ABSTRACT

Floyd Ernest Bell, Jr. THE MATURITY ONSET RELATIONSHIP OF PROSTAGLANDIN $F_{2\alpha}$ IN RABBIT SERUM. (Under the direction of Dr. William James Smith) Department of Biology, May 1976.

The purpose of this study was to determine if there exists a direct relationship between peripheral serum prostaglandin concentration and age. Past research has indicated a strong connection between prostaglandins and the reproductive process. The biologically active group of fatty acids has been the subject of many studies during the last 40 years, but until this time, there has been no study of the relationship of prostaglandin level to sexual maturity.

Using past research as a basis for investigation, experiments were arranged to investigate the possibility of a connection between puberty and a rise in serum prostaglandin level. The New Zealand White rabbit (Oryctolagus cuniculus) was the experimental animal and radioimmunoassay was the procedure for the determination of prostaglandin level. The rabbits were tested at ages varying from 1 day to 2 years and the prostaglandin level of the animals was compared to known solutions of prostaglandin. This method was used to determine the prostaglandin concentration in nanograms per milliliter of blood serum from animals of different ages. These results were correlated to determine if there was a direct relationship between increasing age and blood serum prostaglandin

level. The data indicated that there was no direct relationship between sexual maturity and serum prostaglandin level, but that other factors probably control the blood prostaglandin level.

THE MATURITY ONSET RELATIONSHIP OF PROSTAGLANDIN $F_{2\alpha}$ IN RABBIT SERUM

A Thesis

Presented to

the Faculty of the Department of Biology

East Carolina University

In Partial Fulfillment

of the Requirements for the Degree

Master of Arts in Biology

by

Floyd Ernest Bell, Jr.

May 1976

THE MATURITY ONSET RELATIONSHIP OF PROSTAGLANDIN $F_{2\alpha}$ IN RABBIT SERUM

by

Floyd Ernest Bell, Jr.

APPROVED BY:

SUPERVISOR OF THESIS W James Smith
Dr. William James Smith

CHAIRMAN OF THE DEPARTMENT OF BIOLOGY

James S McDaniel
Dr. James S. McDaniel

DEAN OF THE GRADUATE SCHOOL

Joseph G. Boyette
Dr. Joseph G. Boyette

ACKNOWLEDGMENT

Grateful acknowledgment is made to Professor William James Smith of the East Carolina University faculty for his untiring efforts in supervising this thesis. His guidance and constructive suggestions have made the completion of this thesis possible.

TABLE OF CONTENTS

LIST OF TABLES AND FIGURES

INTRODUCTION

The biochemical group of substances known as prostaglandins have been studied since 1913 when Battez and Boulet found that fresh human prostate tissue caused depressor action on the blood pressure of dogs. This work was followed by the experiments of Jurzrok and Lieb in 1930 when they found a substance in human seminal fluid that had extraordinary smooth muscle contracting ability. In 1934, through the work of a Swedish scientist, U.S. Von Euler, the establishment of a new group of naturally occurring substances took place (E. W. Horton, 1972). Von Euler gave the new group of substances the name of prostaglandins due to the fact that they were located in the seminal fluid and thought to be from the prostate gland. Von Euler suggested in 1947 that Professor Sune Bergstrom try to purify prostaglandins which he did in 1960 (E. W. Horton, 1972). In 1959 it was discovered by fractionation of ejaculates, that human seminal prostaglandin was secreted mainly by the seminal vesicles and not in the prostate (Eliasson, R., 1959). This would have made a major difference in the terminology for these substances had the discovery occurred prior to the widespread use of the term prostaglandin. With the purification of prostaglandin in 1960 it was discovered that two different compounds existed and that they could be separated. One compound was more soluble in ether and was called prostaglandin E (PGE) while the other was more soluble in phosphate

buffer (in Swedish spelled with an "F") and was called prostaglandin F (PGF). These compounds displayed the empirical formulae $C_{20}H_{34}O_5$ and $C_{20}H_{36}O_5$ respectively (Bergstrom and Sjovall, 1960 a&b). Since these early experiments at least 14 different prostaglandins have been discovered and chemically identified (table 1). The primary prostaglandin configurations and their precursor molecules are shown in table 2.

The prostaglandins are cyclic, oxygenated, C_{20}, fatty acids based on the prostanoic acid skeleton (N. Anderson, 1971). They are produced by an enzyme system which catalyses the conversion of unsaturated fatty acids to prostaglandins. The enzyme is known as prostaglandin synthetase and is found in many tissues including some from prostate, seminal vesicle, lung, intestine, uterus, thymus, heart, liver, kidney, pancreas, brain, stomach and iris (Ramwell, 1973). The fact that prostaglandin synthetase has been isolated in so many different areas of the body has led to countless hours of research on the origin, stimulation, and concentration of prostaglandins in body tissues. Knowledge of prostaglandins is being further extended by the demonstration of the 15 epi PGA_2 compounds even in coral (Weinheimer and Spraggins, 1969).

THE NATURAL PROSTAGLANDINS

Table 1

PGE_1	$PG(E^{\alpha\alpha})_1$	11α, 15α-dihydroxy-9-oxo-13-trans-prostenoic acid
PGE_2	$PG(E^{\alpha\alpha})_2$	11α, 15α-dihydroxy-9-oxo-5-cis, 13-trans-prostadienoic acid
PGE_3	$PG(E^{\alpha\alpha})_3$	11α, 15α-dihydroxy-9-oxo-5-17-cis, 13-trans-prostatrienoic acid
$PGF_{1\alpha}$	$PG(^{\alpha\alpha\alpha})_1$	9α, 11α, 15α-trihydroxy-13-trans-prostenoic acid
$PGF_{2\alpha}$	$PG(^{\alpha\alpha\alpha})_2$	9α, 11α, 15α-trihydroxy-5-cis, 13-trans-prostadienoic acid
$PGF_{3\alpha}$	$PG(^{\alpha\alpha\alpha})_3$	9α, 11α, 15α-trihydroxy-5, 17-cis, 13-trans-prostatrienoic acid
PGA_1	$PG(A\Delta^{\alpha})_1$	15α-hydroxy-9-oxo-10, 13-trans-prostadienoic acid
19-hydroxy-PGA_1		15α, 19α-dihydroxy-9-oxo-5, 10-cis, 13-trans-prostatrienoic acid
PGB_1	$PG(B\text{-}^{\alpha})_1$	15α-hydroxy-9-oxo-8(12), 13-trans-prostadienoic acid
19-hydroxy-PGB_1		15α, 19α-dihydroxy-9-oxo-8(12), 13-trans-prostadienoic acid
PGB_2	$PG(B\text{-}^{\alpha})_2$	15α-hydroxy-9-oxo-5, 8(12)-cis, 13-trans-prostatrienoic acid
19-hydroxy-PGB_2		15α, 19α-dihydroxy-9-oxo-5, 8(12)-cis, 13-trans-prostatrienoic acid

THE PRIMARY PROSTAGLANDINS AND THEIR PRECURSORS

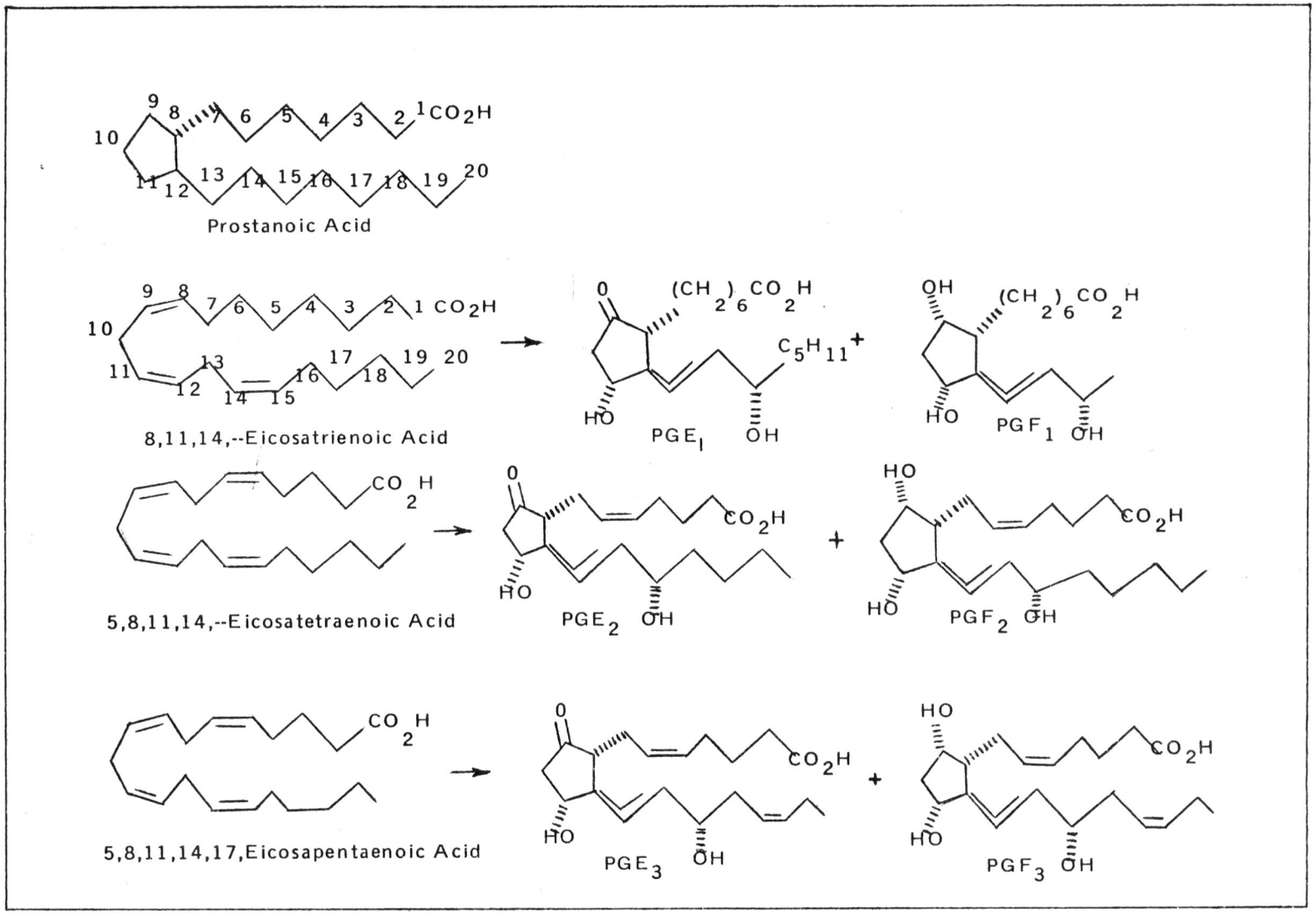


Table 2

The widespread presence of prostaglandins was matched only by the wide range of reactions that the chemicals were found to affect. One of the first effects discovered was enormous smooth muscle tissue contraction. Small quantities of prostaglandins have been found to bring on contraction of uterus, jejunum, ileum, tracheal, stomach fundus and myometrial smooth muscle. Kurzrok and Lieb did extensive research on the contractility of uterine muscle from patients who had undergone successful pregnancies, and from those who had had a long history of sterility. This was a logical line of investigation due to the fact that the prostaglandins were first discovered in large concentrations in the seminal fluid and there was question as to their effect on the reproductive process. It was found that the uterine strips from patients who had undergone successful pregnancies responded in relaxation, but the uteri from women who had had a history of sterility were stimulated into steady and strong contractions with very small amounts of prostaglandins. The conclusion from these findings was that the seminal fluid was its high concentration of prostaglandins caused the myometrium of the sterile women to contract after copulation and this led to the expulsion of the zygote before implantation had occurred (Kurzrok and Lieb, 1967). Karim and Delvin discussed the origin of prostaglandins in the amniotic fluid and proposed that quantitation of prostaglandins in the fluid might allow prediction of labor. Of the E_1, E_2, E_3 and F_1 and F_2 classes

of prostaglandins, the latter two classes, F_1 and F_2, stimulate uterine muscle in vitro and it was found that these two classes were increased in amniotic fluid during labor. It was therefore surmised that the prostaglandins F_1 and F_2 worked in conjunction with the oxytocin built up to initiate partuition (Karim and Delvin, 1967). This original research led to extensive studies of prostaglandins as contraceptives which primarily led to an ex post facto type of birth control. It seemed that introducing large amounts of prostaglandins into the uterus would cause expulsion of the embryo.

From these early experiments with prostaglandins many new activities were discovered. It was found that PGF caused a rise in blood pressure, while PGE caused a lowering of blood pressure (Bergstrom and Sjovall, 1960 a&b). PGA_1 and PGA_2 were found to cause a lowering of blood pressure by direct peripheral arteriolar dilation associated with a reflex increase in cardiac output and a fall in total resistance (Lee et al, 1965). In addition it was found that infusion of PGA_1 or PGA_2 into a hypertensive patient would lead to an increase in renal plasma flow and sodium and water excretion, but that this subsequently fell when the blood pressure was reduced to a normatensive level. This research led to studies of the prostaglandins effects on ion transport and osmotic pressures. It was found that in the toad bladder PGE_1 inhibited the osmotic water flow response induced by vasopressin and theophylline but not by cyclic AMP.

In contrast to the effects of water flow, PGE_1 stimulated sodium transport across the bladder. This stimulation of transport was potentiated by the addition of theophylline, a finding that suggests an action of PGE_1 on adenyl cyclase and consequent stimulation of sodium transport by cyclic AMP. The fact that PGE_1 increased the amount of cyclic AMP in the transporting cells and stimulated adenyl cyclase in vitro led to this confirmation by Lipson in 1971.

Prostaglandins have been found to stimulate many parameters of thyroid gland metabolism, which may mean that the prostaglandins play a big role in the regulating of thyroid function. The method by which the prostaglandins effect the thyroid metabolism has been attributed to the adenyl cyclase-cyclic AMP system (Ahn and Rosenberg, 1970).

