57.3	146	5.2	-
32	2	8	49	13.8	235	93	47	2	2	0.653	40	1	2	-	-	-	-	46
32	2	8	53	13.2	-	-	-	1	2	1.327	21	1	2	-	-	-	-	-
1	1	1	09	3.6	-	-	-	1	1	2.392	22	1	2	-	-	-	-	-
1	1	1	11	3.0	-	-	-	1	1	1.600	50	1	2	-	-	-	-	-
1	1	1	15	6.5	-	-	-	1	1	0.080	50	1	1	63.5	65.0	-	-	-
1	1	1	17	7.5	-	-	-	1	1	0.878	32	1	1	69.3	69.0	-	-	-
1	1	1	19	9.4	-	-	-	1	1	0.049	44	1	1	69.4	70.0	-	-	-
1	1	1	21	10.3	-	-	-	1	1	0.095	44	1	1	68.8	66.5	-	-	-
1	1	1	23	11.0	132	-	-	1	1	0.371	50	1	1	72.2	74.7	-	-	-
1	1	1	25	13.3	-	-	-	1	1	0.181	33	1	1	71.4	71.4	131	4.8	-
1	1	1	25	13.3	216	130	90	2	1	0.337	26	1	1	71.4	71.4	-	-	40
1	1	1	27	12.3	192	-	-	1	1	0.335	50	1	2	75.4	76.3	-	-	-
1	1	1	29	11.6	156	-	-	1	1	1.213	10	1	2	73.6	77.1	-	-	-
1	1	1	29	11.2	192	-	-	1	1	3.048	18	1	2	74.0	75.8	-	-	-
1	1	1	29	11.2	204	135	85	2	1	1.642	15	1	2	74.0	75.8	-	-	50
1	1	1	31	11.6	150	-	-	1	1	7.524	26	1	2	-	-	-	-	-
1	1	1	33	11.2	120	-	-	1	1	7.307	21	1	2	72.6	76.9	134	4.2	-
1	1	1	34	11.4	110	-	-	1	1	4.969	29	1	2	72.2	76.2	-	-	-
1	1	1	34	11.4	204	105	80	2	1	-	-	1	2	72.2	76.2	-	-	25
1	1	1	37	11.2	80	-	-	1	1	1.269	46	1	2	67.3	73.9	151	5.0	-
1	1	1	37	11.2	190	122	68	2	1	0.078	13	1	2	67.3	73.9	-	-	54
1	1	1	39	11.5	100	-	-	1	1	0.667	35	1	2	69.6	71.5	-	-	-
1	1	1	41	10.7	110	-	-	1	1	0.730	36	1	2	59.5	60.2	127	3.9	-

Data Set II. (continued)

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1	1	1	41	10.7	200	125	97	2	1	6.037	16	1	2	59.5	60.2	-	-	28
1	1	1	43	10.9	110	-	-	1	1	2.531	17	1	2	64.0	64.4	-	-	-
1	1	1	45	10.8	110	-	-	1	1	1.185	29	1	2	54.8	55.6	141	4.5	-
1	1	1	45	10.8	180	77	40	2	1	1.316	17	1	2	54.8	55.6	-	-	37
1	1	1	47	10.5	100	-	-	1	1	0.840	44	1	2	60.1	58.0	-	-	-
1	1	1	49	10.1	90	-	-	1	2	1.375	36	1	2	46.9	47.6	136	4.4	-
1	1	1	49	10.1	150	78	45	2	2	0.913	30	1	2	46.9	47.6	-	-	33
1	1	1	51	11.0	140	-	-	1	2	1.276	23	1	2	55.0	55.1	-	-	-
1	1	1	53	10.4	100	-	-	1	2	0.327	16	1	2	47.0	45.7	143	5.2	-
1	1	1	53	10.4	200	100	50	2	2	13.677	47	1	2	47.0	45.7	-	-	50
1	1	1	55	10.4	100	-	-	1	2	2.557	48	1	2	61.4	60.0	-	-	-
1	1	1	57	10.9	130	-	-	1	2	0.313	42	1	2	55.4	58.6	155	4.5	-
1	1	1	57	10.9	180	-	-	2	2	17.314	40	1	2	55.4	58.6	-	-	-
1	1	1	59	11.2	110	-	-	1	2	4.331	21	1	2	62.0	62.0	-	-	-
1	1	1	61	10.8	-	-	-	1	2	0.791	50	1	2	-	-	-	-	-
28	2	7	09	2.4	-	-	-	1	1	0.237	45	1	2	70.2	69.5	-	-	-
28	2	7	11	2.7	-	-	-	1	1	0.213	23	1	1	68.4	68.6	-	-	-
28	2	7	13	3.9	-	-	-	1	1	2.992	47	1	1	72.4	72.6	-	-	-
28	2	7	15	5.0	-	-	-	1	1	0.164	22	1	1	75.0	73.5	-	-	-
28	2	7	17	6.6	190	-	-	1	1	0.176	41	1	1	-	-	-	-	-
28	2	7	19	7.8	200	-	-	1	1	0.526	43	1	1	67.6	68.9	-	-	-
28	2	7	21	9.1	170	-	-	1	1	0.279	48	1	1	78.3	74.6	-	-	-
28	2	7	23	11.0	160	-	-	1	1	0.458	11	1	1	72.6	75.0	-	-	-
28	2	7	25	11.4	160	-	-	1	1	0.029	38	1	1	78.5	81.1	142	4.6	-
28	2	7	27	10.9	120	-	-	1	1	4.855	44	1	2	72.2	76.2	-	-	-
28	2	7	27	10.9	180	130	82	2	1	2.530	47	1	2	72.2	76.2	-	-	48
28	2	7	29	10.1	100	-	-	1	1	5.208	29	1	2	71.0	74.2	131	3.9	-
28	2	7	31	9.9	100	-	-	1	1	0.334	45	1	2	65.9	69.0	-	-	-
28	2	7	33	9.1	80	-	-	1	1	1.230	20	1	2	69.3	69.0	147	3.9	-
28	2	7	33	9.1	180	111	80	2	1	1.137	16	1	2	69.3	69.0	-	-	31
28	2	7	35	8.7	100	-	-	1	1	0.501	40	1	2	65.5	62.5	-	-	-
28	2	7	37	8.7	100	-	-	1	1	1.320	48	1	2	61.5	61.2	146	4.4	-

