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The Influence of Sodium Phenobarbital on the Hepatic Adenosine Triphosphatase Activity and the Development of this Enzyme in the Rat

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THE INFLUENCE OF SODIUM PHENOBARBITAL ON THE HEPATIC ADENOSINE TRIPHOSPHATASE ACTIVITY AND THE DEVELOPMENT OF THIS ENZYME IN THE RAT

BY

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_____ Thesis Adviser Date

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INTRODUCTION

It has long been understood that the living cell is a homeostatic entity existing in dynamic equilibrium with its environment. The expended energy, metabolic transformations, enzymatic processes, and transport mechanisms involved in maintaining constant the composition of the extracellular fluid, the milieu interieur, have been under constant scrutiny by researchers for many decades. One facet of the research has recently been vigorously directed at the energy involvement in maintaining the cellular electrolyte balance. The term "electrolyte pump" has been used to describe the operation of maintaining the cellular electrolyte balance. The analogy to a mechanical pump is apt, in that energy is utilized to move material, in this case ions, against an opposing gradient. In all cases studied, the source of energy for this work has been adenosine triphosphate.

Invariably the problem of directing feasible studies at measuring the parameters of adenosine triphosphate production and consumption confronted the researchers. Finally in the early 1950's studies provided evidence that the liver endoplasmic reticulum, or its fragment microsomes, contain an enzyme system capable of converting various drugs and aromatic substances to more polar compounds (1-3). At present many different reactions are known to be catalyzed by liver microsomes (4-11). Among these reactions are enzyme catalyzed reactions such as demonstrated by adenosine triphosphatase. Thus, since 1957 when Skou (12) first demonstrated

the presence in crab nerve of a sodium-potassium-activated magnesium-dependent adenosine triphosphatase enzyme, it has been found to occur in a wide variety of tissues, including brain (13), red cell membranes (14), kidney (15), ciliary body and retina of the eye (16), avian salt glands (17), electric organ of Electrophorus electricus (18), and heart muscle (19). Therefore, a great deal of attention has been directed at isolating microsomal adenosine triphosphatase activity and measuring this activity in an attempt to correlate the effects with the "electrolyte pump."

There is abundant evidence (13, 20-23) that the sodium-potassium-activated adenosine triphosphatase plays a major role in converting the metabolic energy of adenosine triphosphate into the osmotic work required to transport monovalent cations across cell membranes. Some knowledge of the mechanism by which sodium-potassium-activates this adenosine triphosphatase would thus be of interest, but the enzyme, which is isolated in the microsomal fraction of tissue homogenates, is invariably particulate and is difficult to study.

Inturrisi and Titus (24) introduced a specific difficulty in measuring adenosine triphosphatase when they found that microsomal fractions contain not only adenosine triphosphatase but also a family of neutral phosphatase activities that require potassium, are inhibited by sodium, and are capable of hydrolyzing a variety of artificial substrates, including p-nitrophenyl phospate, acetyl phosphate, and carbamyl phosphate. Nevertheless, even with obvious

difficulties many studies have been undertaken in an effort to attain more explicit information concerning various aspects of the adenosine triphosphate energy system as catalyzed by adenosine triphosphatase. Of particular interest to researchers has been the response of microsomal adenosine triphosphatase in heart, muscle, brain, and liver of both adult and infant animals.

It is known that newborn and young are more sensitive than adults to most drugs introduced into the body. For instance, phenobarbital is more toxic to newborn than to adult rabbits and rats (25). Thus, age is given utmost consideration prior to administration of drugs. This increased sensitivity could possibly be due to the differences in hepatic drug metabolizing enzymes. It has been shown that the microsomal enzymes concerned with drug metabolism are inactive in the newborn and increase in activity until the onset of maturity (26,27). Jondorf et al. (28) showed that tissues of fetal and newborn mammals are known to be deficient in a number of enzymes, and that the activity increases after the animal is born.

Beyth and Gutman (29) demonstrated that microsomal magnesium-adenosine triphosphatase activity increases progressively with the development of the rabbit, both during intra-uterine and extra-uterine growth. The largest increase in activity occurs between the first and tenth day of life. On the other hand sodium-potassium-dependent adenenosine triphosphatase activity is not significantly changed during the first three stages of development (fetus to ten-day-old rabbit). Adenosine triphosphatase activity

of newborn rabbit muscle rises to a maximum in 8 to 10 days after birth according to Holland and Perry (30).

Differences between hepatic microsomal drug metabolizing enzymes present in newborn and adult animals are indicated by several studies. It was reported that the hepatic microsomal fractions of newborn guinea pigs and mice are characterized by an absence of certain metabolizing enzymes (28). Infant guinea pigs are devoid of the enzymatic systems that N-demethylate aminopyrine, O-dealkylate phenacetin, oxidize hexobarbital, and conjugate phenolphthalein as the glucuronide. Although these enzyme systems can not be demonstrated at 24 hours after birth, they begin to appear during the first week and activity increases until the animals are about two months old.

Species differences have also been shown to affect the results of hepatic microsomal enzyme activity. Guinn et al. (31) showed that the level of hepatic microsomal drug-metabolizing activities is species as well as strain dependent. Cram and Fouts (32) discussed the fact that phenobarbital will stimulate hepatic drug-metabolism to a different extent in different strains of animals. Even within the same animal strain, different hepatic microsomal enzymes can be stimulated to different degrees by phenobarbital, and some pathways do not respond at all.

