THE EFFECT OF PARATHYROIDECTOMY ON CARBOHYDRATE AND PHOSPHATE METABOLISM OF RAT LENS

A Thesis

Presented to

the Faculty of Graduate Studies

University of Manitoba

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

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by

Lorne Malcolm Golub

Department of Oral Biology

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Abstract. The effects of parathyroidectomy on certain aspects of carbohydrate and phosphate metabolism in the lens were studied under aerobic and anaerobic conditions. Lenses from control and parathyroidectomized rats were incubated for four hours at 37°C in a Krebs Ringer bicarbonate buffer (pH 7.4 - 7.5) containing added glucose and P^{32}. The pre and postincubation wet weights, the dry weight, and the percentage of water of the lenses from control and parathyroidectomized rats were determined. The pre and postincubation wet weights were found to be essentially identical and the wet weight, therefore, was unaffected by the incubation procedures. Parathyroidectomy had no effect on the wet weight, dry weight or lens water content. The changes in metabolic activity resulting from parathyroidectomy, which throughout this study have been expressed relative to the preincubation lens wet weight were, therefore, true changes and not due to differences in wet weight of lenses from control and parathyroidectomized rats. Under both aerobic and anaerobic conditions, glucose uptake, lactate production, and P^{32} incorporation into nucleotide were positively correlated and decreased with increasing rat weight. Under anaerobic conditions, glycolysis was stimulated indicating that the lens was capable of oxidizing glucose. Parathyroidectomy reduced the glucose uptake, lactate production, and P^{32} incorporation into the acid soluble and nucleotide fractions under both aerobic and anaerobic incubation conditions. Because anaerobic conditions did not abolish these

effects, oxidative metabolism was not required for the effects of parathyroidectomy on the metabolism of the rat lens in vitro. As the P^{32} content of the non-nucleotide fraction was not changed by parathyroidectomy, the reduction of the P^{32} content of the acid soluble fraction was due solely to an effect upon the nucleotide fraction. Parathyroidectomy also reduced the percentage of acid soluble phosphate occurring as nucleotide phosphate but did not affect the nucleotide content of lenses either before or after incubation. The data suggested that parathyroidectomy resulted in a lowered ATP/ADP ratio. It was argued that this lowered ratio did not produce, but resulted from, the reduced glycolysis. A reduction in Pi concentration was thought to be the initial change following parathyroidectomy that resulted in the observed effects on glycolysis and on the ATP/ADP ratio. This must have involved a reduction in the intralenticular phosphate concentration because it was shown that changes in the extralenticular phosphate concentration were not likely to cause the observed effects.

ACKNOWLEDGMENTS

Sincere gratitude is expressed to Dr. C. M. Dowse for contributing his effort and time, most generously, while supervising the author's progress.

Thanks are also extended to Dr. I. Kleinberg for similar contributions; to Mrs. S. Shuster for typing the manuscript; to Mr. E. Trout for technical assistance; and to Mr. D. Lane for the art work.

The author was supported by a Research Fellowship from the Associate Committee on Dental Research, National Research Council of Canada.

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CHAPTER I

INTRODUCTION

Purpose of the Study

This study was conducted in an effort to evaluate the effects of parathyroidectomy on the carbohydrate and phosphate metabolism of the lens. The control of calcium and phosphate metabolism by parathyroid hormone is well documented. Alterations in carbohydrate metabolism resulting from hormonal changes have also been noted. However, no present hypothesis on the mode of action of parathyroid hormone relates the above effects to one another in a satisfactory manner.

Whereas the main sites of action of parathyroid hormone are generally thought to be the skeleton, intestine, and kidney, effects on a variety of other tissues have been reported. Such studies, to be described in Chapter II, indicate that the hormone may have a general action on the carbohydrate and mineral metabolism of all cells to a variable degree. The lens was the tissue studied because:

1) an effect of parathyroidectomy on this tissue would support the concept of a general action of the hormone on many types of tissues.
2) the lens is easily dissected out of the rat as a complete organ.
3) in situ, the lens has no blood supply but is dependent on the

aqueous humour for metabolic requirements. In the incubation procedure employed, therefore, oxygen and nutrients were supplied under conditions similar to those existing in vivo. 4) the lens has been shown to respond to parathyroidectomy and to the addition of parathyroid hormone in vitro.

In brief, the aims of the study were:

1) to demonstrate and examine the effects of parathyroidectomy on lens glycolysis and phosphate metabolism.
2) to determine whether these alterations in phosphate metabolism preceded, or followed, the effects on glycolysis.
3) to determine whether the effects of parathyroidectomy on glycolysis and phosphate metabolism were mediated by oxidative mechanisms, as recent studies have emphasized a direct action of the hormone on mitochondria.

Definition of the Terms Used

PTX - parathyroidectomy. The name of the operation that removes or destroys the parathyroid glands.

Control - animal or group of animals with parathyroid glands intact.

μmole - micromole.

N - normality.

cpm - counts per minute.

P^{32} - the radioactive isotope of phosphorus.

DPN and TPN - diphosphopyridine and triphosphopyridine nucleotide.

DPNH and TPNH - reduced DPN and TPN.

ATPase - adenosine triphosphatase.

λ - wavelength.

mμ - millimicron.

O.D. - optical density.

S.E.M. - standard error of the mean.

Organization of the remainder of the study. The following chapter reviews the literature concerned with parathyroid hormone effects on a variety of tissues. Literature describing the morphology, metabolism, and reactivity to parathyroid hormone of the lens is also reviewed in Chapter II. The general methods employed throughout this study are detailed in Chapter III. Chapter IV describes experiments designed to study the effects of parathyroidectomy and incubation on water content of the lens. The effects of parathyroidectomy on aerobic and anaerobic glycolysis of the lens are described in Chapter V. Chapter VI details experiments concerned with:

1) The relationship of glycolysis to nucleotide P^{32} in the lens.

2) The effect of parathyroidectomy on the P^{32} incorporation into several lens fractions during both aerobic and anaerobic incubation.

3) The effect of parathyroidectomy on nucleotide phosphate in vitro and on nucleotide content of the lens before and after incubation.

Chapter VII concludes this report with a summation and discussion of the findings.

CHAPTER II

HISTORY OF THE LITERATURE

Section 1. Parathyroid Hormone.

The hypothesis of Albright and Reifenstein (1948), which stated that an initial phosphaturia produced the observed effects of parathyroid hormone, was the last theory to receive widespread acceptance. This hypothesis was disproved in 1953 when Talmage found that parathyroidectomy produced a prompt fall in the plasma calcium of nephrectomized rats. Since then the demonstration of parathyroid hormone effects on bone, intestine, kidney, mammary gland, lens, salivary gland, erythrocyte and muscle, as well as on liver and kidney mitochondria has made it abundantly clear that the action of the hormone is widespread throughout the body.

Effects of parathyroid hormone on bone. Although several early papers related alterations in parathyroid hormone to various bone changes (Jaffé and Bodansky, 1930; Shelling, 1930; and Thomson and Collip, 1932), Barnicot (1948) was the first to demonstrate a direct action of parathyroid hormone on bone. He transplanted parathyroid tissue in direct contact with membranous bone of the skull. Bone resorption occurred on the bone surface next to the parathyroid tissue and bone deposition occurred on the opposite

surface. Subsequent studies verified the finding that parathyroid hormone directly stimulated the process of bone resorption (Chang, 1951; Engfeldt and Zetterstrom, 1954; and Gaillard, 1959).

Several reports have related parathyroid hormone to bone carbohydrate metabolism. Neuman and Neuman (1957) suggested that the stimulation of citric and lactic acid production by parathyroid hormone may be responsible for the dissolution of bone mineral. Firschein et al. (1958) reported that parathyroid extract administration produced a rise in the serum citrate levels prior to a rise in serum calcium. Martin et al. (1964) found that parathyroid extract increased the utilization of glucose and the production of lactate and citrate by calvaria. However, Dowse et al. (1963) felt that purified parathyroid polypeptide acted on rat calvaria in vitro through a system other than glucose metabolism, as they found no significant changes in citrate production or utilization, glucose uptake, respiration, or levels of calcium or inorganic phosphate in the medium. The only significant finding was an increased production of lactate and this occurred only under aerobic conditions. Dowse et al. (1963) suggested that the hormone may act by influencing the transport of phosphate in and/or out of the cell.

The stimulation of bone resorption by parathyroid hormone may involve other metabolic processes. Rasmussen, Arnaud and

Hawker (1964) found that actinomycin D, a compound that blocks DNA-directed RNA synthesis and thus protein synthesis, did not modify the effects of injected parathyroid hormone on the renal excretion of calcium and phosphate in parathyroidectomized rats. Actinomycin D did, however, inhibit the effect of parathyroid hormone on the bone of these rats, as measured by calcium mobilization. The effect of the hormone on bone is generally thought to involve osteoclast cells. The authors stated that a fundamental action of parathyroid hormone could be the stimulation of RNA and protein synthesis in bone cells resulting in the formation of osteoclasts.

Effects of parathyroid hormone on kidney and intestine.

In a review of the relevant literature, Rasmussen (1961) stated that there was abundant evidence in favour of a hormonal regulation of calcium and phosphate excretion by the kidney. Since then, studies have revealed a variety of hormonal effects on kidney tissue. Parathyroid hormone stimulated calcium release (Deluca et al., 1962) and phosphate uptake coupled to augmented respiration (Fang and Rasmussen, 1964) in kidney mitochondria. Purified parathyroid hormone added *in vitro* stimulated the respiration of oligomycin-inhibited rat kidney tubules (Arnaud and Rasmussen, 1964). Injected parathyroid extract increased the incorporation of subsequently injected P^{32} into the total phosphate, acid-soluble phosphate,

inorganic phosphate, acid soluble organic phosphate, phospholipid and nucleic acid phosphate fractions of rat kidney (Egawa and Neuman, 1964)..

According to Rasmussen (1961) most early investigators felt that the parathyroid hormone had little or no influence on the intestinal absorption of calcium. Talmage and Elliot (1958), however, found that parathyroidectomy significantly decreased the rate of absorption of radioactive calcium from an isolated loop of intestine in vivo. Rasmussen (1959) and Cramer (1963) obtained similar results. Borle et al. (1963) found that the addition of parathyroid extract increased the influx of phosphate in duodenal loops of rats in vitro.

Effects of parathyroid hormone on mitochondria. Recently, emphasis in the field has been focused on mitochondrial effects in an attempt to gain some insight into the mode of action of parathyroid hormone at a subcellular level. Liver mitochondria have been the most widely used, and effects on these organelles indicate that the hormone has a widespread activity throughout the body.

Parathyroid hormone and vitamin D, added in vitro and acting synergistically, stimulated the release of calcium by mitochondria (Deluca et al., 1962). Vitamin D was able to function independently in this regard while parathyroid hormone required the presence of vitamin D. Mitochondrial uptake of calcium, which required ATP,

Mg^{++} and oxidizable substrate, was independent of vitamin D and parathyroid hormone.

Parathyroid hormone was shown to stimulate mitochondrial accumulation of phosphate, sulfate and arsenate ions (Sallis et al., 1963). This translocation of anions was coupled to a respiratory stimulation (Rasmussen et al., 1964) and some authors (Deluca et al., 1962; and Sallis et al., 1963) believed that a primary action of the hormone was to regulate the translocation of phosphate across cellular and subcellular membranes.

More complex effects of parathyroid hormone on mitochondria have also been shown. Under certain incubation conditions, parathyroid hormone stimulated respiration without producing a net accumulation of phosphate or magnesium (Fang and Rasmussen, 1964). In oligomycin - inhibited mitochondria supplied with glutamate or succinate as substrate, Fang et al., (1963) found that parathyroid hormone could stimulate respiration that was uncoupled from phosphorylation. Electron transport inhibitors, for example cyanide, blocked the stimulation of non phosphorylative oxidation. Uncouplers of oxidative phosphorlyation, for example dinitrophenol, stimulated respiration in this system and the subsequent addition of parathyroid hormone did not further increase oxygen consumption.

