REGULATION OF 3-HYDROXY-3-METHYLGLUTARYL COENZYME A REDUCTASE, MEVALONATE KINASE, 5-PHOSPHOMEVALONATE KINASE AND 5-PYROPHOSPHOMEVALONATE DECARBOXYLASE IN TURKEY LIVER AND KIDNEYS

By

CAROLYN FRANCES HUNTER

Bachelor of Science

Henderson State University

Arkadelphia, Arkansas

1974

Submitted to the Faculty of the Graduate College
of the Oklahoma State University
in partial fulfillment of the requirements
for the Degree of
DOCTOR OF PHILOSOPHY
July, 1978

Thesis
1978D
H945r
cop. 2

OKLAHOMA STATE
UNIVERSITY
LIBRARY

Dedicated to

MY MOTHER AND FATHER

for their love and gentle discipline

MY SISTERS AND BROTHERS

for their laughter and moral support

MY FRIEND, PHILLIP,

for making everything worthwhile

1016581

REGULATION OF 3-HYDROXY-3-METHYLGLUTARYL COENZYME A REDUCTASE, MEVALONATE KINASE, 5-PHOSPHOMEVALONATE KINASE AND 5-PYROPHOSPHOMEVALONATE DECARBOXYLASE IN TURKEY LIVER AND KIDNEYS

Thesis Approved:

Earl D Mitchell Jr
Thesis Adviser

Eldon Nelson

Roger E. Koeppe

K D Berlin

George V. Odell

Norman N Durham
Dean of the Graduate College

ACKNOWLEDGMENTS

This thesis has been aided over the past four years by many people, some with impressive scholarly credentials and others with an abiding amateur interest in its success. I am most grateful for their help and would like to thank each of them.

I gratefully acknowledge Dr. Earl D. Mitchell, Jr., my major professor, for his sage and pertinent criticisms during the course of these studies and in the preparation of this thesis. Thanks are extended to my committee members, Dr. R. E. Koeppe, Dr. G. V. Odell, Dr. E. C. Nelson and Dr. K. D. Berlin for their suggestions.

I extend a very special and sincere thanks to my parents, Mr. and Mrs. Bert F. Hunter, for their uncompromising principles that led me into intellectual pursuits, encouragement, assistance, both financial and otherwise which ultimately made this thesis possible.

I extend a warm and special thanks to a wonderful friend, Phillip Dokes, for his patience and understanding during the course of this study.

Special thanks are extended to: Dr. Rollin Thayer who made a significant contribution to this thesis by providing the turkeys for experimentation; Ms. Lyndia Clemmons and Dr. Haskel Jones who encouraged me to attend graduate school; and Ms. Gloria Perry for encouraging me throughout my endeavors in the field of biochemistry.

I would like to thank: Gene Murray, Leon Bloom and Ed Smith for

taking care of, and sacrificing the turkeys; Judy Chang, Chalong Konantakieti, Janie Glass and Neil Gunn for the sunshine they brought to the laboratory and for their technical assistance; and Ms. Cherry Christian for her typing expertise.

A special gratitude and appreciation is extended to the Oklahoma Agricultural Experiment Station and Department of Biochemistry, for financial assistance and use of facilities during these investigations.

TABLE OF CONTENTS

Chapter Page

Chapter Page

LIST OF TABLES

Table Page

LIST OF FIGURES

Figure Page

NOMENCLATURE

HMG-CoA	3-Hydroxy-3-Methylglutaryl Coenzyme A
MVA	Mevalonic Acid
MVAP	5-Phosphomevalonic Acid
MVAPP	5-Pyrophosphomevalonic Acid
ATP	Adenosine Triphosphate
IPP	Isopentenylpyrophosphate
NADP	Nicotinamide Adenine Dinucleotide Phosphate
DTT	Dithiothreitol
EDTA	Ethylene Diamine Tetraacetic Acid
SCP	Sterol Carrier Protein
HDL	High Density Lipoprotein
LDL	Low Density Lipoprotein
VLDL	Very Low Density Lipoprotein