Prostaglandins have also been found to cause a decrease in gastric acid secretion and act as a potential mediator of the inflammatory response. Ramwell and Shaw employing a perfusion technique observed a reduction in gastric acid formation in rats with the use of PGE_1. Co-workers showed that pyloric-ligated rats with PGE_1 administered subcutaneously either by injection or infusion showed inhibited gastric acid secretion (Lippmann, W., 1970). These results were of great interest to there studying gastric ulcers, and the possibility of prostaglandin treatment for over-production of gastric juices is presently being researched. Kaley and Weiner researched the effect of PGE_1 on the inflammatory response.

They showed that very small doses of prostaglandins, notably PGE_1, would greatly increase vascular permeability in the skin. Crunkhorn and Willis showed that PGE and PGF compounds induced weal and flare responses in human skin (Crunkhorn and Willis, 1971). Beitch and Eakins in 1969 demonstrated that the intraocular injection of PGE compounds resulted in a marked increase in intraocular pressure, most likely mediated by an increase in the permeability of the blood-aqueous barrier. With these findings it was the conclusion of Kaley and Weiner in 1968 that the prostaglandins could reproduce some of the cardinal features of the acute inflammatory response, providing a possible explanation for the pathogenesis of inflammation.

The research mentioned above was of great help in the quest to understand the full range of effects of the prostaglandins; but most experiments with these ubiquetous fatty acids have been in the field of reproduction, especially the female reproductive system.

Early research with prostaglandins showed that these fatty acids had a negative effect in vivo on luteal activity in animals in a stage of active luteal metabolism and progesterone secretion (Pharriss and Wyngarden, 1969). Specifically it has been shown that $PGF_{2\alpha}$, $PGF_{1\alpha}$ and $PGE_{2\alpha}$ can cause rapid irreversible destruction of the progesterone synthesizing mechanism of luteal tissue of pregnant rabbits and rats (Gutknecht et al, 1970a), hamsters (Gutknecht et al,

1970b), and rhesus monkeys (Kirton et al, 1970), pseudo-pregnant rabbits and rats (Pharriss and Wyngarden, 1969) and cycling guinea pigs (Blatchley and Donovan, 1969). This research led to ideas of birth control in humans through induction of menses by removing the ovarian hormonal support with prostaglandin treatment. Another method of birth control would be an oxytocic-like effect on the uterus to deliver its contents. Knowing the smooth muscle contracting ability of the prostaglandins, research was led in a direction of uterine contraction with prostaglandin treatment. Kirton et al did research on rhesus monkeys to determine the pregnancy termination cause when monkeys were treated with prostaglandins (Kirton et al, 1970). The results were that in animals injected with $PGF_{2\alpha}$ prior to day 40, pregnancy termination was associated with a heavy bloody vaginal discharge. This effect was initiated within 48 hours after the first injection and diminished in flow for 3 or 4 days. Treatment at later stages of gestation was not accompanied by the heavy vaginal bleeding. In these animals the fetus and placenta were expelled intact, with no postpartum complications (Kirton et al, 1970). These results led to the conclusion that the method of termination differed for the monkeys treated in early pregnancy from the monkeys treated in later pregnancy. The hypothesis was that the early pregnancy terminations were due to a luteolytic effect while the termination in the older animals was due to an oxytocic-like effect

on the uterus. Uterine contractility was monitored, and it showed a marked increase in uterine activity after treatment with the prostaglandins. With the establishment of a strong oxytocic effect on the uterus by the prostaglandins it was evident that there was a strong possibility for birth control and abortion in human patients through use of these chemicals. Use of prostaglandins as abortifacients in humans was documented for more than 100 cases in 1970 (Wiqvist and Bygdeman, 1970; Karim and Filshie, 1970; and Embrey, 1970). Nearly all of these studies were conducted with intravenous infusions, which led to complete expulsion of the fetus and placenta. Karim demonstrated the same action with oral and vaginal application in the rhesus monkey. The interruption of human reproductive processes with prostaglandins by vaginal or oral administration would greatly enhance their usefulness as agents for widespread population control (Pharriss and Kirton, 1969).

The above mentioned research disclosed the wide range of activities attributed to the prostaglandins and also proved that the 20-carbon fatty acids could have even more activity in yet unresearched body functions.

The fact that prostaglandins are widespread throughout the body led to extensive research on the analysis of prostaglandins from tissue and serum samples. Several methods for reaching a quantitative figure on the amount of prostaglandin present in a sample have been discovered. Bioassays, thin

layer chromatography, gas chromatography, and mass spectrometry have all been used effectively to isolate and identify prostaglandins. The most specific method for detection of prostaglandins has been use of radioimmunoassay procedures. A radioimmunoassay of prostaglandins provides a simple and useful technique for identification of prostaglandins in tissue homogenates and serum. The first work on development of an immunoassay led to a method for detection of prostaglandins down to nanogram and picogram levels (Levine and Vunakis, 1970). Effectiveness of the immunoassay is limited only by the specificity exhibited by the antibodies toward their individual prostaglandins. Although some members of the prostaglandin family have different functional groups on the cyclopentane ring, others differ by the degree of unsaturation on the aliphatic chains or exist as isomers because of the multiple asymetric centers in the molecules. Therefore, if accurate determination of quantity for specific prostaglandins is required, the amount of cross-binding had to be determined. This was done in 1971 by Lawrence Levine, R.M. Cerrosek, and Helen Vunakis when they discovered that there was cross-reaction with the specific antibodies, but the anti-$PGF_{2\alpha}$ bound best with its homologous antigen $PGF_{2\alpha}$. There was some cross-reaction with $PGF_{1\alpha}$ but the percentage of cross-binding with the other prostaglandins, PGE_1, PGA_1, PGB_1 was negligible. Therefore with the use of anti-$PGF_{2\alpha}$ a prostaglandin of F type could be identified as $PGF_{1\alpha}$ or $PGF_{2\alpha}$

although the only structural differences are the number of double bonds in the side chain. With the specificity of anti-$PGF_{2\alpha}$ established, it became one of the primary substances used in the research of prostaglandins. With better methods of determining presence of prostaglandins, further research was made to determine exactly where these chemicals were found and in what concentrations. The research proved that prostaglandins were present in nearly all types of body tissues, but more importantly it was found that they can be produced in several areas. Distelkotter and Vogt found in 1968 that the release of prostaglandins from isolated gastrointestinal tracts of frogs was estimated and compared with the prostaglandin content of the tissue. During the one hour the intestinal tract liberated about four times as much prostaglandin into the surrounding bath fluid as was contained in the tissue. There was a formation de novo during the incubation. The formation of prostaglandin was increased by immersing the intestine into distilled water instead of Ringer's solution, and it was inhibited with cocaine. This indicated that the biosynthesis of prostaglandin was variable and could be enhanced by stimuli from the nervous system (Distelkotter and Vogt, 1968). The fact that several body tissues were able to produce prostaglandins, and the fact that the prostaglandins were found in the bath solution of the above mentioned research led interested scientists to the conclusion that the blood probably carried prostaglandins.

It was logical to conclude that if the de novo produced prostaglandins were found in the bath solution in vitro, then the prostaglandins produced in vivo could very well be released into the blood plasma.

Unger, Stamford, and Bennett in 1968 devised a method of extracting foreign prostaglandins from human blood and with better quantitative methods of determining their presence they were able to detect small quantities of these fatty acids in all fresh blood samples. With these new techniques of determining the presence of prostaglandins in microquantities, researchers were able to establish a standard for the venous blood content of male humans. Results indicated that for a normal healthy adult male the amount of venous blood prostaglandins was 1.6± 0.9 ng/ml(Unger et al, 1968). This indicated a very small quantity with a fairly large variance, but it did establish the fact that blood serum contained the prostaglandins.

The fact that many body tissues could produce prostaglandins and that the serum level remained extremely low led to the question; "Why doesn't the level of serum prostaglandin increase with the tissue production of prostaglandins?" This question was answered by several different researchers. Hamberg and Samuelsson in 1969 found that when 17, 18 - ^{3}H - PGE_2 was injected intravenously into male subjects, about 50% could be recovered in the urine during the first 5 hours and less than 3% during the following 12 hours

(Hamberg and Samuelsson, 1969). In 20 hours there had been 53% in the urine and 10% in the feces. High concentrations of tritium were found in the kidneys and liver, though the concentrations found in the lungs, adrenals, ovaries, uterus and the pituitary gland were only slightly above the plasma level. Further studies with the labelled PGE_1 in the rat showed that in as little as 2 days the PGE_1 was completely metabolized to more polar derivatives. This research showed that the prostaglandin level in the blood stream was kept at a fairly constant value by the fact that newly produced prostaglandins were being metabolized at a rate very similar to the rate of production, allowing very little build up of prostaglandins in the blood serum. The rapid biological inactivation of the prostaglandins seemed to preclude action of these fatty acids in distant organs and favored the thought that the actions take place at the site of synthesis. The fact of the wide distribution of prostaglandin dehydrogenase led to the above mentioned hypothesis (Samuelsson et al, 1970).

The research done on prostaglandins has been varied and intense over the last 20 years, but until this date there had been no research on the prostaglandin content in immature specimens. Extensive research has been carried out on the prostaglandin level in tissue and blood of adult frogs, humans, rats, monkeys and many other animals as well as research on the prostaglandin build up in the amniotic fluid

before parturition. All this research has been of extreme value to the better understanding of the location and action of the prostaglandins but there remains the question of the functions of prostaglandins between birth and adulthood. The fact that prostaglandins seemed to be so imminently involved with the reproductive system naturally raised questions as to what occurred at the critical time of puberty.

The purpose of the following experiments was to study the relationship between $PGF_{2\alpha}$ and maturity onset through the use of a radioimmunoassay. The procedure was that of examining rabbits of varying ages from newborn to adulthood and determining the blood serum prostaglandin level from each age group. This would suggest existing correlations between age and serum prostaglandin levels.

MATERIALS AND METHODS

BINDING PERCENTAGES AND PROCEDURES

To obtain a radioimmunoassay sensitive enough to detect nanogram quantities of prostaglandins, it was necessary to determine the binding power of the antiserum. The initial antiserum was furnished generously by Dr. J. E. Pike of the Upjohn Company, Kalamazoo, Michigan. The anti-$PGF_{2\alpha}$ was produced in goats by injections of prostaglandin $F_{2\alpha}$ which was conjugated to a larger protein carrier molecule. The anti-$PGF_{2\alpha}$ antibody was isolated and lyophilized. This antibody was used in conjunction with a second antibody, lyophilized anti-goat IgG, purchased from Miles Laboratories.

The anti-goat IgG was reconstituted with 5 ml. of sterile distilled water and was diluted with Tris-HCl buffer (ph 8.00) to the following dilutions: 1-5, 1-8, 1-10, 1-12, 1-14, and 1-16. The anti-$PGF_{2\alpha}$ was reconstituted in distilled water in the ratio of 83 ng/ml which equaled serum concentration. The solution was further diluted with Tris buffer to the following dilutions: 1-100, 1-200, 1-300, 1-500, 1-750, and 1-1000. Each dilution of anti-goat IgG was then matched in duplicate to each dilution of anti-$PGF_{2\alpha}$ to test for the best binding solutions.

The samples were prepared by pipetting .5 ml of a dilution of anti-$PGF_{2\alpha}$ into a test tube, .2 ml of Tris buffer and .1 ml of a dilution of tritiated $PGF_{2\alpha}$. The tritiated $PGF_{2\alpha}$

was purchased from New England Nuclear and had a specific activity of 10.7 c/mM. The $^{3}H\text{-}PGF_{2\alpha}$ solution was prepared by placing 10 lambdas of the $^{3}H\text{-}PGF_{2\alpha}$ in 50 ml of Tris buffer. The control tubes were prepared by adding .7 ml of buffer to .1 ml of the $^{3}H\text{-}PGF_{2\alpha}$ solution. All the tubes were allowed to incubate at 23°C for one hour. Following the one hour incubation period, .1 ml of a dilution of anti-goat IgG was added to all the sample tubes and .1 ml of buffer was added to the control tubes. All the tubes were then allowed to incubate overnight at 4°C. After incubation all the tubes were placed in an International centrifuge with a multi-tube head and spun at 2500 R.P.M. for twenty minutes. Upon completion of centrifugation the tubes were carefully removed and the supernate from each tube was placed in a corresponding plastic scintillation vial. Care was used in removing the .5 ml of supernate so as not to accidently decant any of the pale white precipitate on the bottom of the tubes. The flours solution was made with .5 grams of dimethyl PPO (Packard) and .2 grams of POPOP (Packard) in one liter of toluene. The presence of water in the samples necessitated the use of the detergent Triton-X (Packard) to prevent cloudiness. The toluene solution was mixed with the Triton-X in a ratio of 2 to 1 respectively. The resulting solution was placed in a 20 ml repipet from Labindustries. Ten ml. of the scintillation flours was added to each of the scintillation vials which contained the supernates from the samples

and the controls. The vials were then placed in a Packard Tri-carb scintillation counter. The window settings were 0.5-10 and the gain was set at 7% which allowed maximum counts. Each vial was placed in the counter for several 5-minute runs, while the results were recorded on the machine tape. The results were obtained from the tape and recorded in graphic form, Appendix 1. Calculations were made to obtain a percentage binding for each combination of anti-$PGF_{2\alpha}$ and anti-goat IgG. These calculations demonstrated clearly where the largest amount of binding occurred. The vials with the highest counts were the control vials where no binding occurred due to the fact that no antibodies were present. All other vials showed a lower number of counts as a result of the antibodies precipitating the ^{3}H-$PGF_{2\alpha}$ and decreasing the amount of free ^{3}H-$PGF_{2\alpha}$ in the supernate.