Data Set II. (continued)

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
28	2	7	37	8.7	180	107	70	2	1	3.850	47	1	2	61.5	61.2	-	-	37
24	1	6	09	4.2	-	-	-	1	1	0.024	25	1	2	66.0	67.0	-	-	-
24	1	6	11	4.0	-	-	-	1	1	0.022	22	1	1	69.5	68.0	-	-	-
24	1	6	13	6.5	-	-	-	1	1	0.007	22	1	1	69.4	70.0	-	-	-
24	1	6	15	6.9	-	-	-	1	1	0.096	22	1	1	65.4	65.4	-	-	-
24	1	6	17	9.0	160	-	-	1	1	0.023	11	1	1	72.2	74.7	-	-	-
24	1	6	19	10.5	168	-	-	1	1	0.051	10	1	1	72.8	71.6	-	-	-
24	1	6	21	11.1	180	-	-	1	1	0.223	32	1	1	75.4	76.3	-	-	-
24	1	6	23	12.4	180	-	-	1	1	-	-	1	1	73.6	77.1	-	-	-
24	1	6	25	14.2	140	-	-	1	1	0.076	40	1	1	-	-	133	4.0	-
24	1	6	27	13.4	140	-	-	1	1	6.017	47	1	2	72.6	76.9	-	-	-
24	1	6	29	12.4	140	-	-	1	1	-	-	1	2	72.2	76.2	-	-	-
24	1	6	29	12.4	264	150	90	2	1	3.860	10	1	2	72.2	76.2	-	-	60
24	1	6	33	12.3	150	-	-	1	1	0.055	32	1	2	65.9	69.0	131	4.8	-
24	1	6	33	12.3	270	160	97	2	1	2.086	39	1	2	65.9	69.0	-	-	63
24	1	6	35	12.0	140	-	-	1	1	18.040	41	1	2	69.3	69.0	-	-	-
24	1	6	37	11.1	130	-	-	1	1	0.572	48	1	2	65.5	62.5	122	4.3	-
24	1	6	37	11.1	250	140	107	2	1	9.273	19	1	2	65.5	62.5	-	-	33
24	1	6	39	11.1	120	-	-	1	1	6.461	38	1	2	61.5	61.2	-	-	-
24	1	6	41	10.8	115	-	-	1	2	5.392	15	1	2	57.3	56.5	134	4.0	-
24	1	6	41	10.8	260	100	75	2	2	1.621	42	1	2	57.3	56.5	-	-	25
24	1	6	43	10.9	130	-	-	1	2	5.082	19	1	2	48.4	48.5	-	-	-
24	1	6	45	11.2	160	-	-	1	2	0.927	19	1	2	53.8	54.4	-	-	-
24	1	6	45	11.2	240	120	70	2	2	4.150	18	1	2	53.8	54.4	-	-	50
24	1	6	47	12.0	110	-	-	1	2	-	-	1	2	57.3	57.3	-	-	-
24	1	6	49	10.6	120	-	-	1	2	0.933	29	1	2	61.8	58.8	134	3.9	-
24	1	6	49	10.6	260	122	80	2	2	9.092	44	1	2	61.8	58.8	-	-	42
24	1	6	51	10.5	90	-	-	1	2	4.028	48	1	2	63.4	62.1	-	-	-
24	1	6	53	11.4	130	-	-	1	2	1.338	48	1	2	52.4	57.3	131	4.5	-
24	1	6	53	11.4	240	115	70	2	2	1.093	29	1	2	52.4	57.3	-	-	45