It is interesting to note that over 200 drugs, insecticides, carcinogens, and various other chemicals have been shown to increase the activity of drug metabolizing enzymes located in the hepatic

microsomes (33). This enzymatic induction, characterized by increased levels of enzyme protein, accelerates biotransformations of natural body constituents such as adenosine triphosphate. Sladek and Mannering in 1969 (34) discussed several ways by which inducing agents can increase enzyme activity: (A) by increasing enzyme synthesis, (B) by causing the synthesis of a more active enzyme, (C) by directly activating existing enzyme, (D) by indirectly activating existing enzyme, or (E) by stabilizing the enzyme. They also specifically outlined four theories for phenobarbital enzyme induction: (A) phenobarbital may cause the synthesis of a unique messenger RNA, (B) phenobarbital might stimulate induction at the transcriptional level of protein synthesis, (C) the messenger RNA involved in phenobarbital stimulation is templated in the nucleus; (D) the enzyme activity resulting from phenobarbital treatment is sensitive to some other cellular effect. Many studies have been conducted in an effort to understand the in vivo response of adenosine triphosphatase to various compounds and thus enable researchers to accept or discard the various theories that are being constantly introduced concerning the adenosine triphospate energy system.

Askari (35) reported that oligomycin not only blocks the activating effect of adenosine triphosphatase but that under proper conditions it can, like adenosine triphosphate, activate p-nitrophenyl phosphatase. The sodium-potassium-activated enzyme system is also inhibited by sulfhydryl-blocking reagents (18,36). In 1965, Skou and Hilberg (37) demonstrated that silver ions, mersalylic acid and

PCMB (p-chloromercuribenzoate) not only reversibly block sulfhydryl groups but inhibit the activity of the sodium-potassium-activated adenosine triphosphate enzyme system. Organic arsenicals (oxophenarsine) which react with two sulfhydryl groups also inhibit the activity of the enzyme system. In addition, the enzyme system, magnesium-dependent adenosine triphosphatase, is specifically inhibited by low concentrations of cardiac glycosides (19). Furthermore, high concentrations of a variety of other agents have been shown to be capable of inhibiting this enzyme activity (19,24,38-47).

The effect of catecholamines on in vitro sodium-potassium-activated adenosine triphosphatase apparently varies from preparation to preparation. Using guinea pig hearts, Lee and Yu (19) found that adrenalin, norepinephrine, and the sympathetic blocking agents depressed the sodium-potassium adenosine triphosphatase activity. In contrast, Bonting et al. (16) found epinephrine and norepinephrine to be without effect on the activity of the sodium-potassium adenosine triphosphatase isolated from several other tissues. Kennedy and Nayler (48) demonstrated that epinephrine, norepinephrine, dilantin, and tetraethylammonium chloride failed to cause changes in the adenosine triphosphatase enzyme system.

Innumerable studies have shown that a wide range of drugs such as barbiturates (49) and chlorinated hydrocarbon-insecticides (50-52) increase the activity of the hepatic microsomal drug-metabolizing enzyme system. In a study on rats, rabbits and squirrel monkeys, Cram and Fouts (32) were able to find that dichloro-diphenyl-trichloroethane (DDT) and chlordane stimulated several hepatic microsomal

drug-metabolizing enzyme systems, including the system that reduces adenosine triphosphate. From such and many other investigations it is becoming increasingly possible to better understand the association of adenosine triphosphate with the activity of the "electrolyte pump."

Fiske and Subbarow (53) were the first to develop an adequate system for measuring the activity of adenosine triphosphatase. A search of the literature revealed that neither studies measuring the development of hepatic adenosine triphosphatase activity in newborn and infant rats, nor studies of chemical stimulation of hepatic adenosine triphosphatase in adult male and female rats have been reported. Therefore, a study was conducted in an effort to determine whether this enzyme system increases in activity with the growth and maturity of the animal, and/or whether adenosine triphosphatase activity can be affected by chemical means. It is known that this enzyme, adenosine triphosphatase, is present in microsomes, mitochondria, and several other soluble fractions of tissue homogenates (12,54,55). A modified system of Fiske and Subbarow (56) was used in these experiments to measure hepatic adenosine triphosphatase activity.

MATERIALS AND METHODS

Sprague-Dawley rats were utilized throughout this study. The rats were maintained in an air-conditioned room (approximately 72° F.) and provided with water and Purina Laboratory Chow ad libitum.

Adult male and female rats were given various dosage levels (30mgm./Kg. to 150mgm./Kg.) of sodium phenobarbital intraperitoneally for three days prior to utilization. Control animals were given an equal volume of normal saline at the same injection intervals as the phenobarbital injected animals. Infant rats were allowed to nurse ad libitum. Adenosine triphosphatase activity was measured by a modified method of DuBois and Potter (56). The method was modified by substituting magnesium chloride for calcium chloride as the enzyme activator in the reaction system. This method relates the enzyme activity to the release of inorganic phosphorous from a system containing an excess of adenosine triphosphate and a rate-limiting amount of adenosine triphosphatase in a buffered medium containing magnesium chloride as an enzyme activator.

