6-1985

Dynamics of Steroid Secretions in Rats

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DYNAMICS OF STEROID SECRETIONS IN RATS.

A Thesis
Presented to the Faculty of the Department of Biological Sciences of the State University of New York College at Brockport in Partial Fulfillment for the Degree of Master of Science

by
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June, 1985

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ABSTRACT

In spite of the vast information available on hormone secretions and their levels in blood, there is a paucity of information in age related changes in the dynamics of steroid metabolism in male rats. Also, few investigations exsist concerning the metabolic clearance rates (M.C.R.) of steroids in blood of female rats.

This thesis consists of two parts, the first part deals with changes in M.C.R. in aging male rats and the second part in pregnant rats. It examined these changes relative to testosterone (T), estradiol (E_2), and progesterone (P), using the non-equilibrium single dose injection method. ^{3}H-steroids were administered via the cannulated jugular vein. Sequential blood samplings were obtained up to 150 min. ^{3}H-steroids were extracted, processed by Sephadex LH-20 column chromatography and the radioactive steroids were quantified with liquid scintillation spectrometer. Results were analysed by the computer using the 'peel-off' method for a two-compartment model.

In aging studies M.C.R. and production rate of T were found significantly higher in young male rats when compared with aged rats. Half-life ($t_{\frac{1}{2}}\beta$) in the outer pool was significantly higher in the old rats than in young rats while $t_{\frac{1}{2}}\alpha$ in the inner pool did not change significantly between both aging groups. However, the M.C.R. for E_2 and P remained unchanged in both groups. Chronically castrated animals of both age groups showed significant decline in M.C.R. of T and increased in $t_{\frac{1}{2}}\beta$ when compared with their respective intact controls. M.C.R. of T in young or old castrated rats previously injected with T were restored to values similar to those young intact rats. From these results it is concluded that the age-related changes in M.C.R. of testosterone are due

to an interaction of testicular dysfunction and androgen status of animal.

In the the second part of the study, non-pregnant, pregnant (D-10 and D-20) and post-partum rats were used. The M.C.R. of E_2 in D-20 rats was found to be significantly higher when compared with other groups. In post-partum rats the M.C.R. of E_2 was significantly higher than those of non-pregnancy or D-10 of pregnancy. All values for $t_{\frac{1}{2}}\alpha$ of E_2 were found to be similar for all groups. $t_{\frac{1}{2}}\beta$ of E_2 in D-20 was greatly lower than both non-pregnancy and D-10 gestation. However, the the M.C.R. of P remained unchanged in all female groups. The fast component ($t_{\frac{1}{2}}\alpha$) of P was significantly higher in post-partum than in D-10, meanwhile it did not change in other groups. The slow component ($t_{\frac{1}{2}}\beta$) of P was higher in non-pregnant rats compared with D-10, other groups remained unchanged. The M.C.R. of T was significantly lower in non-pregnant rats than in post-partum rats, other groups remain unchanged. $t_{\frac{1}{2}}\alpha$ of T was singificantly higher in post-partum rats than in D-20 and D-10 of pregnancy. There was no change in $t_{\frac{1}{2}}\beta$ of T between all groups. It is evident that changes in metabolic clearance rates of different steroids vary according to their respective roles in pregnancy reflecting physiological demands.

ACKNOWLEDGEMENTS

I would like to express my best appreciation to Dr. Stephen W. Chan for his guidance, patience and advisement in this research. I am grateful to Faith Rogers-Aubel for her kind assistance in typing this thesis. Finally, I would like to thank my family and my wife Nelly who gave me the love and the encouragement throughout my work.

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INTRODUCTION

Since hormones are the major regulators of the body function, studies of aging on endocrine control mechanisms and reproductive functions are particularly attractive to gerontologists. In the past years there has been considerable discussion among gerontologists as to whether the deterioration of reproduction is due to age-related alterations in gonadal, pituitary, or hypothalamic-neural systems.

i. AGING MALES:

Aging in mammals is characterized by a progressive decrease in sexual activity, and by a deterioration of the organism after maturity, which increases the probability of death. Bishop (1970) reported progressive testicular atrophy and degeneration in aging males. However, it is clear that there are individual differances in testicular function, and also reported individual males are fertile at advanced age.

Many physiological functions decline gradually after cessation of growth (Everitt, 1973) and tissues become more susceptible to disease. According to one theory, there is an intracellular determinant, and an extracellular stochiastic determinant. Intercellular determinant includes those processes which occur in animals regardless of environmental influences. On the other hand stochiastic processes are the results of environmental influences (Strechler, 1977). It has been demonstrated that the influences of the environment increase the lifespan. This effect may very simply reflect a reduction in the growth rate and not a slower rate of cellular aging (Berg and Simms, 1960).

In vitro cultured normal human cells derived from embryonic tissue have a proliferative capacity of 35 to 63 doublings, but fibroblasts derived from adult donors showed a diminished proliferative capacity of

14 to 29 doublings (Hayflick, 1965). Other investigators showed a significant difference in lifespans between cells derived from young vs. old animals cultured in vitro.

Other investigators think that senescence is a part of the cell's genetic program. According to the theory of intrinsic cell aging, the damage is irreversibly and accumulation of errors in the cell's genetic materials, lead to progressive dysfunctions. Orgels (1963) suggested that mutations occur in protein systhesis which involve changes in the genetic code or a disturbance in the processes of replication, transcription, translation, or DNA repair system. These errors would be increased or multiplied with each division and death finally follows.

The immune system is an attractive target for studying the aging process. Immunologic functions decline with age in mammals. This decline is associated with an increase in the incidence of infections, autoimmune and immune complex diseases, and cancer. Various aspects of the aging immune system that lead to decreased antibody production should be examined. It has been shown in mice and humans that the thymus undergoes involution and atrophy during aging (Weksler, 1981), which is evidence that 'T' cells function decline with age. Thymus size increases in fetal and early post natal life to a maximum in mouse and human, after which it rapidly involutes. Thymectomy in the mouse in the neonatal period leads to a deficiency in both 'T' and 'B' cell activities. There is also a general involution of lymphoid tissues at about sexual maturity, followed by a decrease in the functional activity of 'T' cells, and decreased production of antibodies. 'T' cells influence the differnetiation and maturation of 'B' cells during development, so there is an interaction between the two cell types. For example, immature 'B' cells transplanted into cultures containing 'T' cells, obtained from aged

animals, were unable to produce normal amounts of antibodies.

Other 'T' cell functions, such as graph rejection or graft versus host reactivity, also decrease with age in some strains of mice (Peterson et al , 1972). The suppressive effects of 'T' cell populations on 'B' cells also decline. Some hypothesized that a decline in the control mechanism regulating an antibody-forming system results in the appearance of autoantibodies against 'self' antigens (Allison et al , 1971).

Everitt (1973) related aging to a decline in neuroendocrine functions. He proposed an 'aging clock' located in the hypothalamus or at the suprahypothalamic level. The physiological functions are genetically programmed. There may be seperate clocks for each body function, for example, a gonadotropin-aging clock controlling the secretion of pituitary gonadotropins and thus regulating the onset of puberty, cycling, and menopause.

Another area of aging studies is the environmental factors. These studies postulate that exposure to low temperatures, stress, and food intake would shorten the lifespan. There are hypothalamic - pituitary - thyroid - adrenocortical factors which may mediate metabolic processes and accelerate aging.

The major and essential steps involved in the metabolism of steroids in vivo are the delivery of the hormones from the sources of production and tranport by the circulation to the external environment to the sites of metabolism in target organs and then transformation by enzymatic reactions in cells. The metabolic clearance rates of steroids change according to sex and physiologic status of the animal. At a cellular level, the mechanisms of steroid action alter with age (Chang and Roth, 1979), and in many cases studied, receptor concentrations have been shown to decrease during aging. Responsiveness to certain hormones and

neurotransmittors is also decreased. The general mechanism of steroid hormone action is the passive diffusion into the cell and binding to receptors in the cytoplasm. The receptor-hormone complexes then become activated and translocated into the nucleus where they bind to the chromatin and activate the gene. The ability of corticosteroids to inhibit glucose oxidation in adipocytes and uridine uptake in lymphocytes is reduced consequently with glucocorticoid receptor concentration during aging in rats.

ii PREGNANCY:

Pregnancy is an anabolic state and is associated with the increased metabolic activities of the maternal organism as well as the fetuses. The distinctive gain in weight is due partly to the growth of the uterus and fetuses, and partly to the increase in blood volume and in the weights of the organs such as the liver and mammary glands. During gestation the elevated body retention of water, protein, and fat is hormonally regulated, and results from the presence of the placenta rather than the fetus. Thus, the increase in the body weight during pregnancy is not just an accumulation of excess reserves to protect the mother against the increasing demand of the fetuses, but results form the physiological adjustments of pregnancy controlled by the changes in hormonal conditions. Production rates and hormonal levels change during gestation which reflect the requirements of the mother and fetus.

During pregnancy the high levels of steroids circulating in the blood are usually bound to serum proteins to protect against disruption of endocrine balance and physiologic side effects. Vermeulen et al (1969) observed an increase in values of testosterone-binding capacity (TeBC) and binding index (BI) of testosterone-binding globulin (TeBG) during pregnancy in humans, but return to low normal values after

delivery. The binding of steroids by serum proteins render them unavailable for metabolism by enzymes that normally use the free steroids as substrates. Therefore, steroids that circulate in the blood in the bound form are not convenient for uptake by target cells until they are released in the free state. Thus the effect of binding proteins is to provide a large pool of steroid that will be slowly released as the free steroid concentration falls.

LITERATURE REVIEW:

METHODOLOGY OF M.C.R.:

i. NON-ISOTOPIC STUDIES:

The early methods used for *in vivo* studies involved the administration of large amounts of a non radioactive steroid. This is due mainly to the difficulties of obtaining pure radiochemicals at that time. For example, large quantities of cortisol were injected and then the fractional turnover rate was calculated by following the decrease in the plasma concentrations with time. This was determined by plotting the logarithm of the plasma concentration versus time. The disappearance of the injected dose could be represented by a single exponential curve. From the semi-log plot of the data, the fractional turnover rate could be estimated from the slope and the volume of distribution from the intercept on the ordinate. Using these parameters, the M.C.R. could be calculated in this single pool model. The secretion rate was determined as the M.C.R. multiplied by the plasma concentration. The disadvantage of this method is that injection of large amounts of steroids probably disturb the normal distribution and metabolism of the steroids. It has been noticed that at a high plasma concentration of cortisol, the clearance rate of the steroid is increased. Another problem is at this high concentration, a single exponential is not valid. Furthermore, the disappearance rate is not accurately represented by this exponential curve, so more than one compartement must be used to analyse this condition and to avoid considerable errors.

ii. ISOTOPIC STUDIES:

A similar method to the non-isotopic studies to calculate the M.C.R.

involved the use of isotopes (Peterson, 1959 and many others). The specific activity was measured after a single injection of a radioactive hormone, a single compartement model was then used to find the slope and the intercept, and consequently the secretion rate could be calculated in the same manner used as before. But in fact the disappearance curve may consist of two exponentials. For aldosterone, progresterone, and many other steroids which are not strongly bound to plasma proteins other than albumin, the disappearance curve must represented by more than one exponential. The metabolism and transport of steroid after a single injection of a large amount must be represented by two pools, one (inner pool) which is in more rapid equilibrium with plasma steroid than the other (the outer pool), which is in relatively slow equilibrium with plasma steroid. The radioactivity transfered from the outer pool to the plasma would increase the volume of distribution and prolong the half-life ($t_{\frac{1}{2}\beta}$) of the later part of the curve, and indicates a lowered M.C.R..

iii. CONTINUOUS INFUSION:

Another method of calculating the M.C.R. is by the continuous infusion method. Labeled steroid was injected at a constant rate until the plasma concentration of the radioactive steroid was constant. According to Tait et al. (1961), a constant concentration of aldosterone occurred between 105-135 min. The continuous infusion method of the radioactive hormone imitates the secretion of the glands. Since the ratio of the rate of radioactive infusion to the final radioactive concentration of hormone will equal the ratio of the secretion rate to the non-isotopic steroid plasma concentration, this ratio is the metabolic clearance rate.

The disadvantages of continuous infusion is that less information

about volumes of distribution is obtained. Studies by Hembree et al. (1969) showed a sharp increase in the M.C.R. of E_2 following a high infusion rate of high physiological dose of E_2 in humans. Also, de Hertogh et al. (1970) reported that the M.C.R. of E_2 increased slowly with increasing infusion rate in immature female rats.

iv. URINARY METABOLITES:

One method of calculating the secretion rates by the use of non-isotopic hormones was based on the urinary metabolites. Large amounts of steroid were injected and the proportion excreted as urinary metabolite was measured. From this ratio and the normal values for excretion of the metabolite, the secretion rate could be calculated.

A second method involved in the use of the isotopic hormones (Pearlman et al. 1954). To calculate the secretion rates, radioactive steroids were administered and the specific activities of the urinary metabolites were measured. Radioactivity injected, divided by the specific activity of the metabolite, then gives the secretion rate.

v. NONSTEADY STATE:

In the steady state, the clearance rate of non-isotopic steroid from the blood is equal at all times to the rate of entry and the concentration of non-isotopic steroid in the blood is constant. Therefore, it is necessary for the subject to be in steady state for the accurate application of the methods over a prolonged time period. But in the nonsteadly state there is a variation in the secretion rate, and the rate of metabolism does not equal the rate of secretion. However, the M.C.R. is still defined as the volume of blood from which the steroid hormone is completely and irreversible removed in unit time. This also assumes that the rate constants and the volumes remain unchanged.

CHANGES IN HORMONE LEVELS IN AGING MALES:

There has been considerable disagreement on the effect of age on testosterone in the human male. No change in blood testosterone concentration in aged men has been reported (Kent and Acone, 1966; Gandy and Peterson, 1968). Although other reporters (Baker, Burger, Kretser, Hudson, O'Conner, Wang, Mirovics, Court, Dunlop, and Rennie, 1976; Vermeulen et al. 1972) have showed a decreased serum testosterone with age. Humans showed a decline in T levels after 65 years of age (Vermeulen, Rubens and Verdonck 1972; Eleftheriou and Lucas 1974). Vermeulen (1969) found that the decrease in serum testosterone was accompanied by an increase in plasma testosterone binding capacity, which resulted in a further reduction in free testosterone in older men.

Other studies in bulls, rats, guinea pigs, and rabbits have shown a decline in T production with aging (Collins, Inskeep, Dreher, Tyler and Casida 1962; Ewing 1967; Ghanadian, Lewis, and Chisholm 1975; Rigaudiere, Pelandy, Robert and Delost 1976; Chan, Leathem, and Esashi 1977; Miller and Reigle 1978). Chan et al. (1977) incubated testis from 4 and 18 months-old Long-Evans rats with labeled progesterone and showed a decreased T production in aged males. Lin et al. (1980) found that plasma T levels were significantly lower in 24 month old males than 3 month old Sprague-Dawley male rats.

In aged mice, no changes in blood testosterone or testicular response to LH have been reported (Eleftheriou and Lucas, 1974; Finch, 1978). On the other hand Leathem and Albrecht (1974) reported a decrease in testicular Δ^5- 3β- hydroxysteroid dehydrogenase activity in aged Long-Evans male rats with 5 days of HCG treatment. After 7 days of HCG

injection Miller and Riegle (1978) showed that T concentration and testicular responses to HCG were similar in young and aged rats and suggested that the testis is not the primary organ responsible for aging decline in reproductive functions. However, the primary cause of reduced gonadal endocrine function with age in the male rats may be due to insufficient hypotholamic stimulation on the pituitary and hence the gonads. Studies by Finch (1978) and others suggested a reduction in neurotransmittors in aged rodents.

Furthermore, purified young Leydig cells produced more T than cells from old rats in response to LH and cyclic AMP _in vitro_ stimulation (Lin _et al._ 1980), and these results suggested defects in old Leydig cells causing reduced T response with cyclic AMP formation.

Some mammals however showed a decrease in testicular weight with lower T production with age. Rats such as Long-Evans (Chan _et al._ 1977), Wistar (Miller and Riegle, 1978) and Spargue-Dawley (Harman _et al._ 1978) showed no testicular weight loss during aging. Fischer rats have been shown to increase in testicular weight as a result of a spontaneous interstitial cell tumor growth (Bethea and Walker, 1979). Kaler and Neaves (1981) observed that plasma testosterone concentration declined in aging male rats and this is due mainly to the dilution of secreted hormone with the expanded volume of plasma in a signigicantly larger body mass. Androgen production by both testes and adrenals decrease in old age, this is a sequence of a decrease in the metabolic clearance rate.

STEROID LEVELS IN MALE RATS:

Steroid hormones as well as gonadotopins concentrations in male rats fluctuate in circadian rhythm. Serum testosterone exhibited a circadian variation in 4 and 18 month old male rats (Chan _et al._ 1977). Peripheral

serum T level in young male rats was 3.9ng/ml and in older rats was 0.93 ng/ml (Chan et al. 1981). In 4 month young rats, the lowest T level of 1.67 ng/ml was observed at midnight and was followed by a rise to 5.88 ng/ml at 0800 hours. At noon it was not significantly different from the level found at midnight. A second peak was noted at 1600 hours (5.5 ng/ml). In 18 month old rats T levels were significantly higher at 0400 hours (1.41 ng/ml), 0800 hours (1.54 ng/ml), 1600 hours (2.15 ng/ml) and at 2000 hours (2.80 ng/ml) than at midnight (0.71 ng/ml).

Testosterone in the plasma of male fetuses at 18 days of gestation was 2.2 ng/ml (Ward and Weisz, 1984). But according to Corpechot et al. (1981), T level was 0.6 ng/ml and dropped to about 0.3 ng/ml at birth and up to the 4^{th} week of age, then increased gradually to reach the adult level of 3 ng/ml by the 8^{th} week.

At age of 3-4 month, the average plasma T concentration fell from 3.14 ng/ml to less than 1.5 ng/ml at 24 months of age (Kaler and Neaves, 1981). Frankel and Mock (1982) showed that serum T was 4.45 ng/ml at 4 months of age, and dropped significantly to 1.44 ng/ml at 7 months and further decreased to 0.91 ng/ml at 24 months of age.

The concentration of estradiol in plasma of fetal male rats increased to a highest level between days 20.5-21.5 to approximately 0.18 ng/ml (Habert and Picon, 1984). E_2 levels have been found to be similar in plasma of young and old male rats (Lin et al. 1981). Chan et al. (1981) found E_2 concentration to be 28.6 pg/ml in young male rats and was 29 pg/ml in peripheral serum of old male rats.

Serum progesterone levels fluctuate in a circadian rhythm too, the lowest levels were found below 100 pg/ml in the morning between 0500-1100 hours and increased to a highest level 400-500 pg/ml between 1630-2130 hours (Kalra and Kalra, 1977). According to Chan et al. (1981), P level

in young male rats was 1.86 ng/ml and was 1.46 ng/ml in serum of old male rats.

FLUCTUATIONS OF HORMONE LEVELS IN CYCLIC RATS:

Mature female rats exhibit a 4-5 day estrous cycle that is hormonally regulated and shows four stages; proestrus, estrus, metestrus and diestrus. Each phase can be differentiated by changes in vaginal cornification. Young females showed a regular cycle, aging females progress from regular to irregular estrus cycles, then a constant estrus or a pseudopregnant-like condition and finally to an anestrus state.

There are changes in the concentrations of the circulating hormones in each stage of the cycle of the ovulating female rat or exhibited a circadian rhythm. On the morning of proestrus and before the rat comes into estrus, the secretion of estradiol from the developing Graafian follicle reaches peak values. This is followed by prolactin, LH and FSH surges on the afternoon of proestrus, and ovulation occurs in the early hours of the following morning. LH peak occurred at 1700 hours with serum values of 35-40 mg/ml (Smith et al. 1975). E_2 levels reached serum values of 40-50 pg/ml by 0900 hours. Progesterone secretions reached the peak just before ovulation occurs in the afternoon of this phase with a value of 45-55 ng/ml serum at 1700 hours. FSH become elevated in late proestrus. Prolactin levels increased from 10 ng/ml serum at 1100 hours on proestrus and reached peak values of 70 ng/ml serum by 1700 hours. Proestrus lasts 8 hours. Chan and Leathem (1977) have reported that P level in proestrus rats was 13.7 ng/ml serum.

Estrus lasts 24-36 hours, mating at this time is likely to result in pregnancy. Progesterone levels start falling down also LH concentration returned to baseline value of 0.5 ng/ml serum by 0700 hour and E_2 levels

remained at low concentration of 7 pg/ml serum. Serum P was found to be 13.5 ng/ml in this phase (Chan and Leathem, 1977).

Metestrus starts after ovulation and lasts 10-14 hours, E_2 levels increased to up to 15-20 pg/ml at 1300 hours. According to Chan and Leathem (1977) P level was 8.3 ng/ml.

Diestrus lasts between 24-48 hours, hormone concentrations were maintained at low levels in this phase. P secretion begins on the afternoon of metestrus and reached a peak value of 25-30 ng/ml by the early morning of diestrus. The patterns of prolactin, LH and FSH secretions were similer in most of the cycle. Chan and Leathem (1977) have found that P concentration in serum of diestrus rats was 3.7 ng/ml.

STEROID LEVELS IN PREGNANCY:

In pregnancy, steroids are secreted in significantly higher amounts. Serum concentration of P in pregnant rats rise from 5-10 ng/ml on day 1 and 2 to about 70 ng/ml on day 4-10 of pregnancy, another increase occurred at day 10 to reach a peak value of 152 ng/ml in day 15, then declined to a value of 10 ng/ml on day 22 (Bridges 1984). Other reports have shown similar patterns of P levels in pregnant rats (Pepe and Rothchild 1972; Morishige, Pepe and Rothchild 1973). According to Pepe and Rothchild (1972) P level was 76.6 ng/ml in serum of day 10 and was 74.4 ng/ml in day 20 of pregnancy, after parturition the mean serum P level dropped to 4.7 ng/ml and on one day post-partum was 44 ng/ml.

Plasma P concentrations showed a diurnal rhythm in pregnant women (Challis *et al.* 1981) followed by a similar circadian rhythm of estradiol (Patrick *et al.* 1979). But there is no significant different in P concentration during gestation and showed little or no pre-partum decline in humans (Csapo *et al.* 1971). P level in the third trimester in

pregnant women was 12.5 µg/ml (Lin et al. 1972).

Serum E_2 levels remained unchanged through the first 12 days of pregnancy in rats, the mean concentration was approximately 30 pg/ml then increased gradually to 80 pg/ml at day 22 (Bridges 1984).

In humans, the mean concentration of unconjugated E_2 was 800 pg/ml at the first 8 weeks of pregnancy, which it is 2-3 times the peak E_2 concentration of the normal menstrual cycle. The highest E_2 level through pregnancy was at the 36th week of pregnancy with a value of 26 ng/ml serum (Loriaux et al. 1972).

The mean concentration of T in serum did not change in the first 10 days of pregnancy in rats (Bridges et al. 1982). On day 10 T was 364 pmol/l or approximately 105 pg/ml, it incrased to a maximum level of 3685 pmol/l or about 1063 pg/ml on day 22 and dropped to 315 pmol/l or about 91 pg/ml after parturition. Similar values were reported by Ward et al. (1984).

In pregnant guinea pigs the plasma concentration of T and DHT increased up to 0.39 and 0.47 ng/ml respectively at day 48 (Rigaudiere et al. 1980). Three days after parturition the concentration of both hormones decrease rapidly to that level of non-pregnant females with values of 38 pg/ml for T and 62 pg/ml for DHT.

In contast to rats, women failed to show any significant diurnal variation in plasma level of T (Southren et al. 1967).

M.C.R. OF STEROIDS IN MALES:

i. TESTOSTERONE:

Contradictory values have been published for the M.C.R. of many steroids in mammals. Some values obtained from the same animal varied in many observations and even by the same investigator. These changes

depended on the techniques they used to administer steroids to animals. For example, Southren et al. (1967) have found the mean M.C.R. of T was 1288 l/day in man by using constant infusion method, while by single infusion technique was 1696 l/day. Saez et al. (1972) found that the M.C.R. of T was 516 l/m^2/day (m^2 refered to body surface area) which was similar to the value derived by Longcope et al. (1969). Vermeulen et al. (1972) have determined that the M.C.R. of T decreased in male senescence, concomitant with a decrease in the production rate of T in men. They found that plasma M.C.R. of T in young subjects was 640 l/m^2/day which was higher than that of old men of a value of 530 l/m^2/day.

In male pigs, Elsaesser et al. (1978) suggested that a rapid disappearance of T from the circulation in the new-borne when compared with adults. This may be due to a comparatively accelerated catabolism of testosterone.

In male Rhesus monkeys, the mean M.C.R. of T measured in whole blood was 140 l/day (Franz and Longcope, 1979).

Smith et al. (1977) reported a decrease in the M.C.R. of T during development in Long-Evans male rats, and may be partially responsible for the increassing in concentrations of steroids observed in deveoping immature males.

A decrease in the M.C.R. of T has been reported in aged Sprague-Dawley rats (Chan et al. 1981). The M.C.R. of T was found to be 55.2 l/day or 175 l/kg/day in plasma of male rats by continuous infusion (Lee et al. (1975). While Wang et al. (1967) used single injection method found the M.C.R. of T in blood 55.8 l/day or 260 l/kg/day.

ii. ESTRADIOL:

The plasma M.C.R. of E_2 in men was 1890 l/day or 3060 l/day in blood as shown by Longcope et al. (1968). Similar results have been observed

by Hembree et al. (1969), they calculated the M.C.R. of E_2 in plasma of male subjects to be 1640 l/day.

In male Rhesus monkeys the M.C.R. of E_2 in blood was 500 l/day (Franz and Longcope, 1979).

The M.C.R. of E_2 and T in blood of developing boars has been reported (Christenson et al. 1984), and found that the M.C.R. of E_2 and T were lower in pre-puberal boars (4808 l/day for E_2 and 3508 l/day for T) than in post-puberal boars (8254 l/day for E_2 and 4973 l/day for T). When the values were corrected for body weights, the M.C.R. of both steroids were higher in pre-puberal (140 l/kg/day for E_2 and 102 l/kg/day for T) than in post-puberal boars (46 l/kg/day for E_2 and 28 l/kg/day for T).

iii. PROGESTERONE:

Little et al. (1966) observed that the M.C.R. of P in plasma of men was 2450 l/day measured after single injection, similer results reported by Lin et al. (1972) using continuous infusion method was 2080 l/day.

M.C.R. OF STEROIDS IN FEMALES:

i. PROGESTERONE:

The plasma M.C.R. of P using the continuous infusion method in various stages of the menstrual cycle found to be 2510 l/day, and has been observed that there was no relation between the M.C.R. and the day of the cycle, it was constant in all women subjects. In pregnant women in the third trimester was 2020 l/day, and in overiectomized women was 2170 l/day. It was concluded that M.C.Rs. of P were similar in all female subjects in spite of large differences in the rates of production of the hormone. However, by using single injection methods, it has been observed that the M.C.R. of P in pregnant women during the third

trimester was 2340 l/day, which was slightly higher than the value obtained by continuous infusion (Lin et al. 1972).

In Rhesus monkeys, Sholl and Wolf (1974) found that the M.C.R. of P in whole blood did not change during pregnancy. They used the single injection method, the M.C.R. of P was 264 l/day on day 22 and was 214 l/day on day 100 of pregnancy. But when it was calculated on the basis of body weights, The M.C.R. declined from 49.5 l/kg/day on day 22 to 34.1 l/kg/day on day 100 of gestation. They showed that $t_{\frac{1}{2}} \alpha$ of the fast component did not change during pregnancy, but $t_{\frac{1}{2}} \beta$ represented by the slower component changed from 17.1 min at day 22 to 29.6 min at day 48.

Comparing non-pregnant and pregnant sheep, Bedford et al. (1972) found the M.C.R. of P to be slightly greater during pregnancy than in the cycle, and it remained constant during gestation. In lactating sheep it was higher than that during pregnancy

Using the single injection method, Robinson et al. (1981) calculated the M.C.R. of P to be 2.9 l/day in blood of pseudopregnant Sprague-Dawley rats. But the M.C.R. calculated by Pepe and Rothchild (1973) in pseudopregnant rats was 30.88 l/day in plasma or equivalent to 56.2 l/day in blood. On day 10 of pregnancy, the M.C.R. of P was 27.8 l/day in plasma or 50.6 l/day in blood, whereas on day 15, the mean value was 34.8 l/day in plasma or 63.3 l/day in blood.

ii. ESTRADIOL:

The plasma M.C.R. of E_2 in women was 1350 l/day or 2280 l/day in blood (Longcope et al. 1968). Similar results has been reported by Hembree et al. (1969) of a value of 1025 l/day.