The same procedures were followed again with anti-goat IgG dilutions of 1-0, 1-2, 1-4, 1-5, and anti-$PGF_{2\alpha}$ dilutions of 1-100, 1-500, and 1-1000. The number of counts per minute was increased by increasing the concentration of ^{3}H-$PGF_{2\alpha}$ to 10 lambdas in only 40 ml Tris Buffer. This procedure led to the concentration combinations that produced the greatest percentage of binding and therefore the lowest number of counts in relation to the control vials, Appendix 2.

PROCEDURES FOR THE PRODUCTION OF THE STANDARD CURVE

To determine the effectiveness of the assay it was necessary to test the antibodies against known concentrations of prostaglandins. Ten milligrams of $PGF_{2\alpha}$ was donated by the Upjohn Company for this purpose. Prostaglandins are long chain fatty acids that are very unstable in conditions where the pH is >8.5 or <3. To insure stability the stock solution was made by reconstituting 4 mg of the $PGF_{2\alpha}$ in 4 ml of 95% ethanol. With this as a stock solution, dilutions were prepared with Tris buffer up to 10, 20, 30, 40, and 50 ng/ml. All solutions were first placed under a stream of Nitrogen gas and then frozen when not in use.

The sensitivity of the assay was ascertained by determining how dilute a solution of unlabelled $PGF_{2\alpha}$ could be used and still display appreciable inhibition of the $^{3}H\text{-}PGF_{2\alpha}$ antibody binding. The greater the amount of unlabelled $PGF_{2\alpha}$ the higher the counts would be due to increased competition between the labelled and unlabelled $PGF_{2\alpha}$ for the binding sites on the antibodies.

The tubes for these tests were prepared by placing .5 ml of the 1-500 dilution of anti-$PGF_{2\alpha}$ in a tube with .1 ml of Tris buffer and .1 ml of a dilution of unlabelled $PGF_{2\alpha}$. Each solution of unlabelled $PGF_{2\alpha}$ was tested with antibodies and each test was run in duplicate. A blank tube was prepared with just $^{3}H\text{-}PGF_{2\alpha}$ and Tris buffer, and the duplicate control tubes were prepared with the antibodies and the

$^{3}H\text{-}PGF_{2\alpha}$ but no unlabelled $PGF_{2\alpha}$. The tubes were allowed to incubate at 23°C for one hour before .1 ml of a 1-2 dilution of anti-goat IgG was added to all the tubes except the blanks which received another .1 ml of Tris buffer. All tubes were then incubated overnight at 4.0°C before they were placed in the centrifuge and spun at 2500 R.P.M. for twenty minutes. Five tenths ml of the supernate was then carefully pipetted from each tube and placed in appropriately marked scintillation vials. Following the addition of the flours solution the vials were placed in the scintillation counter for several 5-minute periods. The amount of inhibition the unlabelled $PGF_{2\alpha}$ had on the $^{3}H\text{-}PGF_{2\alpha}$ could easily be seen as a straight line correlation when plotted on graph paper. The correlation was a direct one in which the greater inhibition of the $^{3}H\text{-}PGF_{2\alpha}$ antibody binding and thus higher counts. As can be seen in Appendix 3, the portion of the curve with the greatest sensitivity was in solutions of unlabelled $PGF_{2\alpha}$ with concentration between 0 and 10 ng/ml. This area of highest sensitivity was examined to a greater extent by making 1, 2, 3, 4, and 5 ng/ml dilutions of the unlabelled $PGF_{2\alpha}$ and repeating the same procedure as above with the results again a straight line as shown in Appendix 4. The sensitivity of the curve remained high and the final standard dilutions of 2, 4, 6, 8, and 10 ng/ml were tested and used for the experimentation procedures with the rabbit serum.

PROCEDURES WITH RABBIT SERUM

The actual testing procedures for the determination of the amount of prostaglandin in the blood serum was similar to the procedure for obtaining the standards.

Blood was drawn from the lateral ear veins of two male rabbits of each age tested above one month. The rabbits below one month of age were bled by cardiac puncture.

The first actual test of serum prostaglandin level was done with blood from two, six month old and two, one month old New Zealand White rabbits. The fresh drawn blood was placed in culture tubes, allowed to clot and then centrifuged at 1500 R.P.M. for twenty minutes. The serum was then decanted.

The prostaglandins have a molecular weight of 310-340 and this was helpful in the purifying process. The serum was placed in an Amicon 50 automatic dialysis apparatus to remove any molecules with a molecular weight above 10,000. This procedure was followed in order to remove free hemoglobin in the serum as the pigment was found to interfere with the effectiveness of the scintillation counter.

The test for the serum prostaglandin level was done in a similar manner as the standards test. Two blank tubes were prepared with .7 ml of Tris buffer and .1 ml of $^{3}H\text{-}PGF_{2\alpha}$. Two control tubes of Tris buffer, $^{3}H\text{-}PGF_{2\alpha}$, anti-$PGF_{2\alpha}$ and anti-goat IgG were prepared as before, and the standard curve was obtained by preparing two tubes with each of the standard

unlabelled $PGF_{2\alpha}$ dilutions which were 2, 4, 6, 8, and 10 ng/-ml. Each sample was measured in duplicate. The tubes with serum were prepared by placing .1 ml buffer, .1 ml serum, .1 ml of $^{3}H\text{-}PGF_{2\alpha}$ and .5 ml of a 1/500 dilution of anti-$PGF_{2\alpha}$ in each tube. It was necessary to place the antibody in the tube last so as not to allow premature binding to the $^{3}H\text{-}PGF_{2\alpha}$ or the unlabelled prostaglandins in the blood serum. If the antibody had been dispensed first and the other chemicals added in sequence it would not have been a valid testing procedure. All tubes were allowed to incubate for one hour at $23^{\circ}C$ before one tenth ml of a 1-2 dilution of anti-goat IgG was placed in all the tubes except the blank tubes which received .1 ml of Tris buffer. The tubes were then incubated overnight at $4^{\circ}C$. They were then centrifuged at 2500 R.P.M. for twenty minutes. Five tenths ml of the supernate was then carefully pipetted off of each tube and placed in the scintillation vials with 10 ml of the flours solution.

The vials were then placed in the scintillation counter for overnight counting and the resultant data was then compiled and averaged. The vials were counted ten times and each test was run in duplicate. All three windows were used on the counter; therefore, allowing three figures for each tube on each five minute run which gave approximately thirty figures to average for each tube. This procedure allowed the determination of the amount of free prostaglandin in the blood serum from the rabbits of one and six months of age.

Each time blood serum was tested, a new standard curve was produced to avoid error due to over-generalization and chemical breakdown of the solutions in use.

With successful completion of the tests on the one and six month old rabbits, it was necessary to conclude the experiments with blood serum tests on rabbits of varying ages. Rabbits were tested at the ages of one through seven months at one month intervals. The same rabbits were used for tests at different ages where possible. The rabbits tested at one and six months were in turn tested again at the ages of two and seven months, etc. Serum tests were run on very young rabbits of 1, 2, 3, 4, 5, 10, 15, 20, and 25 days of age; and 2 three year old male breeders. Each serum sample was plotted against a standard curve produced during the same test to indicate the concentration of prostaglandin in the peripheral blood serum of the rabbits at each age tested. These graphs are recorded as Appendices 5 through 8.

RESULTS

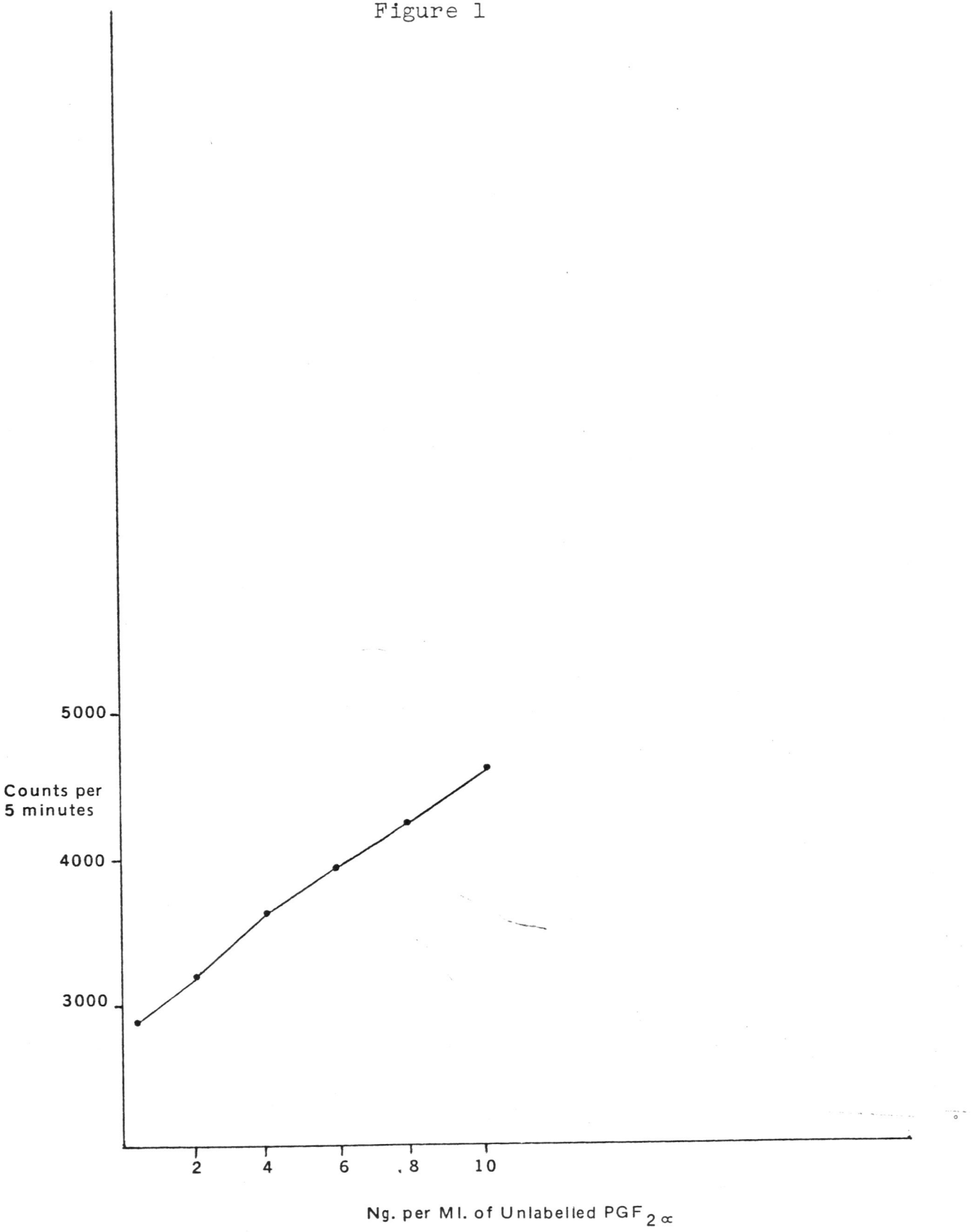
The first assay with the $^{3}H\text{-}PGF_{2\alpha}$ the anti-$PGF_{2\alpha}$ and the anti-goat IgG was an effort to find the dilutions of the antibodies best suited for a binding mechanism of maximum percentage. The dilutions of 1:5, 1:8, 1:10, 1:12, 1:14, and 1:16 anti-goat IgG were combined with the anti-$PGF_{2\alpha}$ dilutions of 1:100, 1:200, 1:300, 1:500, 1:750, and 1:1000. The antibodies were mixed in all possible combinations with 1/10 ml of a dilution of $^{3}H\text{-}PGF_{2\alpha}$ and the resulting data from the Packard Tri-carb counter was plotted on (Figure 1 & 1A). The resultant line showed the greatest percentage of bound $^{3}H\text{-}PGF_{2\alpha}$ to be in the tubes containing the anti-$PGF_{2\alpha}$ dilution of 1:1000 and the anti-goat IgG dilution of 1:5. Following the above mentioned experiment it was decided to try the same method again but with different dilutions. In this experiment the anti-goat IgG prepared with 1:0, 1:2, 1:4, and 1:5 dilutions which were matched with 1:100, 1:500, and 1:1000 dilutions of anti-$PGF_{2\alpha}$. In order to increase the number of counts involved, the $^{3}H\text{-}PGF_{2\alpha}$ was prepared with 10 lambdas in 40 ml of distilled H_2O instead of 50 ml. The counts were recorded from the scintillation counter and prepared in graphic form, Appendix 2. The resulting line indicated the best combination of antisera would be the 1:2 dilution of anti-goat IgG with the 1:500 dilution of anti-$PGF_{2\alpha}$. These dilutions were used for the rest of the experiments due to the high binding potential.

The standard curve was produced with the use of the antibody dilutions decided on and explained in the above section. In order to know the effectiveness of these antibodies it was necessary to test these antibodies against known concentrations of prostaglandins. A stock solution of $PGF_{2\alpha}$ was diluted to concentrations of 10, 20, 30, 40, and 50 ng/ml. The resulting counts showed an expected linear increase in counts with an increase in concentration of unlabelled $PGF_{2\alpha}$, Appendix 3. The increased amount of unlabelled $PGF_{2\alpha}$ increased the competition with the $^{3}H\text{-}PGF_{2\alpha}$ for binding sites on the anti-$PGF_{2\alpha}$. This fact was evidenced by the increased number of counts recorded in the vials with the higher concentrations of unlabelled $PGF_{2\alpha}$. The increased number of counts in the supernate was due to the fact that more unlabelled $PGF_{2\alpha}$ was binding to the anti-$PGF_{2\alpha}$ producing the precipitate and; therefore, leaving more $^{3}H\text{-}PGF_{2\alpha}$ in the supernate. The range of counts per 5 minutes ranged from 617, the number of counts obtained from the tubes with no unlabelled $PGF_{2\alpha}$ to 1308, the number of counts in the vial with no antibodies. The greatest increase in counts occurred in the range from 0-10 ng/ml of unlabelled $PGF_{2\alpha}$. The 186 count increase in this range represented 41.64% of the total increase of 449 counts over the entire range from 0-50 ng/ml of unlabelled $PGF_{2\alpha}$. These figures were noted and it was evidenced that the most sensitive portion of the curve was that part between 0-10 ng/ml of unlabelled $PGF_{2\alpha}$. The

report on "The Extraction of Prostaglandins from Human Blood" by Unger, Stamford and Bennett in 1971 showed that the average amount of prostaglandin per ml of blood in the human was 1.6± .9 ng/ml and thus it was necessary to set up more dilutions to make the assay more specific. Subsequent dilutions of the unlabelled $PGF_{2\alpha}$ were made at concentrations of 1, 2, 3, 4, and 5 ng/ml. The counts were unmistakably linear in nature as was evidenced by the data represented in Appendix 4. The possibility of a high concentration of prostaglandin in the serum from the young rabbits was noted and this was rectified with the test being run again with unlabelled $PGF_{2\alpha}$ dilutions of 2, 4, 6, 8, and 10 ng/ml. The counts again resulted in a linear direct proportion of higher counts with a higher concentration of unlabelled $PGF_{2\alpha}$, Figure 1. The above mentioned dilutions were settled upon as the dilutions to develop the standard curve used with each test on blood serum.