The hormonal stimulation of oxygen consumption or phosphate

uptake by oligomycin - inhibited mitochondria required the addition of ATP (Sallis et al., 1963; and Fang et al., 1963). Aurbach et al. (1964) found that,in oligomycin - inhibited mitochondria, parathyroid hormone stimulated both the formation of $^{14}CO_2$ from radioactive succinate and the oxidation of DPNH. Added ATP was not required to demonstrate these effects. Thus this effect occurred under conditions reported to prevent the hormonal stimulation of phosphate uptake and oxygen consumption.

Parathyroid hormone has also been shown to stimulate mitochondrial ATPase activity (Sallis and Deluca, 1964). However, under the incubation conditions necessary for the maximal hormonal stimulation of this ATPase activity, there was no effect of parathyroid hormone on phosphate accumulation by the mitochondria.

It is difficult at this time to devise a unifying hypothesis to explain these in vitro effects of parathyroid hormone on mitochondria, and to relate them to the in vivo action of the hormone. Because of this lack of a suitable hypothesis, together with the fact that relatively large amounts of hormone are needed to produce these mitochondrial effects, the results of these studies are of questionable physiological significance.

Section 2. Lens Metabolism Studies

Morphology and special features of the lens. Lerman (1965)

discussed the unique features of the lens. The lens is a completely encapsulated, ectodermally derived organ. It is devoid of a blood supply and depends upon the aqueous humour for its metabolic requirements. By the 35th day of ovulation the lens capsule is completely formed, surrounds the whole organ and prevents the exfoliation of cells from the lens to the surrounding media. This results in a unique growth pattern with the worn out cells being pushed into the centre of the lens.

The lens has only one layer of true epithelial cells which are located along its anterior surface immediately under the capsule. The rest of the lens consists mainly of anuclear fibres. As mitochondria are found only in the epithelial layer, the Krebs cycle and electron transport system are confined to this region of the lens.

Lens carbohydrate metabolism. Many workers, including Pirie and Van Heyningen (1956) and Kinoshita et al. (1961) have concluded that glucose is the prime energy source in the lens. Although most workers in the field agree with Kinoshita (1962) that the bulk of glucose entering the lens is metabolized by the Embden Meyerhof pathway, the overall importance of oxidative pathways in lens carbohydrate metabolism remains controversial. The hexose monophosphate shunt exists in the lens and appears to be the principal oxidative pathway (Kinoshita 1955; Kinoshita and Wachtl, 1958;

Kuck, 1961; and Van Heyningen, 1962). The few mitochondria in the lens and its relatively anaerobic state in situ suggest that the citric acid cycle is of minor importance in this tissue (Lerman, 1965; and Christiansen and Leinfelder, 1952). Its presence was questioned by Kinoshita (1955) who established that the lens was unable to use pyruvate.

There is, however, evidence that the citric acid cycle is operative in the lens. Oxygen uptake by the lens has been reported by several workers (Harris, Hauschildt and Nordquist, 1954; Lewis, Talman and Harris, 1960; and Sippel, 1962). Cytochrome C, cytochrome oxidase and flavoprotein have been found in low concentrations in the lens. They were confined almost exclusively to the epithelium (Kinsey and Frohman, 1951). Lens homogenates have been found to be capable of oxidizing various members of the citric acid cycle (Ely, 1951).

Lens phosphate metabolism. Lenticular phosphate uptake is demonstrably linked to active metabolic processes. Palm (1948) showed that 40% of the P^{32} taken up by the lens in a ten minute incubation period was incorporated into the seven-minute hydrolyzable phosphate fraction which consisted mainly of ATP and glucose-1-phosphate. Müller et al. (1956) reported that the metabolic inhibitors, potassium cyanide, iodoacetic acid and sodium fluoride, greatly

inhibited lens phosphate uptake and the amount of P^{32} incorporated into ATP. In addition, they found that the lens was still able to incorporate P^{32} into ATP after removal of the epithelial layer. This incorporation into ATP was decreased by glycolytic inhibitors but not by potassium cyanide, suggesting that it occurred solely through glycolysis.

The effect of parathyroid hormone on lens metabolism.

Clark (1939) found that parathyroid extract added *in vitro* prevented lenticular calcium uptake. Firschein (1962) showed that parathyroidectomy reduced the *in vitro* glucose uptake and lactate production by lenses from 100 gram rats. Parathyroid hormone, added *in vitro*, restored glycolysis to control levels.

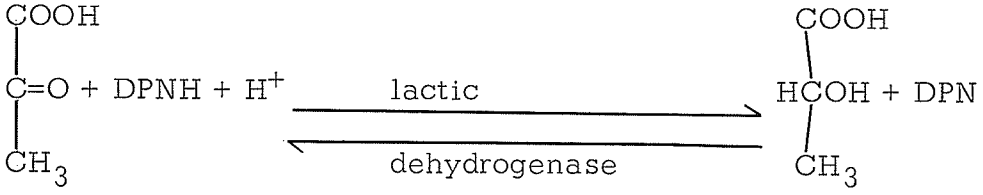
CHAPTER III

GENERAL METHODS USED

The first five sections of this chapter describe techniques employed in the chemical analysis of rat serum, lens fractions, and metabolites in the incubation media. The last section deals with the procedures for animal preparation, and for lens preparation and incubation.

1. Determination of Lactic Acid

Principle of the method. In the presence of excess DPN, and at high pH, lactic dehydrogenase catalyzes the oxidation of lactic acid to pyruvic acid.



pyruvic acid lactic acid

The DPNH formed is directly proportional to the amount of lactic acid present and may be readily measured, spectrophotometrically, at 366mμ.

Experimental procedure. A modification of the microassay method of Cohen and Noell (1960) was used. A stock solution

of 6 millimolar L(+) lactic acid (zinc salt) was prepared. All the samples were made up to 200 µl volume, in 10 x 75 mm test tubes, using distilled water as diluent. Standard tubes contained 0, 10 and 25 µl of stock lactate solution. 50 µl aliquots of postincubation media were found to be suitable for analysis.

The following reagents were added to each tube:

1) 500 µl of glycine - NaOH buffer (pH 10.5), containing semicarbazide hydrochloride.
2) 100 µl of 15 mg/ml DPN.$4H_2O$ solution.
3) 25 µl of a 1 in 4 dilution of lactic dehydrogenase with 6% $NaHCO_3$.

The tubes were sealed with parafilm, agitated in unison and allowed to stand at room temperature for at least two hours. The samples were read at 366 mµ on a Beckman DU spectrophotometer against distilled water.

Materials used.

L(+) Lactic acid: zinc lactate. Water of crystallization was 12.9%. Pfansteihl Laboratories, Inc., Waukegan, Ill.

Glycine-NaOH buffer: pH 10.5. Prepared by mixing a 6% solution of semicarbazide with 0.25M glycine.

β-Diphosphopyridine nucleotide: 98% assay. Corrected for $4H_2O$ per mole when prepared. The solution contained 15mg DPN.$4H_2O$/ml.

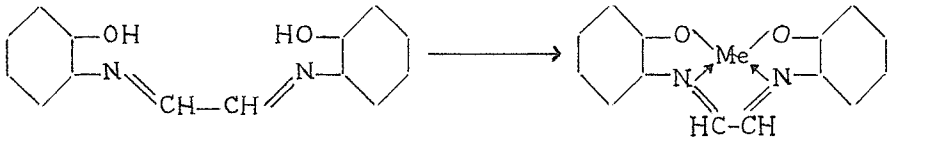
Sigma Chemical Company, St. Louis 18, Missouri.

Lactic dehydrogenase: type II. Crystalline. Substantially free of pyruvate kinase. $(NH_4)_2SO_4$ suspension. 10 mg/ml. Sigma Chemical Company, St. Louis 18, Missouri.

Accuracy of the method. In a group of 15 separate analyses the mean difference between duplicate determinations of standard lactic acid samples was 0.181 µg ± 0.042 µg (S.E.M.) at the 5.3 µg lactate level, and 0.197 µg ± 0.071 µg (S.E.M.) at the 13.3 µg level.

2. Direct Microdetermination of Serum Calcium

Principle of the method. According to Fisher Scientific Company technical data sheet (TD-162), Glyoxalbis (2-hydroxyanil) is a Schiff base which forms coloured inner-complex salts with metals in the following way:



SCHIFF BASE COLOURED COMPLEX

The red calcium complex can be extracted with chloroform, whereas the masking colour complexes formed by other metals are either

not dissolved, or can be removed by addition of alkaline carbonate, cyanide, or an alkaline buffer. The ease with which the masking effects can be eliminated makes Glyoxal-bis specific for calcium. Goldstein and Stark-Mayer (1958), who developed the original qualitative method, tested the reagent with a wide range of metal ions.

Experimental procedure. The following solutions were made up using deionized water:

1) A stock calcium standard solution (100 µg calcium/ml). 0.2498 grams $CaCO_3$ were dissolved in 7 ml of N HCl and diluted to 1 litre with water.
2) Working calcium standard solutions of 12.5, 10.0 and 5.0 µg calcium/ml by dilution of the stock solution.
3) Glyoxal-bis (2-hydroxyanil). 0.5 gram Glyoxal-bis was dissolved in 100 ml of absolute ethyl alcohol with vigorous shaking.
4) Stock 2N NaOH (carbonate free). 50% NaOH was made up in water. The precipitate of sodium carbonate which formed was removed by centrifugation. The NaOH was diluted to 2 N, standardized against potassium hydrogen phthalate using phenolphthalein indicator, and corrected to 2 N.
5) Working NaOH solutions of 0.5N and 1.2N by dilution of above with water.

Blanks and standards consisted respectively of 200 µl deionized

water and 200 µl of standard solutions containing 5,10 and 12.5 µg calcium/ml. Serum samples were prepared by adding 20 µl of test serum to 200 µl of deionized water. Reaction tubes were 10 x 75 mm round Coleman cuvettes kept cold in an ice bath. 200 µl of Glyoxal-bis were added to all the tubes, which were then agitated on a vortex mixer. The tubes were replaced in the ice bath for a few minutes and were then re-agitated. 50 µl of 0.5 N NaOH were added to the blanks and standards of 50 µl of 1.2 N NaOH were added to the serum samples. The tubes were agitated individually, replaced in the ice bath, and the colour was allowed to develop for 15 minutes. The colour was a pale yellow or a pale pink at first. 0.5 ml of chloroform (kept at $0^{o}C$ in a flask within an ice block) was added to each tube from a fixed syringe. All the tubes were stoppered with clean, calcium free, white rubber stoppers. The tubes were carried into a cold room and each tube was shaken vigorously twenty to thirty times. The tubes were centrifuged in the cold just long enough to separate the phases. The tubes were then placed in a water bath at 10^{o} to $15^{o}C$, wiped dry, and read at 540 mµ in a Klett-Summerson colorimeter (industrial model) fitted with a microadaptor.

Accuracy of the method. In a group of 14 separate analyses the mean difference between duplicate determinations of standard calcium samples was 0.037 µg $\pm$ 0.010 µg (S.E.M.) at the 1.0 µg calcium level, and 0.038 µg $\pm$ 0.016 µg (S.E.M.) at the 2.5 µg calcium level.

3. Determination of Glucose

Principle of the method. Kingsley and Getchell (1960) stated that the following reaction occurred in the glucose oxidase method:

$$\text{glucose} + O_2 + H_2O \xrightarrow{\text{glucose oxidase}} \text{gluconic acid} + H_2O_2$$

$$H_2O_2 + \begin{matrix}\text{o-tolidine} \\ \text{o-dianisidine}\end{matrix} \xrightarrow{\text{peroxidase}} \text{colour}$$

Many workers have used the oxidation of glucose by molecular oxygen in the presence of β-glucose oxidase as the basis for glucose methods. The amber colour of the o-dianisidine reaction mixture changes to a deep pink after the addition of concentrated sulfuric acid (McComb & Yushok, 1958). The intensity of this colour is proportional to the amount of glucose present in the sample.

Experimental procedure. A modification of the method of Kingsley & Getchell (1960) was used. All the samples, blanks and standards were made up to 200 µl volume, in 10 x 75 mm test tubes, using distilled water as diluent. Standard tubes contained 0, 10, 25 and 50 µl of a freshly prepared solution of glucose (0.4 µg/ml). 10 µl aliquots of media before and after incubation were taken for analysis.

The following reagents were added to each tube:

1) 800 µl peroxidase buffer reagent: This was stored frozen and

at the time of usage was thawed and heated to 60°C.