In adult female Rhesus monkeys, the M.C.R. of E_2 in blood found to be 167 l/day, which was higher than the M.C.R. in immature females of a value of 69 l/day (Hotchkiss, 1983). When values were corrected for body

weights, the mean M.C.R. was higher in immature females of a value of 48.4 l/kg/day which was higher than that of adult females of a value of 27.7 l/kg/day (Hotchkiss, 1985).

Similar results of the M.C.R. of E_2 has been reported in developing female gilts (Elsaesser et al. 1982). They showed lower M.C.R. values in immature gilts (1481 l/day) than in mature gilts (2102 l/day) but when corrected for body weight, the M.C.R. of E_2 was higher in immature (81 l/kg/day)than in mature gilts (33 l/kg/day).

In adult female Wistar rats, the plasma M.C.R. of E_2 calculated by continuous infusion method was 16.2 l/day or 29.5 l/day in blood (de Hertogh et al. 1970). In ovariectomized rats, the mean M.C.R.of E_2 in serum was 17.8 l/day (Ball et al. 1983).

iii. TESTOSTERONE:

The M.C.R. of T in plasma of women was 275 l/m^2/day (Longcope et al. 1969). Saez et al. (1972) found that the plasma M.C.R. of T was 304 l/m^2 /day during pregnancy.

In female rabbits, blood M.C.R. of T was determined to be 259 l/day (Wang et al. 1967).

INFLUENCE OF TESTOSTERONE BINDING GLOBULIN (TeBG) ON THE M.C.R. OF T

It has been shown that the M.C.R. increased after acute increase of plasma T level by administration of a high amount of T in human subjects (Vermeulen et al. 1969). TeBG bound testosterone is not readily metabolized, thus a decrease in TeBG is paralleled by an increase in the M.C.R.. A higher level of testosterone binding capacity (TeBC) and binding index (BI) of testosterone in adult females than in males was observed, also a higher value during pregnancy than in non-pregnant or at delivery.

Sex hormones are important determinants of the TeBG levels, before puberty values are similar in both sexes, after puberty male TeBG values are significantly lower than the females. It has been observed that the testosterone binding capacity of TeBG increases with advancing age, and as a sequence free T level and the M.C.R. values decreased.

MATERIALS AND METHODS:

ANIMALS:

Sprague-Dawley rats originally purchased from Charles River Laboratory (Charles River, Mass.) were bred in our laboratory to produce a colony used in this research. Animals were kept at a constant temperature of 25 °C and a 14 hr light, 10 hr dark regimen. They were fed Purina Laboratory Chow and water ad libitum .

i. AGING STUDIES:

Male rats were isolated from female rats and kept in a seperate room at the time of weaning (22 days of age) and were caged singly or doubly and identified as to birth dates. Male rats used for castration studies were castrated at 2 months of age, intact or castrated rats were used at 4 months (young) or 18-24 months (old).

In a second sub-group of castrated males, young and old rats were given daily subcutaneous injections of testosterone (100 µg/0.1 ml in sesame oil) for 8 days. On day 9, blood samples were taken to check for basal T level in each rat before metabolic clearance rates (M.C.R.) determinations.

ii. FEMALE STUDIES:

Virgin female rats of 3-4 months old were used. Vaginal smears of the untreated females were examined daily in the mornings, only proestrous were mated with male studs. The day when the presence of sperms was observed was considered day 1 (D-1) of pregnancy. Pregnant females were then seperated. Body weights of all animals (males or

females), and the vaginal smears of the non-pregnant rats were recorded on the day of the experiment. In these studies non-pregnant, pregnant (D-10 and D-20) and post-partum animals were used.

Animals bearing tumors or any overt signs of pathologies were discarded.

STEROIDS:

Radioactive steroids [1,2,6,7-^{3}H(N)] testosterone (s.a. 93.9 Ci/m mol),[2,4,6,7-^{3}H(N)] estradiol (s.a. 93.0 Ci/m mol), [1,2,6,7-^{3}H(N)] progesterone (s.a. 101.0 Ci/m mole), ^{14}C-testosterone (s.a. 51.9m Ci/m mol), ^{14}C-estradiol (s.a. 57.0 m Ci/m mol) and ^{14}C-progesterone (s.a. 57.2 m Ci/m mol) were purchased from New England Nuclear, Boston, Mass. ^{3}H-steroids were brought to a volume of 5 ml with a solution of benzene : ethanol (9:1 v/v) and stored at 4^{o}C. Aliquots of isotopes were dried under a stream of nitrogen and purified one day prior to the injection. They were spotted on thin-layer chromatography using pre-coated plastic plates of silica gel (60 F 254, layer thickness 0.2 mm) in solvent system consisting of benzene: methanol (8:2 v/v). Purified isotopes were recovered by eluting with absolute methanol. 1.55 μCi of each of ^{3}H-T, ^{3}H-E_2 and ^{3}H-P were mixed in one small 1 ml vial, dried under $N_2$and redissolved in 200 μl heparinzed-saline contained 20 μl ethanol. They were injected in one dose via the left jugular vein (see below).

CANNULATION OF THE JUGULAR VEIN:

The jugular vein is a very thin and a delicate blood vessel, so cannulation procedures were done under a magnifying lens with great care.

Rats were anesthetized with ether. The skin just above the left clavicle was cut approximately 1-1.5 cm in length. Tissues and blood

vessels were spread by forcing the points of a haemostat between tissues and let them slowly opened, so that damaging the surrounding tissues and blood vessels was avoided. The jugular vein was then exposed. Two ligatures were place about 1 cm apart around the vein. The vein was then pinched open and two heparinized polythethylene cannulae (I.D. 0.86 mm, O.D. 1.27 mm, Clay-Adams, Parsippany, New Jersey) were inserted into the points of ligature and tied. The cannula at the end towards the heart was used for isotopes and saline injections, the other towards the head was used for sequential blood collection. The clamped forcept was released from under the vein. The opened cut of the skin was kept moistened by covering it with gauze rinsed with physiological saline (0.9% NaCl).

A single injection of the mixture of isotopes i.e. ^{3}H-T, ^{3}H-E_2 and ^{3}H-P was given, followed immediately by injection of 200 µl heparinized physiological saline to wash down all the isotopes that are left in the cannula into the circulation.

After injection, blood was collected in a 400 µl polyethylene micro-test tubes (Bio-Rad. Laboratories, Richmond, Calf.) at the following times 3, 6, 9, 12, 15, 20, 30, 45, 60, 90, 120, and 150 min. Hematocrit reading was also taken with each sample by using heparinized precalibrated micro-Hematocrit tubes (VWR Scientific, USA). Before each sample was collected the first 2-3 drops of blood were discarded, and after each blood sampling the rats were replenished with the equal volume of physiological saline. Blood samples were then centrifuged for 5 min at 13,000 rpm at 4 °C using Bechman Microfuge B, and plasma samples were seperated. 2000 cpm each of ^{14}C-T, ^{14}C-E_2 and ^{14}C-P were added to each sample as internal standard (I.S.) for correction of procedural losses, plasma samples were stored at -70 °C until assayed.

EXTRACTION PROCEDURE:

Plasma samples were brought to a volume of 200 µl with dist. water. 2 ml of methylene chloride (Fisher Scientific Company) were added to each sample and vortexed for about 30 sec. and allowed to stand at room temperature for 30 min. The samples were chilled to freeze the aqueous phase (plasma) and the organic phase was decanted and collected. This procedure was repeated once more and the two extracts were combined. The organic phase was evaporated under a stream of N_2 immediately. To assure that all the steroids were collected in the bottom of the culture tubes, samples were washed down with ethanol and were dried under N_2 and stored at -70 ^{o}C until further purification by sephadex LH-20 column chromatography.

SEPHADEX LH-20 COLUMN CHROMATOGRAPHY:

The three radioactive hormones ^{3}H-T, ^{3}H-E_2 and ^{3}H-P were seperated and purified from their metabolites by column chromatography of sephadex LH-20 (Sephadex, N.J.).

A disposable 10 ml serological pipettes with mouth pieces cut off to 20 cm length, plugged with glass fibers at the tips. One gram of sephadex LH-20 was suspended in 4 ml slurry solvent (Benzene: Methanol 9:1 v/v), transfered into the column, the sephadex column was initially washed with 7 ml twice of slurry solvent to get rid of impurities which interfere with the assay and to ensure a compact packing, without letting the column to run out form the solvent and just before the slurry solvent reached the sephadex surface, the first solvent system (iso-octane: benzene: methanol, 85:10:5 v/v/v) was added by pouring it down the sides of the column so that to avoid disturbing the packed sephadex. The

columns were conditioned by passage of additional solvent (7 ml x 2). Before the samples were applied to the columns, an elution profile was run through two typical columns by using ^{3}H-steroids, and the peaks of the fractions of the hormones were determined (Figure 2). Samples obtained from the extraction procedure were redissolved in 200 µl of the first solvent and transferred to the columns by applying to the top of the sephadex. To minimize losses, each sample was rinsed again with 0.2 ml same solvent and applied to the column. The samples were let to drain into the column before adding ony solvent. The first solvent system was added. P was collected in fractions 4-7 and T in 13-17. To elute E_2 a second solvent system (iso-octane: benzene: methanol, 55:20:25, v/v/v) was used and E_2 was collected in fractions 25-29. Each fraction was collected in a 20 ml counting vial. The fractions were dried immediately under N_2.

These columns were reusable. Each time after using the columns they were rinsed with 7 ml of the first solvent and were kept in this solvent without aggitation or let to dry. Before the next samples were used, the columns were reconditioned with the first solvent (7 ml x 3) to assure that all the residual radioactivities were removed. A column can be used 8-10 times before discarded.

T RADIOIMMUNOASSAY:

Blood samples were centrifuged and the plasma samples were seperated and extracted with methlene chloride. T was seperated by column chromatography as previously described. Radioimmunoassay (RIA) was performed on testosterone using T-antiserum (prepared by Dr. S.W.C. Chan) CTS-1 diluted to a concentration 1:25,000. The procedure for RIA was as follows:

Samples collected after column chromatography were dried under N_2 and aliquots were taken and redissolved in 100 µl of buffer solution (PBS-0.1% gel). One hundred µl of each of ^{3}H-T and T-antiserum were added to all samples, then incubated at 37 ^{o}C for 1 hr in shaking incubator. The tubes were then transferred to an ice bath for 15 min. Charcoal/dextran solution (0.3 ml) was then added, and the samples were vortexed and let sit in ice for 10 min. An aliquot of the supernatant (0.3 ml) was aspirated to which 3 ml of counting fluid contianing ominfluor and triton-x (see appendix) was added. The samples were counted in Packard Tri-carb Liquid Scintillation Counter.

Data were calculated and plotted on a 'semi-log' (2x10 or 3x10) graph paper. The amounts of T in ng were extrapolated from the standard curve constructed using known amounts of steroid standards (Figure 33).

LIQUID SCINTILLATION COUNTING:

Ten mls liquid scintillation fluid (4g PPO/0.1g POPO/L. Tolune) were added to each sample and counted with a Packard Tri-carb Liquid Scintillation Spectrometer Model 3320. Counting efficiencies of single label were approximately 58% for ^{3}H and 85% for ^{14}C. For double labelling (^{3}H + ^{14}C), counting efficiencies were about 30% for each one. Figure 1 summarizes the major procedures involved in this research.

CALCULATION OF THE DATA:

Radioactivities in the samples were measured by a double label counting method. Procedural losses were corrected by addition of ^{14}C-internal standards. From these values, cpm/ml serum were calculated and expressed as fraction of injected dose/liter blood at the various times of sampling. Hematocrit readings were used to convert serum to

whole blood values (see appendix). The data were plotted as a fraction of injected dose/liter blood against time (min).

The disappearence of curve of radioactivity concentration in blood can be represented as

$$X = A e^{-\alpha t} + B e^{-\beta t} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

where X = percent injected radioactive dose per liter blood at a time t for each hormone.

The data as plotted on semi-log graph (Figure 3) showed two straight lines with slopes α and β . B is the y intercept of the second (slow) component, A+B is the corresponding intercept form the first (slow + fast) component of the curve.

The linear regression of β (and hence B) was determined by the 'peel off' method (Gurpide E, 1975). Using data from samples at the first component, the slope α was determined after subtraction of the β component for each value. From this information the values of the following were derived:

half-lives of the hormones in blood for

fast component $$t_{\frac{1}{2}}\alpha = \frac{\ln 2}{\alpha} \text{ (min)} \ldots\ldots\ldots (2a)$$

slow component $$t_{\frac{1}{2}}\beta = \frac{\ln 2}{\beta} \text{ (min)} \ldots\ldots\ldots (2b)$$

metabolic clearance rate, $$M.C.R = \frac{\alpha\beta}{A\beta + B\alpha} \text{ (liters/day)} \ldots (3)$$

Production rate, $$P = M.C.R. \times i \text{ (mass/day)} \ldots (4)$$

where i is the concentration of the hormone in blood.

initial volume of distribution (or 'inner pool'),

$$V = \frac{I}{A + B} \quad \text{(liters)} \quad \ldots\ldots\ldots (5)$$

outer volume ('outer pool '), where Q is the total hormone content

$$V_{a=0} = \frac{Q}{(A+B)(A\beta + B\alpha)} \quad \text{(liters)} \quad \text{[assuming metabolism of hormone in the inner pool (a) is zero (a=0)]; . . .(6)}$$

$$V_{e=0} = \frac{Q}{(A+B)} \cdot \frac{(\alpha - \beta)^2}{(A\beta + B\alpha)^2} \text{(liters)} \quad \text{[assuming metabolism of hormone in the outer pool (e) is zero (e=0)]; . . . (7)}$$

Equations (3), (6), and (7) were multiplied by 1440 (time units of days),

Calculations of the data were performed on computer (Prime 750). The programs were designed by Dr. S.W.C.Chan. Storage of the data as data files. Examples are listed as follows:

```
Ok, ED

INPUT

3       1347      .554
6       1256      .570
9       1277      .597
12      952       .619
15      828       .625
20      654       .627
30      450       .641
45      365       .653
60      192       .640
90      242       .631
120     48        .625
150     169       .650

EDIT

File P-D20-RAWDATA-5 (Name of data file)
```

The program for calculation of α, β, A and B is by MINITAB for multiple regressions is listed as follows:

```
READ FROM FILE 'FILE NAME' INTO C1, C2, C3
LET C4= (C2 * C3)/ INJ. DOSE
PLOT C4 VS C1
PICK (late times)C1, PUT INTO C5
PICK (late times)C4, PUT C6
LOGE C6, PUT C7
REGR C7 ON 1 PRED. IN C5
EXPO (LNB) INTO K1
PICK (early times)C1, PUT INTO C8
LET C9=K1*(EXPO(β*C8))
PICK (early times)C4, PUT C10
LET C11=C10-C9
LOGE C11, PUT C12
REGR C12 ON 1 PRED. IN C8
PRINT C1-C12
EXPO (LNA) INTO K2
```

An example is listed in the appendix.

M.C.R., $t_{\frac{1}{2}\alpha}$, $t_{\frac{1}{2}\beta}$ and volumes of distribution were calculated by a BASIC program written by Dr. S.W.C. Chan (see appendix).

STATISTICAL TESTS

Statistical analysis using students' 't' tests were performed using the MINITAB Program, statistical significance was assumed at $P \leqslant 0.05$ level.

RESULTS:

CHANGES IN THE M.C.R. IN AGING MALE RATS:

i. CHANGES IN THE M.C.R. OF T:

The mean M.C.Rs. of T in blood of young intact male rats (52.67 ± 2.10 l/day) was significantly higher (P<0.001) than in young castrated rats (12.43 ± 1.81 l/day), and were also significantly higher than old intact male rats (27.60 ± 4.90 l/day). When T was injected into young castrated rats, the mean M.C.R. value was restored to the intact level (62.01 ± 4.17 l/day) and was significantly higher than the value in the young non-injected castrated rats (P<0.001). In old intact male rats the mean was significantly higher (P<0.02) than in old castrated rats (10.80 ± 2.23 l/day). Similarly M.C.R. was significantly higher in old castrated rats injected with T (55.06 ± 8.76 l/day) than in old intact male rats (P<0.05) and old non-injected castrated rats (P<0.001).

When the M.C.Rs. were expressed as l/kg body weight the mean value in young intact male rats (115.93 ± 5.42 l/kg/day) was significantly higher (P<0.001) than in young castrated rats (26.26 ± 4.22 l/kg/day), and was significantly higher (P<0.001) than in old intact male rats (53.73 ± 6.20 l/kg/day). In young castrated rats injected with T the M.C.R. (137.18 ± 13.25 l/kg/day) was significantly higher than in young castrated rats (P<0.001). It was also significantly higher (P<0.05) than in old castrated rats injected with T (89.86 ± 11.41 l/kg/day). Old intact male rats showed significantly higher value (P<0.01) than in old castrated rats (20.93 ± 4.09 l/kg/day), but was significantly lower when compared with old castrated rats injected with T (P<0.05).

The mean half-life of the fast component ($t_{\frac{1}{2}}\alpha$) of T in young

intact male rats was 6.89 ± 0.51 min, in young castrated rats was 8.22 ± 0.74 min, and in young castrated rats injected with T was 6.44 ± 1.10 min. The mean value in old intact male rats was 7.96 ± 0.58 min which was significantly lower ($P<0.01$) than in old castrated rats injected with T at 11.34 ± 0.44 min. The mean value of the slower component ($t_{\frac{1}{2}\beta}$) of T in young intact male rats (44.12 ± 3.12 min) was significantly lower ($P<0.01$) than that of young castrated rats (152.67 ± 24.80 min). Old intact male rats showed significantly higher value (72.78 ± 3.60 min) than in young intact rats ($P<0.001$). Young castrated rats injected with T showed significantly lower value (37.26 ± 2.94 min) than in young castrated rats ($P<0.01$). Old castrated rats injected with T was 97.07 ± 6.64 min, and old castrated rats (95.90 ± 5.85 min) showed a significantly higher value ($P<0.01$) than in old intact rats (see table 1 and figures 4-9).

Our data was used to dertermine the sizes of the two metabolic pools of the hormones. Inner metabolic pool (V^I) of T in young intact male rats was 0.93 ± 0.05 l, in young castrated rats was 0.82 ± 0.12 l; both were significantly higher than V^I in old castrated rats at 0.41 ± 0.06 l. Young castrated rats injected with T has V^I value of 1.09 ± 0.07 l which was significantly lower ($P<0.01$) than old castrated rats injected with T (1.66 ± 0.14 l). Old intact rats (1.00 ± 0.19 l) was significantly higher ($P<0.02$) than old castrated rats.

The size of the outer pool ($V^Q_{a=o}$) of T metabolism in young intact male rats (1.75 ± 0.16 l) was significantly higher ($P<0.05$) than old intact rats (1.19 ± 0.16 l). The means of the sizes of the outer pools ($V^Q_{e=o}$) of T in young intact rats and in old intact male rats were similar. In old intact rats was significantly ($P<0.02$) lower than in old castrated rats injected with T (see table 7).

Production rate of T in young intact rats (205.41 ± 8.10 μg/day) was significantly greater (P<0.001) than that of aged intact rats (25.64 ± 4.53 μg/day).

ii. CHANGES IN THE M.C.R. OF E_2:

There was no significant difference in the M.C.R. of E_2 in male rats (l/day values). The mean M.C.R. of E_2 in young intact males was 23.89 ± 1.36 l/day, in young castrated rats was 16.98 ± 4.55 l/day, in young castrated rats injected with T was 17.67 ± 3.91 l/day. M.C.R. values for old intact male rats was 27.46 ± 2.02 l/day, old castrated rats was 35.70 ± 5.94 l/day and old castrated rats injected with T was 22.18 ± 2.36 l/day.

When the means of the M.C.Rs. of E_2 were corrected as l/kg body weight, the mean M.C.R. value of E_2 in young intact male rats was 52.21 ± 1.98 l/kg/day, in young castrated rats was 35.29 ± 10.89 l/kg/day and in young castrated rats injected with T was 41.00 ± 11.26 l/kg/day. The M.C.R. in old intact rats (49.69 ± 3.39 l/kg/day) was significantly lower (P<0.05) than that of old castrated rats (71.13 ± 9.25 l/kg/day) and in old castrated rats injected with T the M.C.R. (36.90 ± 3.41 l/kg/day) was significantly lower than that of old castrated rats (P<0.01).

The mean $t_{\frac{1}{2}}\alpha$ of E_2 in young intact male rats was 9.57 ± 2.47 min, in young castrated rats (4.12 ± 0.22 min) was significantly lower (P<0.01) than in young castrated rats injected with T (8.70 ± 0.79 min). In old intact rats $t_{\frac{1}{2}}\alpha$ of 6.37 ± 1.01 min was significantly lower (P<0.02) than in old castrated rats injected with T at 10.13 ± 0.79 min. Also, old castrated rats has $t_{\frac{1}{2}}\alpha$ value of 4.58 ± 1.21 min which was significantly lower than in old castrated rats injected with T (P<0.01).

The slower component ($t_{\frac{1}{2}}\beta$) of E_2 in young intact male rats (91.08 ± 7.82 min) was significantly faster (P<0.001) than in young castrated

rats (215.29 ± 16.62 min) and young castrated rats injected with T (177.26 ± 16.56 min). $t_{\frac{1}{2}}\beta$ in young castrated rats was significantly higher ($P<0.01$) than in old castrated rats (97.20 ± 16.72 min). The value for old intact rats was 106.70 ± 11.09 min which was significantly faster ($P<0.01$) than old castrated rats injected with T at 259.41 ± 42.48 min (see table 2 and figures 10-15).

The size of the inner metabolic pool (V^I) of E_2 in young intact male rats was similar to those of old intacts. Old intact male rats has V^I value of 1.30 ± 0.16 l was significantly lower ($P<0.05$) than old castrated rats injected with T. The mean size of the outer metabolic pool ($V^{\varphi}_{\alpha=o}$) of E_2 in young intact rats was significaltly lower ($P<0.01$) than young castrated rats. The mean value of the size of the outer metabolic pool ($V^{\varphi}_{e=o}$) of E_2 in young rats (0.82 ± 0.10 l) was signifcantly lower than both young castrated rats (1.97 ± 0.09 l) and old intact rats (1.21 ± 0.14 l). See table 8.

Production rate of E_2 in young intact male rats was 683.42 ± 38.81 ng/day and in old intact rats was 796.31 ± 58.68 ng/day. There was no significant difference between the two values.

iii. <u>CHANGES IN THE M.C.R. OF P:</u>

It has been found that the mean M.C.R. of P in the blood of young intact male rats (73.81 ± 7.81 l/day) was significantly higher ($P<0.001$) than in young castrated rats injected with T (26.04 ± 2.67 l/day). The M.C.Rs of P were similar in other groups. When the means of M.C.Rs. of P were expressed as l/kg body weight, the value in young male rats (164.97 ± 15.34 l/kg/day) was significantly higher ($P<0.001$) than in young castrated rats injected with T (57.87 ± 7.69 l/kg/day). Also was significantly higher than old intact rats ($P<0.05$). Other groups showed no significant differences in the M.C.Rs.

It has been observed that there were no significant changes in the means of $t_{\frac{1}{2}\alpha}$ of P comparing all groups of aging male rats. Young intact male rats showed significantly faster $t_{\frac{1}{2}\beta}$ than that of old intact rats ($P<0.01$). See table 3 and figures 16-20.

There were no significant changes of the mean sizes in the inner metabolic pools (V^I) of P in aging male rats. The sizes in the outer metabolic pools ($V^{Q}_{a=0}$ or $V^{Q}_{e=0}$) of P in young male rats was significantly lower than young castrated rats injected with T (3.01 ± 0.16 l). Young intact rats were significantly lower than old intact rats (see table 9).

Production rate of P in young intact male rats (137.28 ± 14.53 μg/day) was found to be significantly greater ($P<0.05$) than that of old intact male rats (92.47 ± 12.64 μg/day).

CHANGES IN THE M.C.R. IN PREGNANT RATS:

i. CHANGES IN THE M.C.R. OF P:

No change has been observed in the M.C.R. of progesterone comparing non-pregnant, pregnant, and post-partum rats. The mean M.C.R. value in non-pregnant rats was 62.70 ± 6.88 l/day, and in pregnant rats on day 10 was 68.20 ± 6.99 l/day, on day 20 was 77.78 ± 8.99 l/day and on post-partum rats was 80.13 ± 4.35 l/day.

When the mean values were expressed as l/kg body weight, there was no significant change in the M.C.R. of P in all groups. The value in non-pregnant rats was 232.19 ± 25.91 l/kg/day, in day 10 was 241.27 ± 24.61 l/kg/day, in day 20 was 215.84 ± 22.85 l/kg/day and in post-partum was 243.10 ± 10.42 l/kg/day.

The mean $t_{\frac{1}{2}\alpha}$ of P in non-pregnant rats was 5.89 ± 0.38 min, on day 10 of pregnancy was 5.20 ± 0.31 min which was significantly lower

(P<0.05) than in post-partum rats at 8.85 ± 1.31 min and was 6.99 ± 1.04 min in day 20 of pregnancy in rats. The mean value of $t\frac{1}{2}\beta$ of P in non-pregnant rats (53.78 ± 4.21 min) was significantly higher (P<0.01) than on day 10 of pregnancy (34.99 ± 1.78 min), the mean value in day 20 was 43.72 ± 5.13 min and was 54.03 ± 9.89 min in post-partum rats (see table 4 and figures 21-24).

The inner metabolic pool was higher in day 20 of pregnancy and post-partum rats (1.49 ± 0.22 l and 1.32 ± 0.20 l respectively) compared with the day 10 and non-pregnant rats at 0.82 ± 0.12 l and 0.81 ± 0.08 l respectively. The outer metabolic pool ($V_{a=o}^{Q}$) of P was higher in the non-pregnant and post-partum rats (2.94 ± 0.39 l and 3.64 ± 0.67 l respectively) than day 10 of pregnancy at 1.89 ± 0.15 l. The outer metabolic pool ($V_{e=o}^{Q}$) of P was higher in non-pregnant rats than day 10 of pregnancy and no change has been observed in other groups (see table 10).

ii. CHANGES IN THE M.C.R. OF E_2:

The mean M.C.R. of E_2 in blood of non-pregnant (17.18 ± 2.03 l/day) and day 10 pregnant rats (20.56 ± 0.92 l/day) was significantly lower than that of day 20 (32.59 ± 2.87 l/day), and post-partum rats (25.66 ± 1.34 l/day). The mean M.C.R. of E_2 expressed as l/kg body weight in non-pregnant rats (62.72 ± 6.94 l/kg/day) was significantly lower (P<0.02) than in day 20 (92.52 ± 8.62 l/kg/day) but there was no significant difference between other groups. In day 10 the mean was 72.89 ± 4.47 l/kg/day and in post-partum rats was 76.38 ± 4.50 l/kg/day.

The mean values of $t\frac{1}{2}\alpha$ of E_2 in all groups were not significantly different. For $t\frac{1}{2}\beta$, the mean value in non-pregnant rats (101.25 ± 6.05 min) was found to be significantly higher than in day 20 (63.45 ± 7.33 min), and post-partum rats (73.90 ± 3.72 min), in day 10 (89.58 ± 4.79

min) was significantly higher than in day 20 and in post-partum rats (see table 5 and figures 25-28).

The mean values of the sizes in the inner metabolic pools (V^I) of E_2 in all female groups were not significantly different. There were no significant differences between the mean values of the sizes in the outer metabolic pools ($V^Q_{a=o}$) and outer metabolic pools ($V^Q_{e=o}$) of E_2 in all groups (see table 11).

iii. CHANGES IN THE M.C.R. OF T:

The only significant difference ($P<0.05$) in the M.C.R. of T in whole blood was observed between non-pregnant (27.72 $\pm$ 5.38 l/day) and post-partum rats (41.27 $\pm$ 1.90 l/day), the mean M.C.R. of T in day 10 was 36.58 $\pm$ 4.19 l/day and in day 20 was 45.08 $\pm$ 6.75 l/day.

When the M.C.R. values were expressed as l/kg body weight, there was no significant difference between any of the groups. The mean value in non-pregnant rats was 92.63 $\pm$ 14.43 l/kg/day, in day 10 was 130.39 $\pm$ 16.22 l/kg/day, in day 20 was 127.64 $\pm$ 17.59 l/kg/day, and in post-partum rats was 123.04 $\pm$ 6.98 l/kg/day.