The first actual test of the rabbit serum was run with 4 male rabbits, 2 each from the ages of 1 month and six months. Two samples of serum were extracted from each rabbit giving 8 test tubes of serum. The serum samples were run against the standard dilutions of unlabelled $PGF_{2\alpha}$ and the Blanks with no antibodies present. The range of counts was from 2167 counts per 5 minutes with no unlabelled $PGF_{2\alpha}$ and the antibodies present, to 5762 counts per 5 minutes with no antibodies. The standard curve ran from 2167 counts/5 minutes

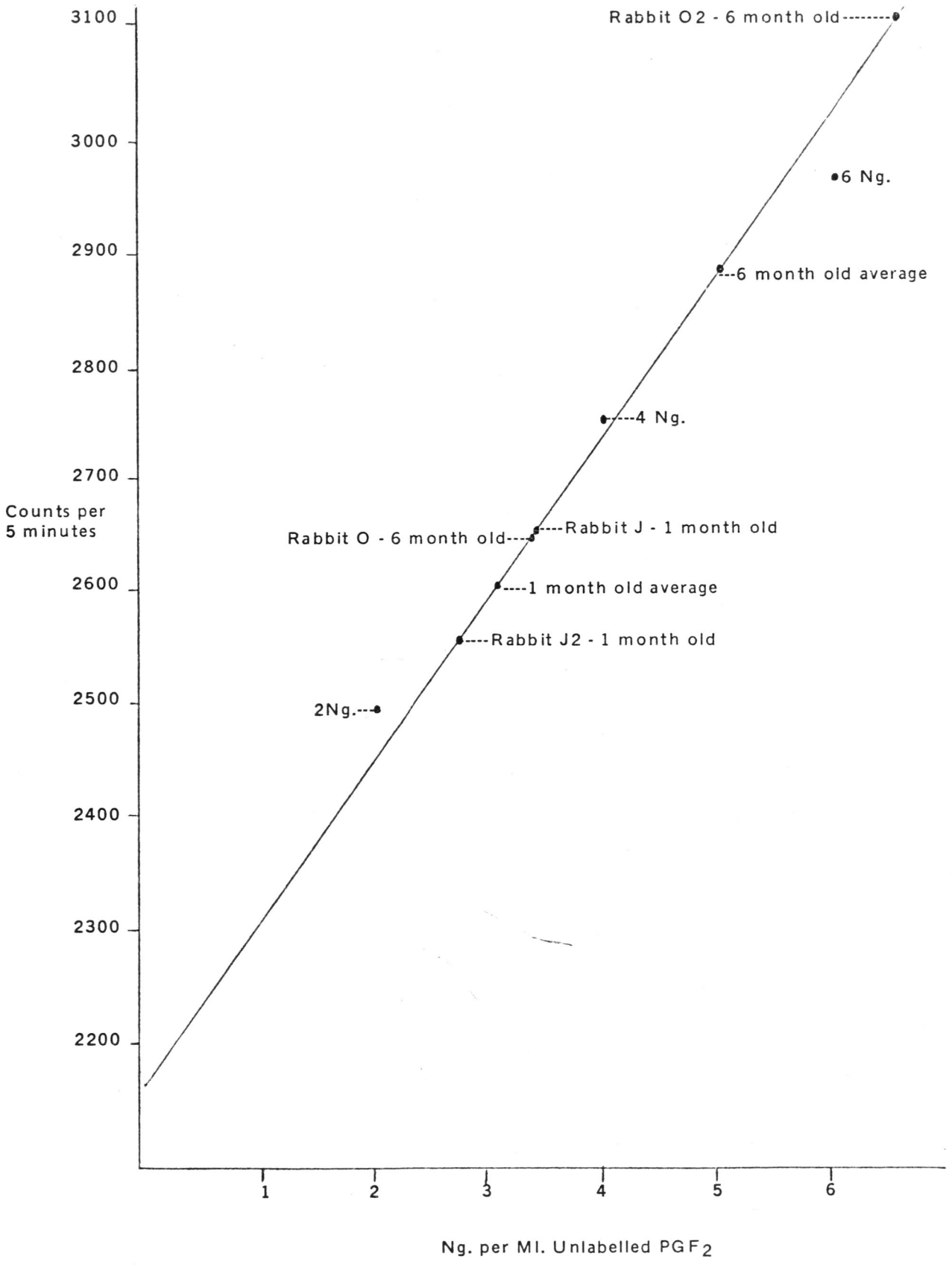
Figure 1


5000
4000
3000
Counts per
5 minutes
2
4
6
8
10
Ng. per Ml. of Unlabelled PGF 2 α

with .1 ml of an 8 ng/ml dilution of unlabelled $PGF_{2\alpha}$. The average counts/5 minutes for the 2 tubes of the first 1 month old rabbit (Rabbit J) was 2649 C/5M while the average for the tubes of second 1 month old rabbit (Rabbit J2) was 2555 C/5M for an overall average of 2602 counts/5 minutes. The 2602 C/5M average of the 1 month old rabbits corresponded to 3 ng/ml on the standard curve. Rabbit J had a prostaglandin level of 3.35 ng/ml while Rabbit J2 had a blood serum prostaglandin level of 2.7 ng/ml for a deviation of +.3 ng/ml, Figure 2. The four tubes prepared from the 6 month old rabbit serum yielded an encouraging high average count of 2882 C/5M with the first six month old rabbit (Rabbit O) having 2646 C/5M and the second (Rabbit O2) having 3118 C/5M. The average figure of 2882 C/5M corresponded with 5.0 ng/ml while Rabbit O represented 3.3 ng/ml and Rabbit O2, 6.5 ng/ml for a very high deviation of +1.7 ng/ml.

Following the tests with the 1 and 6 month old rabbits the same procedure was followed with 3 and 4 month old rabbits with the expectation of a linear upsweep from the 1 month average of 3 ng/ml to the 5 ng/ml average of the 6 month old rabbits. The tests placed the 3 month old rabbits average of prostaglandin in blood serum at a relatively high level of 6.1 ng/ml, while the average blood prostaglandin content of the 4 month old rabbits was 1.9 ng/ml, Appendix 5. The high concentration determined to be the blood serum prostaglandin mark in the 3 month old rabbits made it necessary

Figure 2


3100
3000
2900
2800
2700
2600
2500
2400
2300
2200
Counts per 5 minutes
Rabbit O2 - 6 month old
6 Ng.
6 month old average
4 Ng.
Rabbit O - 6 month old
Rabbit J - 1 month old
1 month old average
Rabbit J2 - 1 month old
2Ng.
1
2
3
4
5
6
Ng. per Ml. Unlabelled PGF2

to run additional 3 month and 4 month old rabbit experiments with the tests on the 2 and 7 month old rabbits. The 7 month old rabbits yielded an average prostaglandin content of 4.65 ng/ml and the 2 month old rabbits indicated a 2.1 ng/ml content. The 3 and 4 month old rabbits included in this experiment were different rabbits than the 4 rabbits used in the preceeding test, but even though the rabbits were identical in age and sex (male), the results were startling different. The 4 month old rabbits showed an average of .65 ng/ml with a deviation of only .15 ng/ml, a considerable decrease from the 1.9 ng/ml mark recorded in the last experiment. Similarly the 3 month olds proved to have less prostaglandin content than the 3 month olds of the first experiment. The average of 4 ng/ml was a full 2.1 ng/ml lower than the results from the preceeding experiment even though the deviation was only .1 ng/ml, Appendix 6.

Similar experiments were conducted on rabbits of 1, 2, 3, 4, 5, 10, 15, 20, and 25 days as well as on 5 month old rabbits and 2 year old rabbits. The results were placed in graphic form in Appendices 7 and 8.

Upon completion of the experiments, it became obvious that the prostaglandin concentration was varying at different ages. Figure 3 contains the code names of all the rabbits tested, their ages, their average $PGF_{2\alpha}$ concentration and the deviations between the rabbits of the same age. The average prostaglandin level for the rabbits of each age was tabulated

PROSTAGLANDIN CONCENTRATIONS IN TEST RABBITS

Figure 3

NAME	AGE	AVERAGE $PGF_{2\alpha}$ CONCENTRATION	DEVIATION
Rabbits A & A2	1 day	2.90 ng/ml	± .10 ng/ml
Rabbits B & B2	2 days	3.35 ng/ml	± .15 ng/ml
Rabbits C & C2	3 days	2.20 ng/ml	± .20 ng/ml
Rabbits D & D2	4 days	0.85 ng/ml	± .20 ng/ml
Rabbits E & E2	5 days	3.10 ng/ml	± .40 ng/ml
Rabbits F & F2	10 days	1.75 ng/ml	± .45 ng/ml
Rabbits G & G2	15 days	4.50 ng/ml	± .20 ng/ml
Rabbits H & H2	20 days	4.75 ng/ml	± .75 ng/ml
Rabbits I & I2	25 days	4.90 ng/ml	± .30 ng/ml
Rabbits J & J2	1 month	3.00 ng/ml	± .30 ng/ml
Rabbits K & K2	2 months	2.10 ng/ml	± .90 ng/ml
Rabbits L & L2	3 months	5.05 ng/ml	± 1.15 ng/ml
Rabbits M & M2	4 months	1.25 ng/ml	± .75 ng/ml
Rabbits N & N2	5 months	0.70 ng/ml	± .05 ng/ml
Rabbits O & O2	6 months	5.00 ng/ml	± 1.70 ng/ml
Rabbits P & P2	7 months	4.65 ng/ml	± 0.15 ng/ml
Rabbits Q & Q2	2 years	1.00 ng/ml	± 0.05 ng/ml

in Figure 4 with the results being an array of peaks and valleys rather than the anticipated linear increase of $PGF_{2\alpha}$ concentration with age. The data shows the low point of serum prostaglandin level to be that of the 5 month old rabbits at .7 ng/ml, and the high point to be 5.05 ng/ml for the 3 month old rabbits.

The above information led to the results tabulated in Appendix 9 showing the averages and the deviations of each set of rabbits tested.

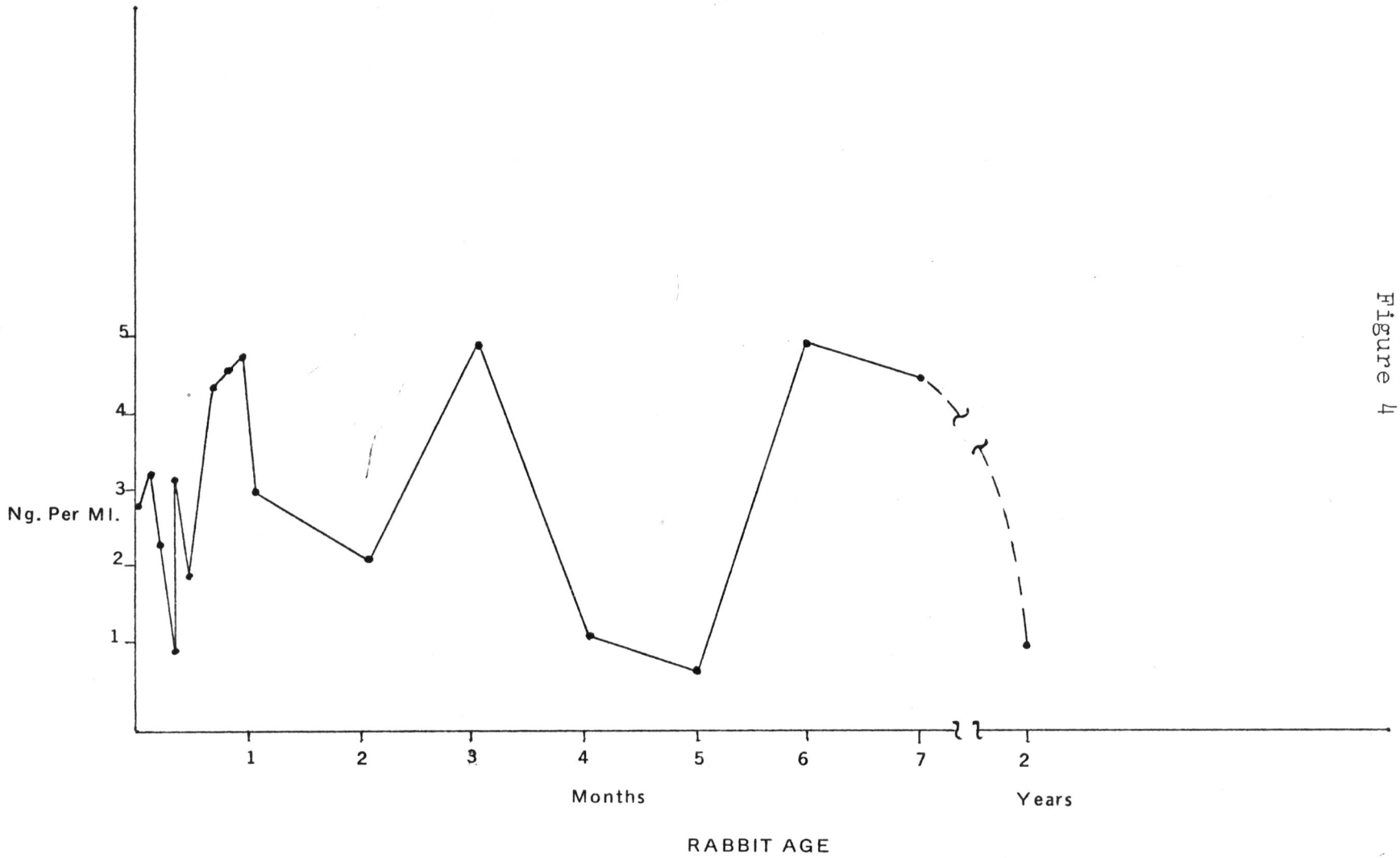


Figure 4

DISCUSSION AND CONCLUSION

The results of the experiments tended to show a variety of points, but the final graph was not linear as predicted.