2) 100 µl glucose oxidase solution (0.1 gram/10 ml).

The tubes were agitated in unison and incubated for one hour at 37°C. After the incubation, 400 µl of 37.4% (v/v) sulfuric acid were added to each tube. The tubes were agitated, and the samples read on a Beckman DU spectrophotometer at 530 mµ.

Materials used.

Glucose oxidase: crude; contains catalase. Sigma Chemical Company, St. Louis 18, Missouri.

Glucose: A.C.S. Anhydrous powder.

o-dianiside: 3,3'-dimethoxybenzidine. 0.250 grams were dissolved in 25 ml of methyl alcohol.

Peroxidase: horseradish type I. Approximately 70 (20 sec) Purpurogallin Units/mg. Sigma Chemical Company, St. Louis 18, Missouri.

Peroxidase buffer reagent: 0.8409 grams of Na_2HPO_4 and 1.1194 grams of KH_2PO_4 were dissolved in distilled water. 5.625 mg of peroxidase were added to the above solution, which was then adjusted to 500 mls. 5.6 ml of o-dianisidine solution were added to the top of the 500 mls.

Accuracy of the method. In a group of 15 separate analyses the mean difference between duplicate determinations of standard glucose samples was 0.148 μg ± 0.031 μg (S.E.M.) at the 4.0 μg glucose level, and 0.268 μg ± 0.053 μg (S.E.M.) at the 10 μg glucose level.

4. Determination of Inorganic Phosphate

Principle of the method. Many sensitive methods for determining phosphorus in biological materials have been based on the colour formed by the reduction of a phosphomolybdate complex. Chen, Toribara and Warner (1956) used ascorbic acid to carry out this reduction and reported that, in spite of the high sensitivity of the method, the colour produced was very stable.

Procedure. A modification of the method of Chen et al. was used. Blanks and standards consisted respectively of 500 μl of water and 500 μl of standard solutions containing 0.5, 1.0 2.0 mg phosphorus/ml. Suitable aliquots of solution to be analyzed were taken and made up to 500 μl with water. 10 x 75 mm test tubes were used. 500 μl of phosphate reagent were added to all tubes. The tubes were stoppered with parafilm, agitated, and placed in a 37°C. water bath for one hour. They were removed from the bath, cooled to room temperature and the absorbance of the solu-

tions read in a Beckman DU spectrophotometer at a wavelength of 820 mμ.

Materials used.

Ammonium molybdate: A.R. 2.5 grams were dissolved in 100 ml water.

Sulfuric acid: C.P. Specific gravity 1.84. 95.5% pure sulfuric acid was diluted to 6.0 N.

Ascorbic acid: powder. A.R.

Phosphate reagent: 5ml of a 2.5% solution of ammonium molybdate, 5 ml of 6.0 N H_2SO_4 and 0.5 gram ascorbic acid were mixed in this order and made up to 25 ml with distilled water.

Accuracy of the method. In a group of 10 separate analyses the mean difference between duplicate determinations of standard inorganic phosphate samples was 0.009 μg ± 0.002 μg (S.E.M.) at the 0.25 μg Pi level, and 0.008 μg ± 0.003 μg (S.E.M.) at the 0.50 μg Pi level.

5. Procedure for Ashing of Lens Extract

Experimental procedure. The ashing procedure employed was similar to one described by Chen et al. (1956).

250 μl aliquots were added to 100 μl of concentrated sulfuric

acid in 10 x 75 mm test tubes, which were then heated on a modified Kjeldahl rack for 4-6 hours. After this time hydrogen peroxide was added drop by drop to the solution until it became colourless and remained so after cooling. The tubes were heated for an additional thirty minutes to ensure the total removal of hydrogen peroxide. Three ml of deionized water were added to each tube and 250 µl aliquots were analyzed for phosphate.

The effect of the sulfuric acid used in ashing on the determination of phosphate by the method of Chen et al. (1956). According to the authors, ascorbic acid will cause reduction of the molybdate in a reagent blank if the acid concentration be below 0.4N. Above 1.0N, even with phosphorous present, no reduction, and therefore no absorbance, occurs. Within the range 0.4N - 1.0N, colour development is proportional to phosphate concentration. They proposed that the sulfuric acid concentration in the phosphate reagent be modified to ensure a final concentration in the reaction tube within this range and, therefore, to eliminate the need for neutralization of the acids carried over from the ashing procedure. The effect of variation in the final sulfuric acid concentration on the modified method used in this study was checked, and findings essentially in agreement with those of Chen et al. were obtained.

6. Animal Preparation, and Lens Preparation and Incubation.

The following techniques are common to many of the experiments described in later Chapters.

Animal preparation. Four to five days prior to an incubation, male rats ranging from 80-160 grams were parathyroidectomized. The animals were anaesthetized with ether. The parathyroid glands were exposed and destroyed by electrocautery. The incision was closed with metal sutures and the animals maintained on the same diet as the control rats. One to two days before the incubation, blood samples from the tail were collected in capillary tubes. The tubes were centrifuged immediately and the serum was analyzed for calcium. Animals with a serum calcium of less than 8 mg per cent were considered to be parathyroidectomized. Figure 1 shows that the reduction of the serum calcium observed in vivo after parathyroidectomy was produced solely by removal of the parathyroid glands, as the addition of parathyroid hormone in vivo restored the serum calcium to normal.

Lens preparation and incubation. The incubation apparatus consisted of:

a) ten ml Erlenmeyer flasks containing three ml of medium (table 1) equivalent in composition to that used by Firschein (1962).

b) a gas distributing system to supply oxygen for respiration and carbon dioxide for maintenance of the CO_2- bicarbonate

buffer system. A preliminary experiment demonstrated that this gassing aparatus was capable of maintaining a medium pH of 7.4-7.5 in a series of flasks incubated at 37°C. for four hours. A mixture of 95% air and 5% CO_2 was used for aerobic incubations. A mixture of 95% nitrogen and 5% CO_2 was used for anaerobic incubations.

c) a mechanical rocker used to minimize local differences in concentration of metabolites.

d) a water bath maintained at 37°C.

The lenses were prepared and incubated as follows. The Krebs-Ringer bicarbonate medium was made up one day before the incubation and stored in a refrigerator. On the day of the incubation the medium was gassed at room temperature with the appropriate gas mixture until a pH of 7.4-7.5 was reached. This usually required two to four hours of gassing. Three ml of medium were added to each Erlenmeyer flask and the flask sealed and weighed. They were then refrigerated until just prior to the addition of the lenses for a time never exceeding one hour.

Two control or two parathyroidectomized rats of approximately the same weight were decapitated. The four eyeballs were removed and immediately placed in a beaker of ice cold saline. The lenses were carefully removed using small tissue forceps and placed in

a beaker of ice cold saline. After the four lenses were collected, they were removed from the saline and blotted on a filter paper just long enough to remove adsorbed aqueous humour, saline, and non-lenticular tissue fragments. The four lenses were then rolled into the incubation flask. The preweighed flask, now containing the added lenses, was weighed to enable calculation of the pre-incubation wet weight of the lenses. The flask was incubated under a constant gas flow for four hours. After incubation the flask was removed from the shaking incubator, the lenses were removed from the flask and the medium was frozen. The lenses were subsequently handled in several ways as described in succeeding Chapters.

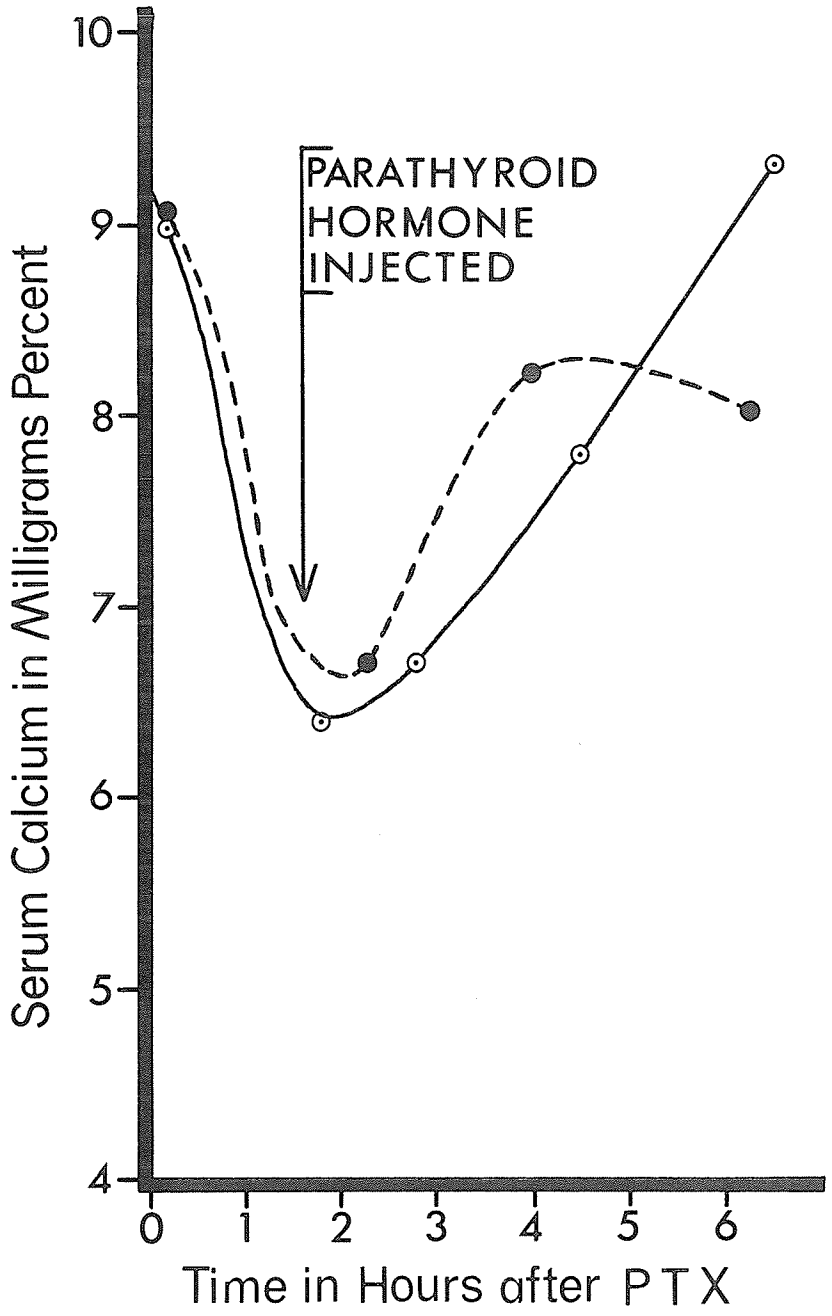


Figure 1. The effect of parathyroidectomy (PTX), and subsequent in vivo administration of parathyroid hormone on rat serum calcium. ●— —● Rat A; .⊙——⊙ Rat B.

Na^+	Cl^-	K^+	Ca^{++}	Mg^{++}	$SO_4^=$	IN μMOLES/ML.	
						$H_2PO_4^-$	HCO_3^-
143.0	128.0	5.92	2.54	1.18	1.18	1.18	25.0
		+1MGM/ML. GLUCOSE PRESENT					

TABLE 1.

IONIC COMPOSITION OF KREBS RINGER BICARBONATE BUFFER

CHAPTER IV

THE EFFECT OF PARATHYROIDECTOMY AND INCUBATION ON LENS WATER CONTENT

Introduction. The growth pattern of the lens is such, that older fibres are pushed in towards the centre of the lens as new fibres are laid down. The reduction in volume of the older fibres due to water loss does not completely offset the appositional growth of the lens and so it continues to enlarge with age. (Van Heyningen, 1962). Green and Solomon (1957) found that young rabbit lenses contained about 70% water while, because of the above mentioned growth pattern, older lenses were 60% water.

The reasons for studying the lens water content under various conditions were:

1) to determine whether parathyroidectomy altered the water content of the lens.
2) to determine whether incubation procedures altered the water content of lenses from either control or parathyroidectomized rats.
3) to determine whether parathyroidectomy altered lens dry weight.