In the fast component ($t\frac{1}{2}\alpha$) of T was metabolized faster in pregnant than in post-partum rats. There were no other significant changes in any of the other groups. The means of $t\frac{1}{2}\beta$ of T in female groups did not change significantly (see table 6 and figures 29-32).

The mean of the size in the inner metabolic pool (V^I) of T was higher in day 20 than in non-pregnant rats. The means of the sizes in the outer metabolic pools ($V^Q_{a=o}$ or $V^Q_{e=o}$) of T in all female groups were not significantly different (see table 12).

T LEVELS IN CASTRATED RATS AFTER T INJECTIONS:

The mean plasma T concentration after T administration into young

castrated rats was 1.87 ± 0.49 ng/ml and was 1.34 ± 0.38 ng/ml in plasma of old castrated rats.

DISCUSSION:

AGING MALE RATS:

i. TESTOSTERONE:

The M.C.Rs. and production rates of steroid hormones have been studied in several species. In these investigations values were reported only for plasma (Little et al. 1966; Southren et al. 1967; Elsaesser et al. 1982) and a few studies focused on whole blood (Longcope et al. 1968; Hotchkiss, 1985). In this research, non-equilibrium method of single dose injection of ^{3}H-steroids was used. It has been observed that the clearance of radioactivities can be represented by a two-compartment model, the first one is in rapid equilibrium with blood and the second compartment is metabolized slowly. This method provided sufficient information concerning M.C.R., half-lives, production rates, and volumes of distribution in whole blood.

To my knowledge, there is no published data concerning the dynamics of T in aging or castrated rats. In these studies, our results indicated that the M.C.R. of T in whole blood of young intact male rats was 52.67 l/day which was similar to the results of Wang et al. (1967) with a value of 55.8 l/day. But it was different when corrected for body weight. The explanation for this is that the average body weight of young males used by Wang's research group was 214 g, while our mean body weight was 456 g. We disagree with the value reported by Lee et al. (1975). They calculated the M.C.R. of T to be 55.2 l/day in plasma or approximately 100 l/day when converted into whole blood value. Also, they used continuous infusion method which, according to Tait (1963) and Little et

al. (1966), should give lower results.

In this research, age-related changes in the M.C.R. of T in male rats have been observed. In young rats the mean of M.C.R. was approximately 2.5 times higher than old male rats. The mean values for castrated rats of both age groups were at the same level, and were significantly lower than their respective intact controls. Interestingly, the mean M.C.R. of T of castrated rats previously injected with T of both age groups increased and were restored to the young intact level. This is in agreement with Vermeulen et al. (1969) in which they observed that the M.C.R. of T increased after acute injection of T into hypogondal men. Also we agree with Southren et al. (1968) who demonstrated an increase in the M.C.R.of T following the chronic administration of a large dose of testosterone propionate. Thus, the M.C.R. of T appeared to be related to the level of circulating testosterone. TeBG bound-testosterone is not biologically active and is not readily available for catabolism. Administration of a high dose of T resulted in a decrease in TeBG binding testosterone which paralleled an increased in the M.C.R. of T. Chronic testosterone treatment might increase the hepatic blood flow and induce testosterone catabolizing enzymes. From our results we concluded that age-related changes in the M.C.R. of T is due to testicular dysfunction in senile rats resulting in decreased T secretion.

Young intact male rats showed faster $t_{\frac{1}{2}}\alpha$ of T when compared with old intact rats. The difference, however, is not statistically significant. Old castrated rats previously treated with T showed significantly higher values when compared with both old castrated rats and old intact rats.

Old intact male rats exhibited higher $t_{\frac{1}{2}}\beta$ than those of young

intact male rats or in other words, aged male rats metabolized T slower than young rats. Young castrated rats showed higher value than their intact young controls. While the mean $t_{\frac{1}{2}}\beta$ in young castrated rats injected with T was quite similar to the intact controls. Old intact rats metabolized T faster than old castrated rats or old castrates injected with T. Comparing the means of $t_{\frac{1}{2}}\beta$ in castrated rats injected with T of both age groups, it was significantly higher in old than in young rats. From the data previously reported by Wang et al. (1967) the half-lives were calculated to be 4.8 min and 13.3 min for α and β respectively. These were lower than our corresponding values.

After acute intravenous injection of ^{3}H-steroids into rats, the radioactive hormones were distributed quickly into the first compartment. Then after this initial distribution, the radioactivities appeared to move slowly into the outer compartment. It has been observed that the initial volumes of distribution (V^{I}) of T in young and old intact male rats were similar, also there were no changes among the three young groups (see table 7). The size of the inner metabolic pool in old castrated rats was significantly lower than those of old intact rats, old castrates injected with T and young castrates. It has been observed that old castrated rats injected with T showed significantly higher value than those of old intacts, old castrates, and young castrates injected with T.

The size of the outer metabolic pool ($V_{a=o}^{Q}$) of T in young intact rats was significantly greater than those of old intacts. The volume in old castrated rats previously injected with T greatly exceded those of old castrates, old intacts, and castrates injected with T. The mean in old intact rats was significantly higher than old castrated rats.

The volume of distribution in the outer pool ($V_{e=o}^{Q}$) of T in old castrated rats injected with T was higher than those of old intacts, old

castrates, and young castrated rats injected with T. Our results indicated that the total volumes of distriubutions ($V_{\alpha=0}^{I+Q}$ or $V_{e=0}^{I+Q}$) of T in old castrated rats injected with T were higher than those of old castrates, old intacts, and young castrated rats injected with T. The means in old intacts were higher than those of old castrated rats. The initial volume of distribution derived from data reported by Wang et al. (1967) was found to be 0.364 liters which was lower than our value.

The production rates of T were calculated on the basis of the M.C.Rs. and the peripheral plasma concentration described here and the plasma concentration determined by studies of Chan et al. (1981). The mean production rate of T in young intact male rats was significantly higher than those of old intacts or castrated rats of both aging groups that were injected with T. We concluded that production rate decline with age in Sprague-Dawley male rats.

ii. ESTRADIOL:

Our results indicated that there were no age-related changes in the M.C.R. of E_2 in male rats. Although when values were normalized for body weight, old castrated rats were significantly higher than those of old intacts or old castrated rats injected with T. In fact, there was a slight increase in the M.C.R. of E_2 in old castrates when compared with their intact controls, but this difference was not statistically significant. In the case of young castrated rats, the mean M.C.R. was slightly decreased when compared with their intact.

It has been observed that there was no age-related change in $t_{\frac{1}{2}\alpha}$ of E_2 comparing intact rats of the two aging groups. The mean value in young castrates previously treated with T was significantly higher than that of young castrated rats. The value in old castrates injected with T greatly exceded that of old intact and old castrated rats. There was no

change in $t\frac{1}{2}\beta$ of E_2 between intact rats of both age groups. But there were variations among other groups. Meanwhile, young castrated rats showed significantly higher value than their intact controls.

There was no change in the initial volume of distribution of E_2 between young and old intact rats. But in the outer pools the mean values were significantly higher in old intacts than those in young intact male rats.

iii. PROGESTERONE:

Blood M.C.R. of P did not change from young to old intact male rats. When values were corrected for body weight, the mean value in young intacts was significantly higher than those of old intact rats. It was observed that there was no change in $t\frac{1}{2}\alpha$ of P in all male groups. The mean of $t\frac{1}{2}\beta$ in old intact rats showed significantly slower value than in young intact male rats. The volume of distribution of P in the inner or the total pool ($V_{a=0}^{I+Q}$) did not change significantly. The mean volumes in the outer metabolic pools ($V_{a=0}^{Q}$ and $V_{c=0}^{Q}$) or in the total pool ($V_{c=0}^{I+Q}$) were significantly higher in old intacts than in young intact male rats.

In aging studies it has been observed that the M.C.Rs. of E_2 and P were metabolized at similar rates regardless of the age of the male rats. We concluded that E_2 and P did not play a major role in aging Sprague-Dawley male rats.

PREGNANCY:

i. PROGESTERONE:

A few studies have been published on the M.C.R. of P in non-pregnant and during early-mid pregnancy in rats (Pepe and Rothchild, 1973; Robinson et al. 1981). Yet there is scanty information concerning the

dynamics of steroid hormones in late pregnancy or post-partum in rats. Changes in serum progesterone levels are reflections of the changes in the secretion rate throughout pregnancy. The parallelism between the two parameters is evidently the remarkable stability in the M.C.R. of P. In this research it has been observed that there is no significant change in the M.C.R. of P comparing non-pregnant, pregnant and post-partum rats. The absence of changes in the M.C.R. of P during pregnancy in rats resembles the same situation in pregnant women (Little et al. 1966; Lin et al. 1972) and Rhesus monkeys (Sholl et al. 1974; Thau et al. 1977). The rat and human contrast with the guinea pig in which the M.C.R. of P decreased 10-20 fold during pregnancy (Illingworth et al. 1970) which most likely is a result of the increase in progesterone binding levels in this species. Binding attribute not only to the increased concentrations of corticosteroid-binding globulin (CBG) but to the presence of progesterone-binding globulin (PBG), a protein unique to the guinea pig.

Our results indicated that the M.C.R. of P increases slightly during pregnancy and was highest in post-partum rats however, these increases were not significant. The rise in these values during pregnancy is apparently associated with an increase in the body weight that is related to the growth of fetuses and mammary glands near term. The means of the M.C.R. of P expressed as l/kg body weight were similar in all groups. My present finding of the M.C.R. of P in whole blood during pregnancy in rats found to be 72.66 $\pm$ 54.3 l/day was similar to those derived by Pepe and Rothchild (1973) of a value of 59.68 l/day after correction for whole blood. According to Robinson et al. (1981), they calculated the M.C.R. of P to be 2.9 l/day for blood in pseudopregnant rats. That was much lower than our value for pregnant rats. Their value for pseudopregnant rats was also lower than that derived by Wang et al. (1967).

Our data indicated that initial half-life ($t_{\frac{1}{2}\alpha}$) of P in D-10 was significantly lower than that of post-partum rats. There was no significnat change between other groups. Progesterone clearance from blood has been reported previously in D-10 of pseudopregnant rats. Robinson et al. (1981) found values of 0.5 min for $t_{\frac{1}{2}\alpha}$ and 11.7 min for $t_{\frac{1}{2}\beta}$. Pepe and Rothchild (1973) reported values of 1.5 min and 9.9 min for $t_{\frac{1}{2}\alpha}$ and $t_{\frac{1}{2}\beta}$ respectively (derived from their values for serum). These values were lower than our corresponding half-lives (5.20 min for α and 34.99 for β) for D-10 pregnant rats. It has been observed that the mean $t_{\frac{1}{2}\beta}$ in non-pregnant rats was significantly higher than that in D-10 of pregnancy, while the mean values of other groups remained unchanged.

The initial volumes of distribution (V^I) of P appeared to increase significantly in late pregnancy (D-20) than those of non-pregnant rats and D-10 of pregnancy. The mean values of the size of P metabolism in the inner pools derived from our results showed higher values than those derived by Pepe and Rothchild (1973). Their mean volume of distribution of P in D-10 of pregnancy was 165 ml blood while our mean value at the corresponding day was 0.82 liters. Robinson et al. (1981) calculated the initial volume to be 5 ml in pseudopregnancy induced by gonadotropins in rats.

The outer volumne of distribution ($V^{Q}_{\alpha=0}$) of P in D-10 pregnant rats was significantly lower than those of non-pregnant and post-partum rats. The mean value of the outer metabolic pool ($V^{Q}_{e=0}$) of P was significantly higher in non-pregnant rats compared with D-10 of pregnancy, but did not change in other groups. The mean of the total volumes of distribution ($V^{Q+I}_{\alpha=0}$) of P was significantly higher in non-pregnant and post-partum rats than those of D-10 of pregnancy. The

mean value of the total volumes of distribution ($V_{\ell=0}^{I+Q}$) of P was significantly higher in D-20 than in D-10 of pregnancy, and was no change between other groups. In general, our means of the volumes of distributions of P in female rats were higher than those of Pepe and Rothchild (1973) and Robinson et al. (1981).

ii. ESTRADIOL:

An age-dependent in estradiol metabolism has been suggested. Elsaesser et al. (1982) reported that the M.C.R. of E_2 declined during maturation comparing immature and pre-puberal female pigs. Thus in addition to an activation of ovarian E_2 secretion, a decrease in the catabolism of E_2 during sexual maturation. This might be important in the effect of estrogen on feedback signal, resulting in the first pre-ovulation LH-surge and in the induction of the first ovulation during puberty.

Using continuous infusion method, de Hertogh et al. (1970) showed that the M.C.R. of E_2 did not depend on the infusion rate in adult Wistar female rats. They have been observed a constant mean value of the M.C.R. of E_2 (16.2 l/day in plasma or 26.4 l/day in blood) in all stages of the estrous cycle. This suggested either that rat plasma lacks specific carrier of E_2 of limited capacity or that specific binding did not protect the hormone against metabolic changes in non-pregnant rats. Their M.C.R. value for whole blood was higher than our mean value (17.18 l/day).

Our results showed that the M.C.R. of E_2 increased throughout pregnancy in rats. In post-partum, the mean M.C.R. started to decline, yet it was higher than those of non-pregnant rats. Although, when mean values were corrected for body weight, the M.C.R. of E_2 in D-20 of pregnancy was significantly higher than those of non-pregnant rats.

Whereas, other groups showed no changes. Absence of changes in $t\ \frac{1}{2}\ \alpha$ between all female groups have been observed. In $t\ \frac{1}{2}\ \beta$, our results indicated that E_2 was metabolized faster in D-20 and post-partum than those of non-pregnant rats. There was no change in the sizes of the inner, the outer or the total metabolic pools of E_2 in all female groups.

iii. TESTOSTERONE:

Under physiological conditions, the M.C.R. of a steroid depends on its specific binding to the plasma proteins (Tait and Burstein, 1964). For example, the M.C.Rs. of E_2 and T that are strongly bound to plasma proteins are lower than the M.C.Rs. of estrone (E_1) and cortisone, which have weaker binding to plasma proteins. The percentage of bound testosterone to plasma proteins is higher in pregnant women than in non-pregnant women. The M.C.Rs. of E_2 and T are higher in men than in women, and it has been suggested that the proportionally decreased binding of these steroids to specific plasma proteins may be responsible for this change in the M.C.Rs.. Sex hormones can affect liver matabolism of steroids and therefore hepatic clearance might contribute to the difference in the M.C.R. between males and females. Our results indicated that the M.C.Rs. of E_2 and T were higher in intact young male rats than in female rats. Transcortin concentrations are not different between males and females, and therefore lack of difference in the M.C.R. of P between males and females is not surprising. This is in agreement with our results using Sprague-Dawley rats.

Our results indicated that the mean M.C.R. of T in post-partum rats was significantly higher than those of non-pregnant rats (in l/day values). When mean values were corrected for body weight, it has been observed that there was no change between all groups. These values contradict with the M.C.R. of T in women which decreased throughout

pregnancy (Saez et al. 1972). For $t_{\frac{1}{2}}\alpha$, post-partum rats showed higher mean value than those during pregnancy, and in other groups remained constant. The means $t_{\frac{1}{2}}\beta$ of T in all female groups were similar. The mean volume of distribution of T in the inner pool for D-20 was significantly higher than those of non-pregnant rats, and was no change when non-pregnant rats were compared with both D-10 and post-partum rats. The mean values of the outer pools or the total pools remain unchanged .

We concluded that changes in the metabolic clearance rates vary with different steroids reflecting physiological demands.

ILLUSTRATIONS: i. Tables

Table 1 : DYNAMICS OF ^{3}H-TESTOSTERONE IN AGING MALE RATS.

		M.C.R. 1/day	M.C.R. 1/kg/day	T½ min α	T½ min β
4M	(7)	52.67 ± 2.10	115.93 ± 5.42	6.89 ± 0.51	44.12 ± 3.12
4M ⊗	(5)	12.43 ± 1.81	26.26 ± 4.22	8.22 ± 0.74	152.67 ± 24.80
4M ⊗ + T	(5)	62.01 ± 4.17	137.18 ± 13.25	6.44 ± 1.10	37.26 ± 2.94
18M	(5)	27.60 ± 4.90	53.73 ± 6.20	7.96 ± 0.58	72.78 ± 3.60
18M ⊗	(7)	10.80 ± 2.23	20.93 ± 4.09	7.61 ± 0.63	95.90 ± 5.85
18M ⊗ + T	(5)	55.06 ± 8.76	89.86 ± 11.41	11.34 ± 0.44	97.07 ± 6.64

Statistical significance: *P < 0.05; ** P < 0.02; *** P < 0.01; **** P < 0.001
All data are expressed as mean ± SEM
Numbers in ( ) refer to numbers of animals per treatment group

Table 2 : DYNAMICS OF ^{3}H-ESTRADIOL IN AGING MALE RATS.

		M.C.R.		$T_{\frac{1}{2}}$ min	
		1/day	1/kg/day	α	β
4M	(7)	23.89 ± 1.36	52.21 ± 1.98	9.57 ± 2.47	91.08 ± 7.82
4M ♂	(2)	16.98 ± 4.55	35.29 ± 10.89	4.12 ± 0.22	215.29 ± 16.62
4M ♂ + T	(5)	17.67 ± 3.91	41.00 ± 11.26	8.70 ± 0.79	177.26 ± 16.56
18M	(10)	27.46 ± 2.02	49.69 ± 3.39	6.37 ± 1.01	106.70 ± 11.09
18M ♂	(5)	35.70 ± 5.94	71.13 ± 9.25	4.58 ± 1.21	97.20 ± 16.72
18M ♂ + T	(5)	22.18 ± 2.36	36.90 ± 3.41	10.13 ± 0.79	259.41 ± 42.48

Statistical significance: * $P < 0.05$; ** $P < 0.02$; *** $P < 0.01$; **** $P < 0.001$
All data are expressed as mean ± SEM
Numbers in ( ) refer to numbers of animals per treatment group

Table 3 : DYNAMICS OF ^{3}H-PROGRESTERONE IN AGING MALE RATS.

	M.C.R.		$T_{\frac{1}{2}}$ min	
	1/day	1/kg/day	α	β
4M (6)	73.81 ± 7.81	164.97 ± 15.34	7.81 ± 1.72	44.43 ± 6.66
4M ⊗ + T(5)	26.04 ± 2.67	57.87 ± 7.69	7.14 ± 1.01	140.98 ± 20.55
18M (8)	63.34 ± 8.66	115.39 ± 14.53	6.37 ± 0.97	86.76 ± 8.45
18M ⊗ (5)	101.93 ± 22.49	206.26 ± 38.67	7.14 ± 1.66	51.65 ± 6.43
18M ⊗ + T(5)	65.93 ± 18.30	109.28 ± 27.59	7.15 ± 0.53	108.27 ± 7.34

Statistical significance: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$
All data are expressed as mean ± SEM
Numbers in ( ) refer to numbers of animals per treatment group

Table 4 : DYNAMICS OF ^{3}H-PROGESTERONE IN PREGNANT RATS.

	M. C. R.		$T_{1/2}$ min	
	l/day	l/kg/day	α	β
N. P. (7)	62.70±6.88	232.19±25.91	5.89±0.38	53.78±4.21 **
D-10 (7)	68.20±6.99	241.27±24.61	5.20±0.31	34.99±1.78 **
D-20 (6)	77.78±8.99	215.84±22.85	6.99±1.04 *	43.72±5.13
P. P. (6)	80.13±4.35	243.10±10.42	8.85±1.31 *	54.03±9.89

Statistical significance: ★ $P < 0.05$; ★★ $P < 0.01$
All data are expressed as mean ± SEM
Numbers in ( ) refer to numbers of animals per treatment group

N.P. = non-pregnant; D-10 = day 10; D-20 = day 20; P.P. = post-partum

Table 5 : DYNAMICS OF ^{3}H-ESTRADIOL IN PREGNANT RATS.

	M. C. R.		$T_{1/2}$ min	
	l/day	l/kg/day	α	β
N. P. (7)	17.18±2.03	62.72±6.94	8.26±1.02	101.25±6.05
D-10 (7)	20.56±0.92	72.89±4.47	6.31±0.76	89.58±4.79
D-20 (7)	32.59±2.87	92.52±8.62	5.36±0.99	63.45±7.33
P. P. (7)	25.66±1.34	76.38±4.50	6.24±0.35	73.90±3.72

Statistical significance: ★ $P<0.05$; ★★ $P<0.02$; ★★★ $P<0.01$; ★★★★ $P<0.001$

All data are expressed as mean ± SEM

Numbers in ( ) refer to numbers of animals per treatment group

N.P.=non-pregnant; D-10=day 10; D-20=day 20; P.P.=post-partum

Table 6 : DYNAMICS OF ^{3}H-TESTOSTERONE IN PREGNANT RATS.

	M. C. R.		$T_{1/2}$ min	
	l/day	l/kg/day	α	β
N. P. (7)	27.72±5.38	92.63±14.43	7.88±2.19	84.56±22.74
D-10 (7)	36.58±4.19	130.39±16.22	5.94±0.21	61.68±6.57
D-20 (6)	45.08±6.75	127.64±17.59	5.83±0.61	59.95±8.97
P. P. (7)	41.27±1.90	123.04±6.98	9.75±1.52	65.22±7.39

★ (N. P. vs P. P.; D-10 vs P. P.; D-20 vs P. P.)

Statistical significance : ★ $P < 0.05$

All data are expressed as mean ± SEM
Numbers in ( ) refer to numbers of animals per treatment group

N.P.=non-pregnant; D-10=day 10; D-20=day 20; P.P.=post-partum

Table 7: CHANGES IN METABOLIC POOL SIZE OF T IN AGING MALE RATS

		Inner Pool	Outer Pool		Total Pool	
		V^I	$V^Q_{a=0}$	$V^Q_{e=0}$	$V^{I+Q}_{a=0}$	$V^{I+Q}_{e=0}$
		(liters)	(liters)		(liters)	
4M	(7)	0.93 ± 0.05	1.75 ± 0.16	0.85 ± 0.10	2.68 ± 0.17	1.78 ± 0.10
4M ⛤	(5)	0.82 ± 0.12	1.09 ± 0.31	0.87 ± 0.27	1.91 ± 0.33	1.68 ± 0.30
4M ⛤ + T	(5)	1.09 ± 0.07	1.64 ± 0.27	0.85 ± 0.23	2.73 ± 0.27	1.94 ± 0.22
18M	(5)	1.00 ± 0.19	1.19 ± 0.16	0.77 ± 0.13	2.20 ± 0.35	1.78 ± 0.32
18M ⛤	(7)	0.41 ± 0.06	0.68 ± 0.14	0.48 ± 0.08	1.09 ± 0.20	0.87 ± 0.14
18M ⛤ + T	(5)	1.66 ± 0.14	4.49 ± 1.15	2.24 ± 0.44	6.14 ± 1.28	3.90 ± 0.57

Statistical significance: * $P<0.05$; ** $P<0.02$; *** $P<0.01$; **** $P<0.001$
All data expressed as mean ± SEM.
Number in ( ) refer to number of animals per treatment group.

Table 8: CHANGES IN METABOLIC POOL SIZE OF E_2 IN AGING MALE RATS

		Inner Pool	Outer Pool		Total Pool	
		V^{I}	$V^{Q}_{a=0}$	$V^{Q}_{e=0}$	$V^{I+Q}_{a=0}$	$V^{I+Q}_{a=e}$
		(liters)	(liters)		(liters)	
4M	(7)	1.14 ± 0.13	1.25 ± 0.16	0.82 ± 0.10	2.39 ± 0.23	1.96 ± 0.13
4M ⛤	(2)	1.40 ± 0.65	2.18 ± 0.07	1.97 ± 0.09	3.58 ± 1.01	3.37 ± 1.05
4M ⛤ + T	(5)	1.46 ± 0.16	1.69 ± 0.37	1.37 ± 0.29	3.15 ± 0.50	2.83 ± 0.43
18M	(10)	1.30 ± 0.16	1.58 ± 0.13	1.21 ± 0.14	2.99 ± 0.20	2.62 ± 0.20
18M ⛤	(5)	1.10 ± 0.21	2.18 ± 0.55	1.70 ± 0.45	3.28 ± 0.40	2.80 ± 0.30
18M ⛤ + T	(5)	1.88 ± 0.16	3.84 ± 0.62	3.18 ± 0.53	5.72 ± 0.70	5.06 ± 0.61

Statistical significance: * $P<0.05$; ** $P<0.02$; *** $P<0.01$; **** $P<0.001$
All data are expressed as mean ± SEM.
Numbers in ( ) refer to numbers of animals per treatment group.

Table 9: CHANGES IN METABOLIC POOL SIZE OF P IN AGING MALE RATS.

		Inner Pool	Outer Pool		Total Pool	
		V^{I}	$V^{Q}_{a=0}$	$V^{Q}_{e=0}$	$V^{I+Q}_{a=0}$	$V^{I+Q}_{e=0}$
		(liters)	(liters)		(liters)	
4M	(6)	1.51 ± 0.21	2.08 ± 0.22	0.99 ± 0.13	3.59 ± 0.25	2.50 ± 0.11
4M ⚥ + T	(5)	1.19 ± 0.08	3.01 ± 0.16 ***	2.42 ± 0.12 ****	4.20 ± 0.16	3.61 ± 0.10 ****
18M	(8)	1.72 ± 0.33	4.25 ± 0.81 *	2.84 ± 0.50 ***	5.97 ± 1.11	4.56 ± 0.79 *
18M ⚥	(5)	1.93 ± 0.45	3.70 ± 0.88	1.76 ± 0.47	5.63 ± 0.86	3.68 ± 0.31
18M ⚥ + T	(5)	1.61 ± 0.25	6.28 ± 2.13	3.57 ± 1.02	7.89 ± 2.30	5.18 ± 1.22

Statistical significance: * $P<0.05$; *** $P<0.01$; **** $P<0.001$
All data are expressed as mean ± SEM.
Numbers in ( ) refer to numbers of animals per treatment group.

Table 10: CHANGES IN METABOLIC POOL SIZE OF P IN PREGNANT RATS

		Inner Pool	Outer Pool		Total Pool	
		V_I	$V^Q_{a=0}$	$V^Q_{e=0}$	$V^{I+Q}_{a=0}$	$V^{I+Q}_{e=0}$
		(liters)	(liters)		(liters)	
N.P.	(7)	0.81 ± 0.08	2.94 ± 0.39	1.33 ± 0.17	4.03 ± 0.37	2.14 ± 0.22
D-10	(7)	0.82 ± 0.12	1.89 ± 0.15	0.86 ± 0.10	2.72 ± 0.25	1.68 ± 0.19
D-20	(6)	1.49 ± 0.22	2.43 ± 0.38	1.14 ± 0.18	3.92 ± 0.56	2.63 ± 0.31
P.P.	(6)	1.32 ± 0.20	3.64 ± 0.67	1.37 ± 0.70	4.96 ± 0.83	2.69 ± 0.45

Statistical significance: * $P<0.05$; ** $P<0.02$
All data are expressed as mean ± SEM.
Numbers in ( ) refer to numbers of animals per treatment group.
N.P.=non-pregnant; D-10= day 10; D-20= day 20; P.P.= post-partum.

Table 11: CHANGES IN METABOLIC POOL SIZE OF E_2 IN PREGNANT RATS.

		Inner Pool	Outer Pool		Total Pool	
		V^I	$V^Q_{a=0}$	$V^Q_{e=0}$	$V^{I+Q}_{a=0}$	$V^{I+Q}_{e=0}$
		(liters)	(liters)		(liters)	
N.P.	(7)	0.61 ± 0.05	1.26 ± 0.19	0.86 ± 0.13	1.87 ± 0.23	1.46 ± 0.16
D-10	(7)	0.73 ± 0.09	1.25 ± 0.07	0.91 ± 0.06	1.98 ± 0.15	1.64 ± 0.13
D-20	(7)	0.79 ± 0.11	1.37 ± 0.18	0.90 ± 0.09	2.17 ± 0.24	1.70 ± 0.17
P.P.	(7)	0.65 ± 0.04	1.39 ± 0.08	0.92 ± 0.06	2.04 ± 0.08	1.56 ± 0.06

All data are expressed as mean ± SEM.
Numbers in ( ) refer to numbers of animals per treatment group.
N.P.= non-pregnant; D-10= day 10; D-20= day 20; P.P.= post-partum.