The results from the very young rabbits showed an absolute trend; however, with the exception of the five day old rabbits, there was a tendency to decrease to a low of .6 ng/ml at 4 days and then reach another high of 4.85 ng/ml at 25 days. The prostaglandins, not being extremely large molecules could be passed to the fetus through the placenta or transferred to the newborn in the milk. With the following in mind a high concentration of prostaglandin immediately following birth could be explained as maternal prostaglandin. The results did tend to substantiate the hypothesis with the 1 and 2 day old rabbits having fairly average concentrations of 2.9 ng/ml and 3.35 ng/ml respectfully. If the majority of the maternal prostaglandin came from the mother transplacentally there should be a subsequent drop in prostaglandin level as the pseudohormone is broken down to 11-hydroxy-9,15-diketoprost-5-enoic acid. With the following in mind, the results from the 3 and 4 day old rabbits seemed to be fairly reasonable with a drop of 1.1 ng/ml from the second day reading to the third day and an additional drop of 1.55 ng/ml from the third day to the fourth. With the peripheral serum prostaglandin level at a low .85 ng/ml at 4 days, the fifth day readings should have either continued on a downward trend indicating the continued degradation of transplacental maternal

prostaglandin or increase as a result of the newborn rabbit producing its own prostaglandin. The latter was the case and the level increased to another peak of 3.1 ng/ml which could not be explained if transplacental maternal prostaglandin was the only factor involved.

Another possibility, however, did exist and that was the increase was due to maternal prostaglandin level in the milk. If colostrun carried prostaglandin was responsible for the sudden increase at the fifth day level, then it had to be supposed that readings up until that age were not effected by milk flow or the rabbit blood was taken at a time not closely following feeding. Since prostaglandin breakdown in the blood to arachidonic acid occurs only after around 3 days the following variable can be virtually eliminated with the supposition that there should have been an increase for the first three days followed by a leveling off effect as the amount of prostaglandin consumed equaled the amount being broken down in the body. With the above in mind, it would be expected that the next reading at the age of 10 days would not necessarily resemble that of the five day reading. This was the case with the level decreasing to a reading of 1.75 ng/ml. Until 10 days the average peripheral blood level of $PGF_{2\alpha}$ had not exceeded 3.75 ng/ml which occurred in the 2 day old and had not been below .85 ng/ml in the 4 day old. This information could not be totally explained by the maturity onset hypothesis, but neither could it totally

discredit the thought since the sexual maturity of the New Zealand White rabbit is approximately 5 to 6 months and the level could be much greater at these ages. The fact that the rabbits at the very young stages demonstrated a highly irregular concentration of serum prostaglandin did not necessarily indicate that there would be no leveling off at the more mature ages. The tests on serum from 15 day old rabbits showed a marked increase to 4.5 ng/ml, the highest level recorded. This was followed by levels of 4.75 ng/ml at 20 days and 4.95 ng/ml at 25 days. These data suggested a definite pattern of linear increase with age, however, a sudden drop at 1 month to 3 ng/ml and a further decrease to 2.1 ng/ml at 2 months was detected. The sudden decrease in serum prostaglandin level at these ages indicated that maturity onset was not the controlling factor in the amount of prostaglandin found in the blood. This still did not prove that there would not be a large increase at the age of 6 months. Two separate tests were run on the 3 month old rabbits with highly irregular results. One test indicated a 6.1 ng/ml level while the second test indicated a 4.0 ng/ml level. These data were very important in drawing conclusions from the experiments. With the wide discrepancy in the serum levels of the rabbits at the same age it became apparent that some other factor besides age determined the serum prostaglandin level. The four and five month old rabbits substantiated this conclusion with extremely low averages of 1.25 ng/ml and .7 ng/ml

respectively. The six month old rabbits were the key rabbits in the puberty phase of the maturity onset hypothesis and they demonstrated some interesting results. The highest serum level recorded for any one rabbit was 6.5 ng/ml which was one of the 6 month old specimens, but at the same time the serum level of the second specimen was 3.3 ng/ml which gave an average of 5.0 ng/ml with a high deviation of 3.2 ng/ml. The 5.0 ng/ml average was relatively high but the deviation was much too great for any final conclusion. One explanation could have been that one rabbit was reaching sexual maturity ahead of the other but other factors may have contributed to the problem. The seven month old rabbits produced relatively high reading of 4.65 ng/ml with a small deviation, but the final tests on the 2 year old breeder rabbits resulted in the final discreditation of the maturity onset hypothesis with an extremely low level of 1.0 ng/ml. The results, therefore, did not bare out the hypothesis, but the experiment was not a total failure because beneficial conclusions could be drawn from the data and interesting questions from this research could lead to important experiments in the field of prostaglandins.

Deviations of as high as 3.2 ng/ml from rabbits led to the conclusion that age was not the only factor involved in the level of prostaglandin in the blood, and this was substantiated further by the fact that the same animals tested at different ages showed no definite trend. These facts

tended to indicate that the level of prostaglandin in peripheral blood was controlled by things other than age and that there could be more than one variable regulating the level. The rabbits in these experiments were kept in a typical animal research facility and were all on the same feed. This eliminated the variable of different diets between the animals being a cause for the deviations in the results, and the fact that they were all in the same type enclosures eliminated the chance of being a different amount of room for exercise for greater metabolic rates. All the rabbits were kept in the same room to insure the same amount of light and dark ratio as to eliminate circadian rhythm as a primary cause of discrepancy. These facts tend to indicate that all the animals were identical but there were some things that could not be controlled in the experiment. The fact that the feed was presented in the typical continuous feed method would have left the question of when the specimen last fed unanswered as well as to when the specimen last drank from the continuous water supply. These factors could have contributed to the discrepancy, however, for the large deviations in the data, physiological differences in the animals at the time of blood collection was the probable cause. Some of the animals were very calm at the time of blood collection while other animals were extremely agitated and were necessarily restrained during the entire procedure. These different emotional states of the specimens could have led to the large

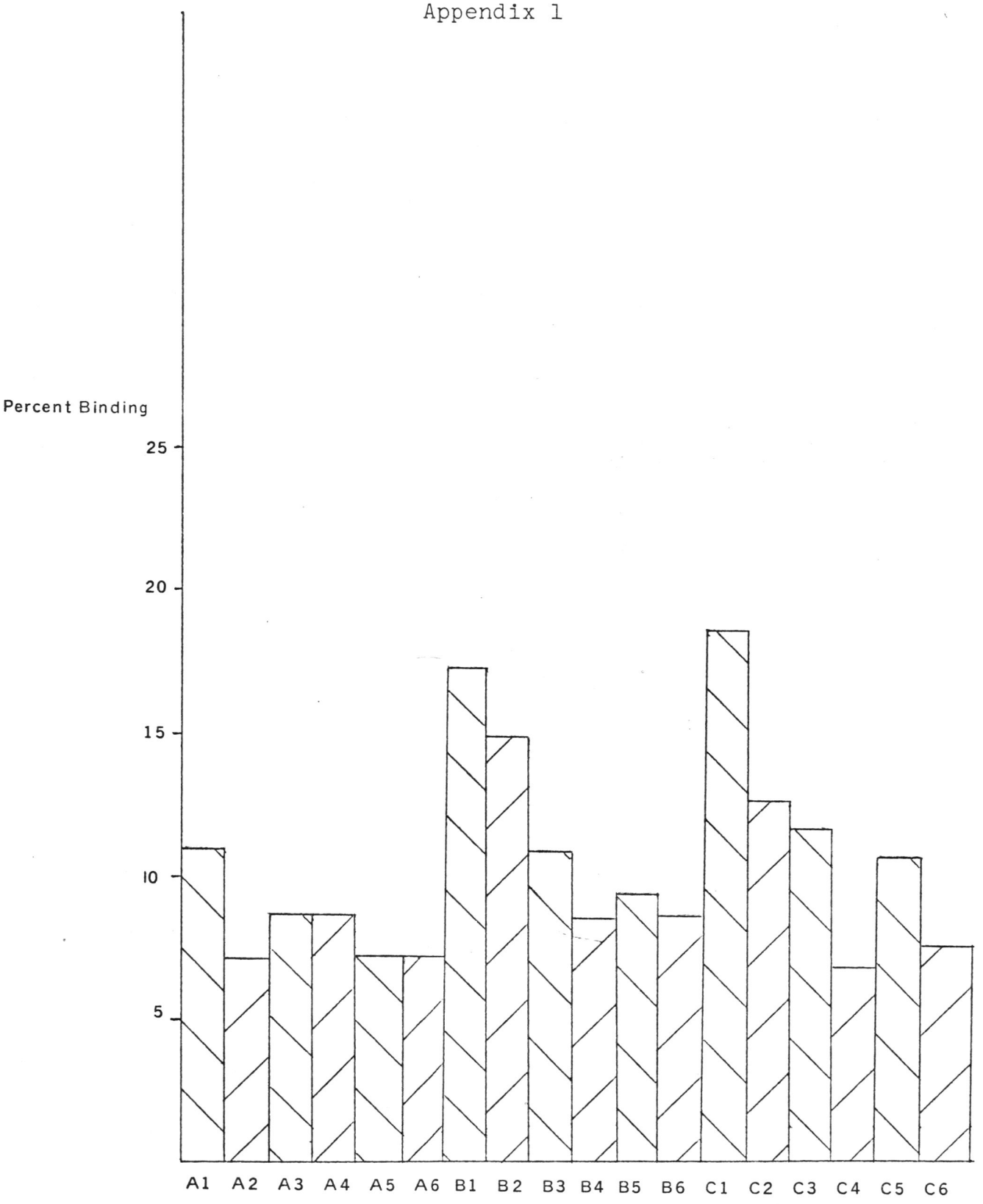
deviation by the differences that would have occurred in the bloodstream during stress conditions. During these stress situations many hormones would have been released into the bloodstream and this could have been the case with the prostaglandin. This would have given a high level in the agitated rabbits and low level in the docile animals. No information was kept on this relationship during the research, but this research could be done by comparing the blood serum prostaglandin level of specimens in a very agitated state against blood serum from animals in a very lethargic state. This research could be done with the use of an EKG to measure heart rate and the deliberate agitation of some animals. The agitation could be physical or drug induced in order to ascertain if the stress situations led to a higher level of prostaglandin in the blood serum.

The overall conclusion from the study was that the maturity onset hypothesis of increasing prostaglandin level with age until puberty was not correct and that several factors other than age were probably involved. The present work did indicate that the prostaglandin level was cyclic in nature rather than linear. This could be a result of physical factors such as developmental changes or physiological factors in relationship to heartbeat, stress, respiration rates, urine output and food and drink intake. Further research will have to be carried out before the question of existing controls for blood serum prostaglandin levels is completely answered.