Procedure. Lenses from control and parathyroidectomized rats were prepared as described in Chapter III. Four lenses from

either control or parathyroidectomized rats were added to a preweighed flask containing the medium. Immediately thereafter, the flask was reweighed to obtain the preincubation wet weight of the four lenses. After the incubation the four lenses were removed from the flask and were placed on a filter paper to blot off adsorbed medium. They were transferred as quickly as possible to a preweighed filter paper to obtain the postincubation wet weight. The lenses were allowed to dry at room temperature for 12 to 15 hours followed by heating in an oven at 75 to 100°C. One hour was found to be adequate to obtain a constant weight. The dry weight of the lenses was then determined. The percentage of water in the lenses was calculated as follows:

$$\frac{W - D}{W} \times 100$$

Where W = preincubation wet weight

and D = postincubation dry weight.

Discussion of the results. Throughout this study metabolic activity is expressed relative to preincubation lens wet weight. The latter was found to increase with increasing body weight, and not to be affected by parathyroidectomy (Figure 2). The changes in metabolic activity resulting from parathroidectomy, therefore, were true changes and not simply due to differences in wet weights

of lenses from control and parathyroidectomized rats.

The changes in wet weight which occurred during incubation of lenses from both control and parathyroidectomized rats were small, and random in direction (Figure 4). There was no difference between the dry weights of lenses from control and parathyroidectomized rats (Figure 3). Neither parathyroidectomy nor incubation, therefore, had any noticeable effect on lens water content.

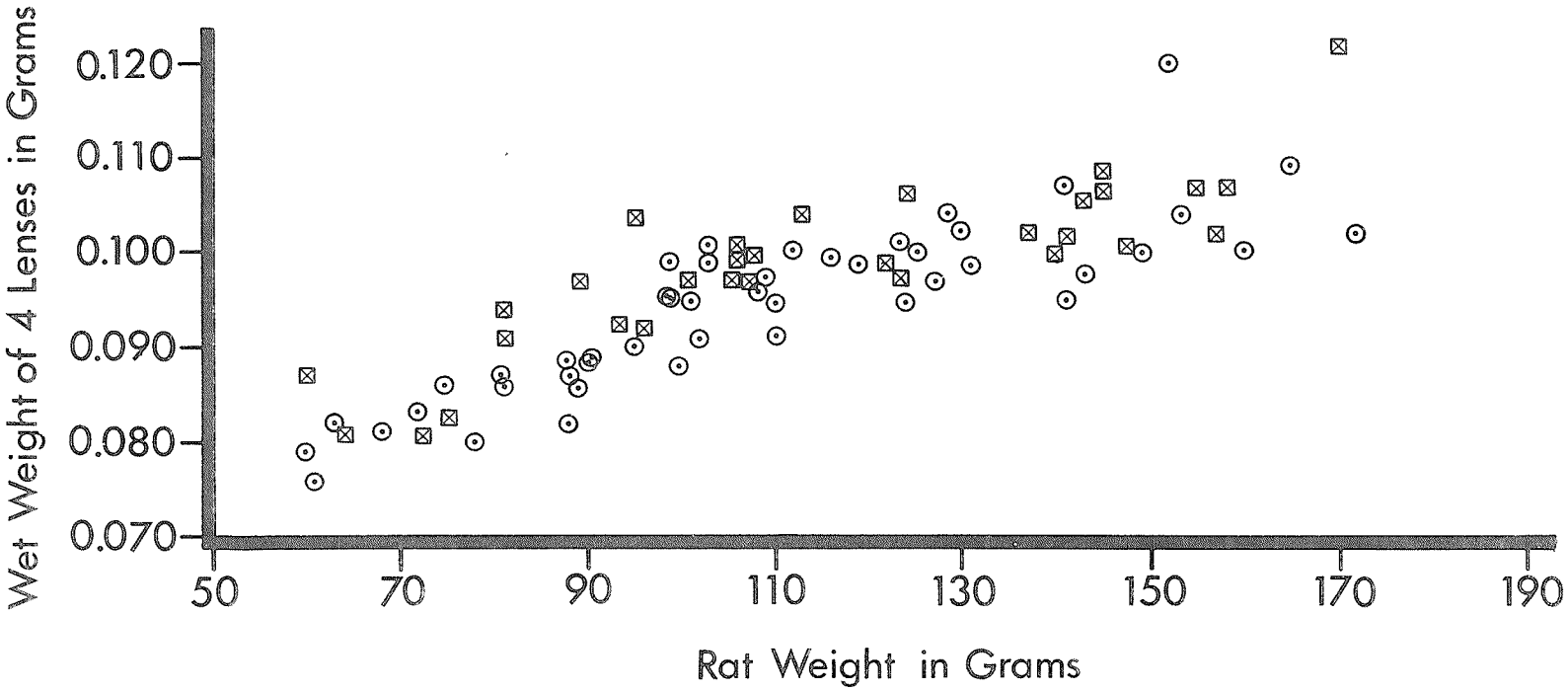


Figure 2. The effect of PTX on the preincubation wet weight of lenses.
⊙ Control; ⊠ PTX.

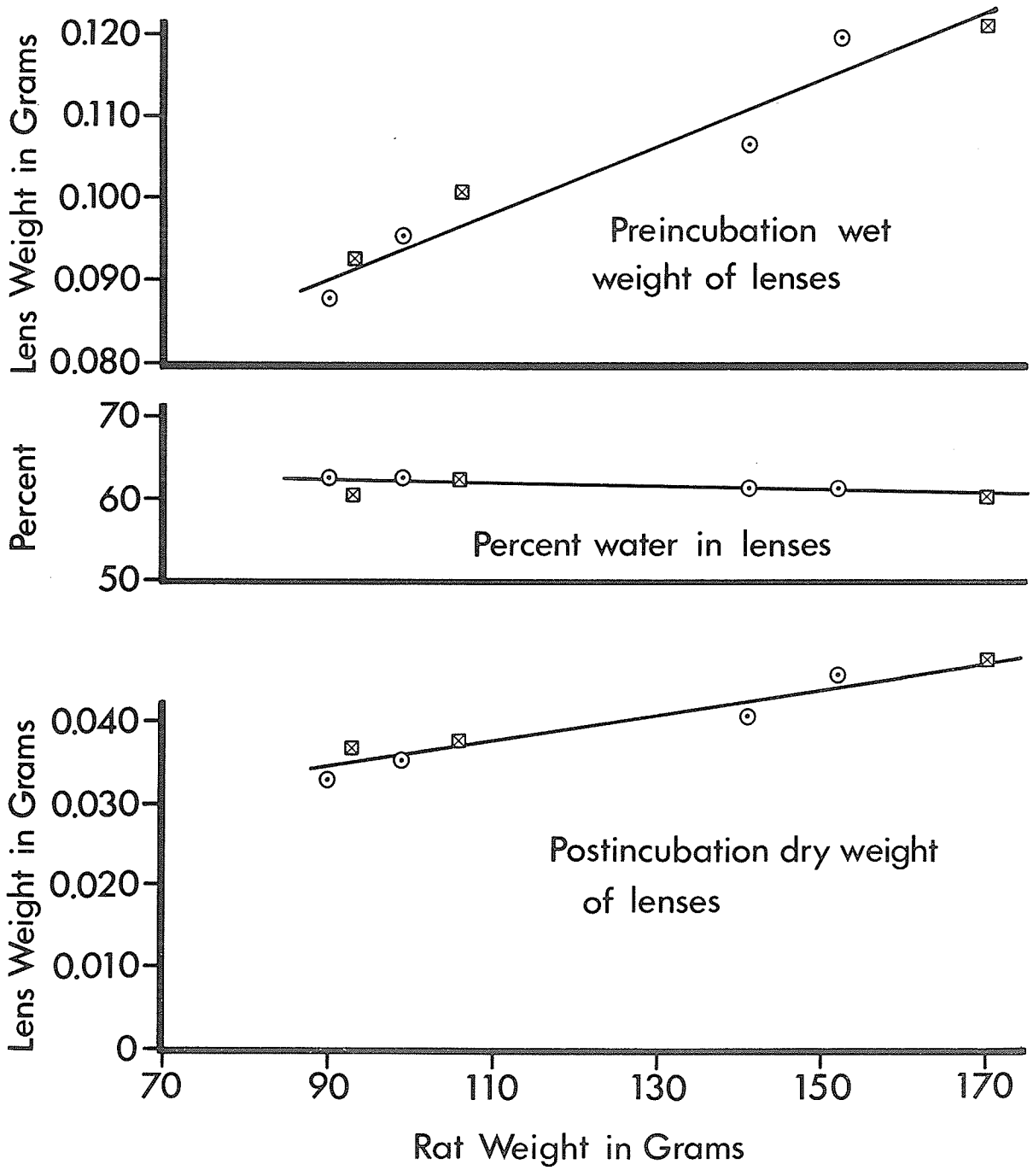


Figure 3. The effect of PTX on lens wet weight, dry weight and water content. ⊙ Control; ⊠ PTX.

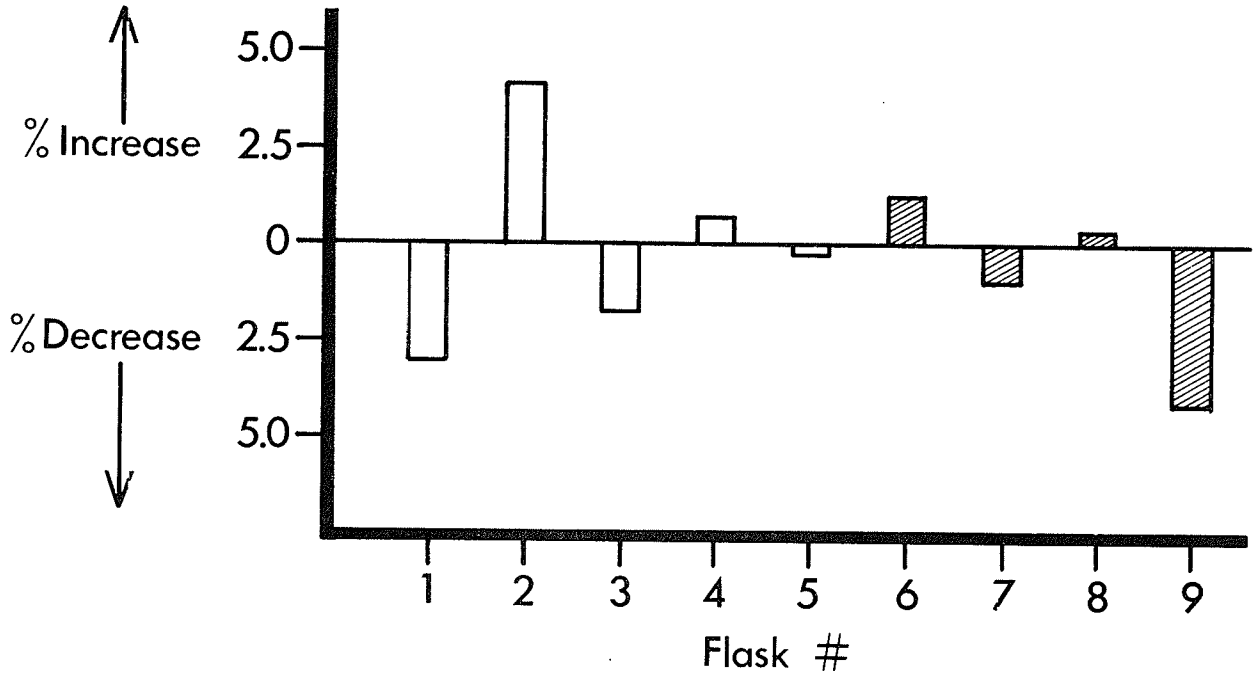


Figure 4. The effect of incubation procedures on lens wet weight.
□ control; ▨ PTX.

$$\% \text{ Difference} = \frac{\text{Preincubation wet weight (grams)} - \text{Postincubation wet weight (grams)}}{\text{Preincubation wet weight (grams)}} \times 100$$

CHAPTER V

THE EFFECT OF PARATHYROIDECTOMY ON LENS GLYCOLYSIS

Introduction. Firschein (1962) reported that parathyroidectomy reduced glycolysis of rat lens *in vitro*, but studied this effect only under aerobic conditions. Dowse et al. (1963) found that parathyroid hormone, added *in vitro*, stimulated the production of lactate by rat calvaria, and Goldhaber (1962) showed that parathyroid hormone, added to bone tissue culture, stimulated resorption.