Table 12: CHANGES IN METABOLIC POOL SIZE OF T IN PREGNANT RATS

		Inner Pool	Outer Pool		Total Pool	
		V^{I}	$V^{Q}_{a=0}$	$V^{Q}_{e=0}$	$V^{I+Q}_{a=0}$	$V^{I+Q}_{e=0}$
		(liters)	(liters)		(liters)	
N.P.	(7)	0.55 ± 0.09	1.55 ± 0.23	0.85 ± 0.18	2.09 ± 0.24	1.40 ± 0.21
D-10	(7)	0.59 ± 0.07	1.84 ± 0.28	1.01 ± 0.15	2.43 ± 0.33	1.60 ± 0.20
D-20	(6)	0.89 ± 0.12 *	2.00 ± 0.52	1.20 ± 0.32	2.89 ± 0.61	2.09 ± 0.40
P.P.	(7)	0.82 ± 0.10	2.04 ± 0.34	0.86 ± 0.44	2.85 ± 0.39	1.68 ± 0.22

Statistical significance: * $P<0.05$
All data are expressed as mean ± SEM.
Numbers in ( ) refer to numbers of animals per treatment group.
N.P.= non-pregnant; D-10= day 10; D-20= day 20; P.P.= post-partum.

ILLUSTRATIONS: ii. Figures

Figure 1

SUMMARY OF M.C.R. PROCEDURES

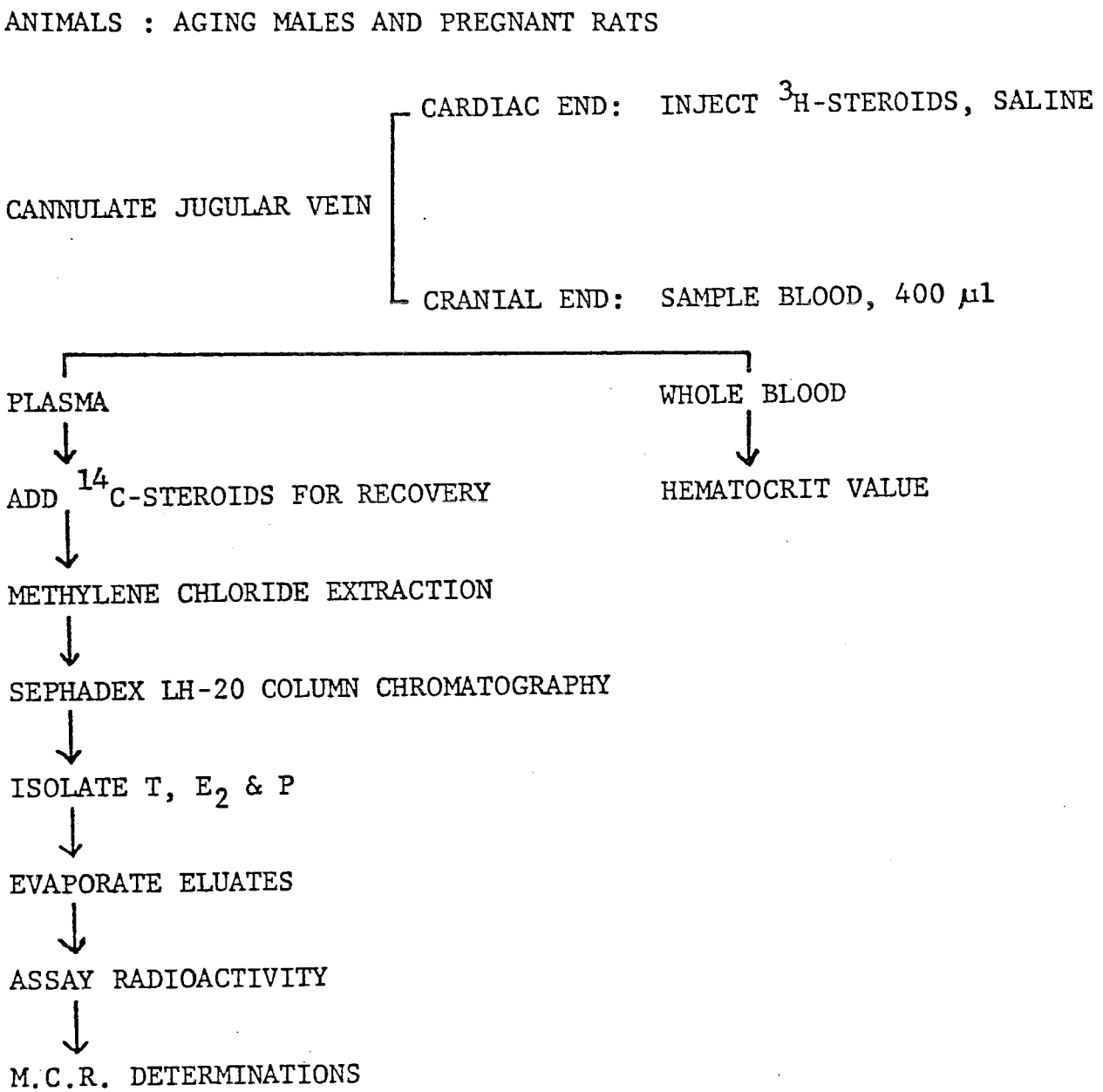


Figure 2: Sephadex LH-20 column chromatography elution profile of ^{3}H-P, ^{3}H-T, and ^{3}H-E_2. Steroids collected in ml fractions were plotted against the activities in cpm. Progesterone (fractions 4-7) and testosterone (fractions 13-17) were eluted by solvent system 1 (85:10:5 v/v/v iso-octane: benzene: methanol). Estradiol (fractions 25-29) eluted by solvent system 2 (55:20:25 v/v/v iso-octane: benzene: methanol).

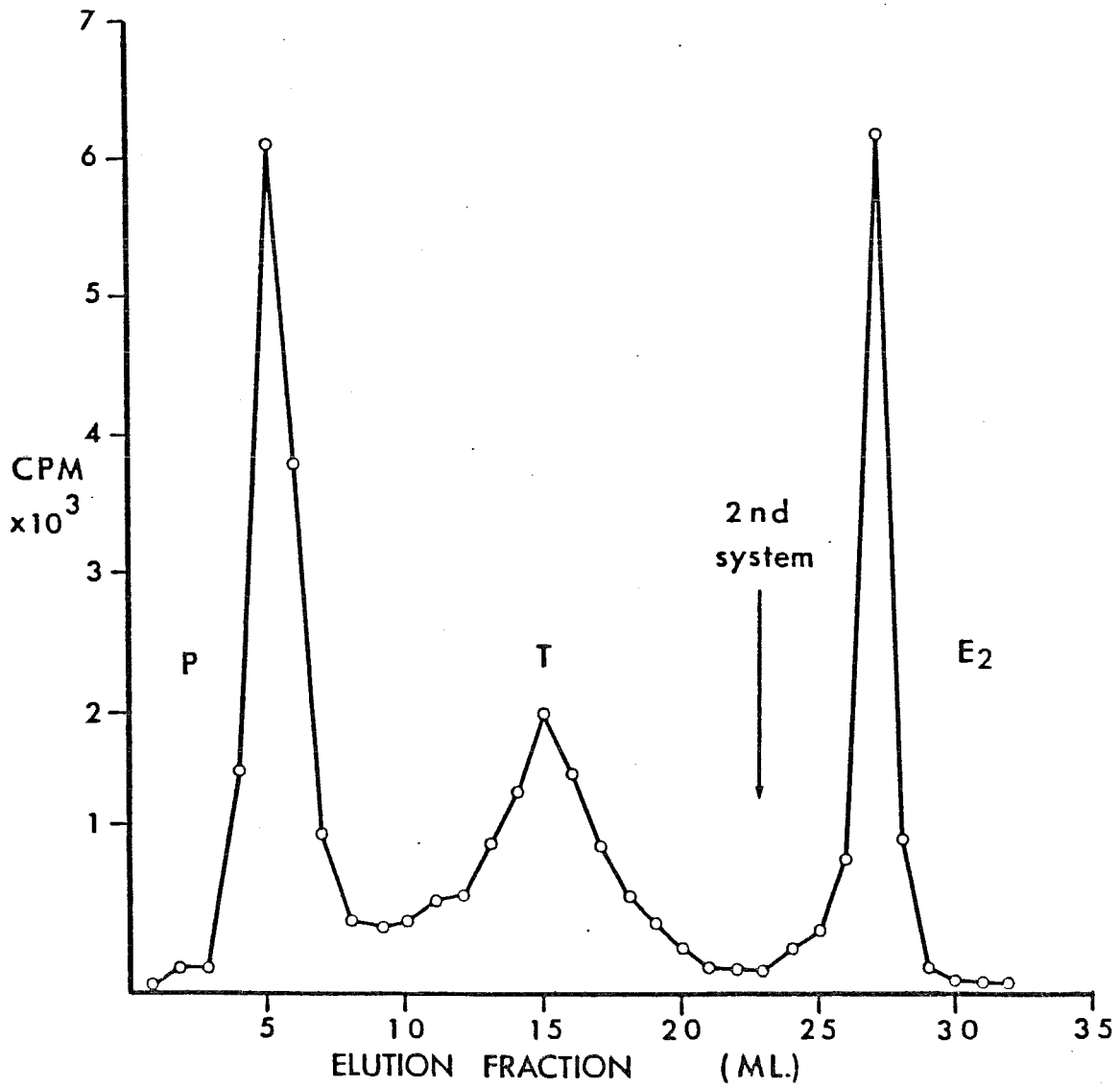

7
6
5
4
3
2
1
CPM
x10^3
P
T
2nd
system
E2
5
10
15
20
25
30
35
ELUTION FRACTION (ML.)

Figure 3: A typical disappearance curve of radioactive steroid from the blood (see materials and methods for details)

Figure 3

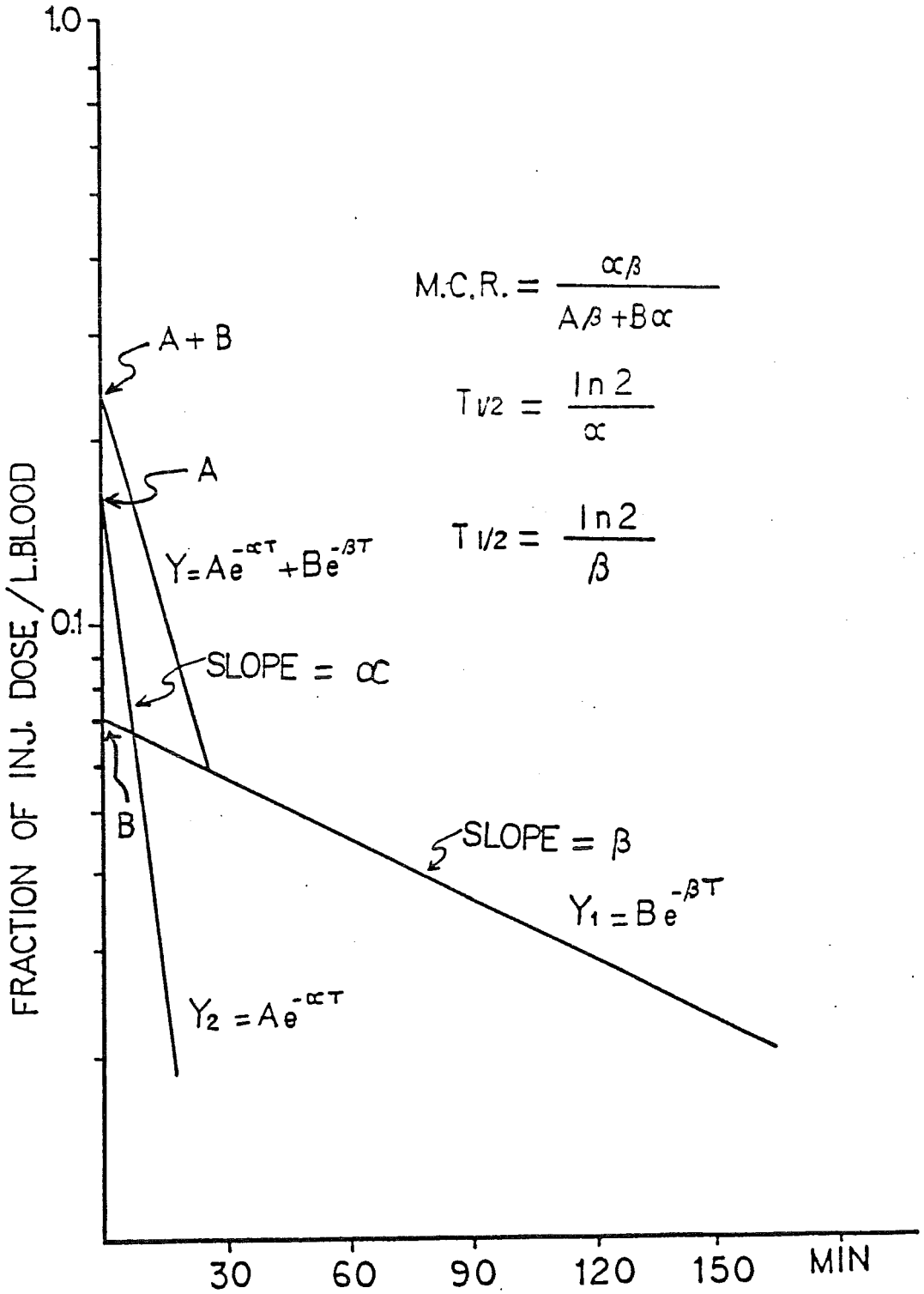

1.0
0.1
FRACTION OF INJ. DOSE / L.BLOOD
A + B
A
B
$Y = Ae^{-\alpha T} + Be^{-\beta T}$
SLOPE = α
SLOPE = β
$Y_1 = Be^{-\beta T}$
$Y_2 = Ae^{-\alpha T}$
$M.C.R. = \frac{\alpha\beta}{A\beta + B\alpha}$
$T_{1/2} = \frac{\ln 2}{\alpha}$
$T_{1/2} = \frac{\ln 2}{\beta}$
30
60
90
120
150
MIN

Figure 4

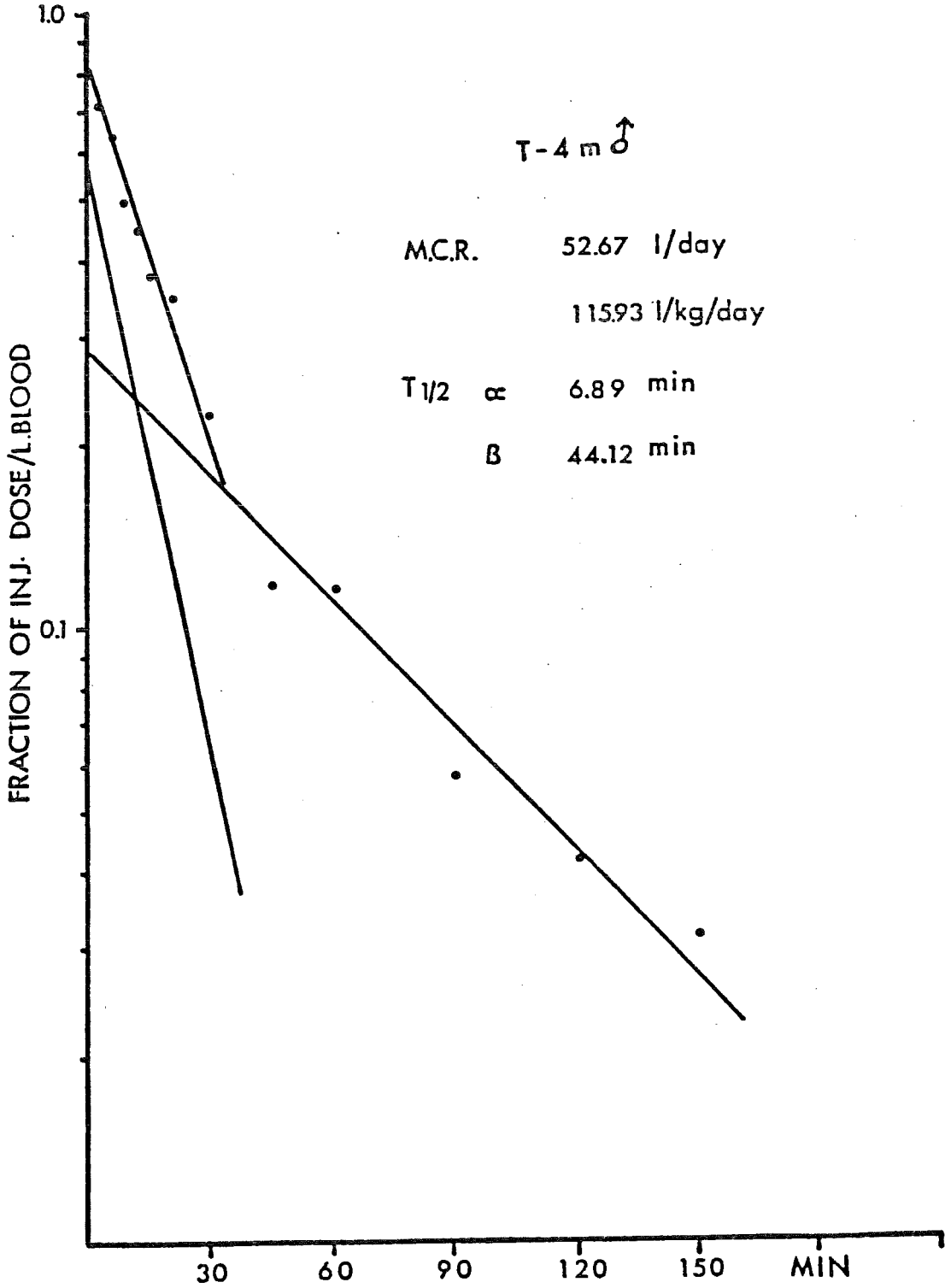

1.0
0.1
FRACTION OF INJ. DOSE/L.BLOOD
T-4 m ♂
M.C.R. 52.67 l/day
115.93 l/kg/day
T1/2 α 6.89 min
β 44.12 min
30
60
90
120
150
MIN

Figure 5

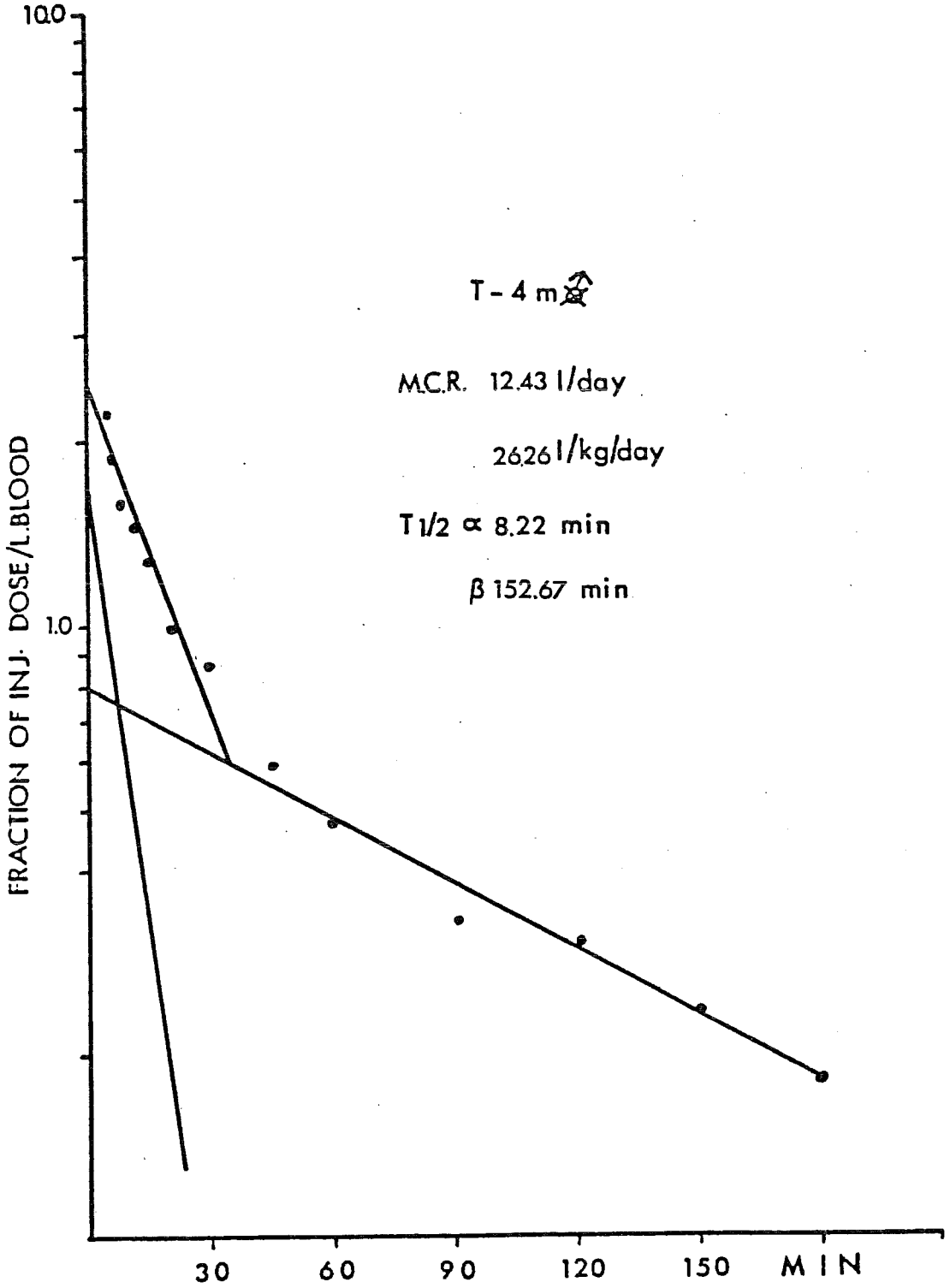

10.0
1.0
FRACTION OF INJ. DOSE/L.BLOOD
T - 4 m
M.C.R. 12.43 l/day
26.26 l/kg/day
T1/2 α 8.22 min
β 152.67 min
30
60
90
120
150
MIN

Figure 6

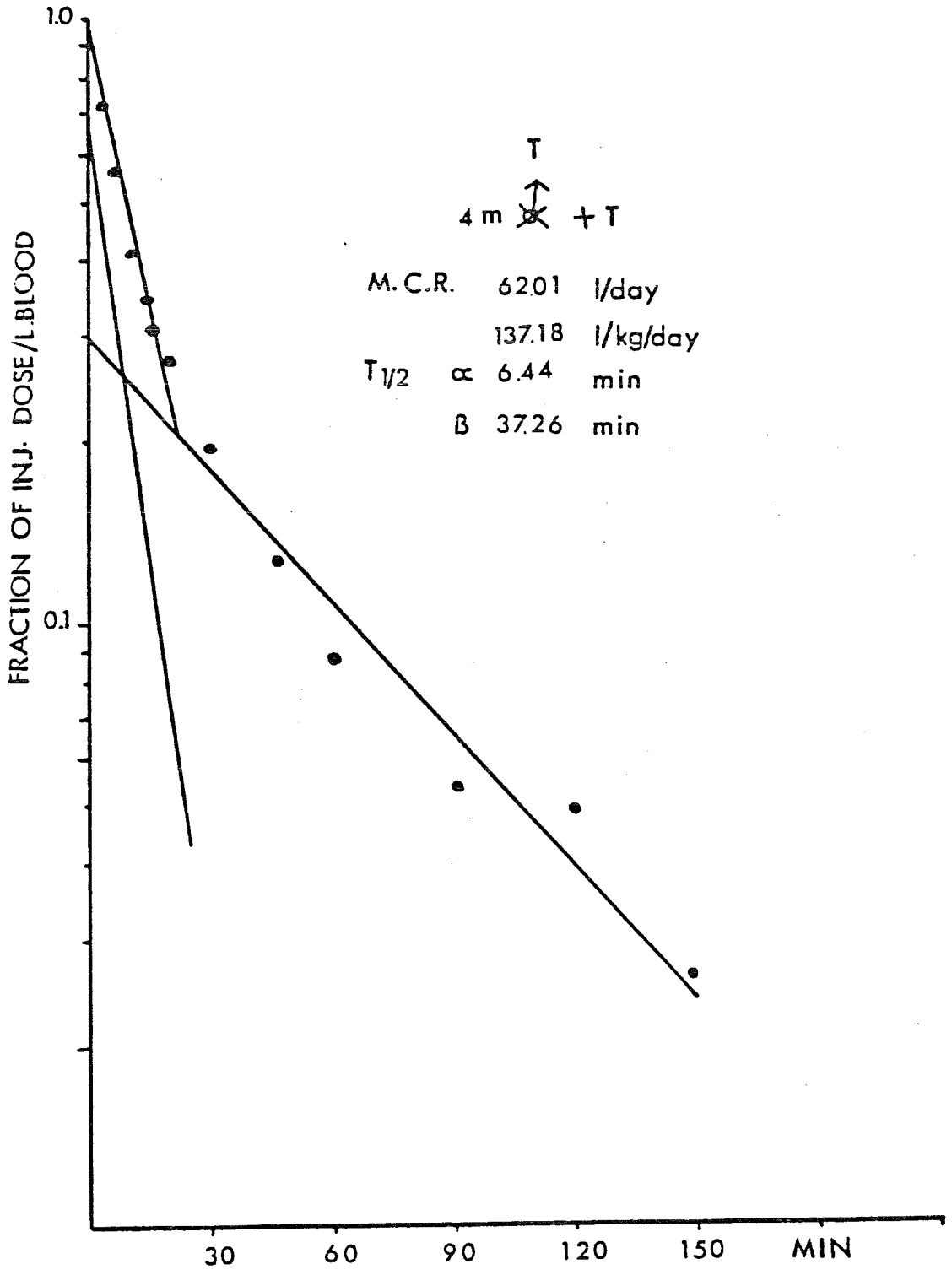

1.0
0.1
FRACTION OF INJ. DOSE/L.BLOOD
T
4 m
+T
M.C.R. 62.01 l/day
137.18 l/kg/day
T1/2 α 6.44 min
β 37.26 min
30
60
90
120
150
MIN

Figure 7

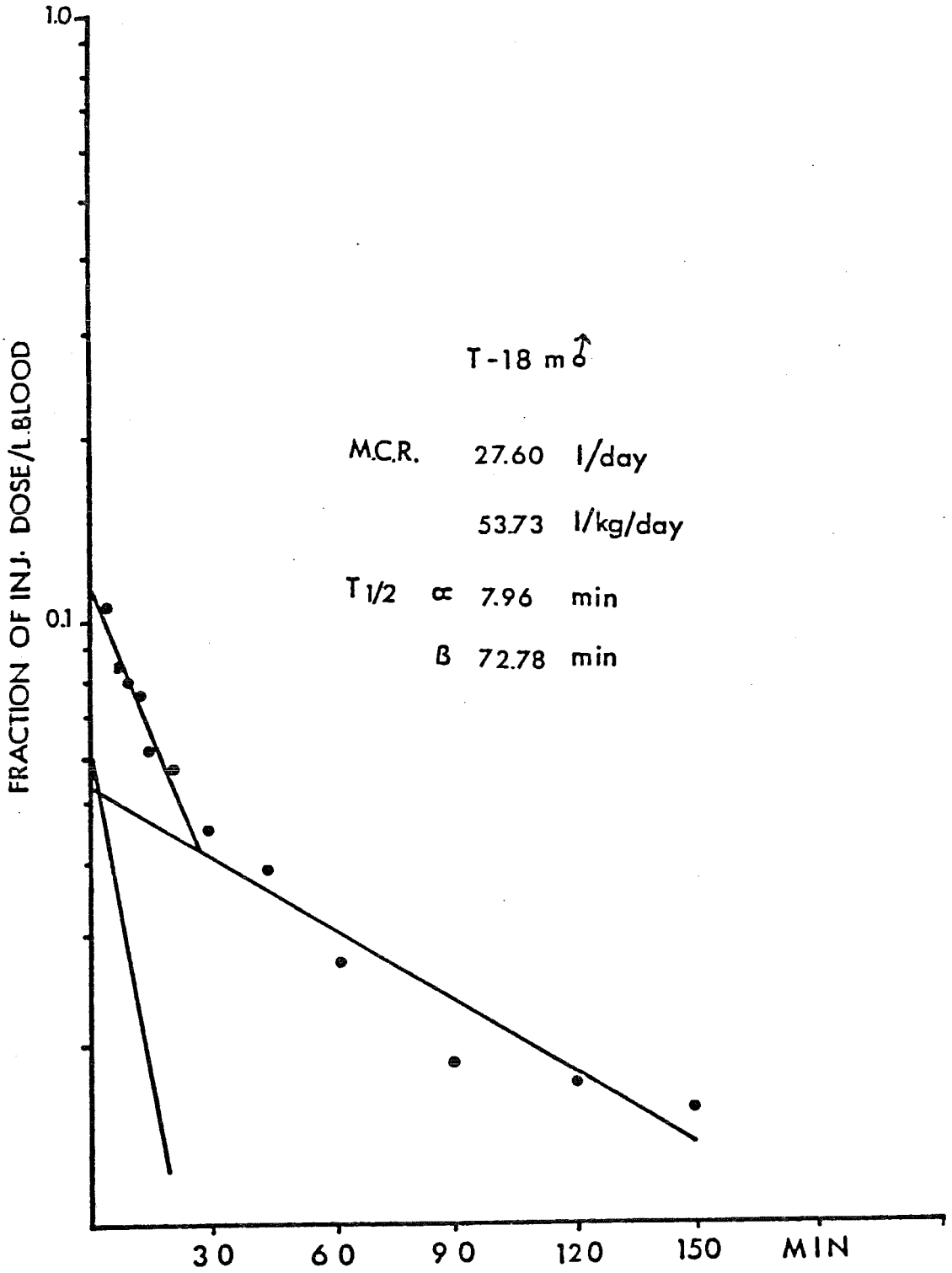

1.0
0.1
FRACTION OF INJ. DOSE/L.BLOOD
T-18 m♂
M.C.R. 27.60 l/day
53.73 l/kg/day
T 1/2 α 7.96 min
β 72.78 min
30
60
90
120
150
MIN