CHAPTER I

INTRODUCTION

Cholesterol is the most ubiquitous and abundant steroid in mammalian tissues. It is a major component of all mammalian plasma membranes and is vital to cell growth and survival. The structure of cholesterol has been known for decades. The biological source of each of its 27 carbon atoms and its oxygen atom was established 15 or more years ago (1-3). Numerous investigators have helped to elucidate the absolute sterochemistry and biological sources of each of its hydrogen atoms (4). Its complete biosynthetic pathway has not been elucidated and some of the intermediates in cholesterol biosynthesis are presently unknown.

Almost all mammalian tissues have the ability to synthesize cholesterol with the exception of the central nervous system. However, the liver is the tissue most actively involved in cholesterogenesis, followed by the intestines. The liver is the major source of plasma cholesterol.

Over the past few years, a tremendous amount of research activity has been centered on the regulation of cholesterol biosynthesis which is primarily due to the close correlations of hypercholesterolemia with the development of arteriosclerosis and coronary artery disease (5). Coronary heart disease is a primary health problem in the United States. The disability and death rates attributable to coronary heart disease in

the western culture exceed those of any other illness (6).

This investigation used turkeys as a model system to study some of the enzymes involved in cholesterogenesis. The rationale for using turkeys as a model system is: 1.) turkeys become atherosclerotic, 2.) turkeys become hypertensive, and 3.) turkeys become symptomatic through dietary manipulations, somewhat similar to humans.

Aortic rupture is a very common occurrence in turkeys. One supposition is that aortic rupture results from a combination of high blood pressure and a weakened aorta. The arterial blood pressure of the turkey is higher than any other vertebrate and has a characteristic increase during the growing period (6 to 20 weeks of age) (7). The presence of atheromatous plaques is a common occurrence in the posterior aorta of the turkey and may provide a weakening effect. Carnaghan (8) and Gibson and de Gruchy (9) have observed plaques at the site of rupture. Ball (10) has observed elastic tissue obliteration and fibrosis in the turkey aorta as early as three weeks of age.

The blood pressure of the turkey is variable. There is evidence for genetic variability in blood pressure in turkeys. Sturkie et al. (11) selected a hypotensive line of chickens on the basis of high and low blood pressures. Krista et al. (12) have shown that it is possible to select for divergent hypertensive and hypotensive strain of turkeys.

In consideration of the importance of dietary cholesterol to many individuals, this study was undertaken to investigate specific enzymes involved in cholesterol synthesis in turkeys. The objectives of this work were as follows: 1.) to determine the activity of 3-hydroxy-3-methylglutaryl Coenzyme A (HMG-CoA) reductase, mevalonate (MVA) kinase, phosphomevalonate (MVAP) kinase and pyrophosphomevalonate (MVAPP)

decarboxylase in livers and kidneys from turkeys fed a control (tallow) diet, 2.) to study the effect(s) of a 1% cholesterol diet on HMG-CoA reductase, MVA-kinase, MVAP kinase and MVAPP decarboxylase activity in turkey liver and kidneys, 3.) to determine if HMG-CoA reductase and the mevalonate activating enzymes exhibits a diurnal rhythm, 4.) to characterize the post-hatched developmental pattern of HMG-CoA reductase and the mevalonate activating enzymes in poult livers, and 5.) to determine the relationship between HMG-CoA reductase and the enzymes involved in mevalonate metabolism in turkey livers and kidneys.