In the two latter studies the hormone was effective only under aerobic conditions. The absence of an effect under anaerobic conditions, taken together with the finding of several direct effects of parathyroid hormone on mitochondria raises the possibility that the action of the hormone requires the presence of aerobic metabolic processes.

In the experiments to be described, the effects of parathyroidectomy on glycolysis of lenses from rats of varying weights were studied under aerobic conditions to confirm and expand Firschein's findings, and under anaerobic conditions to determine whether oxygen is required for the effects of parathyroidectomy on glycolysis of the lens *in vitro*.

Procedure. Male rats ranging from 80 to 160 grams in weight

were parathyroidectomized four to five days prior to an incubation. The serum calcium of each rat was checked to determine whether the operation had been successful.

On the day of the incubation the lenses were prepared, and incubated under aerobic or anaerobic conditions for four hours at 37°C. The incubation was stopped by removing the lenses from the medium. Duplicate 50 µl aliquots of the medium in each flask were removed for the analysis of lactic acid and duplicate 10 µl aliquots were removed for the analysis of glucose. The preincubation medium was also analyzed for glucose to permit calculation of the glucose uptake.

Results and discussion. The results are presented in figures 5 and 6. Each point in these figures represents the metabolic activity per gram wet lens in one to five flasks each containing four lenses from two rats of about the same weight. For example, lenses from the group of ten control rats weighing 120 to 129 grams had a mean lactate production, over a four hour aerobic incubation period, of 56 µmoles lactate per gram wet lens. The rat weight plotted was 125 grams.

The lactate production and glucose uptake per gram wet lens decreased with increasing weight of the rats. These findings are in agreement with those of Green and Solomon (1957) who found

that the metabolic activity per unit weight of the rabbit lens decreased with increasing age. From the results presented in figures 5 and 6 it is clear that, over a range of rat weights from 80 to 160 grams, parathyroidectomy reduced lens glycolysis under aerobic incubation conditions. The curves for both glycose uptake and lactate production of lenses from parathyroidectomized animals were parallel to, but about 30% lower than, the corresponding control curves.

Parathyroidectomy also reduced lens glycolysis under anaerobic conditions. Thus oxygen was not required for the effect of parathyroidectomy on carbohydrate metabolism in the rat lens in vitro.

Anaerobiosis did, however, alter lens carbohydrate metabolism by increasing the glucose uptake and lactate production in both control and parathyroidectomy groups by about 50%. Although the bulk of glucose utilized by the lens is metabolized in the glycolytic pathway, the existence of this Pasteur effect demonstrates that glucose is oxidized in the lens.

The interrelationship of parathyroid hormone effects on glycolysis and on extracellular concentrations of calcium and phosphate. Alterations in parathyroid hormone levels are known to affect carbohydrate metabolism in vivo. Because the concentrations of calcium and phosphate in the extracellular fluid of the parathyroidectomized

animal are different from normal it is possible that the hormonal effects on carbohydrate metabolism are due to these changes in ion concentrations. With respect to the lens, this possibility is made highly unlikely by the following evidence.

1) Lenses incubated in six media of differing calcium and phosphate ion concentrations showed no differences in glycolytic activity. These media contained either 0, 1.18 or 6.0 μmoles $H_2PO_4^-$/ml together with either 0 or 2.54 μmoles calcium ion/ml.
2) Merola, Kern and Kinoshita (1960) found that calcium concentrations ranging from 4.6 to 13.8 mg percent did not change the glucose uptake and lactate production by calf lenses.
3) Parathyroidectomy was found to reduce glucose uptake and lactate production in vitro (Figures 5 and 6); lenses from both control and parathyroidectomized rats were incubated in media identical in calcium and phosphate ion concentration.
4) Firschein (1962) was able to restore glycolysis of lenses from parathyroidectomized rats to the control level by the addition of parathyroid hormone in vitro; all lenses (both groups) were incubated in media identical in calcium and phosphate ion concentrations.

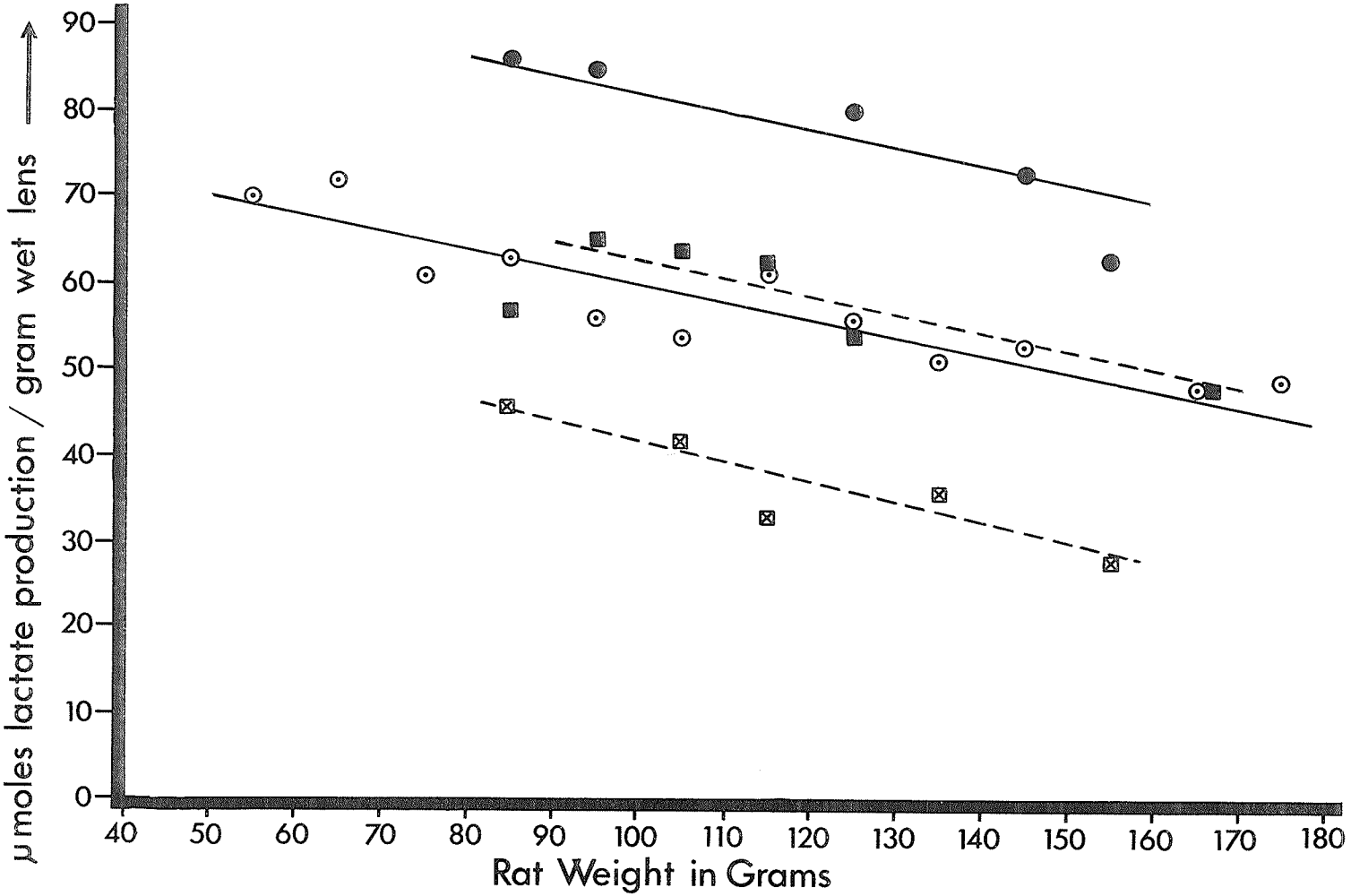


Figure 5. The effect of PTX on aerobic and anaerobic lactate production of lens in vitro. aerobic ⊙—⊙ anaerobic ●—● Control; aerobic ⊠---⊠ anaerobic ■---■ PTX

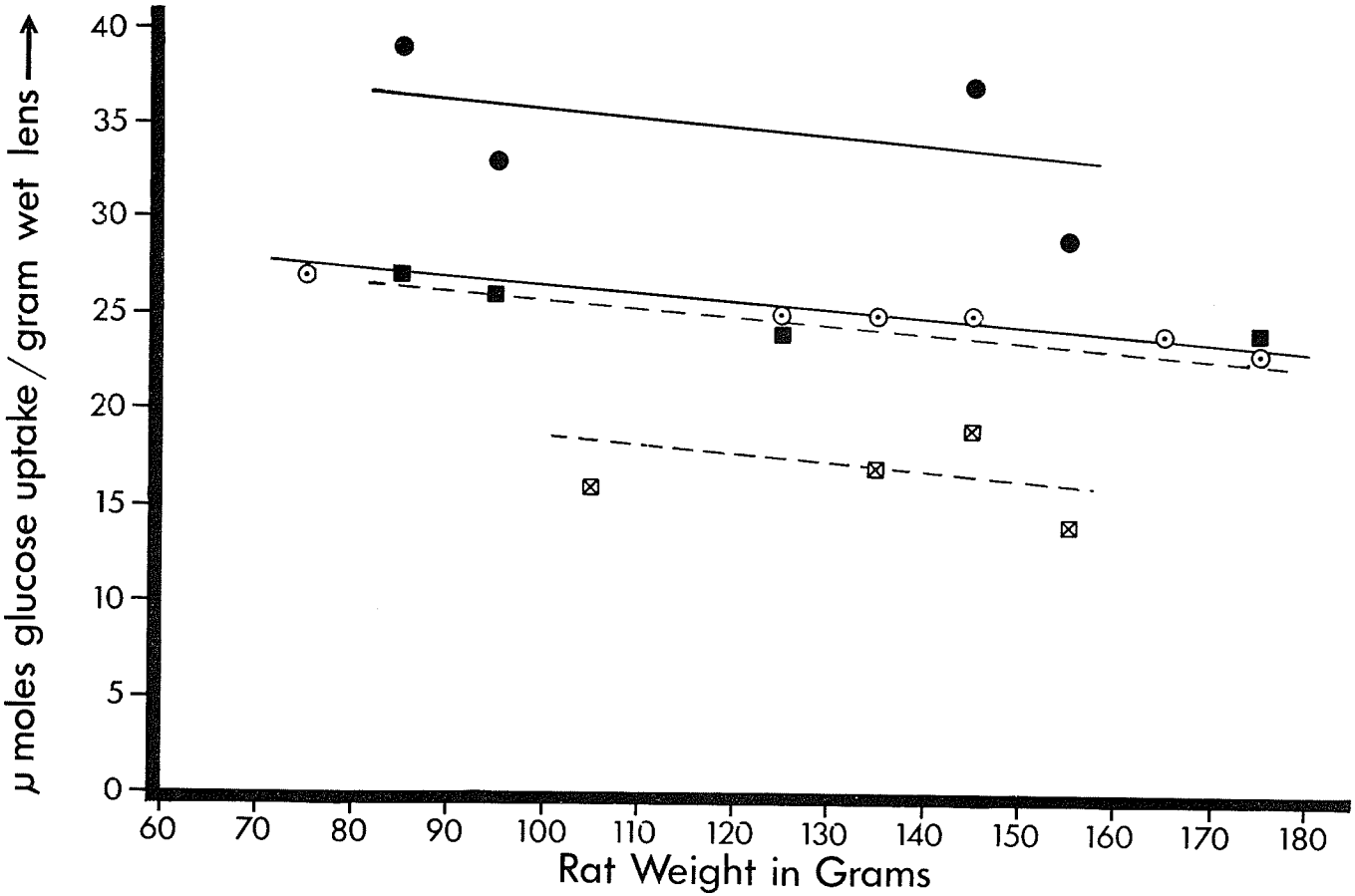


Figure 6. The effect of PTX on aerobic and anaerobic glucose uptake of lens *in vitro*. Control: aerobic ⊙——⊙, anaerobic ●——●; PTX: aerobic ⊠- - -⊠, anaerobic ■- - -■

CHAPTER VI

THE EFFECT OF PARATHYROIDECTOMY ON LENS PHOSPHATE METABOLISM

Introduction. Because parathyroidectomy was shown to reduce glycolysis in the lens (Chapter V) and because phosphate metabolism has been linked to glycolysis in many tissues, the following questions naturally arise.