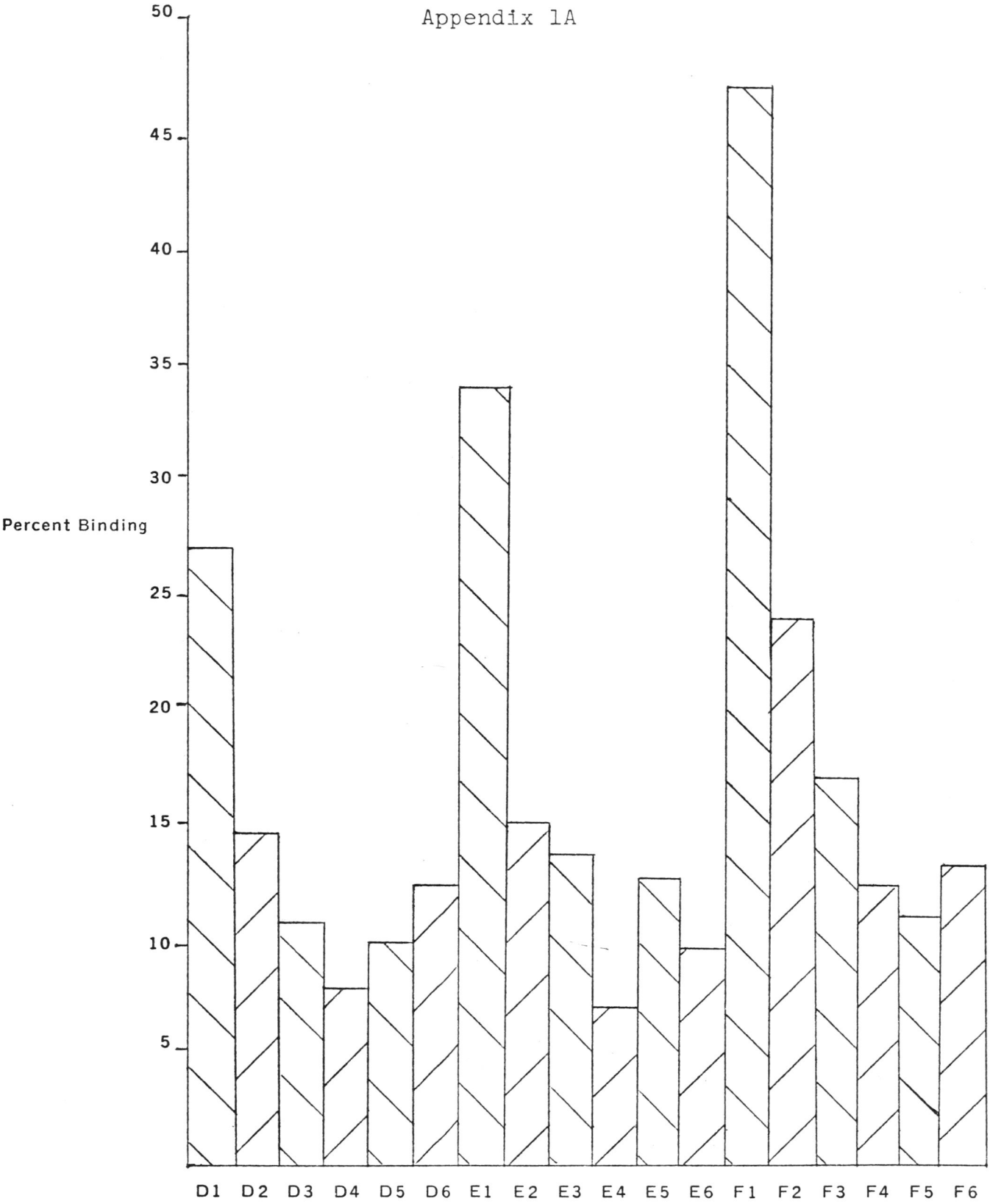
ANTIBODY DILUTIONS

Index (Appendices 1 & 1A)

Appendix 1


Percent Binding
25
20
15
10
5
A1
A2
A3
A4
A5
A6
B1
B2
B3
B4
B5
B6
C1
C2
C3
C4
C5
C6

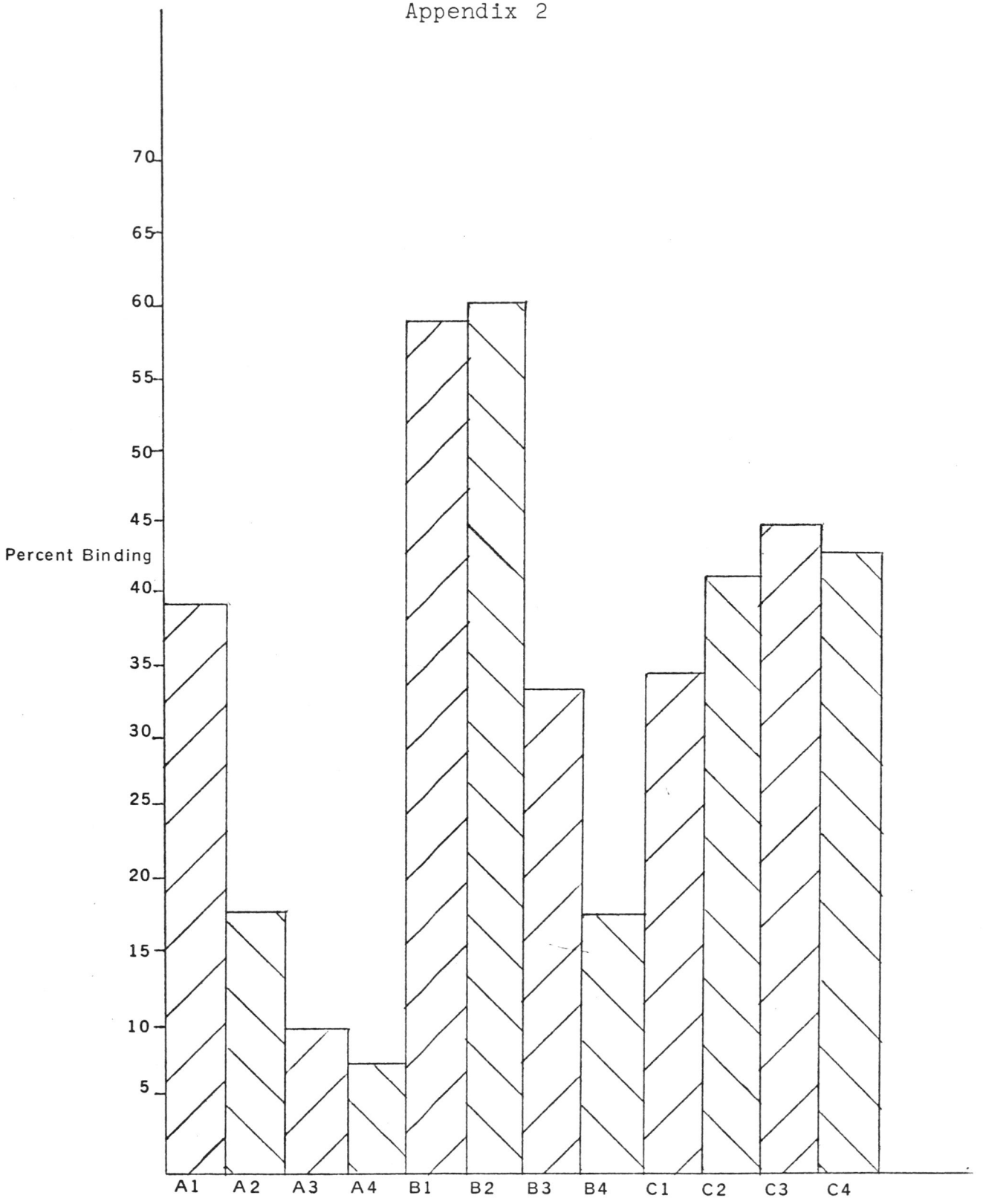
Appendix 1A


50
45
40
35
30
25
20
15
10
5
Percent Binding
D1 D2 D3 D4 D5 D6 E1 E2 E3 E4 E5 E6 F1 F2 F3 F4 F5 F6

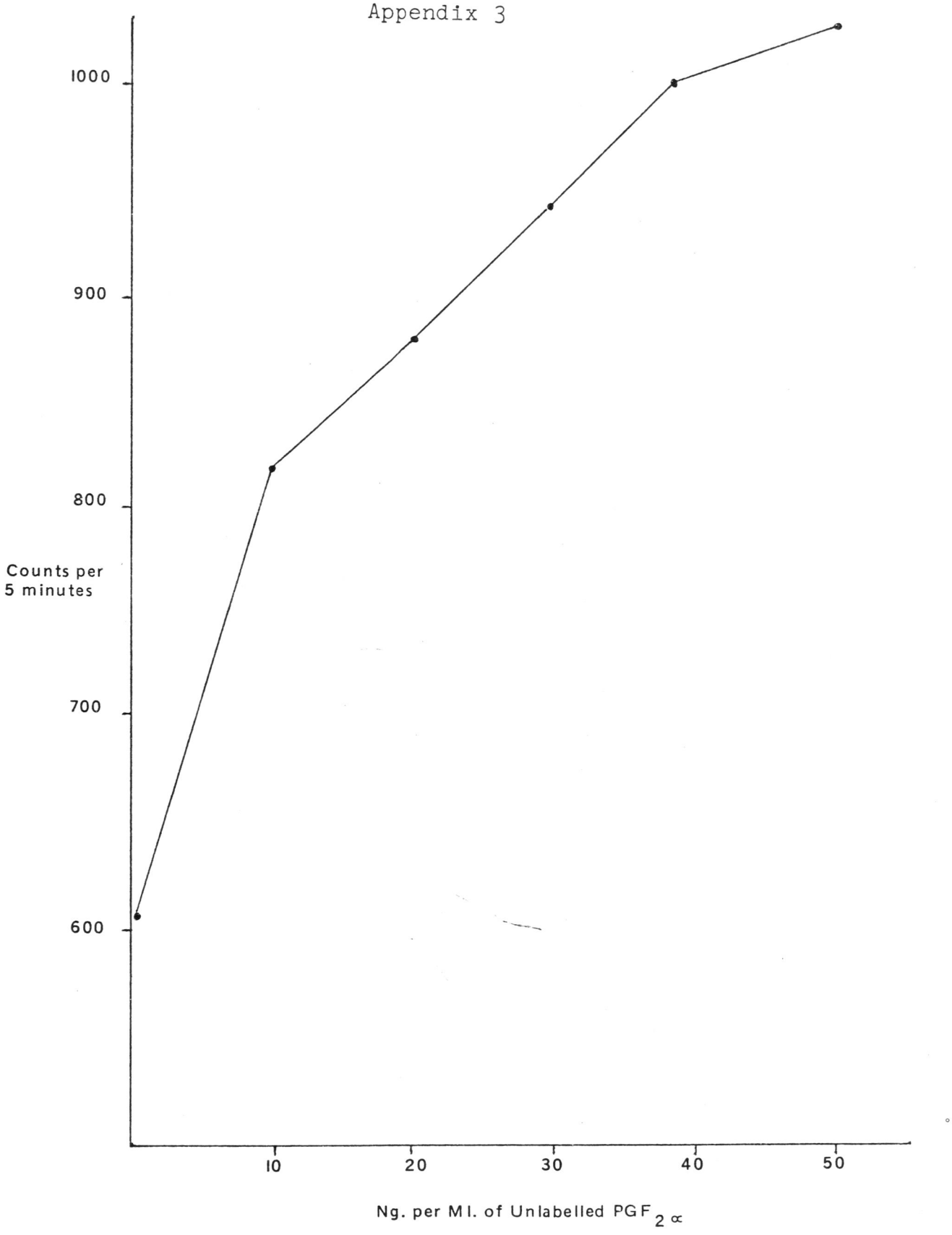
ANTIBODY DILUTIONS

Index (Appendix 2)

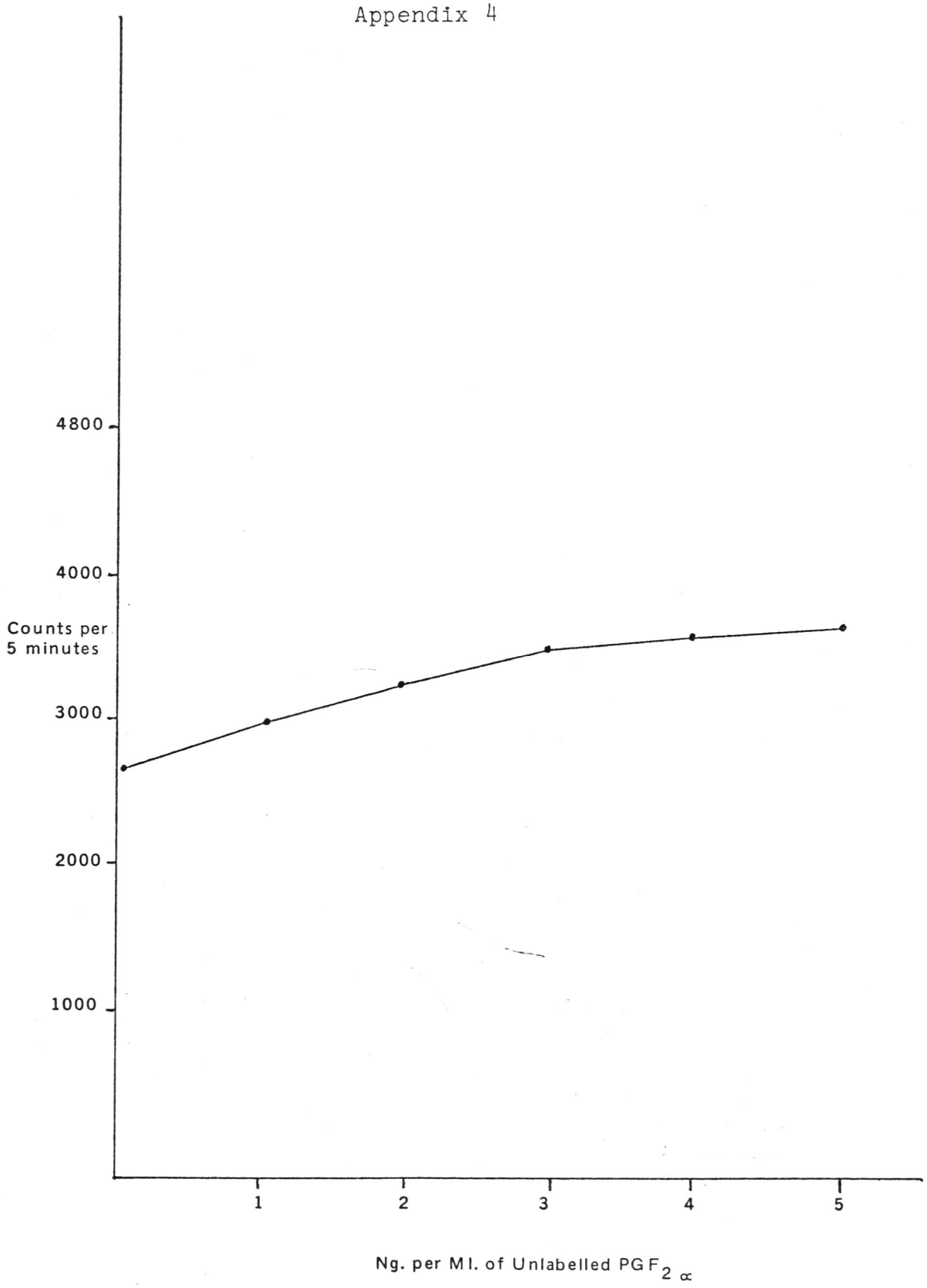
Appendix 2


Percent Binding
70
65
60
55
50
45
40
35
30
25
20
15
10
5
A1
A2
A3
A4
B1
B2
B3
B4
C1
C2
C3
C4