Figure 8

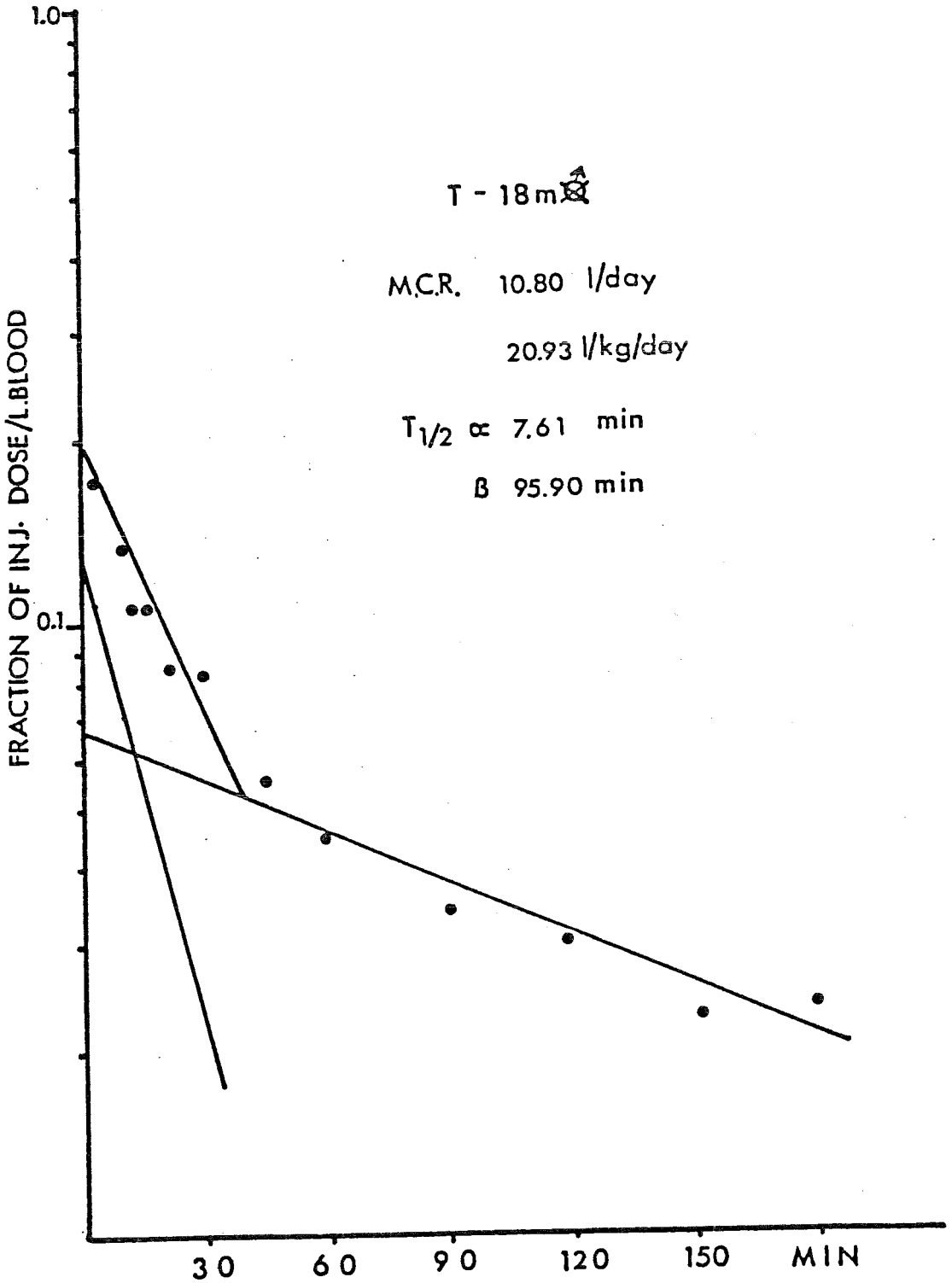

1.0
0.1
FRACTION OF INJ. DOSE/L.BLOOD
T - 18m
M.C.R. 10.80 l/day
20.93 l/kg/day
$T_{1/2}$ α 7.61 min
β 95.90 min
30
60
90
120
150
MIN

Figure 9

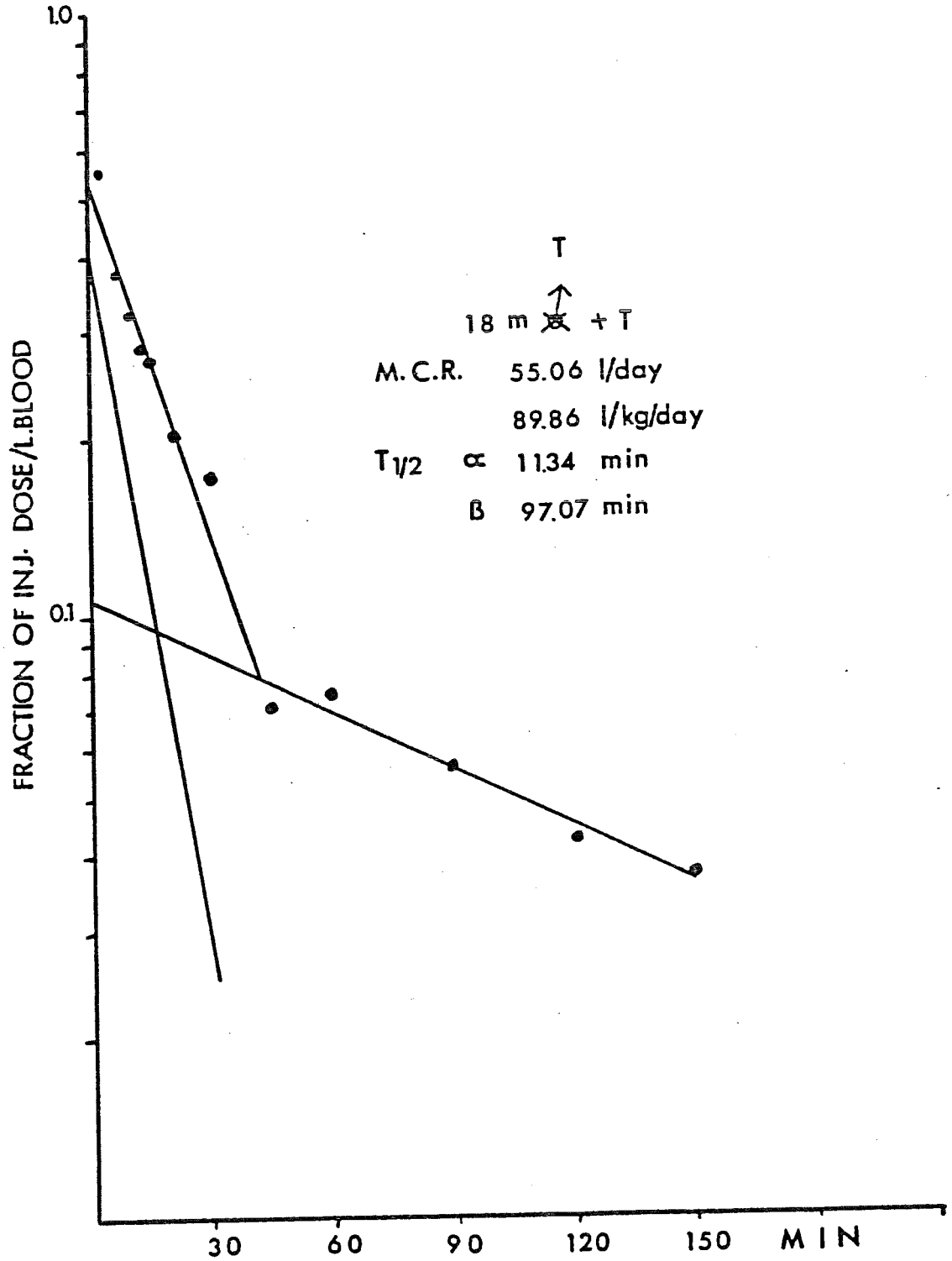

1.0
0.1
FRACTION OF INJ. DOSE/L.BLOOD
T
18 m ⊼ + T̄
M.C.R. 55.06 l/day
89.86 l/kg/day
T1/2 α 11.34 min
β 97.07 min
30
60
90
120
150
MIN

Figure 10

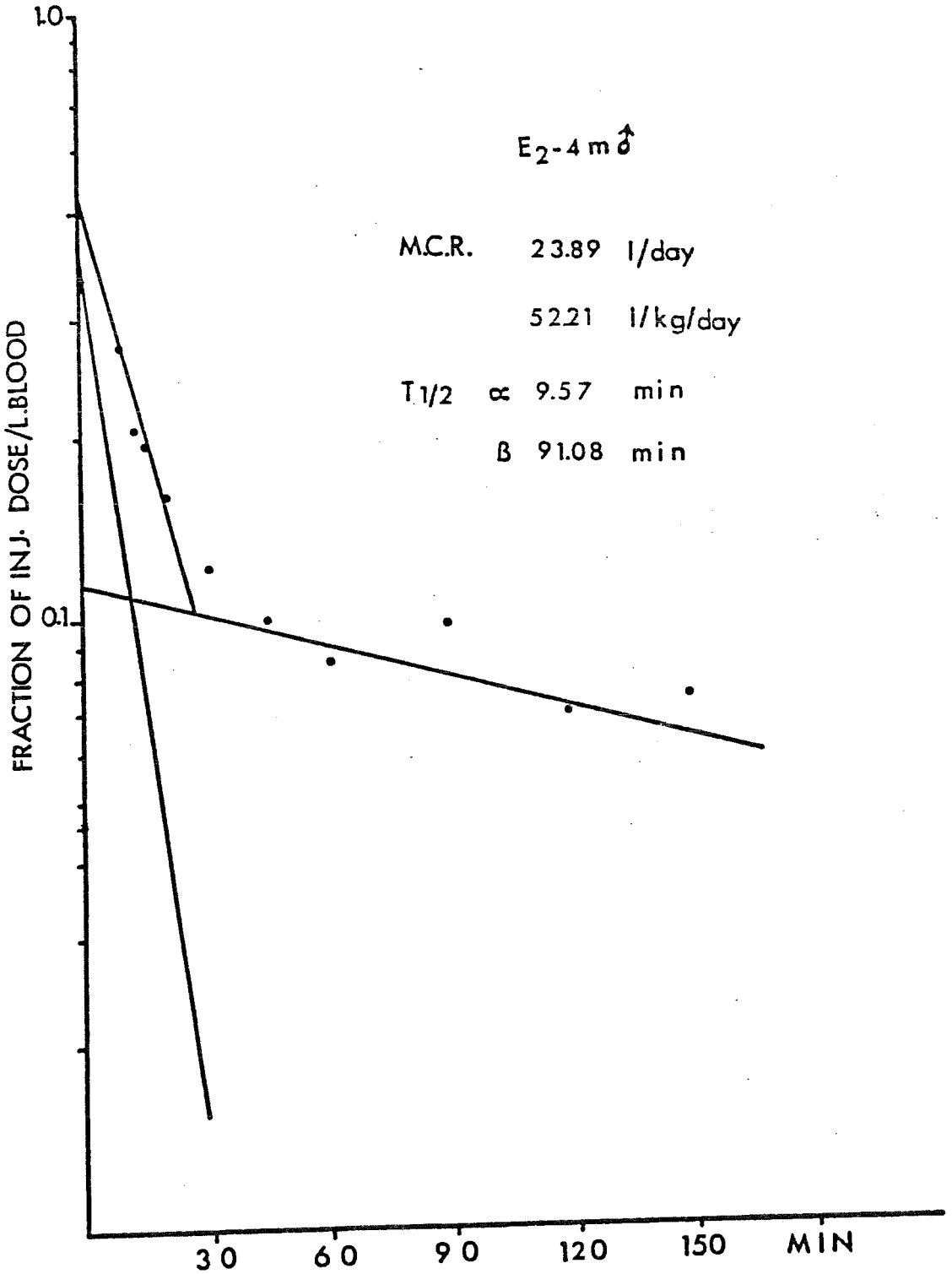

1.0
0.1
FRACTION OF INJ. DOSE/L.BLOOD
E_2-4 m ♂
M.C.R. 23.89 l/day
52.21 l/kg/day
T1/2 α 9.57 min
β 91.08 min
30
60
90
120
150
MIN

Figure 11

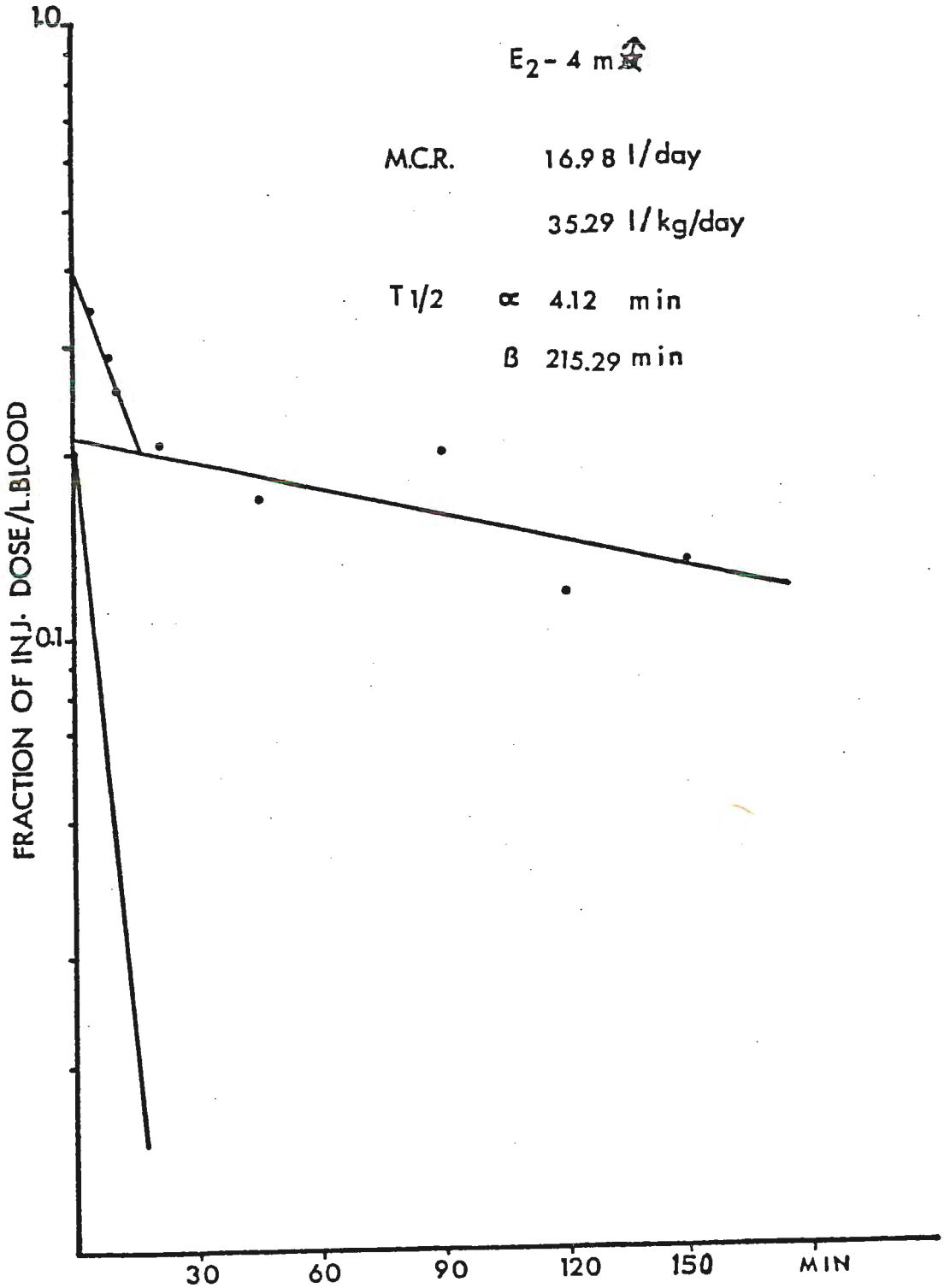

1.0
0.1
FRACTION OF INJ. DOSE/L.BLOOD
E_2 - 4 m
M.C.R. 16.98 l/day
35.29 l/kg/day
T 1/2 α 4.12 min
β 215.29 min
30
60
90
120
150
MIN

Figure 12

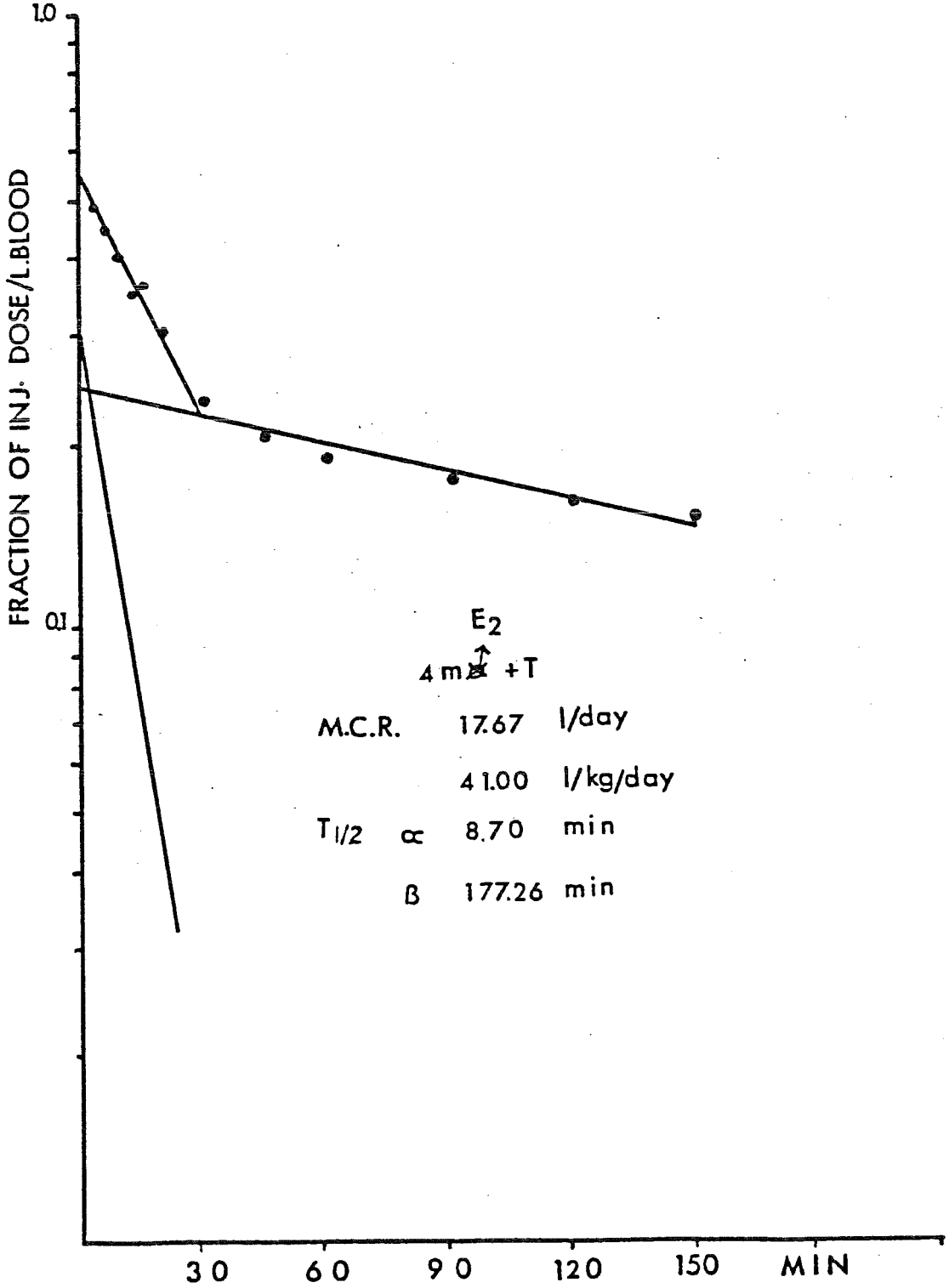

1.0
0.1
FRACTION OF INJ. DOSE/L.BLOOD
E2
4 m +T
M.C.R. 17.67 l/day
41.00 l/kg/day
T1/2 α 8.70 min
β 177.26 min
30
60
90
120
150
MIN

Figure 13

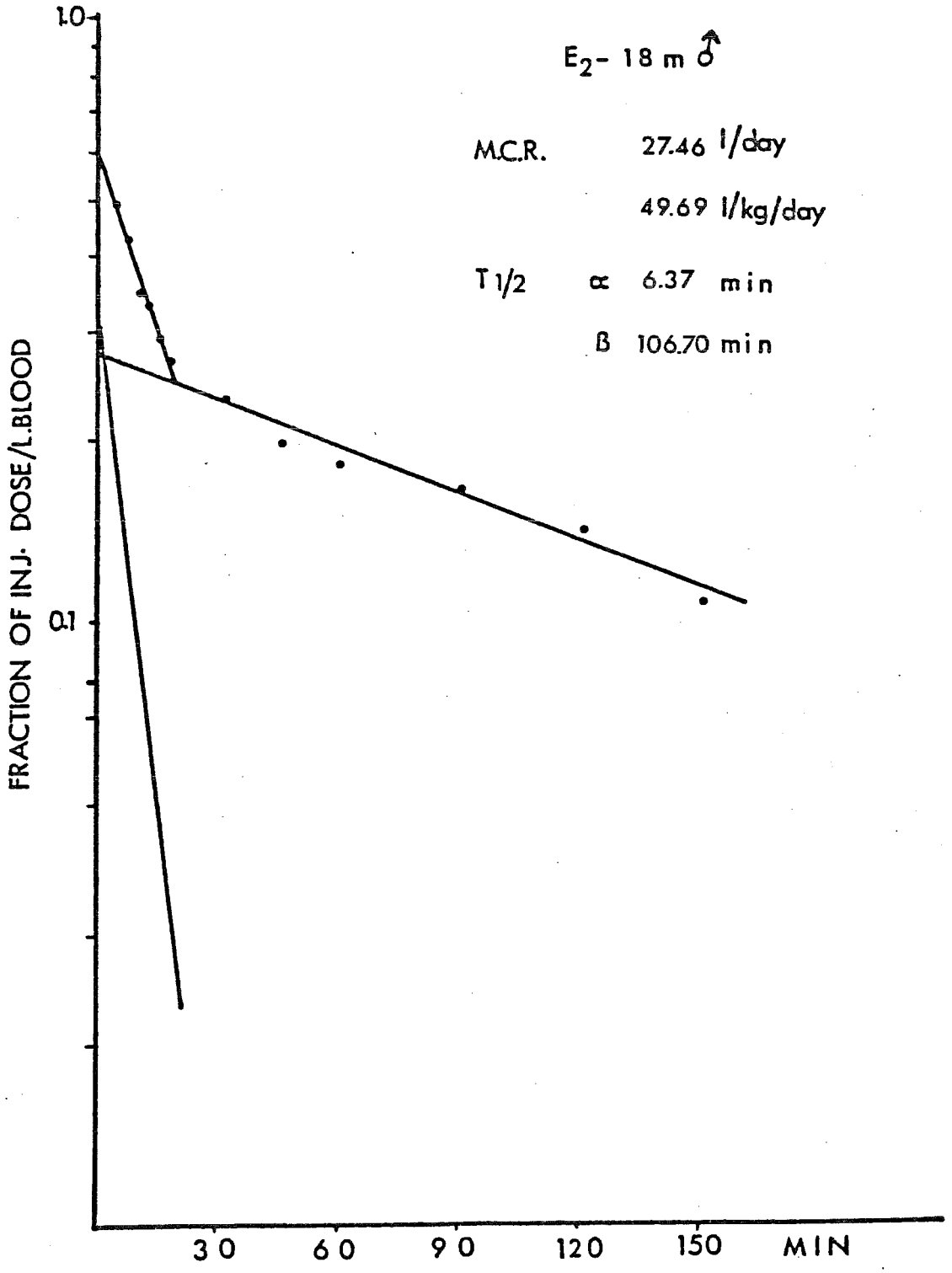

1.0
0.1
FRACTION OF INJ. DOSE/L.BLOOD
E_2- 18 m ♂
M.C.R. 27.46 l/day
49.69 l/kg/day
T 1/2 α 6.37 min
β 106.70 min
30
60
90
120
150
MIN

Figure 14

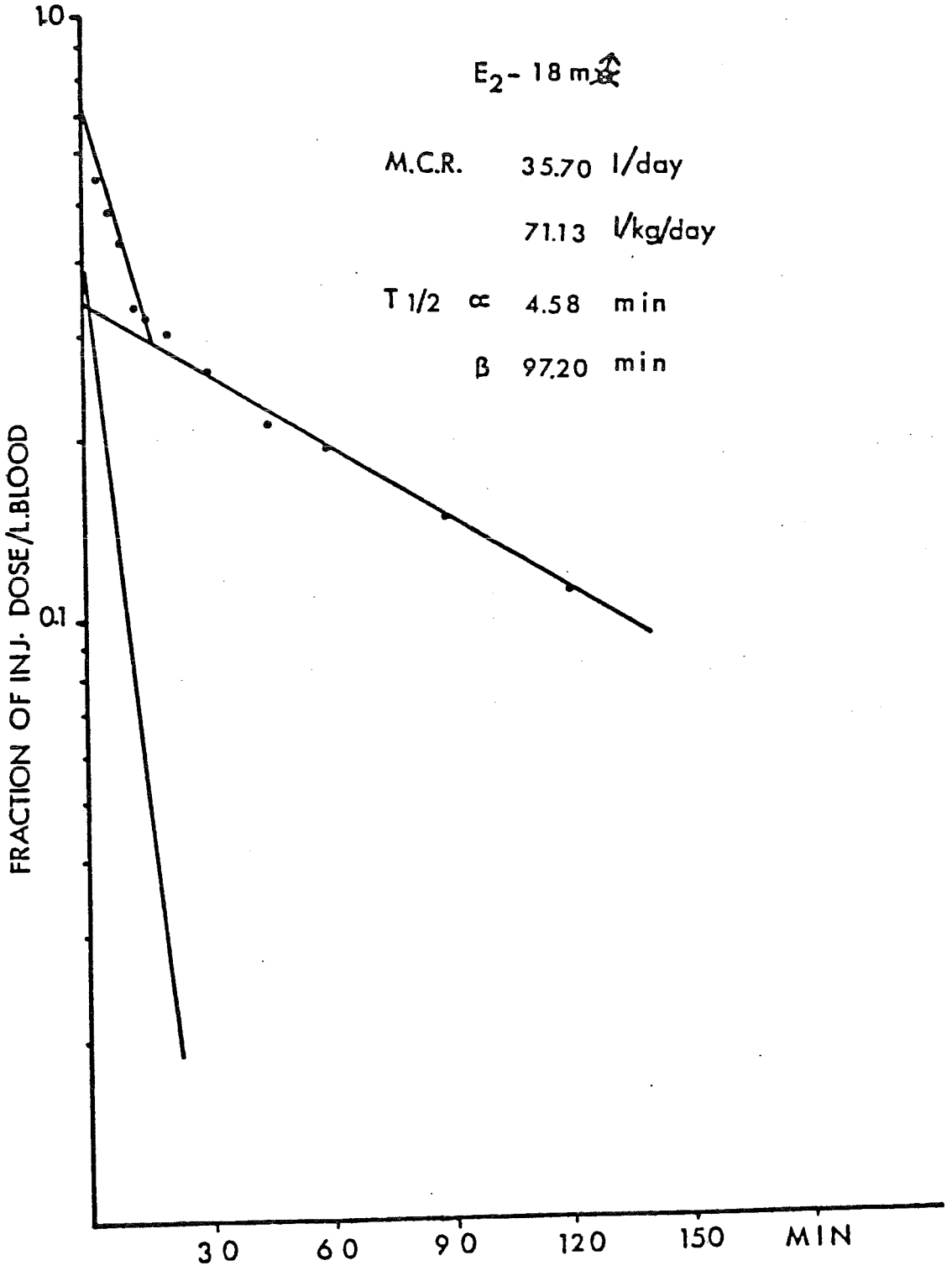

1.0
0.1
FRACTION OF INJ. DOSE/L.BLOOD
E_2 - 18 m
M.C.R. 35.70 l/day
71.13 l/kg/day
T 1/2 α 4.58 min
β 97.20 min
30
60
90
120
150
MIN