CHAPTER II

LITERATURE REVIEW

A. Cholesterol Biosynthesis

Schoenheimer and Breusch were the first to present evidence that intact animals are capable of de novo synthesis of cholesterol. This finding was later confirmed in 1937, with the observation that deuterated water was rapidly incorporated into cholesterol in rats (13). Block and Rittenberg (14) in 1942, showed that acetate leads to cholesterol synthesis in vivo and in liver slices. Bucher and McGarrahan (15) showed that a preparation capable of converting acetate to cholesterol is obtained by using a loose fitting homogenizer. These results, together with the discovery by Tavormina, Gibbs and Huff (16) that mevalonic acid is an excellent precursor of cholesterol, have been key developments in the investigation of cholesterol biosynthesis. The fact that mevalonic acid is formed by a rat liver homogenate system was first observed by Knauss, Porter and Wasson (17). It was hypothesized that the pathway for the biosynthesis of mevalonate occurred through the reduction of HMG-CoA and this fact was later substantiated in the case of the yeast enzyme system (18, 19). Bucher, Overath and Lynen (20) were the first to demonstrate the existence of HMG-CoA reductase from a mammalian source.

Much of the research on cholesterol biosynthesis regulation was

performed in the 1950's and early 1960's. Many of the early studies have become classic works in a difficult area. These studies laid the necessary foundation for many of the current approaches on this problem.

The works of Block, Lynen, Cornforth and Popják led to the elucidation of the biosynthetic pathway of cholesterol from acetate (21). The first step of the pathway is the conversion of HMG-CoA to mevalonate. Mevalonate is phosphorylated to 5-phosphomevalonate, 5-phosphomevalonate is phosphorylated to 5-pyrophosphomevalonate and 5-pyrophosphomevalonate is decarboxylated to isopentenyl pyrophosphate. An isomerization, with loss of 4-S^{3}H from mevalonate (2-R^{3}H of isopentenyl pyrophosphate), is observed in the formation of dimethylallyl pyrophosphate. A head to tail condensation of isopentenyl pyrophosphate and dimethylallyl pyrophosphate results in the formation of the acyclic monoterpene pyrophosphate.

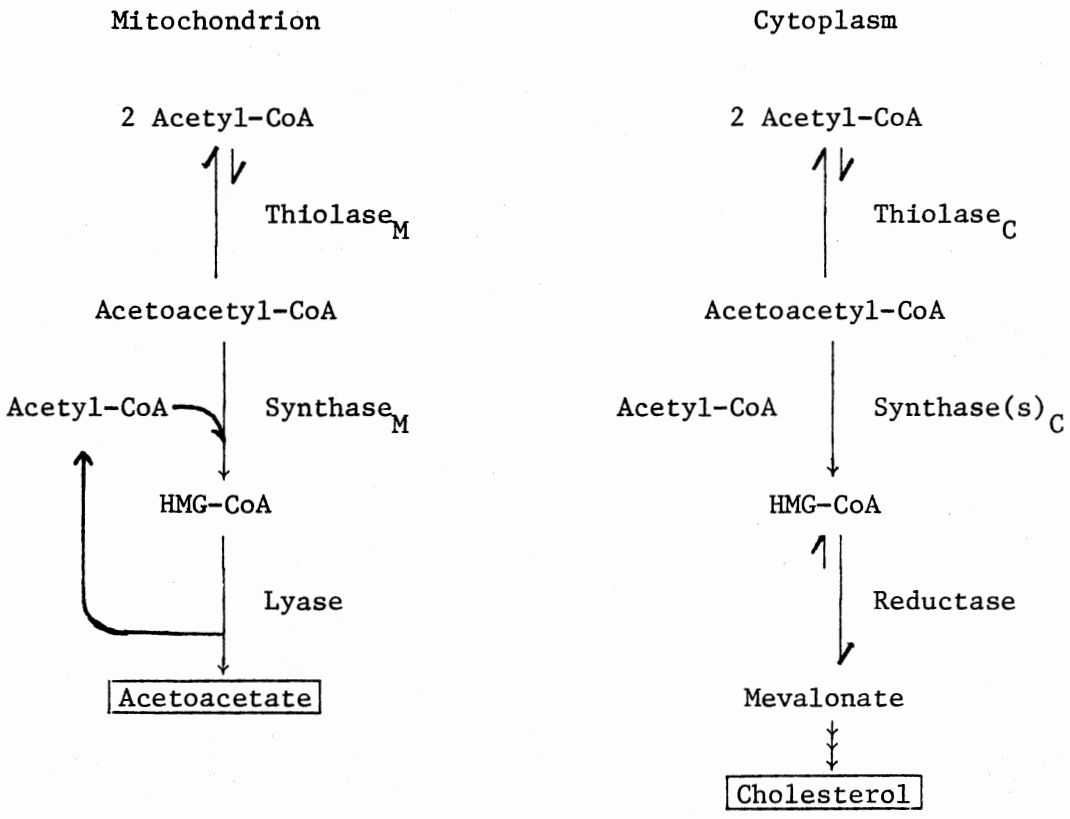
Recently, there have been several important preliminary breakthroughs in the area of cholesterol biosynthesis regulation which hopefully will lead to the rapid elucidation of the regulatory mechanisms. Investigations discussed here were chosen to reflect the current state of the art in this area.