1) Does parathyroidectomy have an effect on phosphate metabolism of the lens?
2) If this effect exists, does it produce, or result from, the effect on glycolysis?

No experimental work to date has demonstrated a direct effect of parathyroidectomy on lens phosphate metabolism. However, several studies have suggested that such an effect could exist. Experimental parathyroidectomy has resulted in cataractous lenses (Brolin, 1953) and Müller et al. (1956) reported that such lenses do not form organic phosphate esters as readily as normal lenses.

The effect of parathyroidectomy on several aspects of lens phosphate metabolism was studied in an effort to answer the above questions.

Experimental procedure. Lenses were incubated aerobically or anaerobically as previously described, except that P^{32} was added to the medium prior to incubation to produce an activity of about 300,00 cpm per ml. After four hours the flasks were removed from the incubator. The lenses were removed from each flask, dropped into a beaker containing two ml of saline and swirled for ten seconds to remove medium adsorbed onto the lenses.

The separation of, and analysis for phosphate in, the acid soluble, nucleotide and non-nucleotide fractions of the lens. After the saline wash the lenses were dropped into a test tube containing three ml of cold 0.58N perchloric acid. All subsequent procedures were carried out at 4°C. to minimize hydrolysis of nucleotides (Van Heyningen and Pirie, 1958). The lenses were homogenized and the homogenate centrifuged. The precipitate was discarded and the supernate (acid soluble fraction) was frozen until it could be analyzed. Aliquots of lens supernate and of the preincubation medium were plated on planchets and counted in a Nuclear Chicago gas flow counter. A 250 µl aliquot of lens supernate was digested with concentrated sulfuric acid and the phosphate content was determined chemically.

A two ml aliquot of lens supernate was pipetted into a 15 ml centrifuge tube and one ml of a suspension of charcoal (Norit A),

containing 30 mg per ml, was added to remove nucleotides. The centrifuge tube was agitated on a vortex mixer for 30 seconds and then allowed to stand for 30 minutes at 4°C. At the end of this time the contents were mixed and transferred to a Millipore filtration apparatus. The suspension was filtered, using positive air pressure. The air flow was continued until the charcoal on the filter was dry. The filter was transferred to a planchet and counted to determine the nucleotide P^{32}.

Aliquots of the filtrate (non-nucleotide fraction) were plated for the assay of P^{32}. Other aliquots were digested with concentrated sulfuric acid and the phosphate content determined chemically. The amount of phosphate in the nucleotide fraction was obtained by subtracting the amount in the non-nucleotide fraction from that in the total acid soluble extract.

A preliminary experiment showed that no substances absorbing in the 250 - 270 mμ range remained in the perchloric acid soluble fraction following Norit A treatment as above. This finding agreed with those of Crane (1958) and of Threlfall (1957) who also demonstrated that non-nucleotide phosphate esters were not adsorbed to Norit charcoal.

Results and Discussion. Values for glucose uptake, lactate production and nucleotide P^{32} content, obtained from four representa-

tive experiments in which rats with body weights in the range 80 - 160 grams were used, are plotted in Figure 7. A positive correlation, which was present under both aerobic and anaerobic incubation conditions, was found to exist between these three metabolic activities.

A reduction in the P^{32} content of the acid soluble and nucleotide fractions occurred with increasing rat weight (Figures 8 and 9). A similar relationship of lens glycolytic activity to increasing rat weight was previously shown (Figures 5 and 6).

A reduction in lens content of acid soluble P^{32} and of nucleotide P^{32}, but not of non-nucleotide P^{32}, was observed as a result of parathyroidectomy (Figure 8). Therefore, the reduction in the P^{32} content of the acid soluble fraction produced by parathyroidectomy was due solely to an effect upon the nucleotide fraction. In addition, in the lenses from parathyroidectomized rats, the percentage of the acid soluble phosphate which was present in the form of nucleotide phosphate was lower than that of corresponding controls (Figure 10). The most predominant nucleotides in the lens, the adenosine phosphates, are principally in the ATP form (Van Heyningen, 1962). The reduction in nucleotide phosphate by parathyroidectomy, therefore, indicated either a lowered ATP/ADP ratio, or a diminished nucleotide content in the lens. Experiments were carried out to settle this

important point. The nucleotide content of lenses from control and parathyroidectomized rats was measured before and after incubation. Two lenses were removed from a control or parathyroidectomized rat immediately after sacrifice. These lenses were added to three ml of perchloric acid and an acid soluble extract was prepared. The O.D. of this extract, measured at 267 mμ, was expressed relative to the lens wet weight and represented the preincubation nucleotide content (Figure 11). The acid soluble fraction of postincubation lenses was prepared from four lenses in three ml of acid and the O.D. of this fraction was measured at 267 and 257 mμ (Figure 12). Parathyroidectomy had no effect on the lens nucleotide content either before or after incubation. The reduction in nucleotide phosphate, therefore, was probably due to a lowered ATP/ADP ratio. Additional evidence for this was a lowering of the postincubation phosphate/nucleotide ratio of the lens nucleotide fraction subsequent to parathyroidectomy (Table 2).

In Chapter V it was pointed out that the effects of parathyroidectomy on glycolysis were not mediated through preliminary alterations in extralenticular calcium and phosphate. This does not exclude the possibility that the reduction, by parathyroidectomy, of lenticular glycolysis and of the ATP/ADP ratio is preceded by a fall in the intralenticular phosphate concentration. This could

be accomplished either by a shift in intralenticular phosphate which made it unavailable to glycolysis, or by a greater release of phosphate through the lens membrane. A parathyroidectomy-stimulated outflow of phosphate is consistent with the well established rise in extracellular phosphate observed *in vivo* subsequent to this operation.

Under anaerobic conditions parathyroidectomy still produced a decreased uptake of P^{32} into the acid soluble and nucleotide fractions (Figure 9) indicating that oxidative processes were not involved in the effect of parathyroidectomy on phosphate metabolism of the lens *in vitro*. These anaerobic studies were carried out using procedures identical with those previously described except that nitrogen took the place of air in the gas mixture.

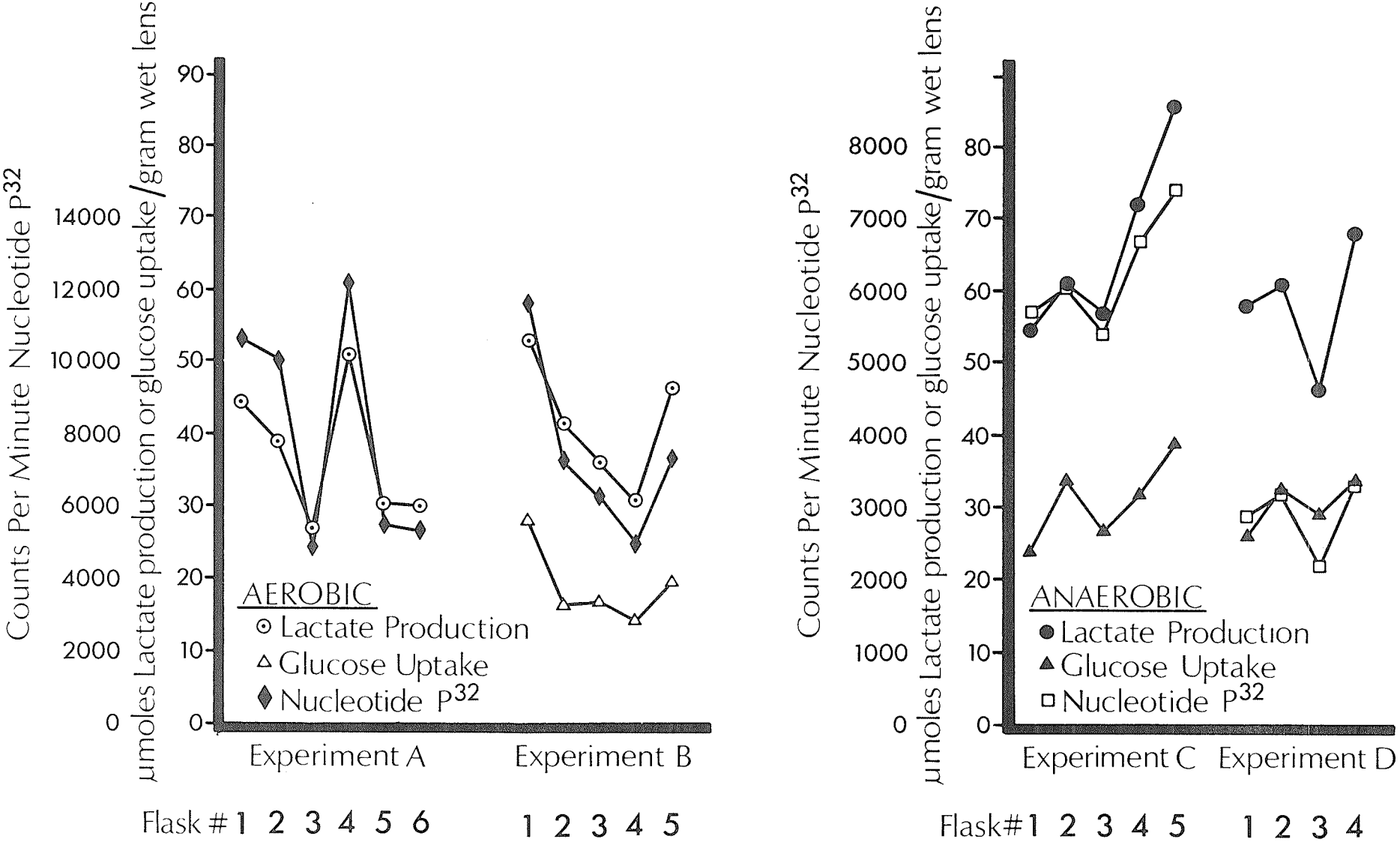


Figure 7. The relationship of lenticular lactate production to glucose uptake and nucleotide P^{32}. The three different types of points in a vertical plane represent the lactate, glucose and P^{32} determinations for one flask.