Figure 15

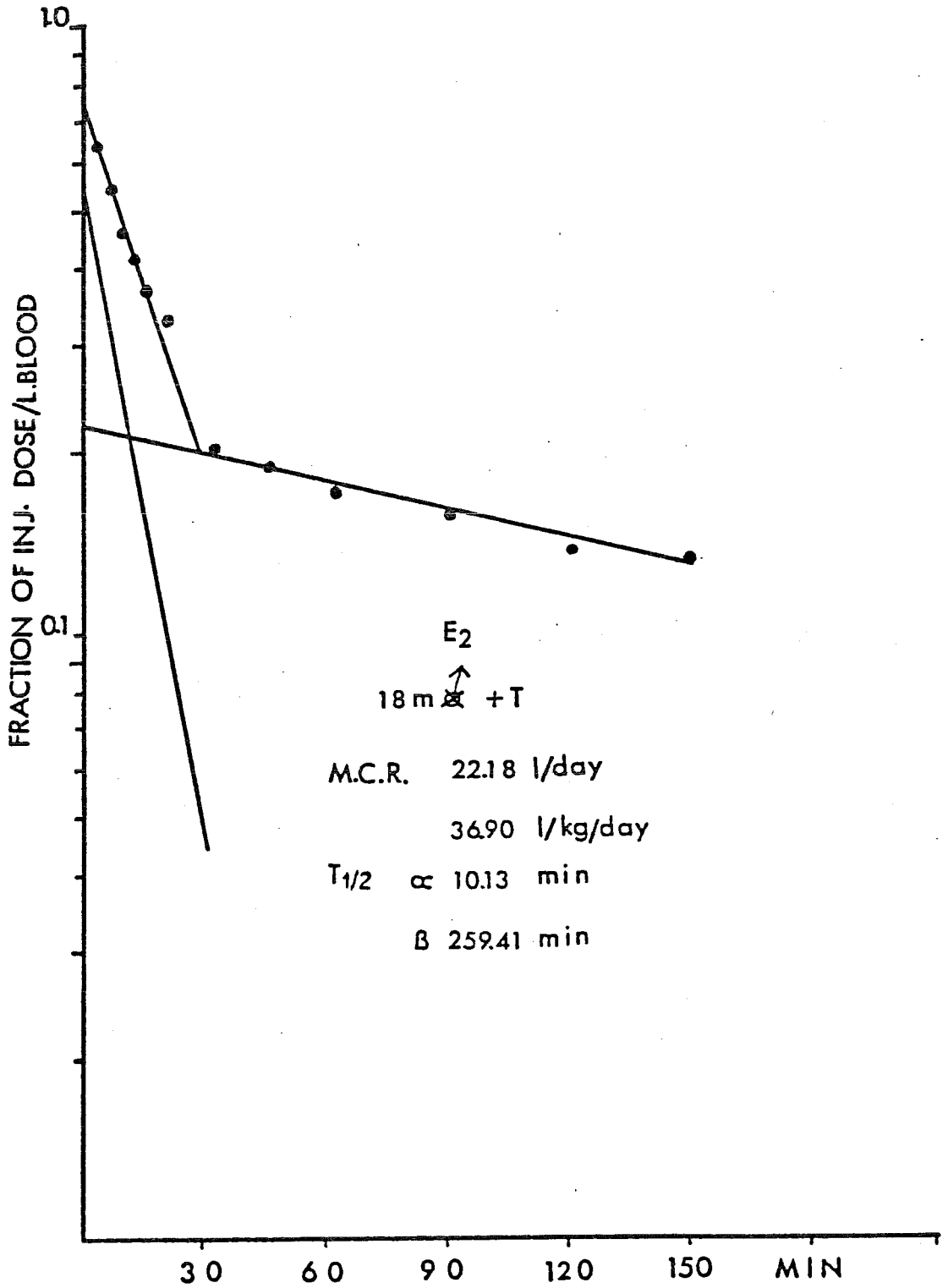

1.0
0.1
FRACTION OF INJ. DOSE/L.BLOOD
E2
18 m
+T
M.C.R. 22.18 l/day
36.90 l/kg/day
T1/2 α 10.13 min
β 259.41 min
30
60
90
120
150
MIN

Figure 16

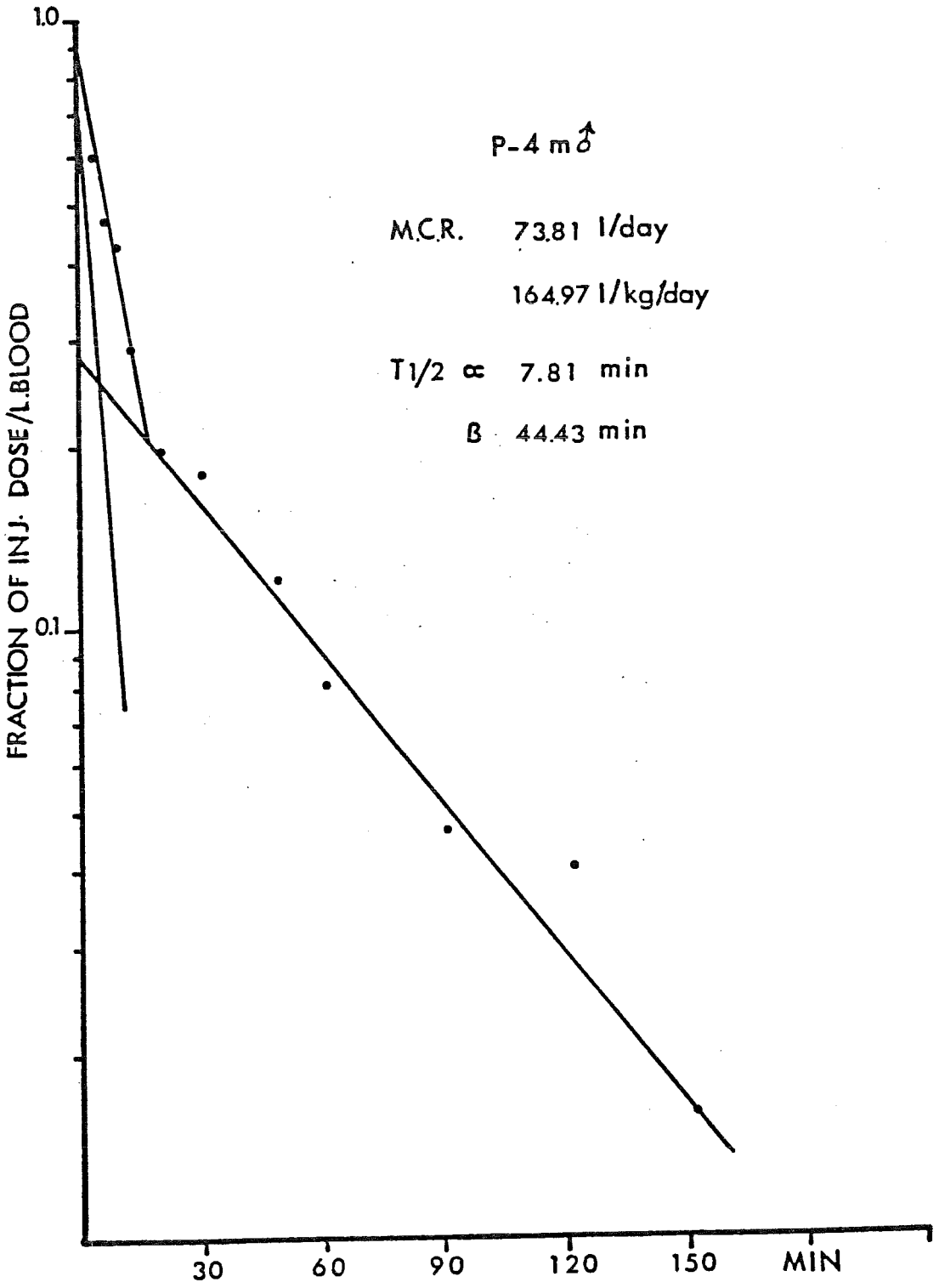

1.0
0.1
FRACTION OF INJ. DOSE/L.BLOOD
P-4 m♂
M.C.R. 73.81 l/day
164.97 l/kg/day
T1/2 α 7.81 min
β 44.43 min
30
60
90
120
150
MIN

Figure 17

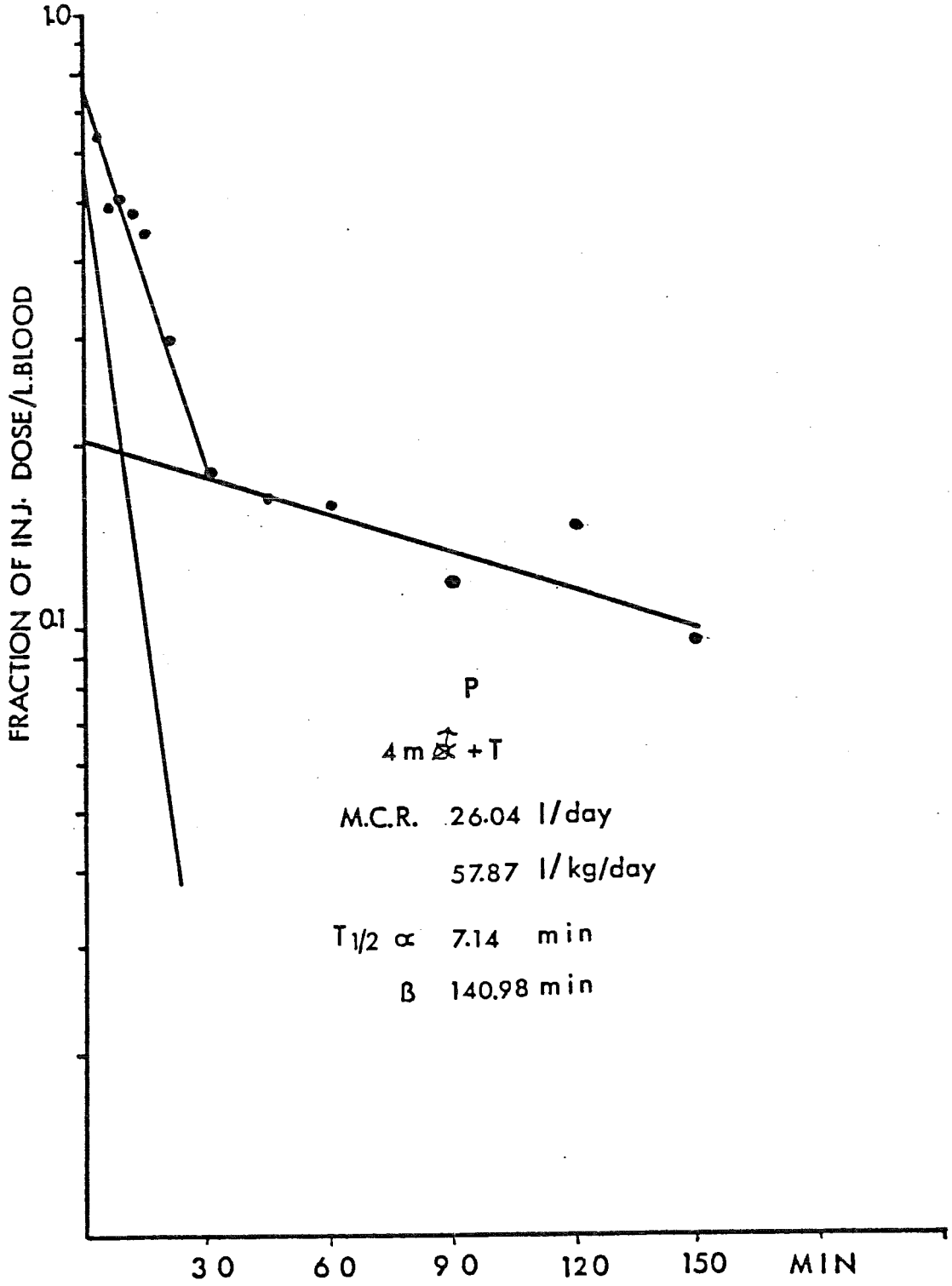

1.0
0.1
FRACTION OF INJ. DOSE/L.BLOOD
P
4 m ∫ + T
M.C.R. 26.04 l/day
57.87 l/kg/day
T1/2 α 7.14 min
β 140.98 min
30
60
90
120
150
MIN

Figure 18

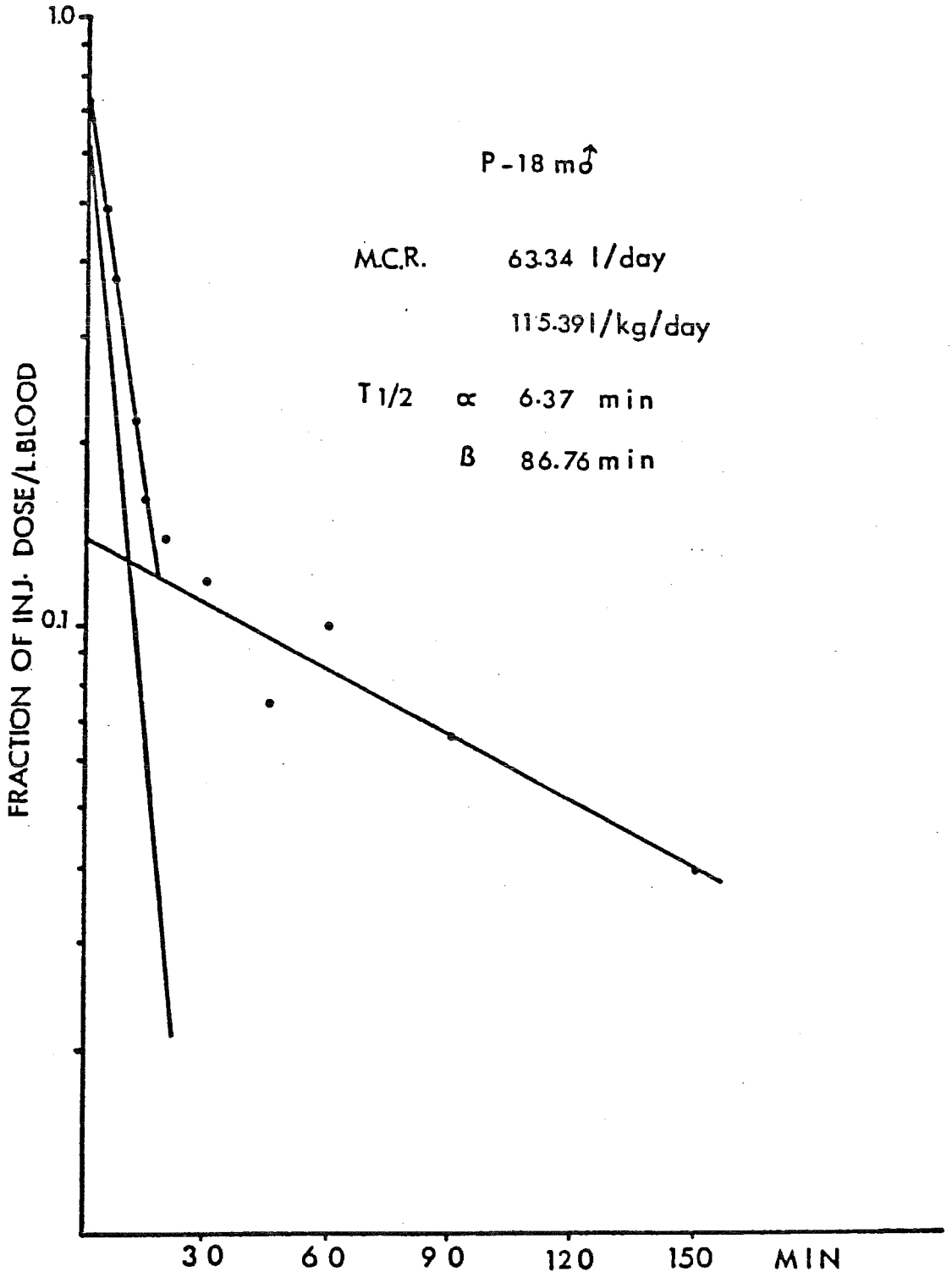

1.0
0.1
FRACTION OF INJ. DOSE/L.BLOOD
P-18 m♂
M.C.R. 63.34 l/day
115.39 l/kg/day
T 1/2 α 6.37 min
β 86.76 min
30
60
90
120
150
MIN

Figure 19

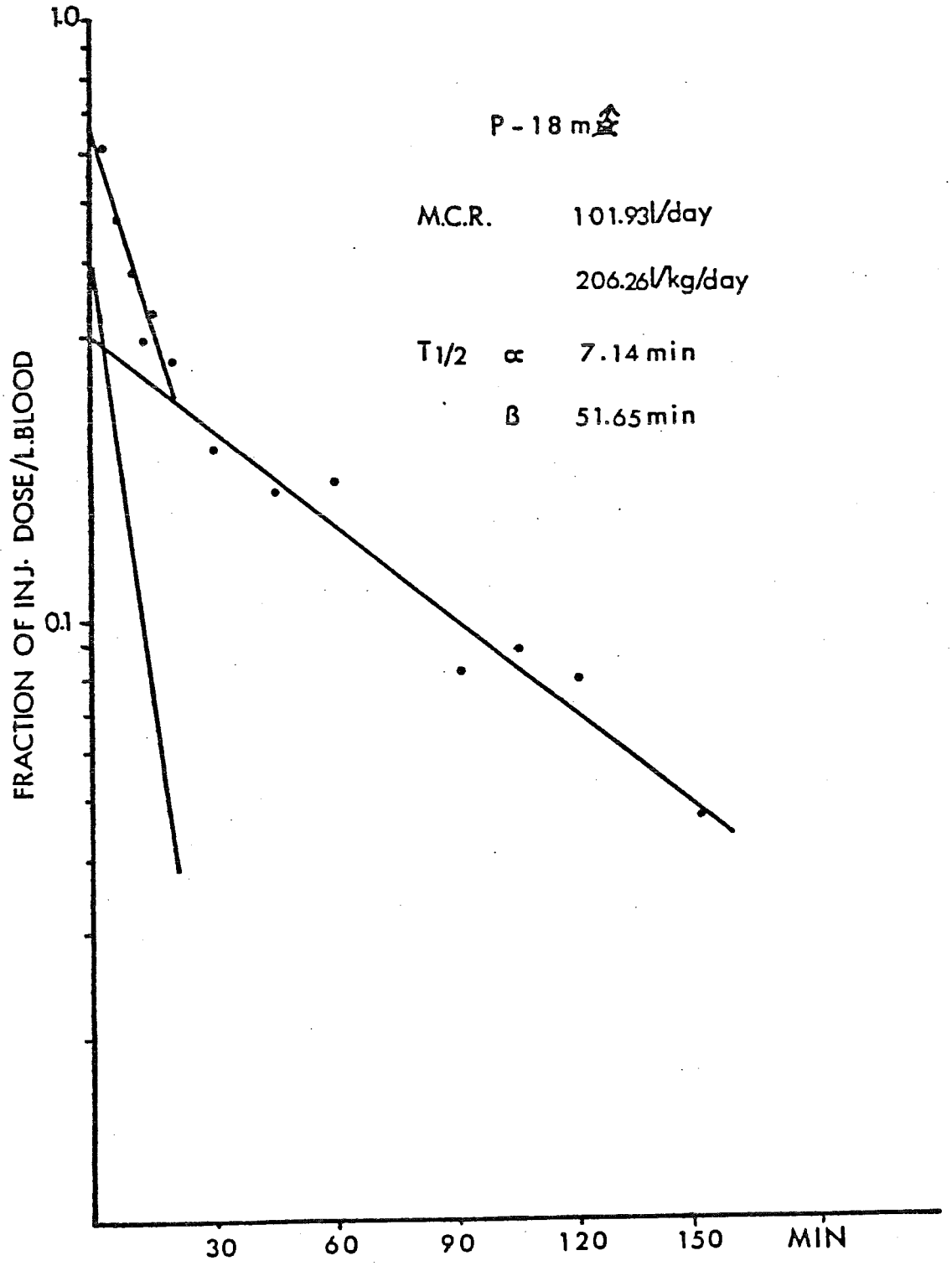

1.0
0.1
FRACTION OF INJ. DOSE/L.BLOOD
P - 18 m
M.C.R. 101.93l/day
206.26l/kg/day
T1/2 α 7.14 min
ß 51.65 min
30
60
90
120
150
MIN

Figure 20

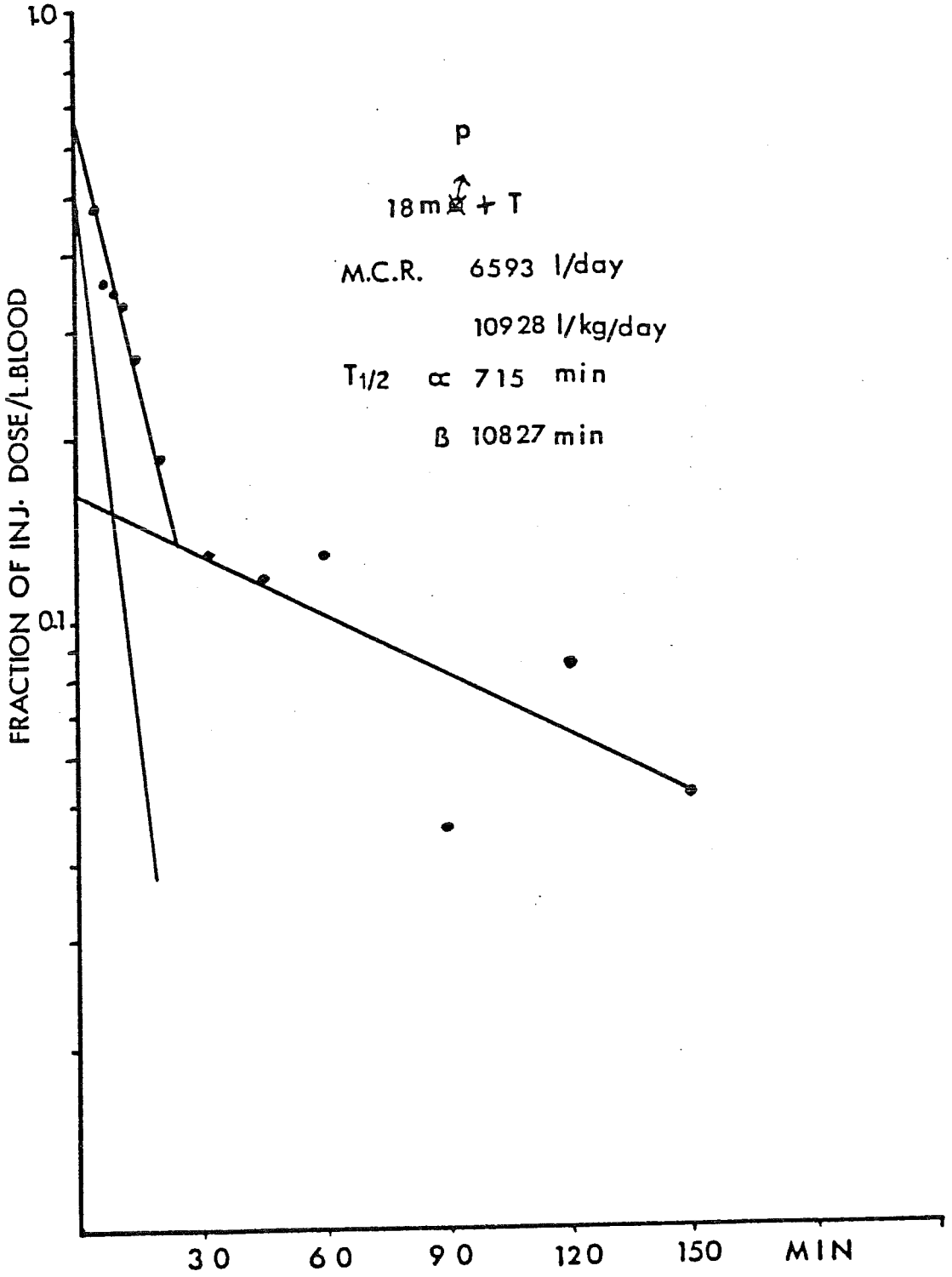

1.0
0.1
FRACTION OF INJ. DOSE/L.BLOOD
P
18 m ☒ + T
M.C.R. 6593 l/day
10928 l/kg/day
T1/2 α 715 min
β 10827 min
30
60
90
120
150
MIN

Figure 21

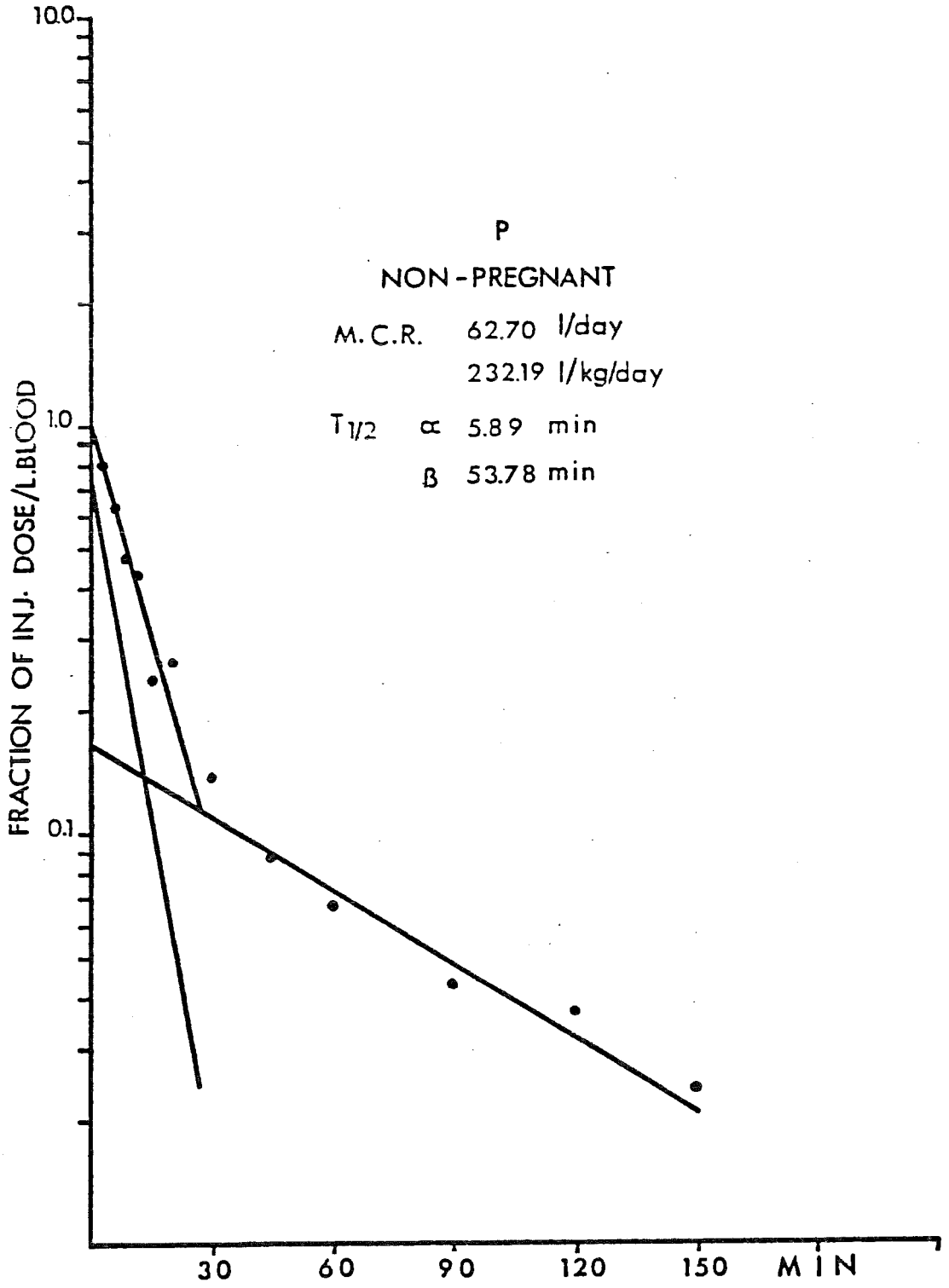

10.0
1.0
0.1
FRACTION OF INJ. DOSE/L.BLOOD
P
NON-PREGNANT
M.C.R. 62.70 l/day
232.19 l/kg/day
T1/2 α 5.89 min
β 53.78 min
30
60
90
120
150
MIN