B. Regulation of Cholesterol Biosynthesis

1. Enzymic Synthesis of HMG-CoA

Acetoacetyl-CoA thiolase [EC 2.3.1.9] and HMG-CoA synthase [EC 4.1.3.5] catalyze the synthesis of HMG-CoA from acetyl-CoA (Figure 1). Lane et al. (22), Clinkebeard et al. (23) and Sugiyama et al. (24) demonstrated that the thiolase and synthase are present in both the

Figure 1. Pathways of HMG-CoA Biosynthesis.


Mitochondrion
2 Acetyl-CoA
Thiolase M
Acetoacetyl-CoA
Acetyl-CoA
Synthase M
HMG-CoA
Lyase
Acetoacetate
Cytoplasm
2 Acetyl-CoA
Thiolase C
Acetoacetyl-CoA
Acetyl-CoA
Synthase(s) C
HMG-CoA
Reductase
Mevalonate
Cholesterol

mitochondrial and cytoplasmic fractions of avian liver. Proof for distinct cytosolic and mitochondrial thiolases in avian liver was the presence of one cytosolic and two mitochondrial enzymes (23). It is probable that one of the mitochondrial thiolases is involved in β oxidation of fatty acids and the other in ketogenesis (23). HMG-CoA lyase is almost exclusively located in the mitochondria (22).

Cholesterol synthesis from acetyl-CoA is exclusively an extramitochondrial process in mammalian liver cells (25). Therefore, it appears that HMG-CoA reductase is not a branch point between ketogenesis and cholesterogenesis and also not the first unique step in sterol synthesis. Further studies are needed to elucidate the mechanism(s) involved in regulating this enzyme complex.

2. Enzymic Reduction of HMG-CoA to Mevalonate

Although a tremendous amount of research is centered in the area of cholesterol biosynthesis regulation, several important questions concerning the control of the rates of cholesterol synthesis remain unanswered. For the past several years, most investigators believed that the regulation of cholesterol biosynthesis was controlled solely by 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase (mevalonate: NADP oxidoreductase [acylating CoA] EC 1.1.1.34). HMG-CoA reductase catalyzes the reduction of HMG-CoA to mevalonate by NADPH (Figure 2). This enzyme catalyzes the first step unique to cholesterol biosynthesis and is both rate-limiting for the over-all sequence of reactions and the site of feedback control of hepatic cholesterol synthesis.

Bucher et al. (26) presented the first evidence for the occurrence of HMG-CoA reductase in rat liver and showed that the enzyme was par-

Figure 2. Enzymes of Cholesterol Biosynthesis.