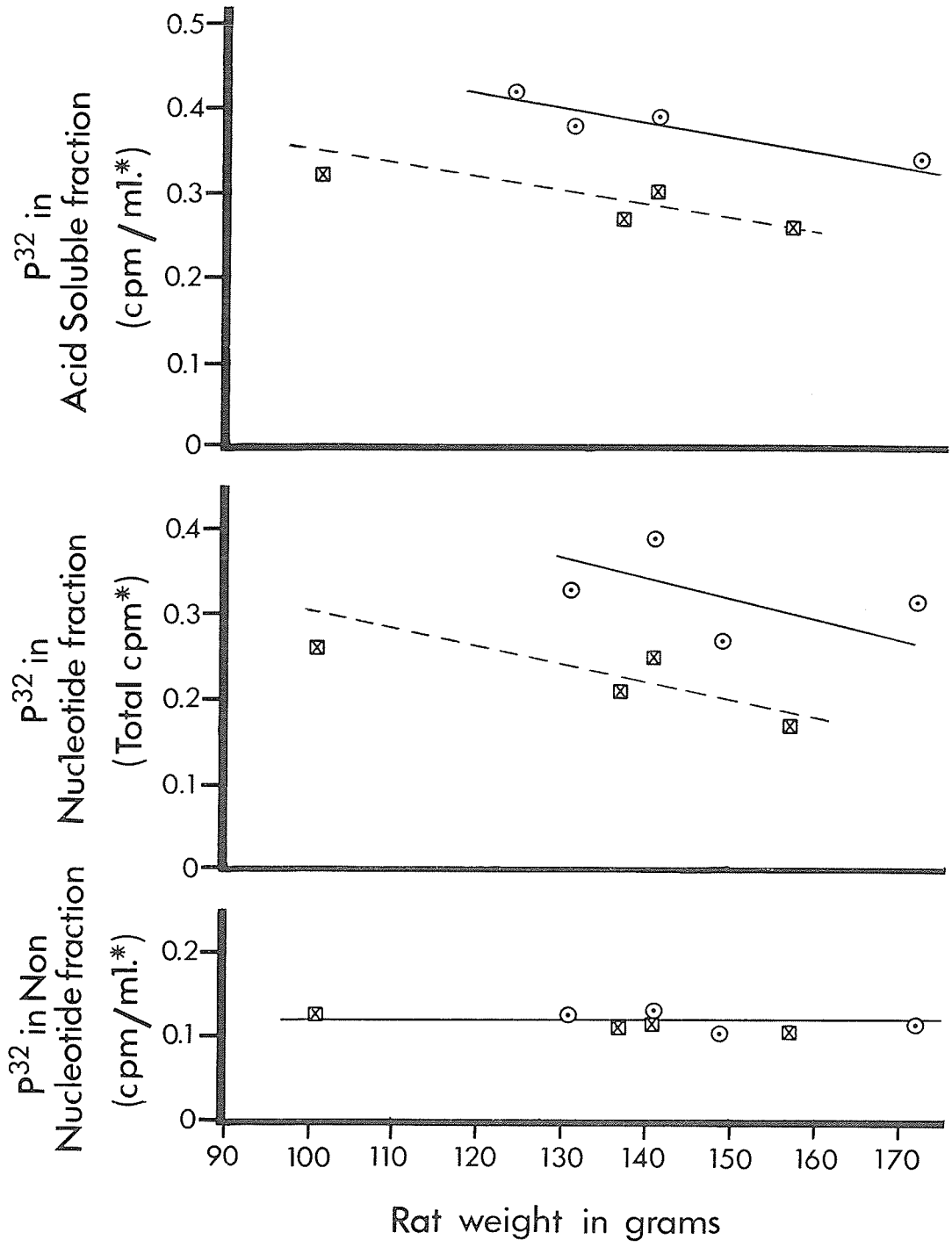


Figure 8. The effect of PTX on P^{32} uptake into lens fractions.
⊙——⊙ aerobic control; ⊠– –⊠ aerobic PTX.

* per cpm/ml media per gram wet lens

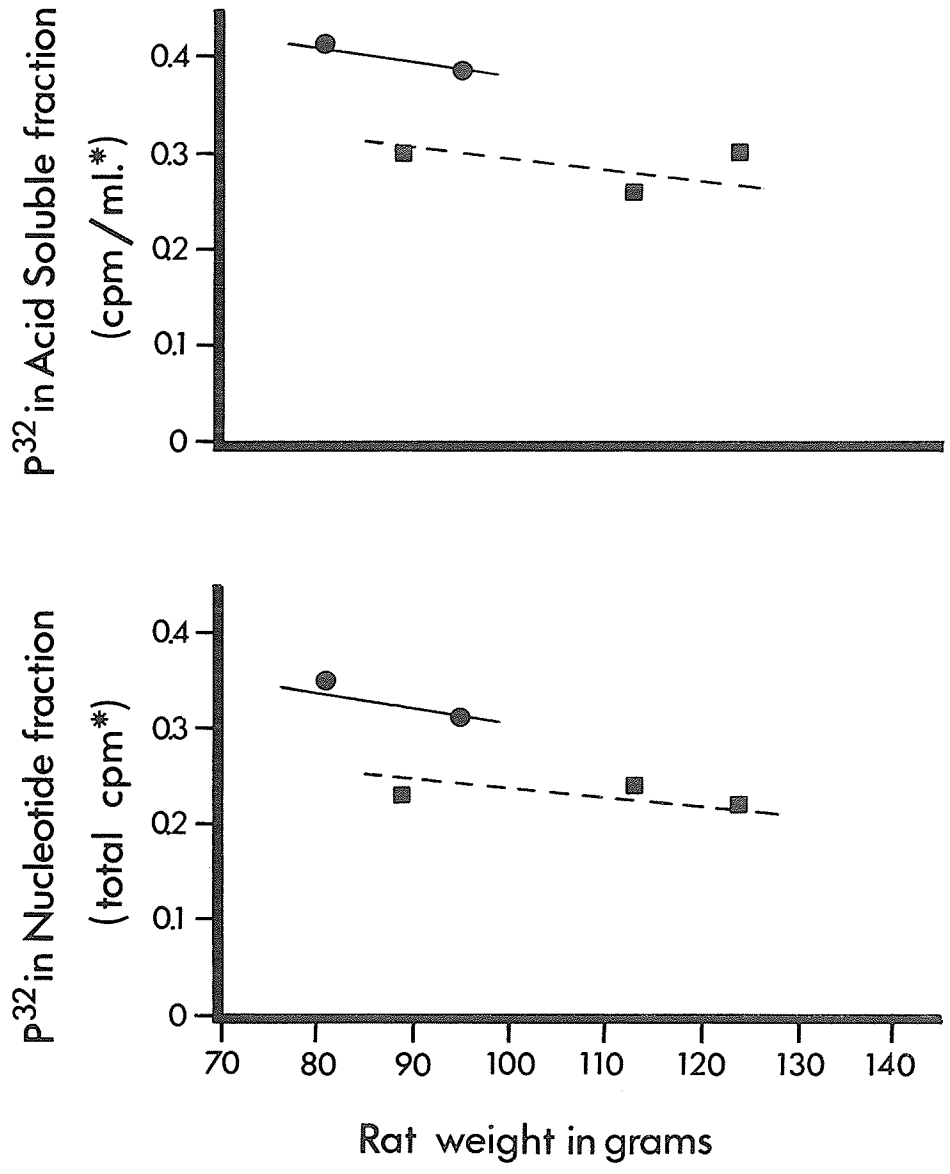


Figure 9. The effect of PTX on P^{32} uptake into lens fractions.
●——● anaerobic control; ■- - -■ anaerobic PTX.

* per cpm/ml media per gram wet lens.

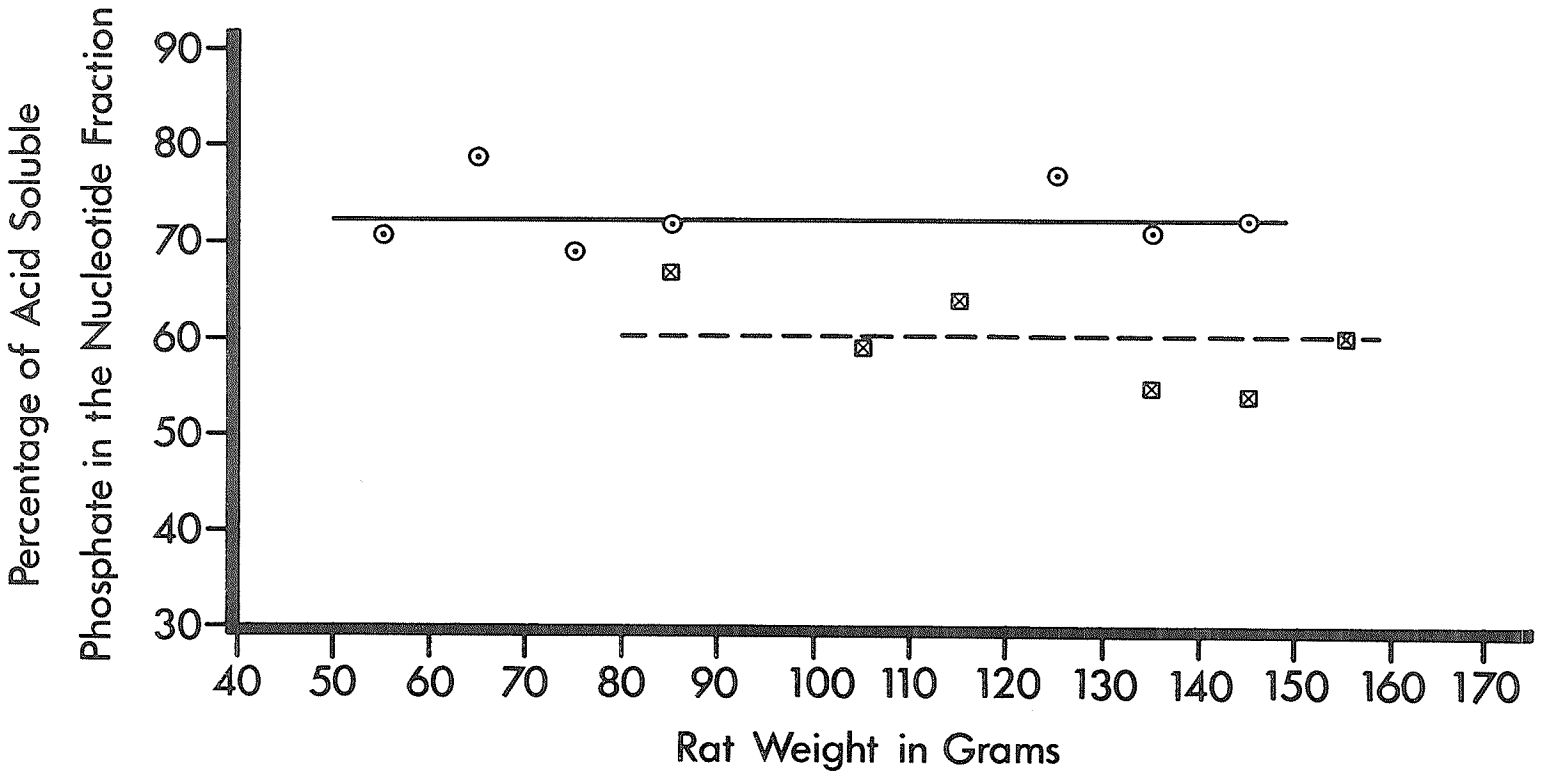


Figure 10. The effect of PTX on the percentage of acid soluble phosphate in the nucleotide fraction. ⊙——⊙ Control; ⊠– – –⊠ PTX.

* calculated as $\frac{\mu \text{ moles nucleotide phosphate per gram wet lens}}{\mu \text{ moles acid soluble phosphate per gram wet lens}} \times 100$

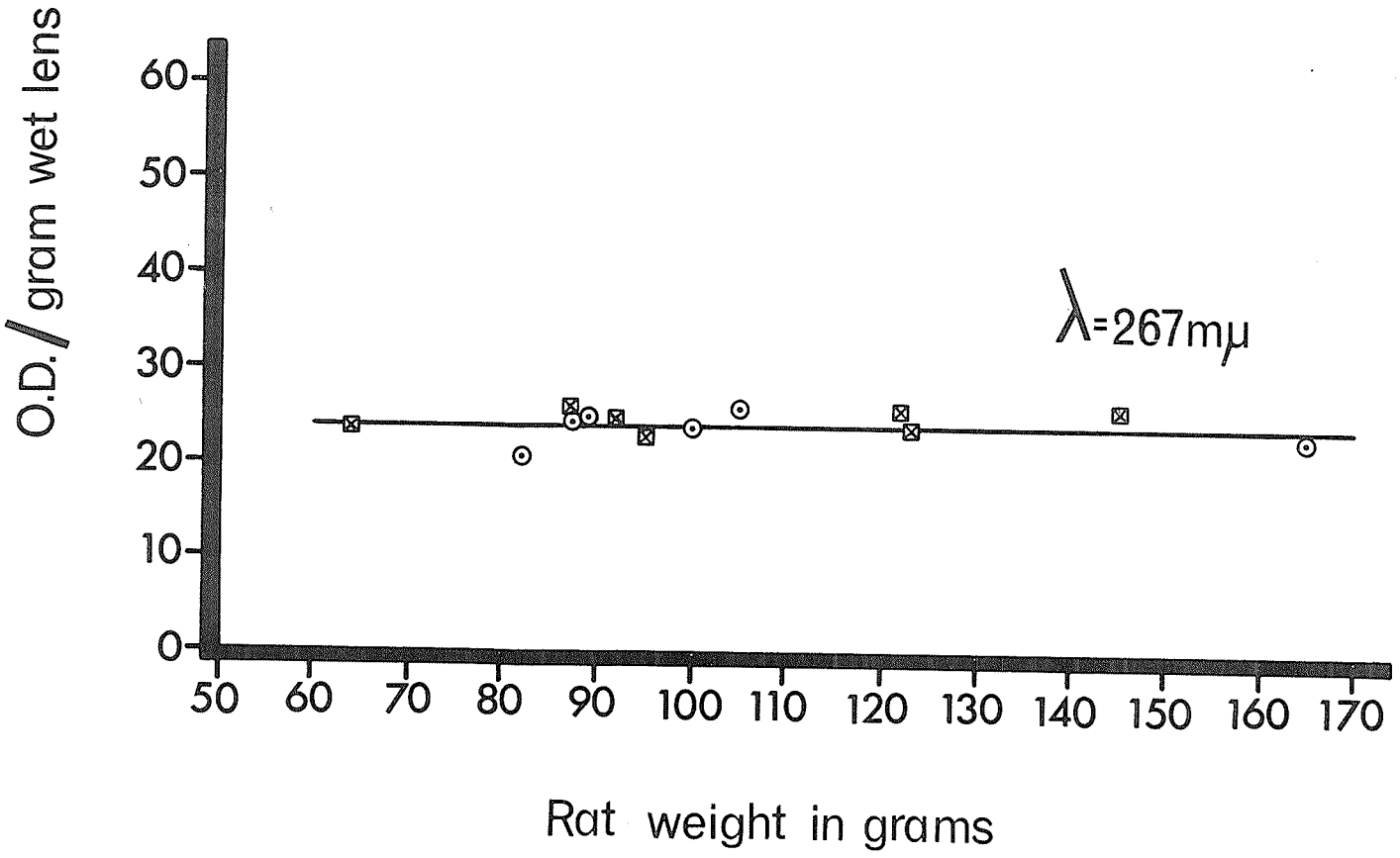


Figure 11. The effect of PTX on nucleotide content of lens before incubation. ⊙ control; ⊠ PTX.