Figure 22

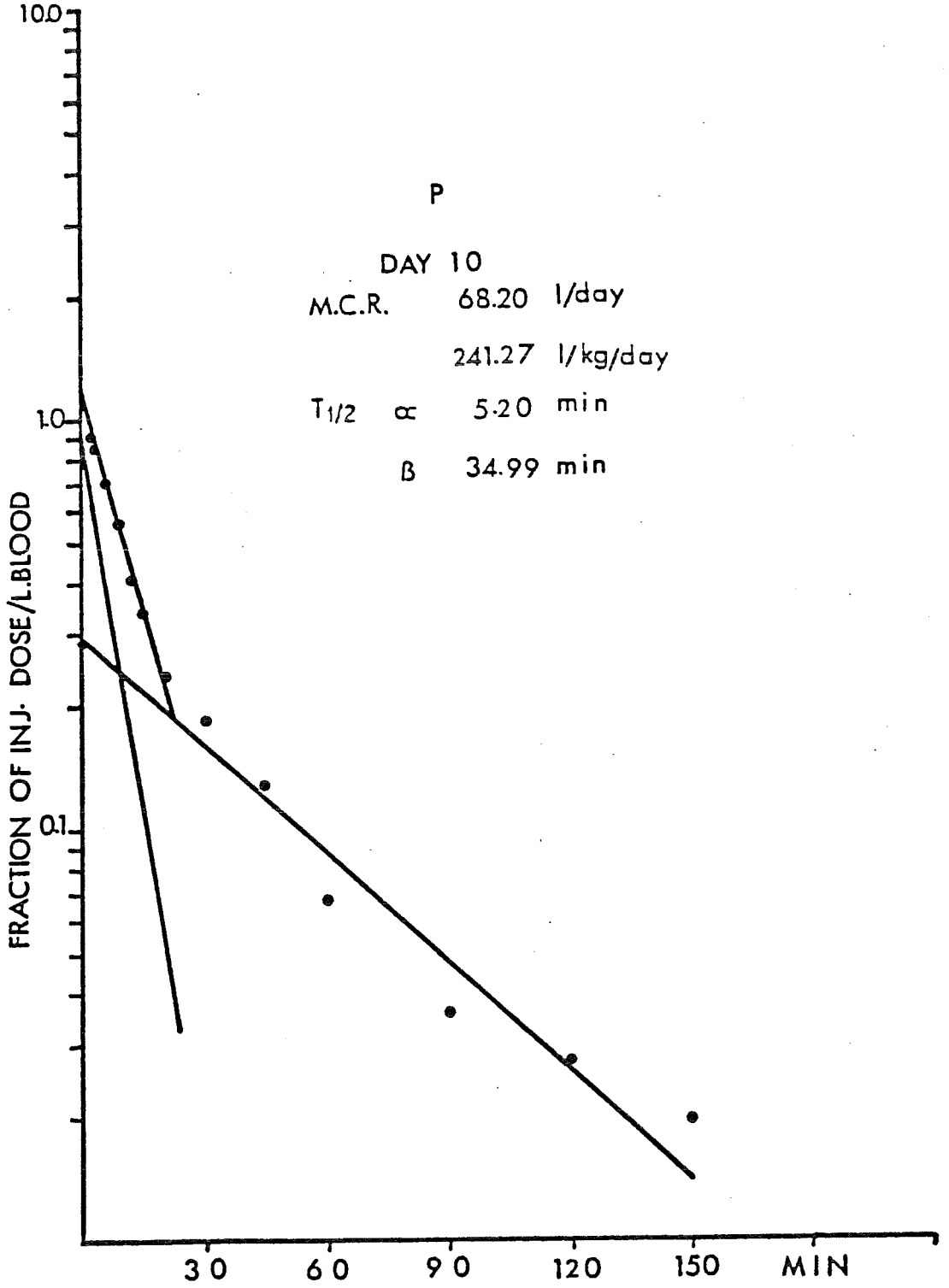

P
DAY 10
M.C.R. 68.20 l/day
241.27 l/kg/day
T1/2 α 5.20 min
β 34.99 min
10.0
1.0
0.1
FRACTION OF INJ. DOSE/L.BLOOD
30
60
90
120
150
MIN

Figure 23

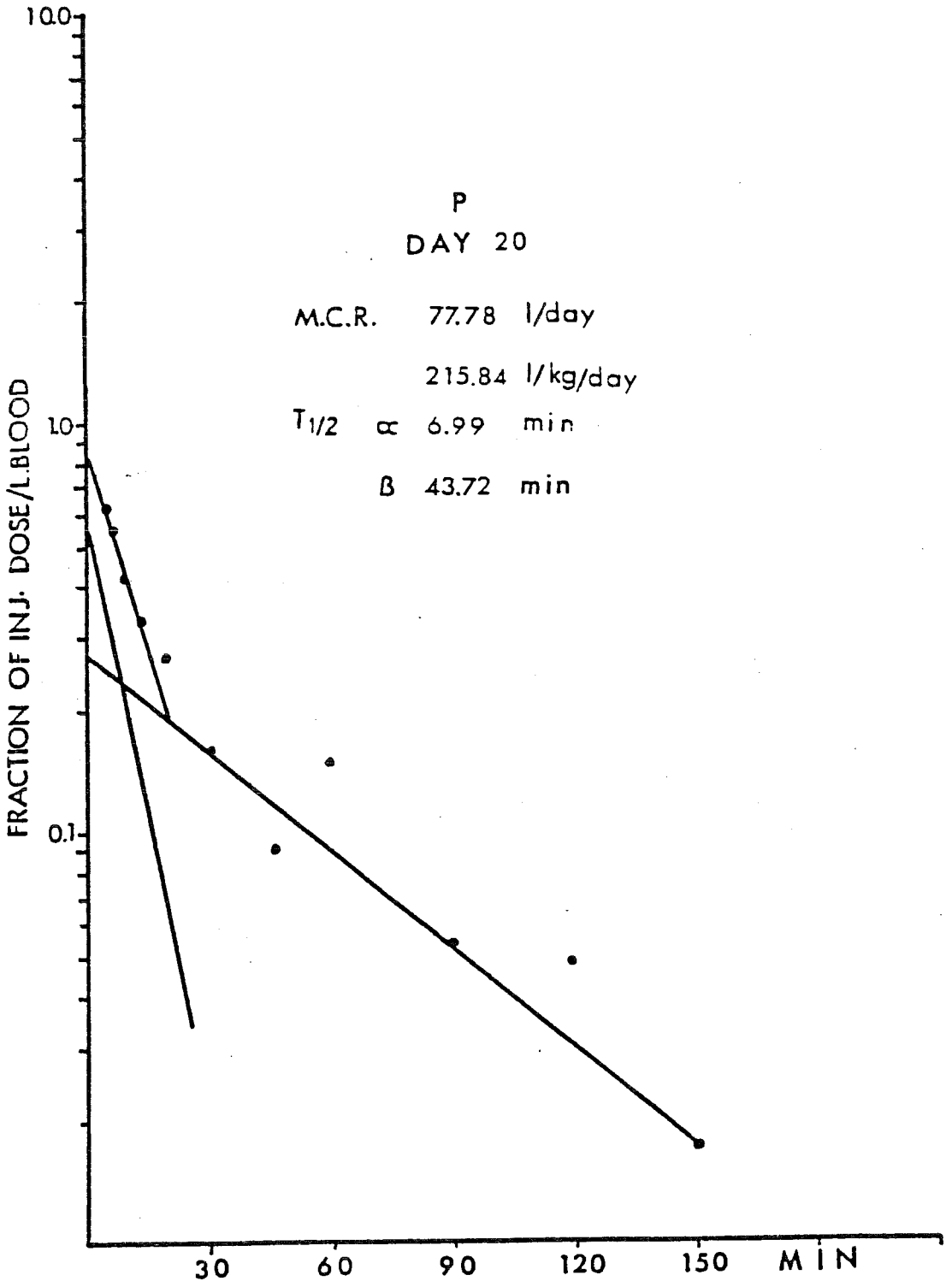

10.0
1.0
0.1
FRACTION OF INJ. DOSE/L.BLOOD
P
DAY 20
M.C.R. 77.78 l/day
215.84 l/kg/day
T1/2 α 6.99 min
β 43.72 min
30
60
90
120
150
MIN

Figure 24

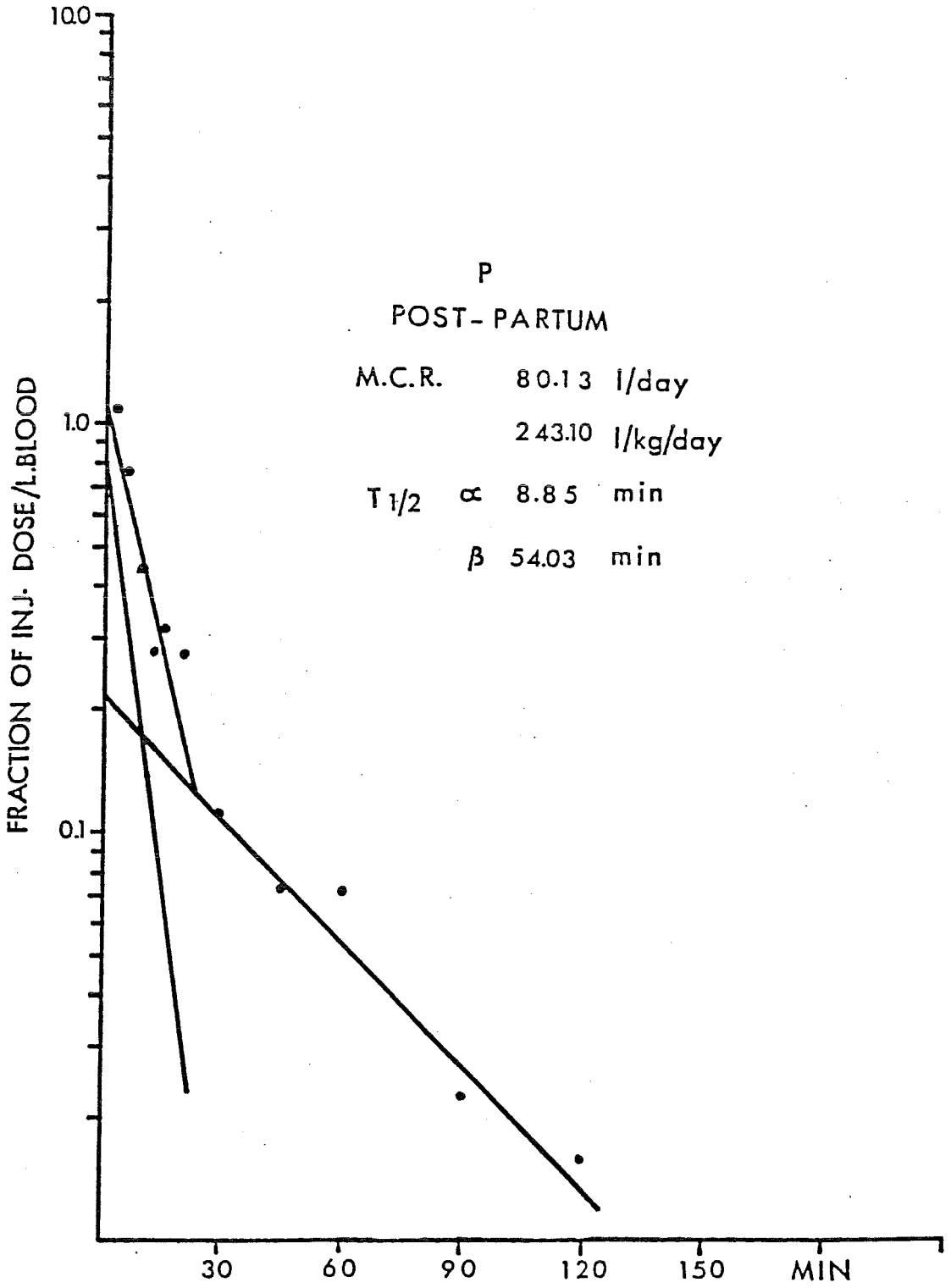

10.0
1.0
0.1
FRACTION OF INJ. DOSE/L.BLOOD
P
POST-PARTUM
M.C.R. 80.13 l/day
243.10 l/kg/day
T 1/2 α 8.85 min
β 54.03 min
30
60
90
120
150
MIN

Figure 25

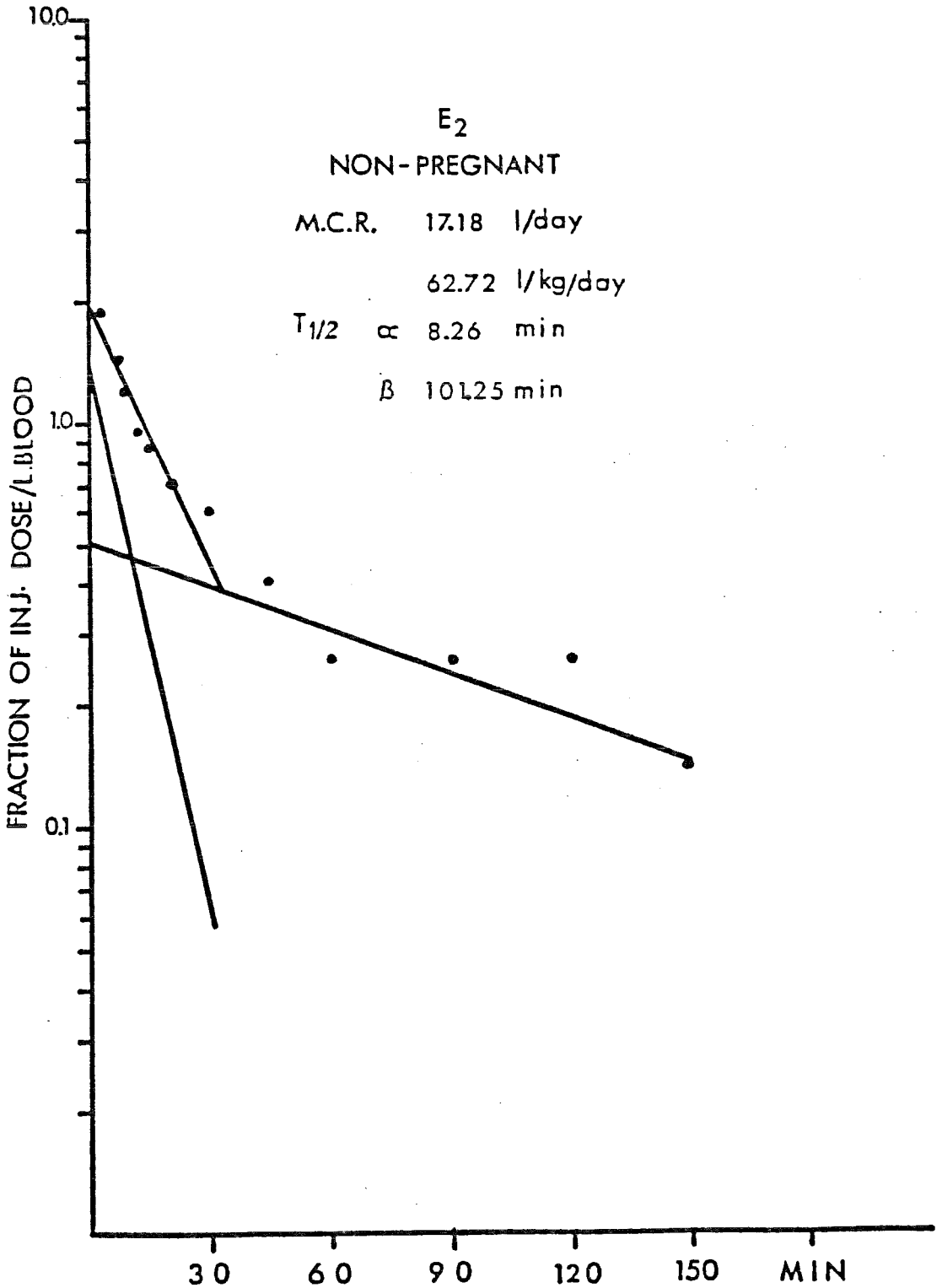

10.0
1.0
0.1
FRACTION OF INJ. DOSE/L.BLOOD
E2
NON-PREGNANT
M.C.R. 17.18 l/day
62.72 l/kg/day
T1/2 α 8.26 min
β 101.25 min
30
60
90
120
150
MIN

Figure 26

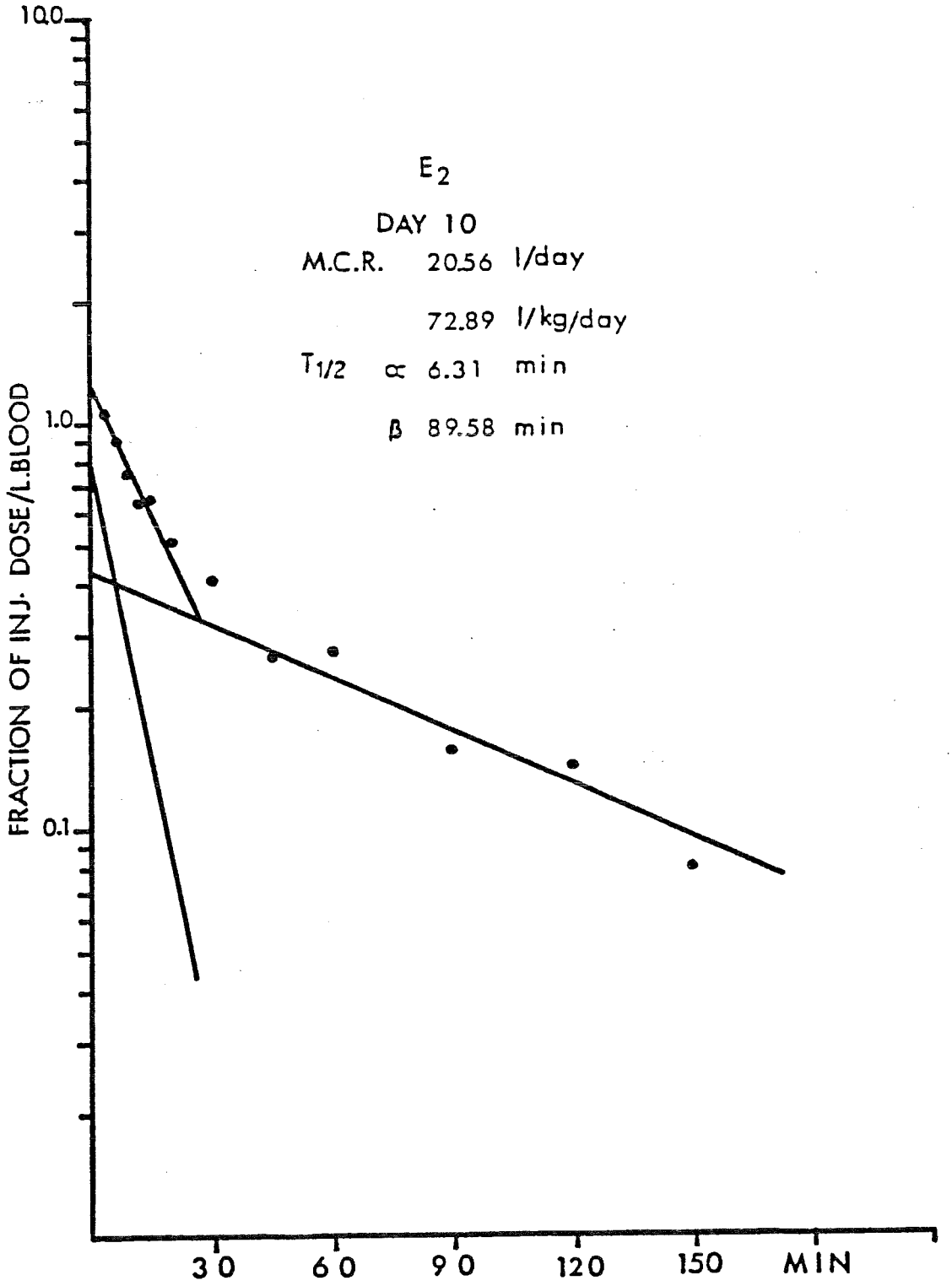

10.0
1.0
0.1
FRACTION OF INJ. DOSE/L.BLOOD
E_2
DAY 10
M.C.R. 20.56 l/day
72.89 l/kg/day
$T_{1/2}$ α 6.31 min
β 89.58 min
30
60
90
120
150
MIN

Figure 27

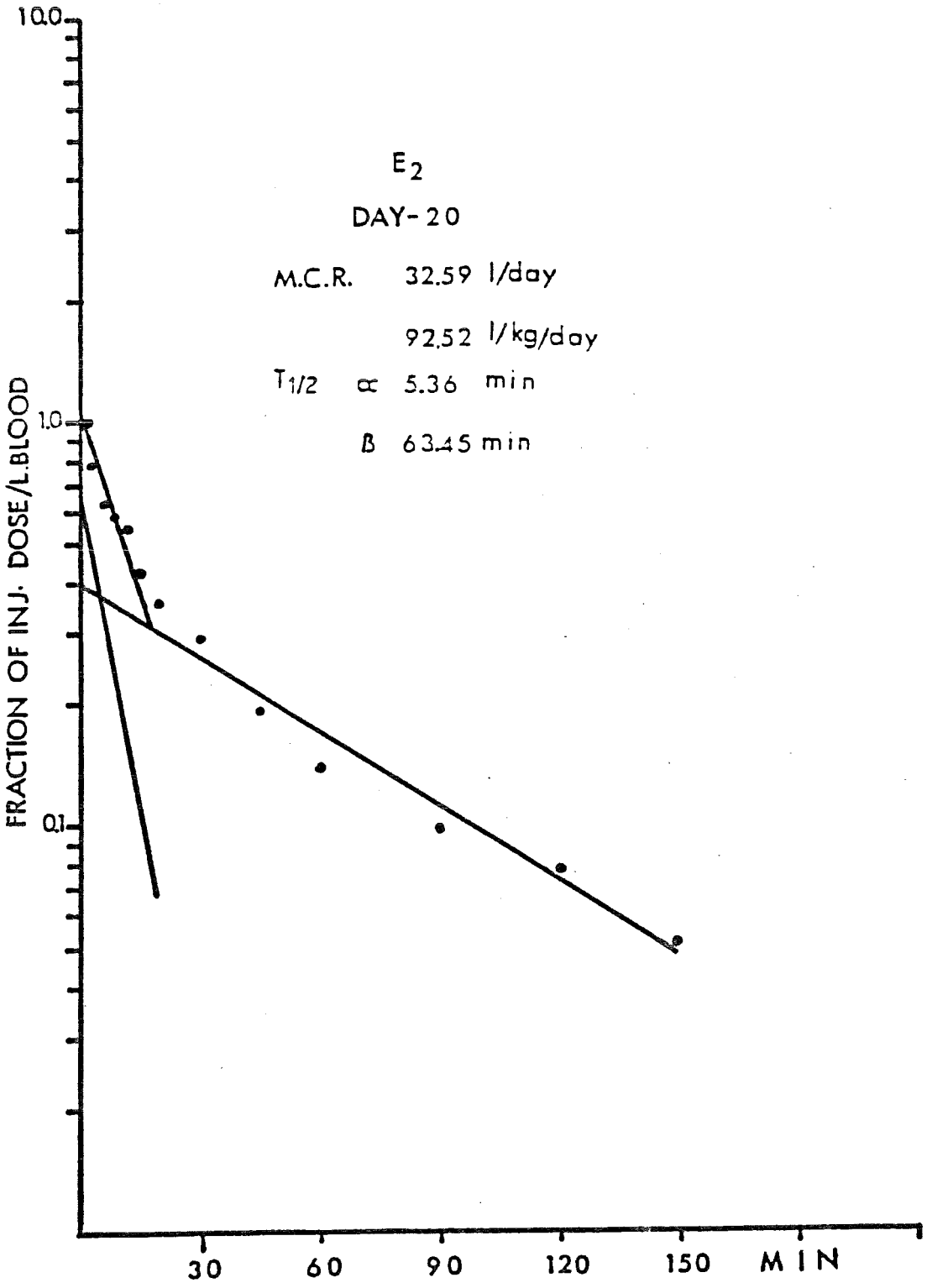

10.0
1.0
0.1
FRACTION OF INJ. DOSE/L.BLOOD
E_2
DAY-20
M.C.R. 32.59 l/day
92.52 l/kg/day
$T_{1/2}$ α 5.36 min
β 63.45 min
30
60
90
120
150
MIN

Figure 28

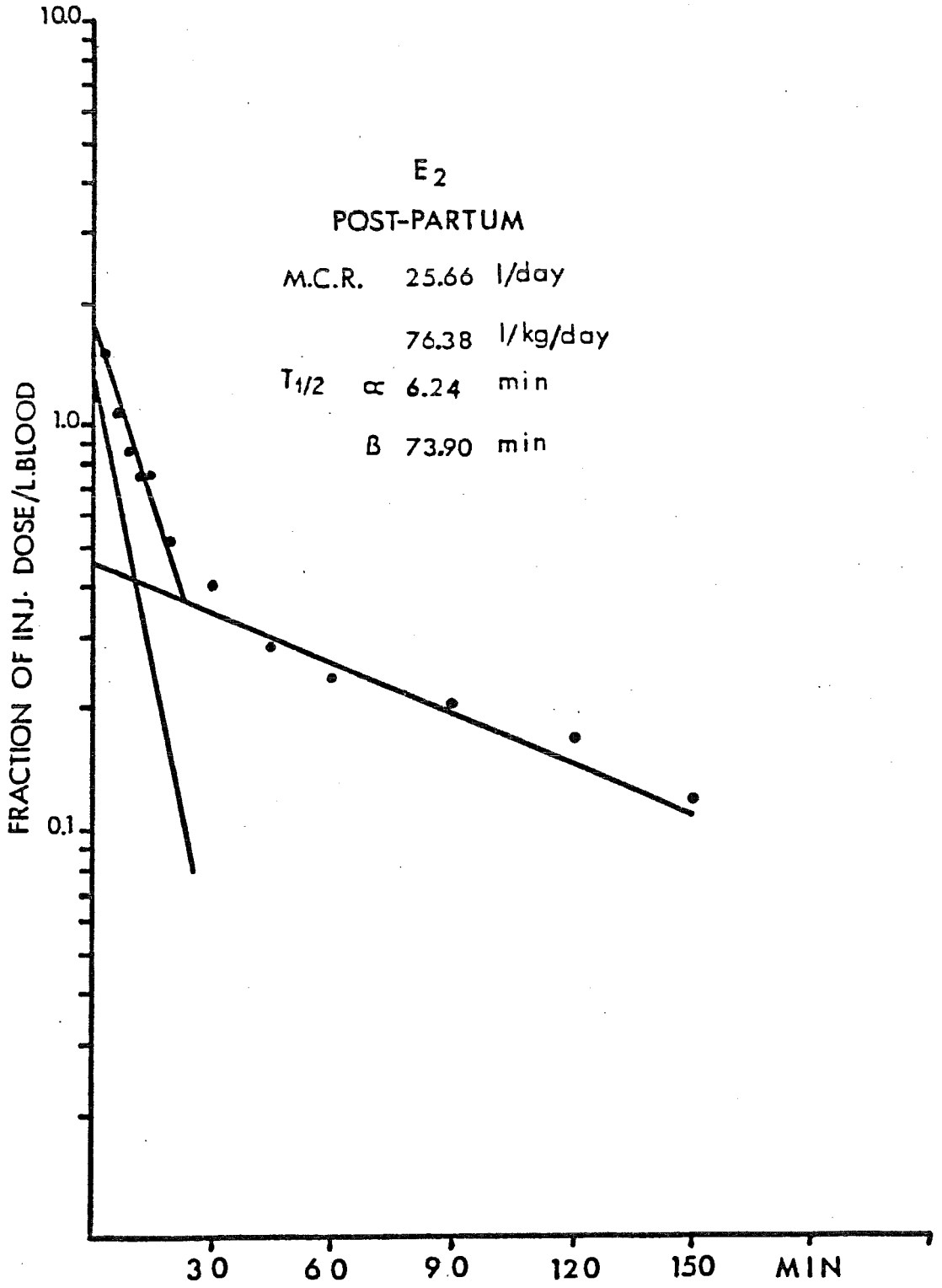

10.0
1.0
0.1
FRACTION OF INJ. DOSE/L.BLOOD
E2
POST-PARTUM
M.C.R. 25.66 l/day
76.38 l/kg/day
T1/2 α 6.24 min
β 73.90 min
30
60
90
120
150
MIN