Adult animals were sacrificed by decapitation without anesthesia and infant animals were anesthetized slightly with ether and their livers were excised as rapidly as possible. The extracted livers were maintained on ice and immediately weighed. Liver homogenates were prepared with a Potter-Elvehjem teflon-glass homogenizer in sufficient cold double-distilled water to provide a 5 per cent homogenate. A portion of the 5 per cent homogenate was further diluted to provide a 0.5 per cent liver homogenate. The test

system contained 0.15 ml. of 0.05 M barbital buffer (pH 7.4), 0.05 ml. of 0.04 M magnesium chloride, 0.15 ml. of 0.013 M adenosine triphosphate, tissue and sufficient water to make a final volume of 0.65 ml. Assays were performed in duplicate in all cases using 0.1 ml. and 0.2 ml. of 0.5 per cent liver homogenates. These volumes represent additions of 0.5 mgm. and 1 mgm. of wet tissue per duplicate assay. The tissues were incubated in a Dubnoff metabolic shaker at 38°C. for 15 minutes and 0.1 ml. of 50 per cent trichloroacetic acid was added to stop the reaction. The mixture was then centrifuged for five minutes and the inorganic phosphorous liberated from adenosine triphosphate was measured using 0.3 ml. aliquots of the supernatant according to the method of Fiske and Subbarow (53). A 0.3 ml. portion of the supernatant was mixed in culture tubes containing 1.0 ml. of a molybdate-sulfuric acid mixture and 4.5 ml. of double-distilled water. A mixture of 1.0 ml. of molybdate-sulfuric acid and 4.8 ml. of distilled water served as the blank. The addition of 0.2 ml. of a reducer mixture (14.7 per cent sodium bisulfite, 0.5 per cent sodium sulfite, and 0.25 per cent aminonaphtholsulfonic acid) gave a final volume of 6.0 ml. and activated the color reaction. After the tubes were shaken, the color was allowed to develop for ten minutes. The color was measured with a Bausch and Lomb Spectronic 20 at 660 mu. The adenosine triphosphatase activity was expressed as units, i.e., the micrograms of inorganic phosphorous liberated from adenosine triphosphate by one milligram of tissue during a 15 minute incubation period.

Prior to the study using adult and infant rats, the following experiments were performed:

Standard Inorganic Phosphorous Curve. An experiment to determine the relationship between the concentration of inorganic phosphorous present in a 0.3 ml. aliquot of a standard phosphate solution and the optical density at 660 mu. was conducted. Identical reaction vessels as those previously described and monopotassium phosphate varying from 0 to 100 micrograms were prepared. The reaction vessels were incubated for 15 minutes and trichloroacetic acid was added to stop the reaction. Three-tenths ml. aliquots were analyzed for inorganic phosphorous content by the previously described method. The results of this experiment are shown in Figure 1. Each point on the standard curve is representative of triplicate determinations. The results of this experiment illustrate that there is a direct relationship between optical density and inorganic phosphorous in the range of 0 to 100 micrograms of a standard phosphate solution. Adenosine triphosphatase activity is directly related to the amount of inorganic phosphorous liberated; therefore, this curve was used to determine the results of this study.

The relationship between tissue levels and the amount of inorganic phosphorous formed was determined by preparing culture tubes containing 10, 5, 2, and 1 milligram of tissue. The same procedure as previously described was used to prepare the reaction vessels and measure the inorganic phosphorous liberated. The results of this experiment are shown in Figure 2. As the graph