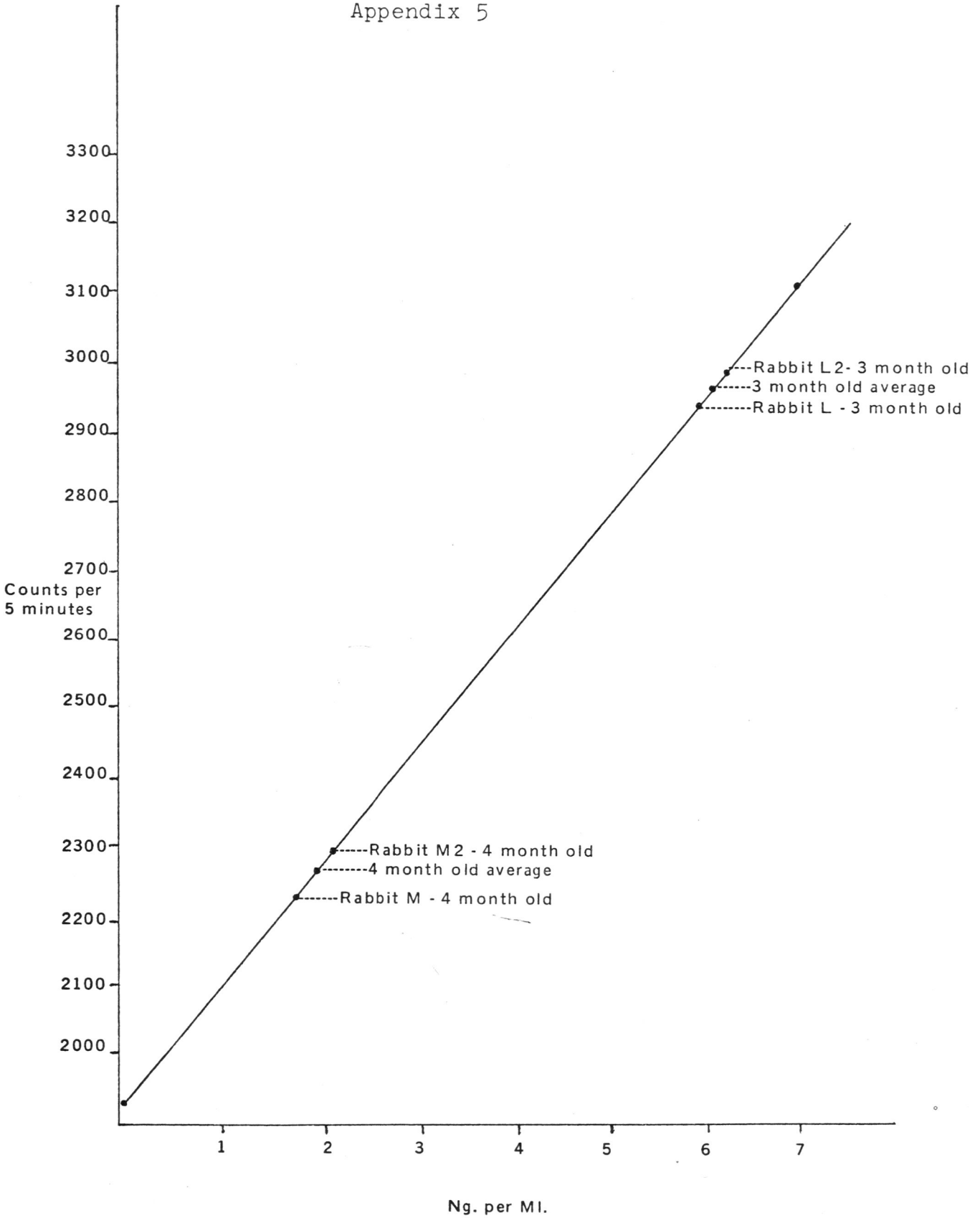
Appendix 3


1000
900
800
700
600
Counts per
5 minutes
10
20
30
40
50
Ng. per Ml. of Unlabelled PGF 2 α

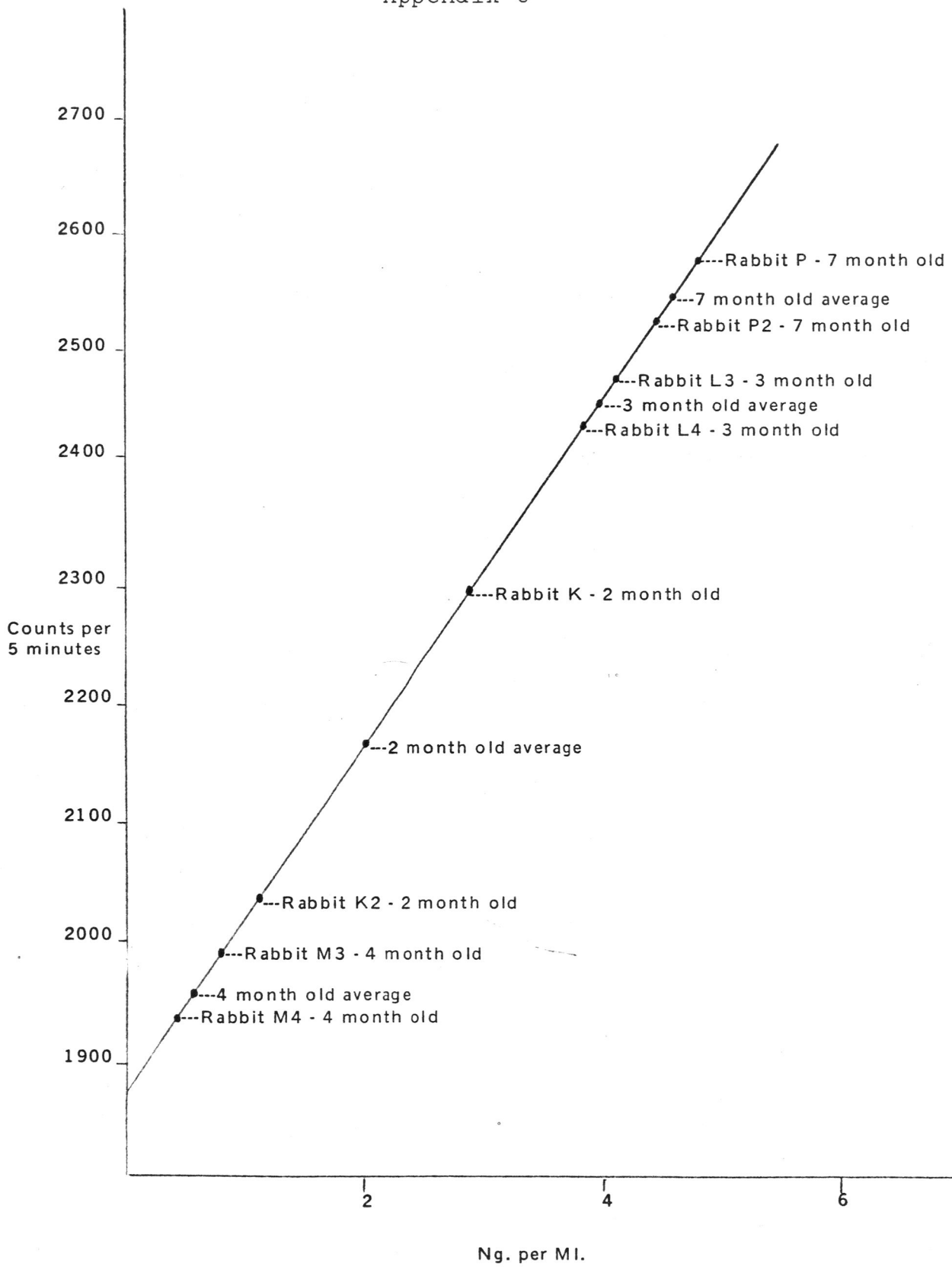
Appendix 4


4800
4000
Counts per
5 minutes
3000
2000
1000
1
2
3
4
5
Ng. per Ml. of Unlabelled PG F2 ∝

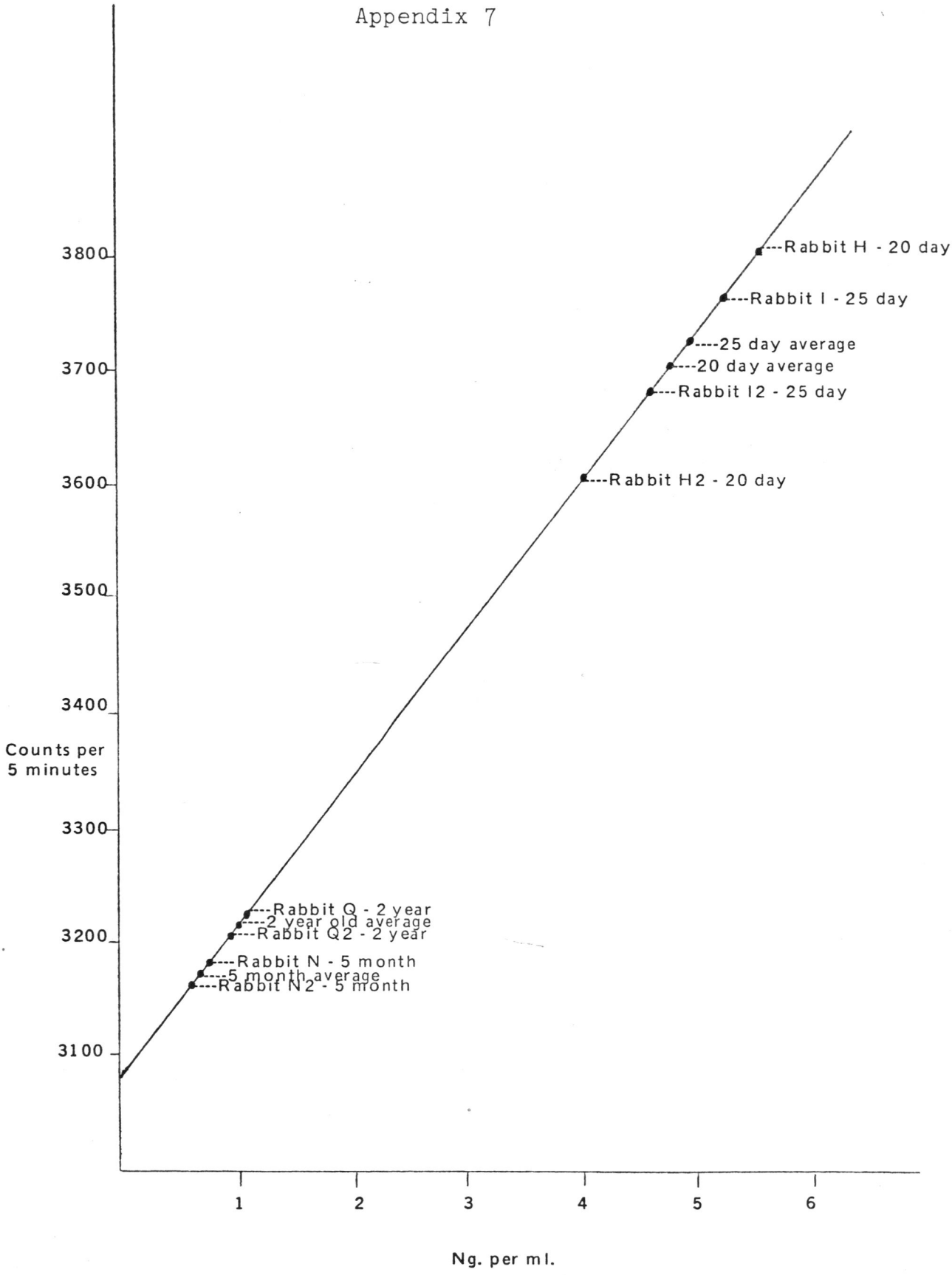
Appendix 5


3300
3200
3100
3000
2900
2800
2700
Counts per
5 minutes
2600
2500
2400
2300
2200
2100
2000
1
2
3
4
5
6
7
Ng. per Ml.
Rabbit L2- 3 month old
3 month old average
Rabbit L - 3 month old
Rabbit M2 - 4 month old
4 month old average
Rabbit M - 4 month old