Figure 29

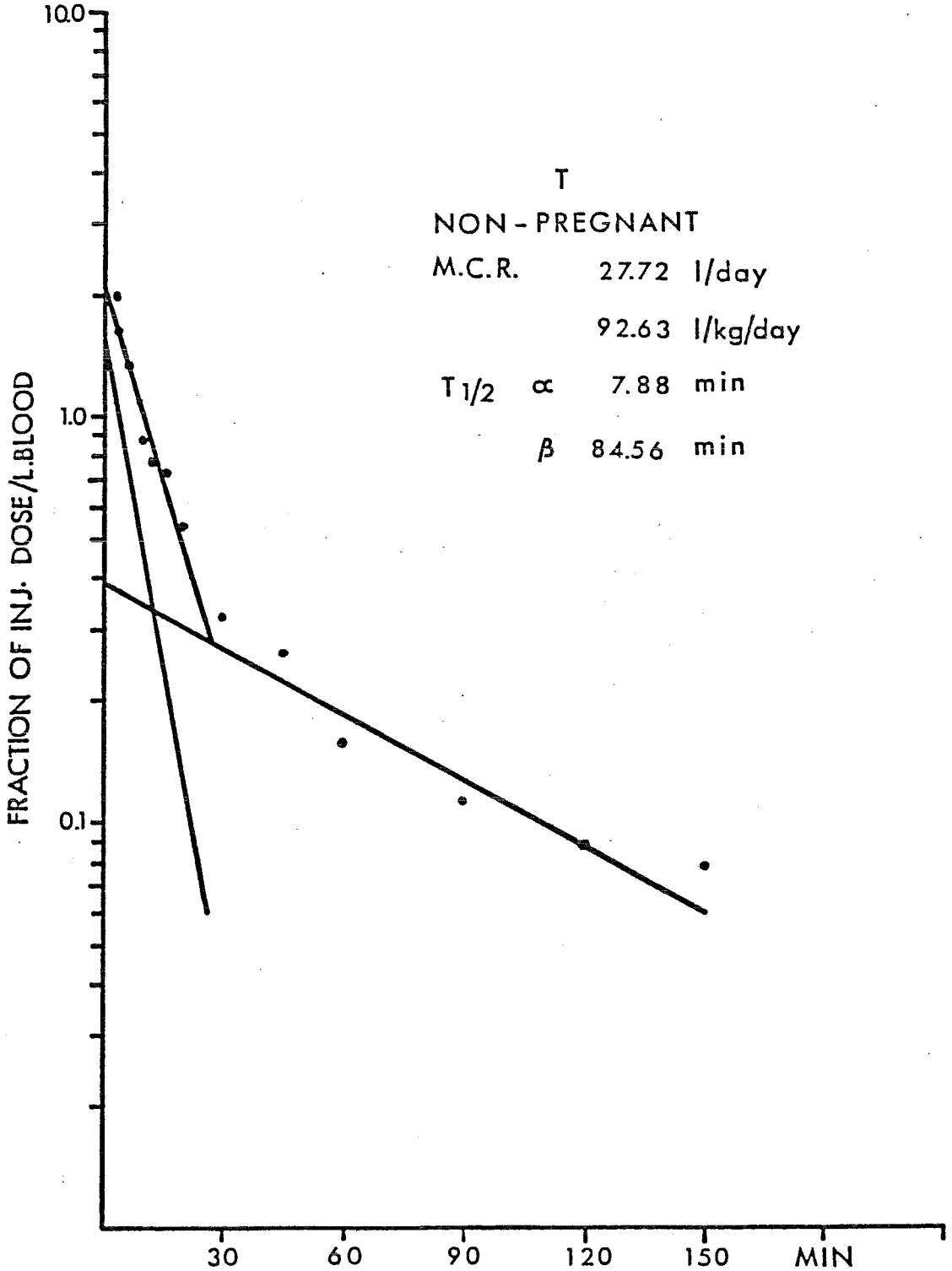

T
NON - PREGNANT
M.C.R. 27.72 l/day
92.63 l/kg/day
T 1/2 α 7.88 min
β 84.56 min
FRACTION OF INJ. DOSE/L.BLOOD
10.0
1.0
0.1
30
60
90
120
150
MIN

Figure 30

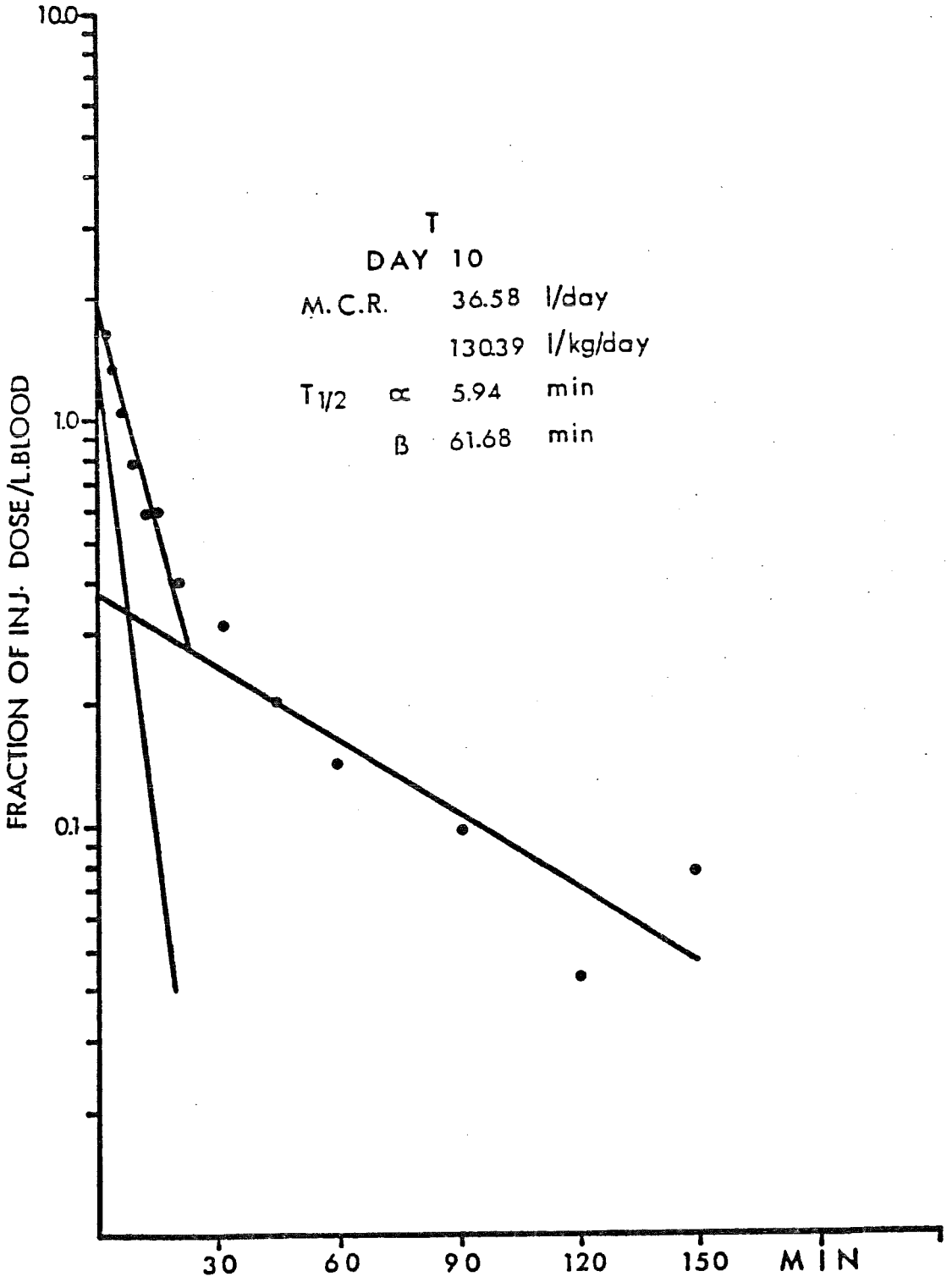

10.0
1.0
0.1
FRACTION OF INJ. DOSE/L.BLOOD
T
DAY 10
M.C.R. 36.58 l/day
130.39 l/kg/day
T1/2 α 5.94 min
β 61.68 min
30
60
90
120
150
MIN

Figure 31

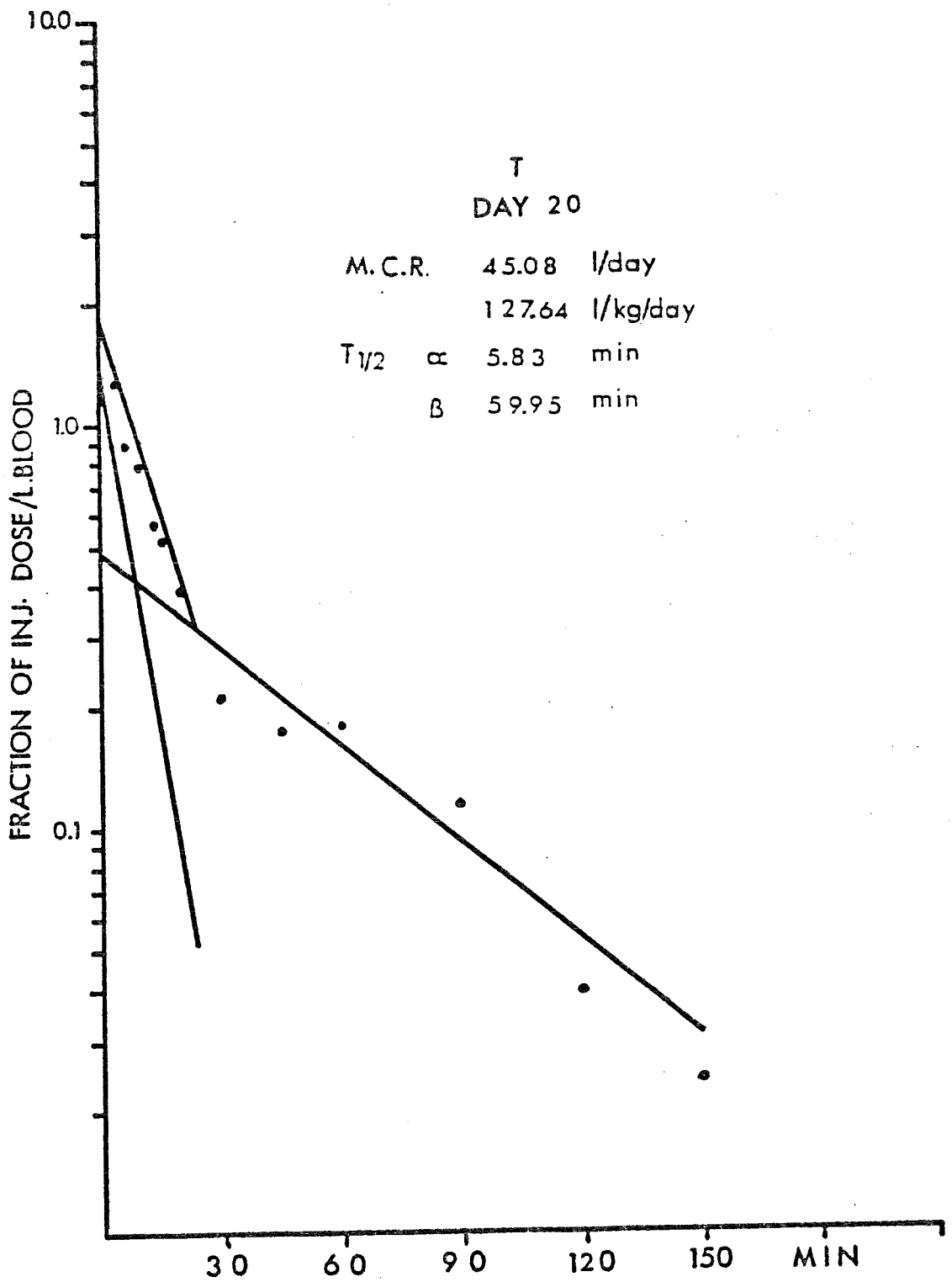

10.0
1.0
0.1
FRACTION OF INJ. DOSE/L.BLOOD
T
DAY 20
M.C.R. 45.08 l/day
127.64 l/kg/day
T1/2 α 5.83 min
β 59.95 min
30
60
90
120
150
MIN

Figure 32

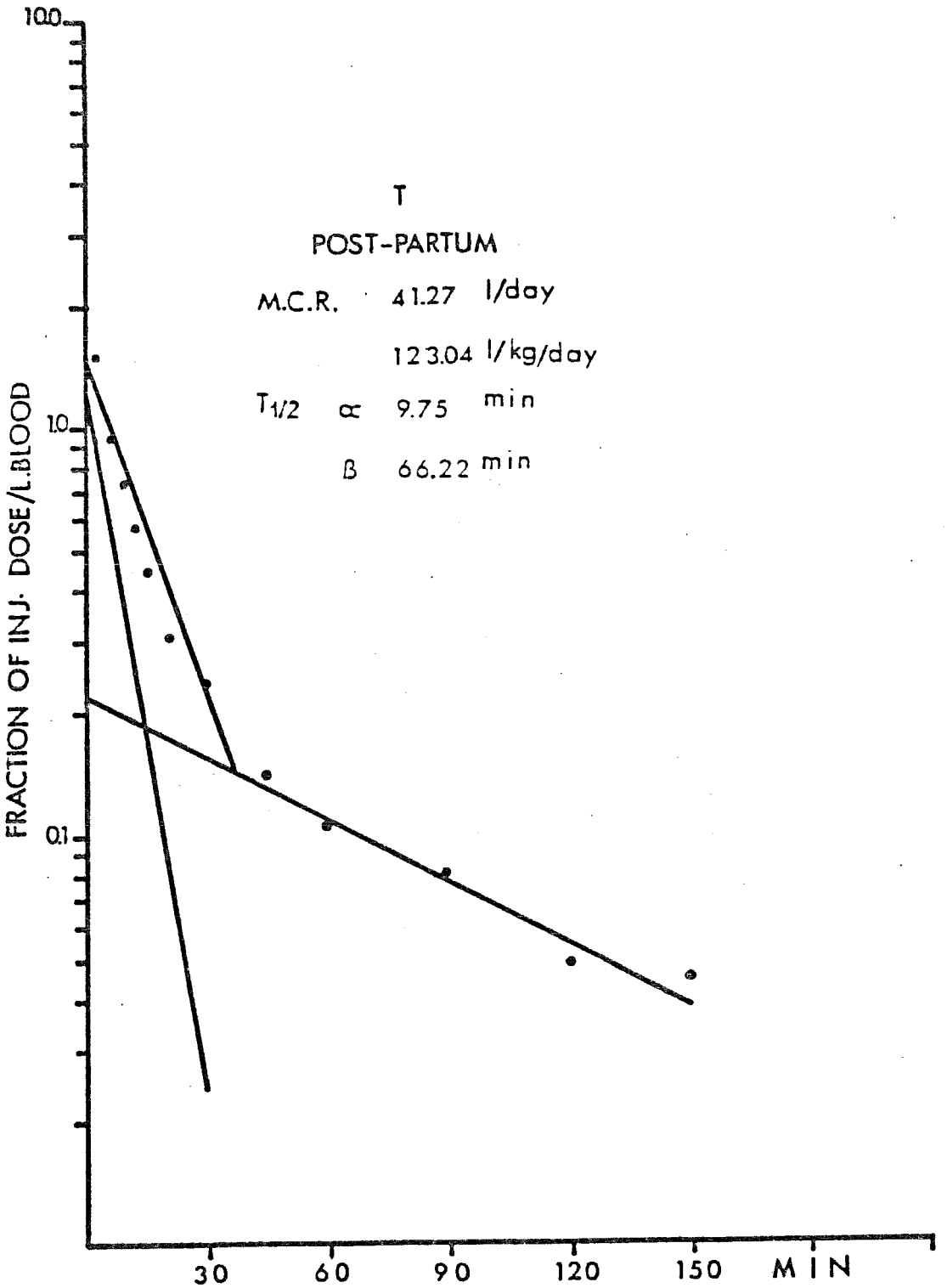
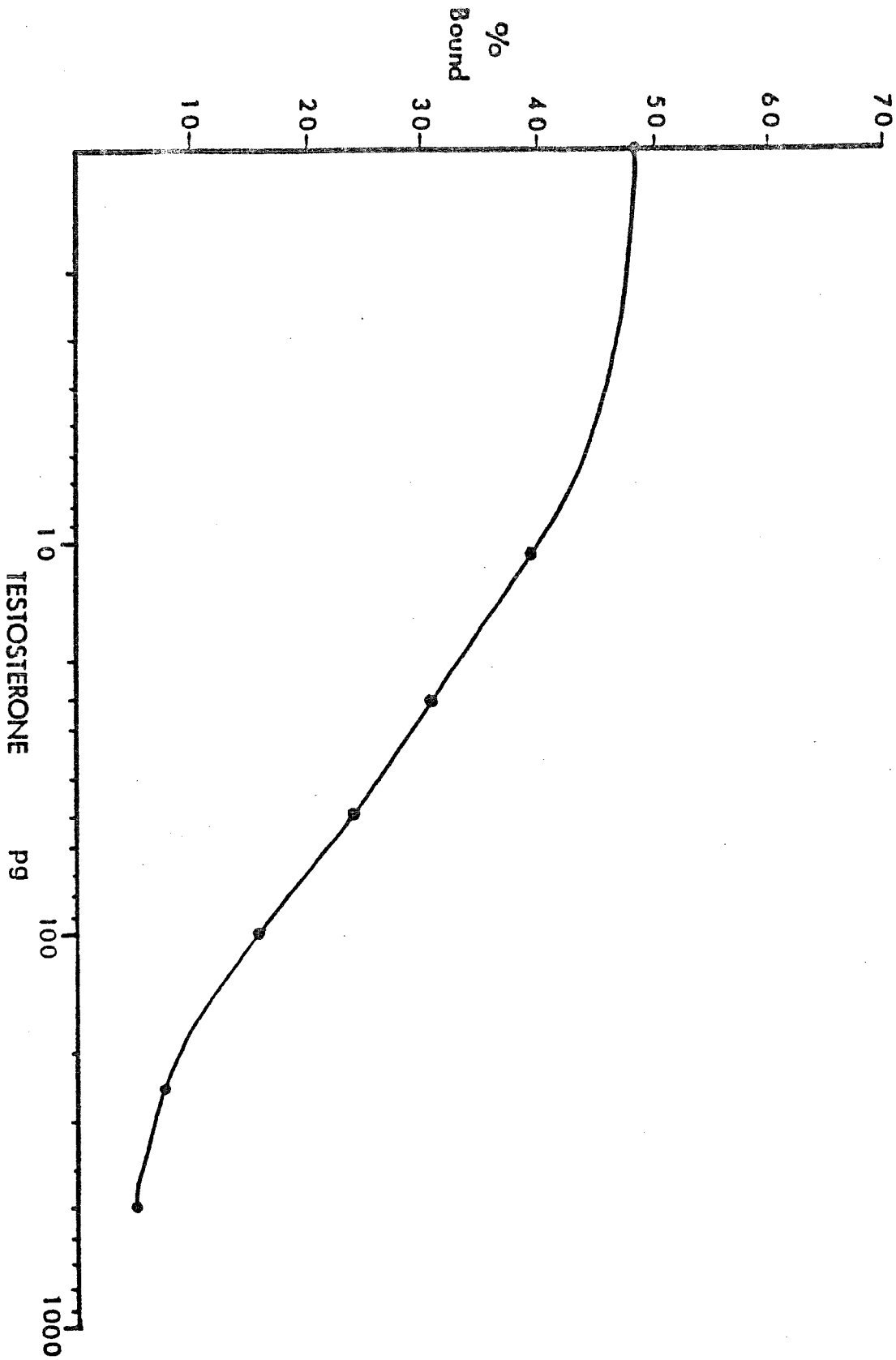

100
1.0
0.1
FRACTION OF INJ. DOSE/L.BLOOD
T
POST-PARTUM
M.C.R. 41.27 l/day
123.04 l/kg/day
T1/2 α 9.75 min
β 66.22 min
30
60
90
120
150
MIN

Figure 33: A typical standard curve of testosterone radioimmunoassay with antiserum dilution of 1:25,000.

Figure 33



APPENDIX

PBS -FOR STEROID RIA ONLY ; pH=7.4

0.87%	Na_2HPO_4	anhydrous
0.54%	$NaH_2PO_4 \cdot H_2O$	
0.1%	Na-azide	
0.9%	NaCl	
0.1%	gelatin (heat and stir to dissolve)	

CHARCOAL/DEXTRAN SOLUTION

0.5%	20 time washed charcoal
0.05%	Dextran T-70

make up fresh in PBS with 0.1% gel

PHYSIOLOGICAL SALINE 0.9%

9 grams NaCl in one liter distilled water

SCINTILLATION COUNTING FLUIDS:

for aqueous samples:

8 grams Omniflour

1 liter Triton X-100

2 liters Toluene

stir before use and store in the dark

for non-aqueous samples:

PPO/POPOP Fluor

4 grams PPO

0.1 gram POPOP

1 liter Toluene

mix and store in the dark

CONVERSION OF SERUM TO WHOLE BLOOD VALUES:

over all equation;

$$\text{fraction of injected dose/l.blood} = \frac{\text{cpm/ml serum}}{\text{injected dose}} \text{X(1 - hem) X 1000}$$

Example of Calculations of α, β, A and B as Performed by the Computer.

```
OK, MINITAB
 MINITAB RELEASE 83.6 *** COPYRIGHT - MINITAB, INC. 1984
 APRIL 23, 1985 *** S.U.N.Y. BROCKPORT - ACADEMIC COMPUTING SERVICES
 STORAGE AVAILABLE 1046576
 MTB > READ FROM FILE 'F-D20-RAWDATA-4' INTO C1,C2,C3
     12 ROWS READ
  ROW      C1      C2      C3

    1       4    2088   0.588
    2       6    1984   0.545
    3       9    1581   0.540
    4      13    1202   0.555
   .   .   .

 MTB > LET C4=(C2*C3)/2000
 MTB > PLOT C4 VS C1

     0.64+
         -       *
 C4      -
         -        *
         -
     0.48+
         -
         -         *
         -
         -
     0.32+           *
         -
         -            * *
         -
         -
     0.16+                 *          *
         -
         -                       *
         -                                    *          *
         -                                                          *
     0.00+
           ----+---------+---------+---------+---------+---------+---------+--C1
               0         30        60        90        120       150

 MTB > PICK 7 12 C1 , PUT INTO C5
 MTB > PICK 7 12 C4 , PUT INTO C6
 MTB > LOGE C6 , PUT INTO C7
 MTB > REGR C7 ON 1 PRED. IN C5

 The regression equation is
 C7 = - 1.31 - 0.0167 C5

 Predictor       Coef      Std.Coef     t-ratio
 Constant     -1.3129        0.3097       -4.24
 C5          -0.016683      0.003342       -4.99
```

```
S = 0.3455      R-sq = 86.172    R-sq(adj) = 82.715

Analysis of Variance

Source        DF          SS          MS
Regression     1      2.9747      2.9747
Error          4      0.4773      0.1193
Total          5      3.4521


Durbin-Watson statistic = 3.13

MTB > EXPO(-1.31) INTO K1
   ANSWER =          0.2698
MTB > PICK 1 6 C1 , PUT INTO C8
MTB > LET C9=K1*(EXPO(-0.0167*C8))

MTB > PICK 1 6 C4 , PUT INTO C10
MTB > LET C11=C10-C9
MTB > LOGE C11 , PUT INTO C12
MTB > REGR C12 ON 1 PRED. IN C8

The regression equation is
C12 = - 0.592 - 0.119 C8

Predictor        Coef      Std.Coef     t-ratio
Constant      -0.5923        0.2446       -2.42
C8           -0.11855       0.01968       -6.02

S = 0.2632      R-sq = 90.070    R-sq(adj) = 87.587

Analysis of Variance

Source        DF          SS          MS
Regression     1      2.5133      2.5133
Error          4      0.2771      0.0693
Total          5      2.7903


Durbin-Watson statistic = 2.49

MTB > EXPO(-0.592) INTO K2
   ANSWER =          0.5532
```

```
MTB > PRINT C1-C12
 ROW     C1     C2     C3          C4      C5          C6          C7     C8

   1      4   2088  0.588    0.613872      30    0.158802   -1.84010      4
   2      6   1984  0.545    0.540640      45    0.088350   -2.42645      6
   3      9   1581  0.540    0.426870      60    0.151944   -1.88424      9
   4     13   1202  0.555    0.333555      90    0.053760   -2.92323     13
   5     15    985  0.550.   0.270875     120    0.049275   -3.01034     15
   6     20    905  0.579    0.261997     150    0.017394   -4.05163     20
   7     30    532  0.597    0.158802
   8     45    285  0.620    0.088350
   9     60    487  0.624    0.151944
  10     90    168  0.640    0.053760
  11    120    146  0.675    0.049275
  12    150     52  0.669    0.017394

 ROW          C9          C10         C11          C12

   1    0.252385    0.613872    0.361487   -1.01753
   2    0.244094    0.540640    0.296546   -1.21555
   3    0.232166    0.426870    0.194703   -1.63628
   4    0.217164    0.333555    0.116391   -2.15080
   5    0.210031    0.270875    0.060844   -2.79944
   6    0.193206    0.261997    0.068792   -2.67667
MTB > STOP.
```

Examples of Calculations of M.C.R., $T\frac{1}{2}\alpha$, $T\frac{1}{2}\beta$, and Volumes of Distributions as Performed by the Computer.

```
OK, BASICV
BASICV REV19.3.1
>OLD FINAL-CALCU
>LIST
FINAL-CALCU      TUE, APR 23 1985          09:55:14

1000 PRINT       M.C.R.      V1        V2          V3          T1          T2

1020 PRINT


1050 FOR E= 1 TO 100
1150 READ I1,S1,I2,S2
1200 LET C=1440*S1*S2/(I1*S2+I2*S1)
1250 LET C=INT(C*1000+.5)/1000
1300 LET V1=1/(I1+I2)
1350 LET V1=INT(V1*1000+.5)/1000
1400LET V2=(I1*S1+I2*S2)/((I1+I2)*(I1*S2+I2*S1))
1450 LET V2=INT(V2*1000+.5)/1000
1500 LET V3=(I1*I2)*((S1-S2)^2)/((I1+I2)*((I1*S2+I2*S1)^2))
1600 LET T1=LOG(2)/S1
1650 LET T1=INT(T1*100+.5)/100
1700 LET T2=LOG(2)/S2
1750 PRINTUSING G$, C, V1, V2, V3, T1, T2
2000 NEXT E
2102 DATA .5294, .141, .5086, .00979
2104 DATA .6005, .161, .3848, .00884
2106 DATA .6683, .115, .4098, .0143
2108 DATA 1.6000, .190, .5627, .0165
2110 DATA .6859, .0645, .2516, .00779
2112 DATA 2.6432, .262, .4194, .0170
2114 DATA .6887, .133, .5822, .00928
2500 END
>RUN
FINAL-CALCU      TUE, APR 23 1985          10:04:42

   M.C.R.        V1          V2          V3           T1          T2

  $25.850     $0.9630     $0.9980    $0.75525880    $4.920     $70.8016
  $30.470     $1.0150     $1.5100    $1.20019306    $4.310     $78.4105
  $41.777     $0.9280     $1.3540    $0.80172806    $6.030     $48.4718
  $33.863     $0.4620     $1.0870    $0.70510626    $3.650     $42.0089
  $33.541     $1.0670     $2.2850    $1.27222628   $10.750     $88.9791
  $41.428     $0.3270     $1.4760    $0.90648772    $2.650     $40.7734
  $21.203     $0.7870     $0.9110    $0.68728474    $5.210     $74.6926
END OF DATA   AT LINE  1150
```

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