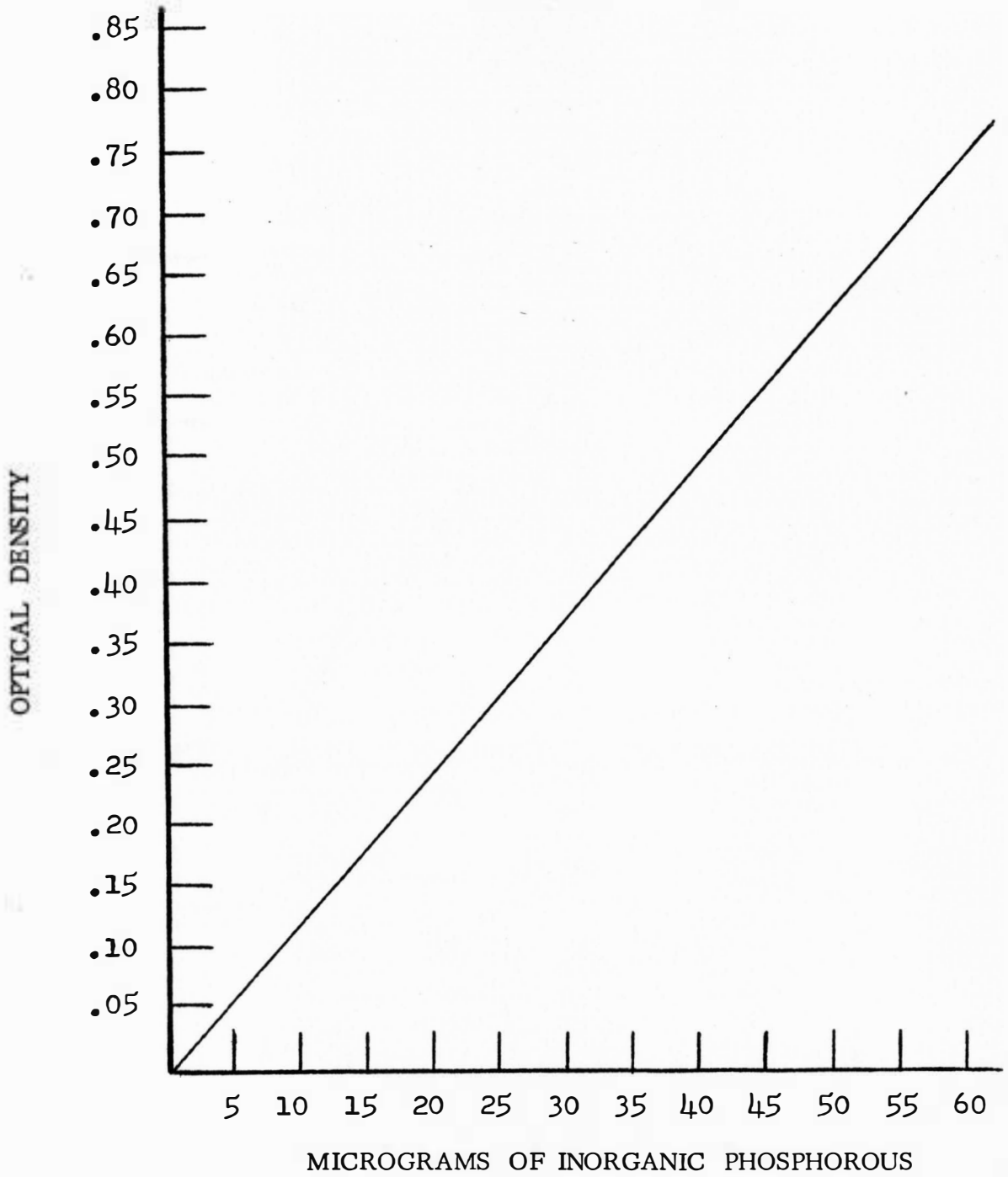


Fig. 1. Standard inorganic phosphorous curve. Optical density versus micrograms of inorganic phosphorous present in a 0.3 ml. aliquot.

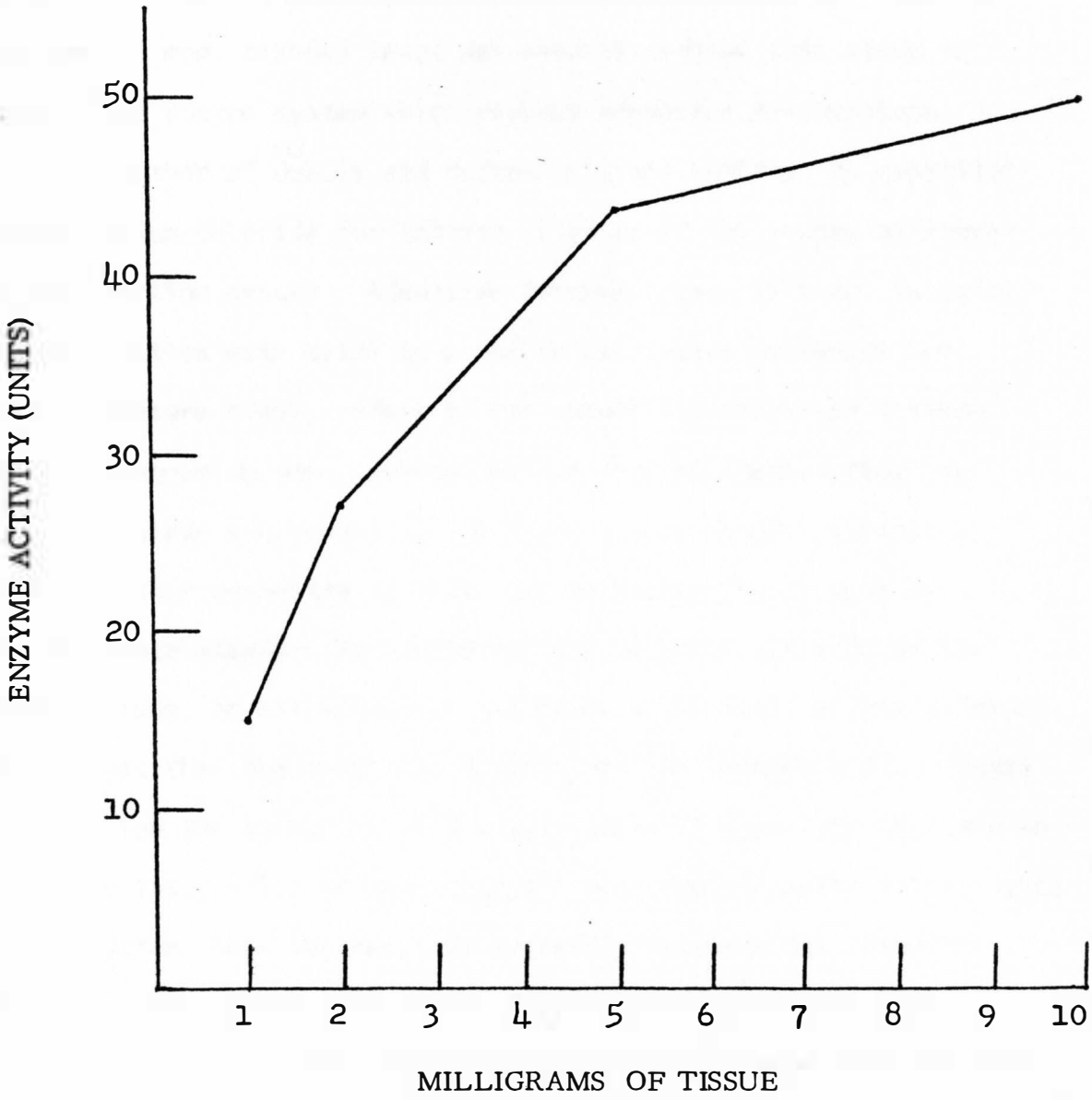


Fig. 2. The activity of the enzyme system which reduces adenosine triphosphate in infant and newborn rats. One unit equals the micrograms of inorganic phosphorous liberated by one milligram of tissue in 15 minutes.

indicates there is a relationship between the amount of inorganic phosphorous formed and the levels of tissue utilized in the reaction system. However, a distinct ten-fold relationship between the 0.5 per cent (1 mgm. tissue) level was used throughout this study to measure the enzyme system which reduces adenosine triphosphate.