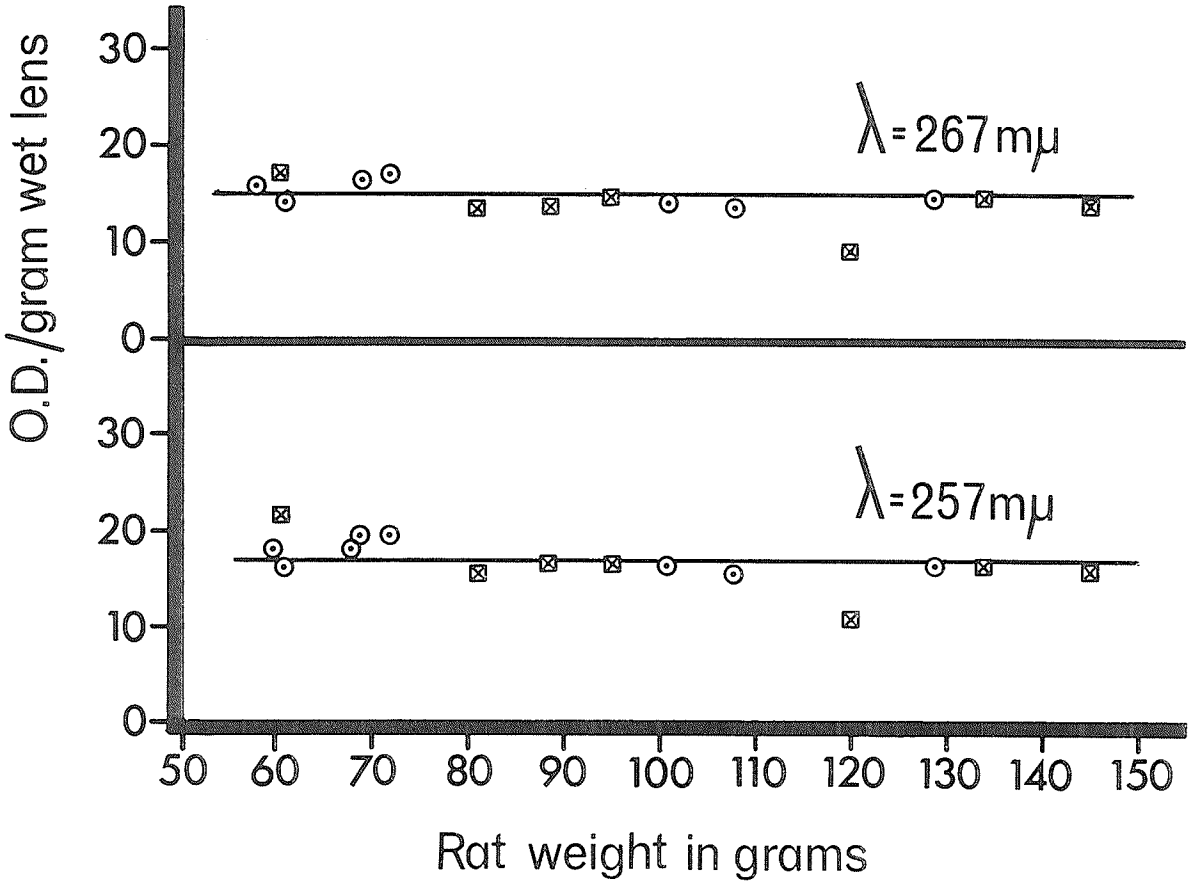


Figure 12. The effect of PTX on nucleotide content of lens after incubation. ⊙ control; ⊠ PTX.

TABLE 2

THE EFFECT OF PARATHYROIDECTOMY ON THE PHOSPHATE*/NUCLEOTIDE** RATIO OF THE LENS NUCLEOTIDE FRACTION

	Control	PTX
Experiment 1.	4.4	3.5
Experiment 2.	4.6	4.2
Experiment 3.	4.7	4.3

* Phosphate as μmoles/gram wet lens

** Nucleotide as μmoles adenosine/gram wet lens, calculated as adenosine. $\varepsilon_{257} = 14.7 \times 10^3$ (O.D./mole/litre) values are means of several determinations

CHAPTER VII

SUMMARY AND DISCUSSION

Discussion. No hypothesis that explains in a satisfactory manner the effects of parathyroid hormone and parathyroidectomy on the metabolism of carbohydrate, calcium and phosphate has yet been devised. The order in which these effects occur is not fully known and the principal aim of this study was to attempt to establish such a sequence. A secondary aim was to identify, if possible, the events that precede the above metabolic effects.

In this study the effects of parathyroidectomy on lens metabolism were a reduction in glycolysis, in the incorporation of P^{32} into nucleotide, and in the percentage of acid soluble phosphate which was present as nucleotide phosphate. It has been argued in the body of the thesis that the phosphate changes were the manifestation of a lowered ATP/ADP ratio.

The concentrations of Pi and of the various forms of adenosine phosphate are known to regulate glycolysis at three sites, each of which involves one of the following enzymes - hexokinase, phosphofructokinase and glyceraldehyde phosphate dehydrogenase. The following studies are among those that have demonstrated such regulation. Passonneau and Lowry (1962) found that a high ATP

concentration inhibited muscle phosphofructokinase activity, and therefore glycolysis, by increasing the concentration of fructose-6-phosphate required for this activity; whereas Pi, AMP and ADP activated the enzyme by reducing the concentration of fructose-6-phosphate required. Passonneau and Lowry postulated that this enzyme possessed two ATP sites, a primary active site and a secondary inhibitory site. The activators (Pi, AMP, fructose-6-phosphate and fructose diphosphate) overcame the ATP inhibition of the enzyme by competing with ATP for this secondary site. ATP concentration inhibits hexokinase, and therefore the rate of glucose utilization, in the erythrocyte (Rose and O'Connell, 1964). Pi stimulates glycolysis by acting as a substrate for glyceraldehyde phosphate dehydrogenase (Wu and Racker, 1959; and Wu, 1965). ATP, being a product of this reaction, would inhibit this enzyme. It follows from the above that the lowered ATP/ADP ratio subsequent to parathyroidectomy was not the cause but rather the result of the reduced glycolytic rate.

Because of the importance of Pi in the control of glycolysis the possibility of a prior action of the hormone on Pi concentration has considerable appeal. Several workers have suggested that the initial target of parathyroid hormone might be the membrane transport of phosphate (Dowse et al., 1963; Egawa and Neuman,

1964; Deluca et al., 1962; and Fang and Rasmussen, 1964).

Glycolysis in the lens was independent of alterations in the extra lenticular concentration of calcium and of phosphate. It would appear that the incorporation of P^{32} into nucleotide was also independent of these alterations in concentration because 1) the glycolytic activity was positively correlated to the incorporation of P^{32} into nucleotide and 2) changes in the incorporation of P^{32} were the result of changes in the glycolytic rate. Therefore, the extra and intralenticular phosphate pools were apparently independent of one another except for active metabolic processes which incorporated extralenticular P^{32} into intralenticular nucleotide. Bartlett (1958) found that the specific activity of intermediates formed during the uptake of P^{32} by the erythrocyte decreased in the following order: extracellular Pi, adenylate phosphorus, hexose phosphorus, intra cellular Pi and glycerate phosphorus. Applying the "precursor criterion" of Zilversmit he concluded that the passage of Pi from the extracellular to the intracellular pool occured through an obligatory intermediate, adenylate phosphorous, and that there was little or no direct contact between the extracellular and intracellular Pi pools.

The effects of parathyroidectomy on glycolysis and nucleotide P^{32} incorporation in the lens could not, therefore, be due to an

alteration in extracellular phosphate concentration. If, then, a reduction in phosphate concentration were responsible for these effects it must have occured in the intralenticular Pi pool. This fall in concentration of intralenticular Pi, whether it be produced by a decreased influx or an increased efflux of Pi, would be consistent with the well known rise in the extracellular phosphate concentration in vivo that results from this operation.

From the following studies it would appear that oxidative metabolism is a requisite for parathyroid hormone effects. Dowse et al. (1963) and Goldhaber (1962) both found that the effects of parathyroid hormone on bone were abolished when oxygen was not present. The addition of parathyroid hormone to mitochondrial preparations has produced a variety of effects including a stimulation of oxidation and of phosphate uptake (Sallis et al., 1963; and Rasmussen et al., 1964).

However, because of the large quantity of hormone required to produce these mitochondrial effects some doubt is cast on their physiological significance. Similar reservations are apparent in the statement of Sallis and Deluca (1964) that "hormone stimulation in vitro of both Pi transport and ATPase activity requires relatively large amounts of the hormone. It seems, therefore, that caution must be exercised before attempting to interpret and correlate these

interesting findings of parathyroid action in vitro to those of its role in the living organizm." In addition, though parathyroid hormone does stimulate mitochondrial oxidation, it can do so without increasing phosphorylation or Pi uptake (Fang and Rasmussen, 1964; and Fang et al., 1963).

In the present study the metabolic effects of parathyroidectomy (a reduced glucose uptake, lactate production and P^{32} incorporation) were still present when lenses from parathyroidectomized rats were incubated in the absence of oxygen. In the lens, therefore, the effects of parathyroidectomy are not dependent on oxidative processes.

If the stimulation of mitochondrial oxidation that occurs in vitro subsequent to the addition of parathyroid hormone is of physiological significance, it may be reasonably concluded that parathyroidectomy would result in an inhibition of mitochondrial oxidation. If this were the case, parathyroidectomy would be expected to produce an increased glycolytic rate. In the lens, however, glycolysis was reduced following parathyroidectomy, further indicating that the observed effects of parathyroidectomy were independent of oxidative processes.

Summary and conclusions. The effects of parathyroidectomy on certain aspects of carbohydrate and phosphate metabolism in the lens were studied under aerobic and anaerobic conditions.

Lens wet weight, dry weight, and water content were not affected by parathyroidectomy or by the incubation procedures. Under both aerobic and anaerobic incubation conditions, glycolysis and P^{32} incorporation into nucleotide were positively correlated, and both metabolic activities decreased with increasing rat weight. A Pasteur effect was demonstrated, indicating that the lens was capable of oxidizing glucose. Parathyroidectomy reduced glycolysis and the incorporation of P^{32} into nucleotide under both aerobic and anaerobic conditions. This operation also reduced the percentage of acid soluble phosphate occurring as nucleotide phosphate, but did not reduce the nucleotide content. The data suggested that parathyroidectomy resulted in a lowered ATP/ADP ratio. This lowered ratio did not produce, but rather resulted from, the reduced glycolysis.

It was concluded that oxidative processes were not required for the effects of parathyroidectomy on lens metabolism and that the event producing these effects was probably a reduction in the intralenticular Pi concentration.

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