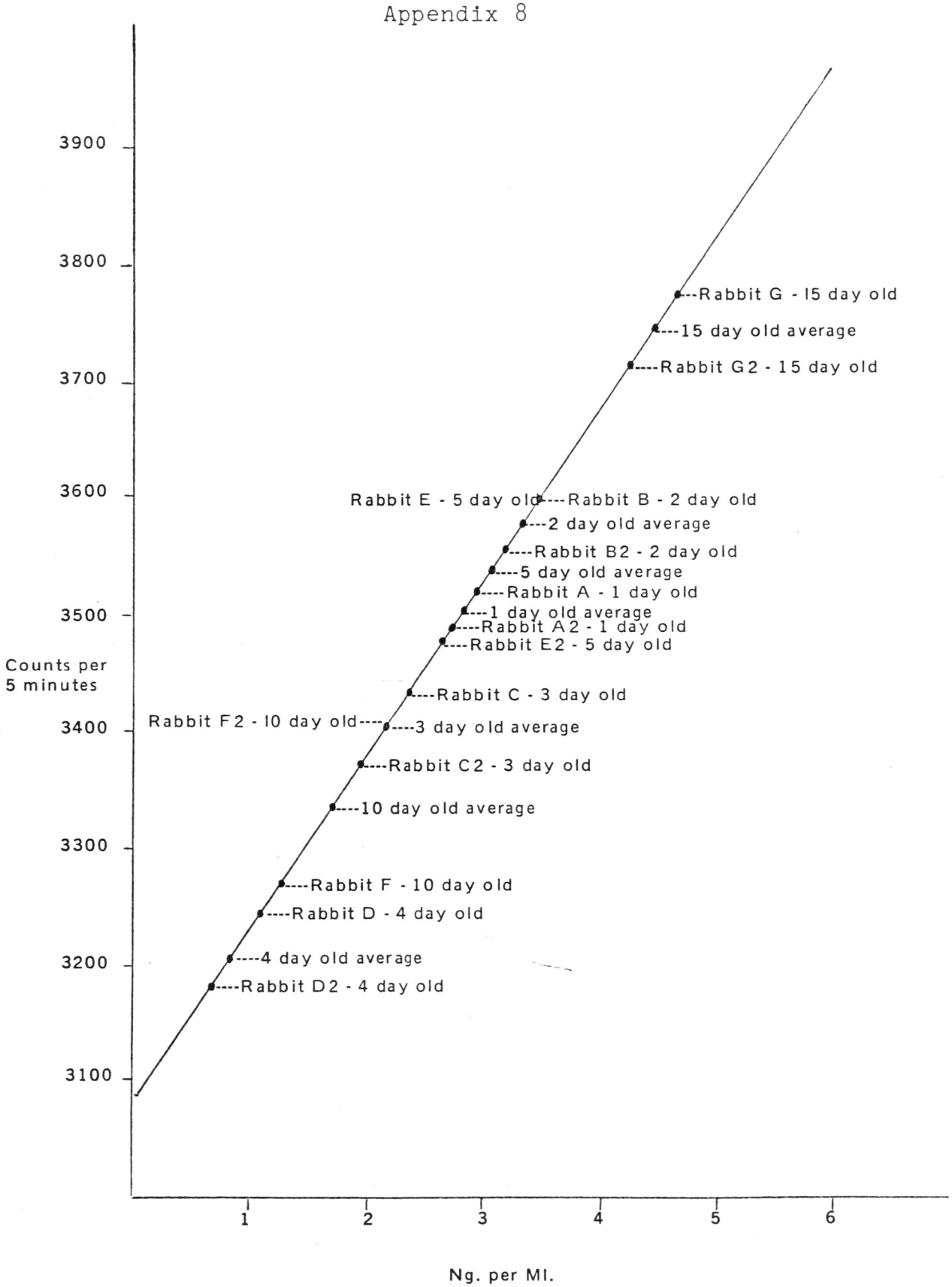
Appendix 6


2700
2600
2500
2400
2300
2200
2100
2000
1900
Counts per 5 minutes
---Rabbit P - 7 month old
---7 month old average
---Rabbit P2 - 7 month old
---Rabbit L3 - 3 month old
---3 month old average
---Rabbit L4 - 3 month old
---Rabbit K - 2 month old
---2 month old average
---Rabbit K2 - 2 month old
---Rabbit M3 - 4 month old
---4 month old average
---Rabbit M4 - 4 month old
2
4
6
Ng. per Ml.

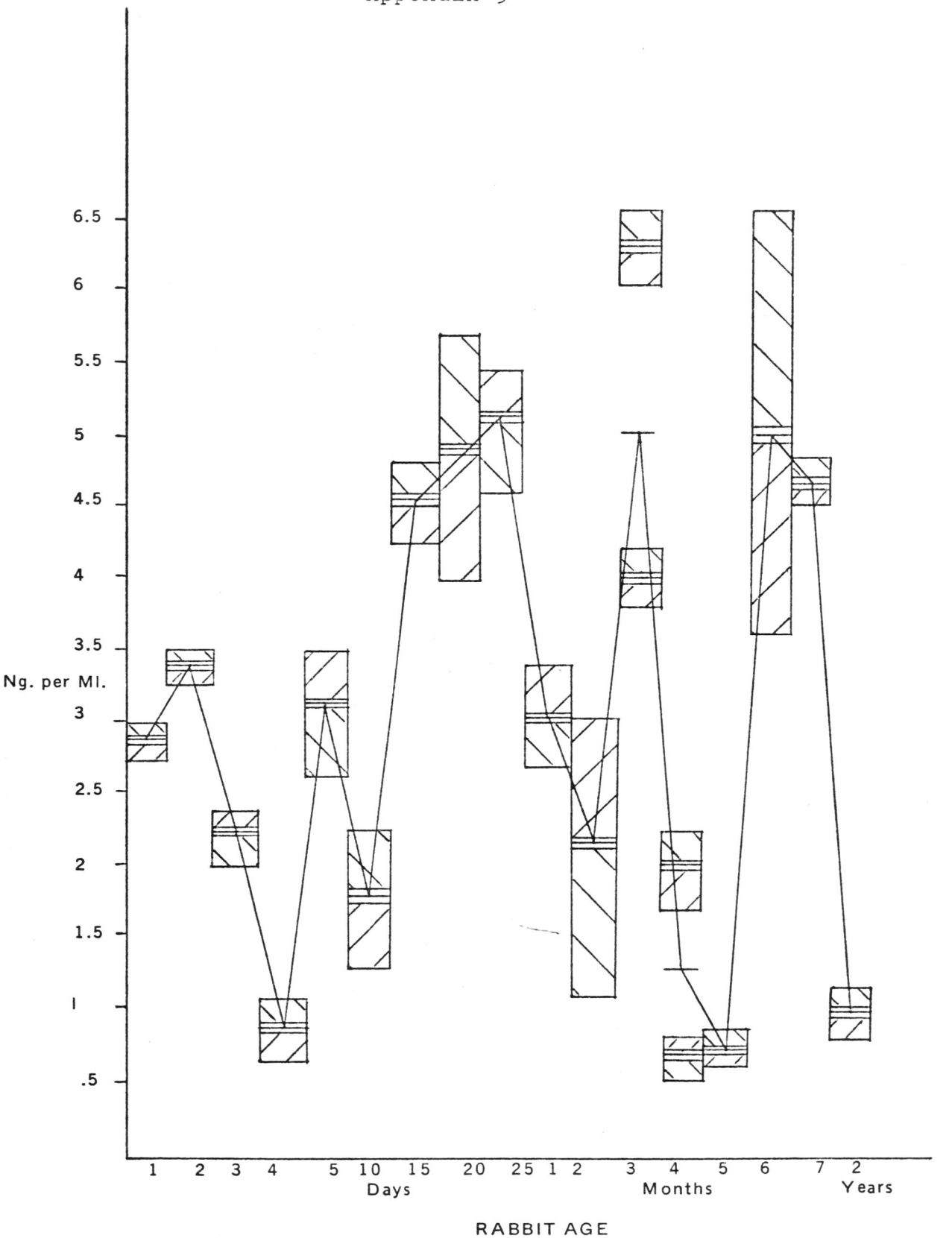
Appendix 7


3800
3700
3600
3500
3400
3300
3200
3100
Counts per 5 minutes
Rabbit H - 20 day
Rabbit I - 25 day
25 day average
20 day average
Rabbit I2 - 25 day
Rabbit H2 - 20 day
Rabbit Q - 2 year
2 year old average
Rabbit Q2 - 2 year
Rabbit N - 5 month
5 month average
Rabbit N2 - 5 month
1
2
3
4
5
6
Ng. per ml.

Appendix 8


3900
3800
3700
3600
3500
3400
3300
3200
3100
Counts per 5 minutes
Rabbit G - 15 day old
15 day old average
Rabbit G2 - 15 day old
Rabbit E - 5 day old
Rabbit B - 2 day old
2 day old average
Rabbit B2 - 2 day old
5 day old average
Rabbit A - 1 day old
1 day old average
Rabbit A2 - 1 day old
Rabbit E2 - 5 day old
Rabbit C - 3 day old
Rabbit F2 - 10 day old
3 day old average
Rabbit C2 - 3 day old
10 day old average
Rabbit F - 10 day old
Rabbit D - 4 day old
4 day old average
Rabbit D2 - 4 day old
1
2
3
4
5
6
Ng. per Ml.

Appendix 9


6.5
6
5.5
5
4.5
4
3.5
Ng. per Ml.
3
2.5
2
1.5
1
.5
1 2 3 4 5 10 15 20 25 1 2 3 4 5 6 7 2
Days
Months
Years
RABBIT AGE

BIBLIOGRAPHY

Ahn, C.S. and I.N. Rosenberg. "Iodine Metabolism in Thyroid Slices: Effects of TSH, Dibutyryl Cyclic 3^1, 5^1-AMP, NaF and Prostaglandin E_1," Endocrinology. 86:396. 1970.

Anderson, N. Program Notes on Structures and Nomenclature. New York Academy of Sciences. Volume 180, P 14. 1971.

Beitch, B.R. and K.E. Eakins. "The Effects of Prostaglandins on the Intraocular Pressure of the Rabbit." Brit. J. Pharmacol. 37:158. 1969.

Bergstrom, S.H. and J. Sjovall. "The Isolation of Prostaglandin F from Sleep Prostate Glands," ACTA Chem. Scand. 14:1693-1700. 1960a.

Bergstrom, S.H. and J. Sjovall. "The Isolation of Prostaglandin E from Sleep Prostate Glands," ACTA Chem. Scand. 14:1700-1709. 1960b.

Blatchley, F.R. and B.T. Donovan. "Luteolytic Effect of Prostaglandins in the Guinea Pig," Nature. 221:1065. 1969.

Crunkhorn, P. and A.L. Willis. "Cutaneous Reactions to Intradermal Prostaglandins," Brit. J. Pharmacol. 41:49. 1971.

Distelkotter, B. and W. Vogt. "Spontaneous Formation of Prostaglandins in Isolated Frog Intestine," Biol. Abstracts. 126463. 1968.

Eliasson, R. "Studies on Prostaglandin Occurence, Formation and Biological Actions," ACTA Physiol. Scand. Suppl. 158, 1-73. 1959.

Embrey, M.B. "Induction of Abortion by Prostaglandin E_1 and E_2," Brit. Med. J. 2:258. 1970.

Gutknecht, G.D.; J.C. Cornette and B.B. Pharriss. "Antifertility Properties of PGF_2," Biol. Reprod. 1:367. 1970a.

Gutknecht, G.D.; L.J. Wyngarden and B.B. Pharriss. "Effect of $PGF_{2\alpha}$ on Ovarian and Plasma Progesterone Levels in the Pregnant Hamsters," Proc. Soc. Exp. Biol. Med. 1970b.

Hambert, M. and B. Samuelsson. "The Structure of the Major Urinary Metabolite of Prostaglandin E_2 in Man," J. Amer. Chem. Soc. 91:2177. 1966.

Horton, E.W. "Prostaglandins," In: William Heinemann, Medical Books Ltd. P 1. 1972.

Kaley, G. and R. Weiner. "Microcirculatory Studies with Prostaglandin E_1," In: Prostaglandin Symp. Worcester Found. Exp. Biol. (P.W. Ramwell & J.E. Shaw, eds.) Interscience, N.Y. P 321.

Karim, S.M.M. and J. Deulin. "Prostaglandin Content of Human Amniotic Fluid During Pregnancy and Labour," J. Obstet. Gynaec. Brit. Comm. 75:230. 1967.

Karim, S.M.M. and G.M. Filshie. "Therapeutic Abortion Using Prostaglandin F_2," Lancet. 1:157. 1970.

Kirton, K.T.; B.B. Pharriss and A.D. Forbes. "Luteolytic Effects of Prostaglandin $F_{2\alpha}$ in Primates," Proc. Soc. Exp. Biol. Med. 133:314. 1970.

Kurzrok, R. and C.C. Lieb. "Biochemical Studies of Human Semen II," Proc. Soc. Exp. Biol. Med. 26:268. 1930.

Lee, J.B.; B.G. Covino; B.H. Takman and E.R. Smith. "Renomedullary Vasodepressor Substance, Medullin; Isolation, Chemical Characterization and Physiological Properties," Circ. Res. 17:57. 1965.

Levine, L.; R.M. Cernosek and H. Van Vunakis. "Specificities of Prostaglandins B_1, F_1, and F_2 Antigen-Antibody Reactions," J. Biol. Chem. 246:26. 1971

Levine, L. and Van Vunakis. Biochem. Biophys. Res. Commun. 41, 1171. 1970.

Lippmann, W. Inhibition of Gastric Acid Secretion in the Rat by Synthetic Prostaglandin Analogues, New York Academy of Sciences. Volume 180, P 332. 1970.

Lipson, L.C. and G.W.G. Sharp. Effect of Prostaglandin E_1 on Sodium Transport and Osmotic Water Flow in the Toad Bladder, New York Academy of Sciences. Volume 180, P 261, 1971.

Pharris, B.B. and K.T. Kirton. "Prostaglandin $F_{2\alpha}$: A New Contraceptive Approach," Exerc. Med. Int. Congr. Ser. No. 207:168. 1969.

Pharriss, B.B. and L.J. Wyngarden. "The Effect of Prostaglandin $F_{2\alpha}$ on the Progesterone Content of Ovaries from Pseudopregnant Rats," Proc. Soc. Exp. Biol. Med. 130:92. 1969.

Ramwell, P.W. The Prostaglandins. Volume I. Plenum Press, New York. P 93. 1973.

Samuelsson, B.; E. Granstrom and M. Hambert. "On the Mechanism of the Biosynthesis of Prostaglanding," In: Prostaglandins, Proc. II Nobel Symp., S. Bergstrom and B. Samuelsson, EDS, Almquist and Wiksell, Stockholm, P 51. 1967.

Unger, W.H.; I.F. Stamford and A. Bennett. "Extraction of Prostaglandin from Human Blood," Nature. Volume 233 p. 336. 1971.

Weinheimer, A.J. and R.L. Spraggins. "The Occurence of Two New Prostaglandin Derivatives (15-epi-PGF_2 and its acetate, methyl ester) in the Gorgonian Plexaura Homomalla," Chemistry of Coelenterates. XV^2 Tetrahedron Letters 59. P 5185. 1969.

Wiquist, N. and M. Bygdeman. "Induction of Therapeutic Abortion with Intravenous Prostaglandin F_2," Lancet. 1:889. 1970.