The method of DuBois and Potter (56) was modified by substituting magnesium chloride for calcium chloride as the enzyme activator in the reaction system. Adenosine triphosphatase is known to exist in complexation with metal ions and in particular magnesium ion. Most literature simply refers to the complex as adenosine triphosphatase-magnesium ion; whereas, Selwyn (57) postulates that the metal ion binds a hydroxyl ion to yield a more complex substance. Selwyn further demonstrated that when magnesium ion is used as the reference standard for measuring the relative activity of the bivalent ions, in all instances the relative activity of the calcium ion is less than one-tenth the activity of the magnesium ion. These findings led the author of this dissertation to postulate that due to the close integration of magnesium ion with the adenosine triphosphate energy system, this ion may then certainly activate the adenosine triphosphatase enzyme. In order to test this theory, reaction vessels identical to the earlier described system were prepared with varying concentrations of magnesium chloride used as the enzyme activator. Magnesium chloride concentrations of 1.0, 0.1, 0.05, and 0.01 M were utilized in each of four culture tubes. The results are graphed in Figure 3. The graph clearly indicates that the

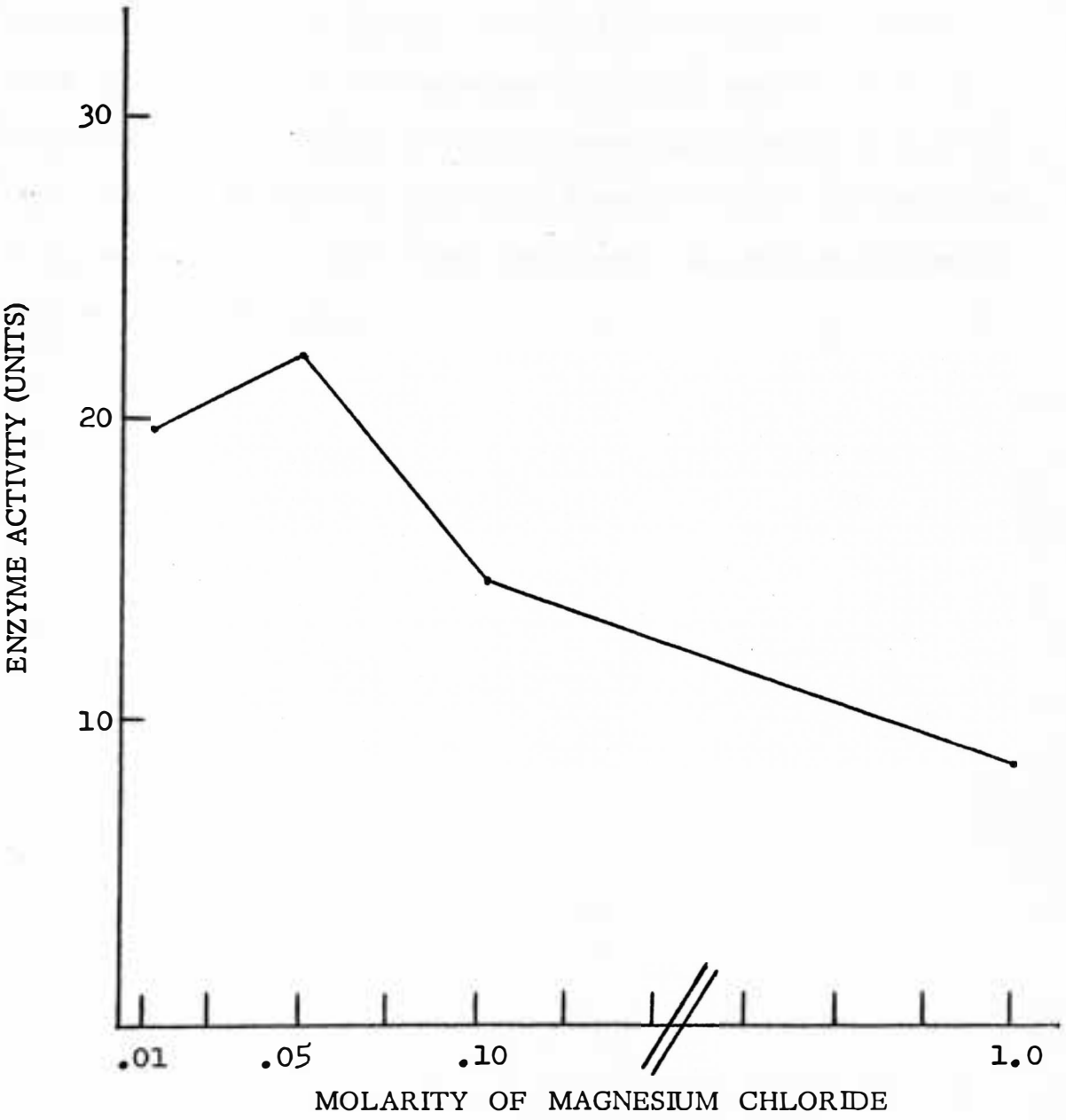


Fig. 3. The effect of various concentrations of magnesium chloride on the adenosine triphosphatase activity of rat liver.

concentration of magnesium chloride definitely affects the amount of inorganic phosphorous formed. A comparison of results received with calcium chloride and magnesium chloride as enzyme activators indicated that the magnesium ion displayed approximately a four-fold increase in activity when using identical tissue concentrations. A concentration of 0.04 M magnesium chloride was used as the enzyme activator in this study.

RESULTS

The effect of sodium phenobarbital on the enzyme system which reduces adenosine triphosphate in adult male and female rats.

Several experiments were conducted to determine the effect of sodium phenobarbital on the hepatic microsomal adenosine triphosphatase activity of adult male and female rats. In triplicate trials, three adult male and female rats were given intraperitoneal injections of 30 mgm./Kg. of sodium phenobarbital for three days prior to sacrifice. An identical number of control animals were given an equal volume of normal saline intraperitoneally at the same intervals. The animals were sacrificed by decapitation, the livers excised and homogenized in glass distilled water (4°C.), reaction vessels prepared as previously described, and inorganic phosphorous measured. The results of these measurements are shown in Table 1 where each value represents the average obtained on the livers of at least four animals plus or minus the standard deviation from the mean. The results of this experiment indicated that adenosine triphosphatase activity of the liver was not affected by sodium phenobarbital at this dosage level. Two trials were conducted with the dosage regimen increased to 60 mgm./Kg. of sodium phenobarbital for three days. The results of this experiment are shown in Table 1. Results of these trials demonstrated that daily injections of 60 mgm./Kg. of sodium phenobarbital did not significantly change the adenosine triphosphatase activity of adult male and female rats.

Table 1

The Effect of Sodium Phenobarbital on the Adult Rat Liver Adenosine Triphosphatase Activity

Injections (Days)	Dose of Sodium Phenobarbital	Number of Animals	Adenosine Triphosphatase Activity (Units)
3	Control	30	41.0 ± 0.78
3	30 mgm./Kg.	9	41.0 ± 2.09
3	60 mgm./Kg.	6	41.5 ± 1.25
3	75 mgm./Kg.	6	40.0 ± 1.12
3	100 mgm./Kg.	5	41.0 ± 1.87
3	150 mgm./Kg.	4	41.5 ± 1.50

An experiment was performed to determine if hepatic male and female rat adenosine triphosphatase activity could be affected by further increasing the dose of sodium phenobarbital. Duplicate trials in which 75 mgm./Kg. and 100 mgm./Kg. of sodium phenobarbital was given for three days to each of two groups of adult male and female rats were performed. The animals were sacrificed on the fourth day and the liver assayed for adenosine triphosphatase activity. The results as shown in Table 1 indicated that there was no significant effect noted in hepatic adenosine triphosphatase activity. An experiment was undertaken to determine the effect of three daily injections of 150 mgm./Kg. of sodium phenobarbital on the hepatic adenosine triphosphatase activity of adult male and female rats. The results, as illustrated in Table 1, again showed that adenosine triphosphatase activity in the liver was not affected by this dosage level of sodium phenobarbital.

It was noted that the rats given higher doses of sodium phenobarbital were sedated for extended periods of time and it is possible that failure to properly consume food and water may have affected the results. Furthermore, the administration of sodium phenobarbital at these higher dosage levels was extremely toxic and in some instances was lethal. Therefore, an experiment was undertaken to determine the effect of two daily injections of 30 mgm./Kg. of sodium phenobarbital for five days on the adenosine triphosphatase activity of the liver of adult male and female rats. Results of this experiment showed that two daily injections of 30 mgm./Kg. of

sodium phenobarbital failed to produce a response in the hepatic adenosine triphosphatase activity.

Measurement of newborn and infant hepatic microsomal adenosine triphosphatase activity. A study was undertaken to determine the rate of development of adenosine triphosphatase activity in the livers of newborn and infant rats. In this study newborn and infant rats were sacrificed at various intervals following birth and their livers assayed for adenosine triphosphatase activity. The results of these studies are presented in Figure 4. Each point on the curve represents the average of duplicate assays of adenosine triphosphatase activity of the liver of six to nine animals. Enzyme activity is expressed as units (micrograms of inorganic phosphorous liberated per milligram of tissue in 15 minutes). Results of this experiment showed that newborn and infant rats demonstrated enzyme activity similar to the adenosine triphosphatase activity of adult rat liver.

In an effort to determine whether adenosine triphosphatase activity is present in rats prior to birth, an experiment was performed using fetal rat livers. The death of a female rat during parturition provided an opportunity for Cesarian surgery. The fetal rats were surgically removed, sacrificed, the livers removed, homogenates prepared, and adenosine triphosphatase activity measured. The results indicated that adenosine triphosphatase activity is present in the fetus at approximately the same activity as is exhibited by adult rat livers.

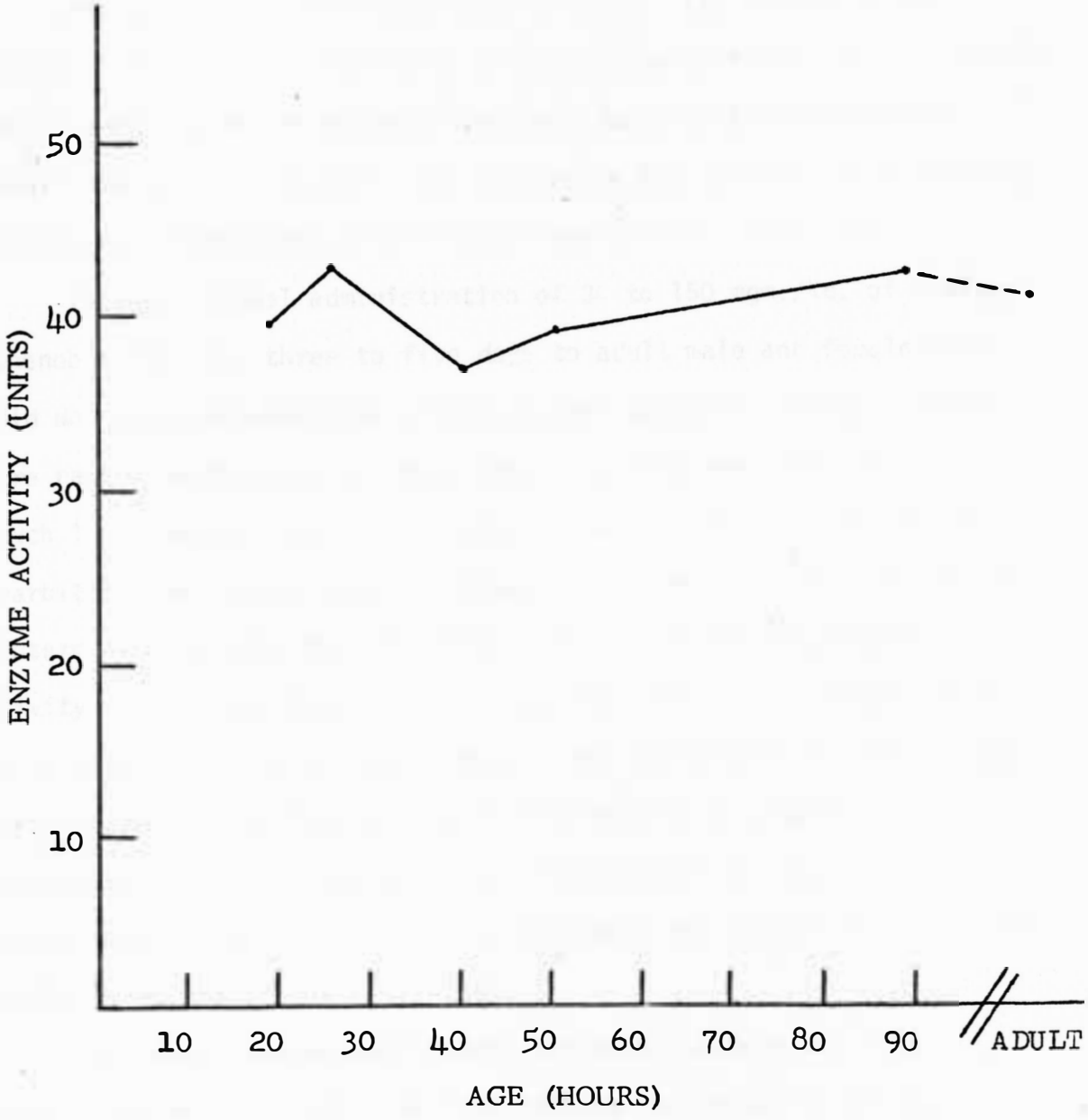


Fig. 4. The activity of the enzyme system which reduces adenosine triphosphate in infant and newborn rats. One unit represents the micrograms of inorganic phosphorous formed by one milligram of tissue in 15 minutes.

DISCUSSION

The purpose of this study was two-fold: (A) To obtain information concerning the influence of sodium phenobarbital on the hepatic microsomal enzyme system which reduces adenosine triphosphate in adult male and female rats; (B) To measure the development of hepatic adenosine triphosphatase activity of newborn and infant rats.

Intraperitoneal administration of 30 to 150 mgm./Kg. of sodium phenobarbital for three to five days to adult male and female rats did not increase adenosine triphosphatase activity. These findings are rather pertinent since many enzyme systems are stimulated by much lower dosage levels of enzyme inducers, such as sodium phenobarbital, over a much shorter induction period. In addition, it is interesting to note that the hepatic adenosine triphosphatase activity of male and female rats is similar. This may indicate that a primary source of metabolic regulation, the androgenic and estrogenic hormones, perform no significant role in regulating hepatic adenosine triphosphatase activity. This effect is rather unusual since many enzymes are hormonally regulated and illustrate increased activity in one of the sexes (58).

The hepatic microsomal enzyme system of newborn and infant rats which reduces adenosine triphosphate is similar to the activity demonstrated in adult rats. These results are contrary to a great deal of the literature concerning stimulation of many enzyme systems by sodium phenobarbital. Furthermore, the fact

that these results showed that newborn and infant rat liver adenosine triphosphatase activity is similar to the adult rat hepatic adenosine triphosphatase activity indicates that this particular enzyme system differs from most enzyme systems since many microsomal enzymes increase in activity until the onset of maturity (26-28). However, it does appear that hepatic adenosine triphosphatase activity reaches its maximum activity very early in life; probably as early as the intra-uterine fetal development. The exact period of fetal development of adenosine triphosphatase activity would be of scientific interest, for few, if any, enzymes exhibit maximum activity early in fetal life.

For reasons such as the preceding discoveries, a great deal of attention has been directed to determining the involvement and possible mechanism of adenosine triphosphate in active transport of ions across cell membranes. Using radioactively labelled ions Skou (13) investigated the mechanism of the adenosine triphosphatase reaction and concluded that the activity associated with the membrane-microsomal fraction had the characteristics of a system which utilizes adenosine triphosphate in the active transport of ions. Hence, drugs such as quinidine, which inhibit adenosine triphosphatase activity may similarly depress the active-ion transport system dependent upon such enzyme activity. However, Kennedy and Nayler (48) reported that drugs which evoked changes in the transmembrane distribution of cations failed to cause changes in the activity of the isolated adenosine triphosphatase enzyme system.

Several pathways for the reduction of adenosine triphosphate by an enzyme system and the production of inorganic phosphorous have been proposed. In order to understand how this enzyme system aids in transporting ions across the cell membrane, it is necessary to know the mechanism of the hydrolysis of adenosine triphosphate by the enzyme system. It is generally considered that the reaction catalyzed by sodium-potassium-dependent adenosine triphosphatase consists of two parts (59,60). Hydrolysis of adenosine triphosphate leads to phosphorylation of a compound in the microsomal particle which contains the enzyme system. From experiments with (^{32}P) adenosine triphosphate as the substrate it was concluded that there is first a sodium-dependent dephosphorylation of the enzyme and then secondly is a potassium-dependent dephosphorylation of the phosphorylated intermediate (59,61). The phosphatase activity which appears in the sodium-potassium adenosine triphosphatase preparation in the presence of magnesium and potassium is known to be similar in many respects to the acitvity in the latter half of the sodium-potassium-activated adenosine triphosphatase reaction (62,63).

Bader et al. (64) and Rendi (65) suggest some kinetics for the sodium-potassium-activated adenosine triphosphatase reaction. Two possible mechanisms which they postulated are dependent on the basis of whether an exchange enzyme is present or not. In the first case the reaction could be schematized as follows:

1. $ATP + E_1 \rightleftharpoons ADP + E_1P$

2. $E_1P + E_2 \rightleftharpoons E_2P + E_1$

3. $E_2P \longrightarrow E_2 + Pi$

---

4. $ATP \longrightarrow ADP + Pi$

E_1= Exchange system

E_2P= Sodium-stimulated phosphorylated intermediate

If the exchange system is not part of the overall adenosine triphosphatase then the partial reactions would be as follows:

1. $ATP + E_2 \longrightarrow E_2P + ADP$

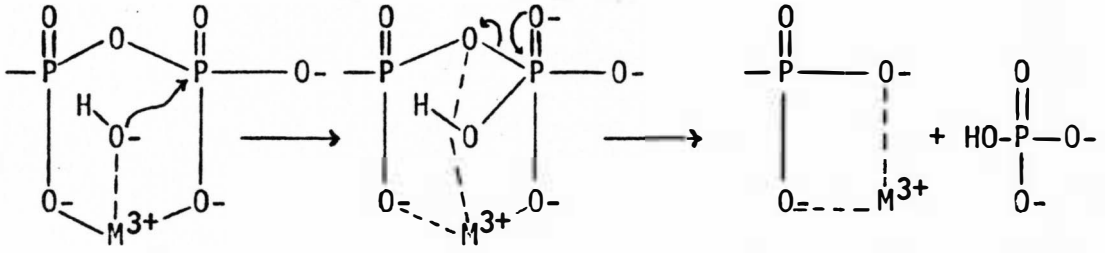
2. $E_2P \longrightarrow E_2 + Pi$

---

3. $ATP \longrightarrow ADP + Pi$

At present no definitive evidence exists to indicate that the exchange enzyme is part of the overall adenosine triphosphatase reaction. Therefore, it is not possible to decide which of the two above schemes is correct. Selwyn (57) later discussed the fact that adenosine triphosphatase has a central role in oxidative phosphorylation and his postulated mechanism indicates that mechanisms proposed for oxidative phosphorylation should involve the elimination of a hydroxyl ion rather than water and the eventual

production of inorganic phosphorous. He further suggested that the metal ion in conjunction with the enzyme is coordinated to a polyphosphate chain and a hydroxyl ion in a favorable position for a nucleophilic attack on the terminal phosphorous atom. A possible mechanism is:



However, even with all the theories and postulations concerning the involvement of adenosine triphosphate and its related enzyme system, adenosine triphosphatase, in ion transport, the exact mechanism of these natural constituents in regulating the "electrolyte pump" has not been elucidated. It is of interest then that this study has shown a significant difference in the activity of the adenosine triphosphatase enzyme system. The significant differences may be expounded upon in this matter: (A) Many enzyme systems demonstrate increased activity in male animals (58), whereas this study showed hepatic adenosine triphosphatase activity to be identical in male and female rats, (B) Failure to alter the activity of the hepatic microsomal adenosine triphosphatase enzyme system in rats by sodium phenobarbital is contrary to many of the studies demonstrating microsomal enzyme inductive effects by this enzyme inducing drug; (C) Demonstration of maximum levels of adenosine

triphosphatase enzyme activity in fetal, infant, and newborn animals is unusual. A comprehensive theory attempting to relegate the significance of these findings is impossible since little work has been done in this particular area.

SUMMARY

1. A modified method of DuBois and Potter (56) was used to determine the effect of sodium phenobarbital on the hepatic microsomal adenosine triphosphatase enzyme system of adult male and female rats; as well as to determine the development of hepatic adenosine triphosphatase activity in newborn and infant rats.

2. Adult male and female rat liver adenosine triphosphatase enzyme activity was identical; thus, this result suggests that this enzyme system is not regulated by specific hormonal activity.

3. Adult male and female rats were given daily intraperitoneal injections of sodium phenobarbital at dosage levels of 30, 60, 75, 100, and 150 mgm./Kg. for three days prior to adenosine triphosphatase determinations of the liver. The drug failed to produce a significant effect on the heptaic adenosine triphosphatase activity. Intraperitoneal administration of 30 mgm./Kg. of sodium phenobarbital twice daily for five days prior to enzyme determination failed to alter the activity of adult male and female rat hepatic adenosine triphosphatase activity.

4. The liver microsomal enzyme system of newborn and infant rats which reduces adenosine triphosphate was similar to adult male and female rat hepatic levels of activity. Removal of fetal rats by Cesarian section demonstrated that hepatic adenosine triphosphatase activity is present prior to parturition.

5. These findings suggest that the hepatic adenosine triphosphatase enzyme system differs from many of the other microsomal

enzyme systems in that it is present at adult activity prior to birth, is not sex-related and is not stimulated by sodium phenobarbital.

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