$$\text{HMG-CoA} + 2\text{NADPH} + \text{H}^{+} \xrightarrow{\text{HMG-CoA Reductase}} \text{MVA} + 2\text{NADP}^{+}$$

$$\text{MVA} + \text{ATP} \xrightarrow[\text{Mg}^{2+}]{\text{MVA Kinase}} \text{MVAP} + \text{ADP}$$

$$\text{MVAP} + \text{ATP} \xrightarrow[\text{Mg}^{2+}]{\text{MVAP Kinase}} \text{MVAPP} + \text{ADP}$$

$$\text{MVAPP} + \text{ATP} \xrightarrow[\text{Mg}^{2+}]{\text{MVAPP Decarboxylase}} \text{IPP} + \text{CO}_2 + \text{ADP} + \text{Pi}$$

ticulate in nature and microsomal in origin. The submicrosomal distribution of HMG-CoA reductase has been studied by numerous investigators. Guder et al. (27) and Shapiro and Rodwell (28), using a discontinuous sucrose gradient technique, obtained data suggesting that 95% of the microsomal enzyme activity is located in membrane from the rough endoplasmic reticulum. Goldfarb (29), also using a discontinuous gradient method, suggested that over 80% of the activity is found in the fraction of smooth membranes composed of smooth endoplasmic reticulum, Golgi apparatus and plasma membrane. Mitropoulos et al. (30) showed that HMG-CoA reductase is in the membranes originating from the endoplasmic reticulum.

In contrast to hepatic tissue, yeast HMG-CoA reductase is located in the mitochondria instead of the endoplasmic reticulum (31, 32).

Investigation of the control mechanisms involving HMG-CoA reductase activity has been hampered by the fact that the enzyme is bound to microsomal membranes in the liver and has therefore been very resistant to purification and study. This enzyme has been solubilized by a variety of procedures in several laboratories. The current four methods of solubilization are: 1.) deoxycholate treatment (33), 2.) acetone powder preparation (34), 3.) high salt, glycerol, or snake venom treatment (35), and 4.) slow freeze-thaw treatment (36). The final specific activity obtained using the deoxycholate treatment is not as high as the specific activity obtained using high salt, glycerol, snake venom or the freeze-thaw method. The preparation obtained by using high salt, glycerol or snake venom is inactivated by chilling to 4°C unless high salt (4M) is present and is fairly heat stable. The slow freeze-thaw preparation is also reversibly inactivated by cold

and is fairly stable.

Reports of purification following the acetone powder solubilization of HMG-CoA reductase have not appeared. However, purification following the other methods (33, 35, 37) have been reported. Tormanen et al. (37) reported a purification procedure that employs Blue Dextran/Sepharose-4B affinity chromatography. Purification can be carried out from microsomal membranes to purified enzyme in 8 to 10 hours. HMG-CoA reductase purified from liver microsomes of rats had an overall purification factor of 2,747-fold. The enzyme was estimated to be 93% pure based upon spectrophotometric scans of polyacrylamide gels.

The molecular mechanism(s) that regulates HMG-CoA reductase activity are not well understood. This enzyme is regulated by such factors as: 1.) time of day, 2.) cholesterol, 3.) fasting, 4.) bile acids and 5.) hormones.

A cyclic rhythm in HMG-CoA reductase was first noted by Kandutsch and Saucier (38). The rhythm appears to be caused by an increase in the rate of reductase synthesis. Factors that affect cholesterol synthesis also affect the diurnal rhythm.

The first direct evidence for reduced reductase activity in microsomes.from livers of cholesterol-fed rats was reported by Linn (39). Although dietary cholesterol causes a rapid decrease in reductase activity, cholesterol does not directly inhibit HMG-CoA reductase activity. The ability of cholesterol to depress the cyclic rise in reductase levels without accelerating the cyclic decline is consistent with the hypothesis that cholesterol suppresses the synthesis of HMG-CoA reductase (40).

Balasubramaniam et al. (41) showed that HMG-CoA synthase and

HMG-CoA reductase are coordinately regulated in the adrenal gland of the rat. Administration of 4-aminopyrazolopyrimidine caused the synthase activity to increase 14 to 29-fold and the reductase activity to increase 50 to 100-fold. Subsequent infusion of low-density lipoprotein suppressed the synthase and reductase activities in parallel. Activities of HMG-CoA synthase and HMG-CoA reductase also exhibited a coordinate pattern of diurnal variation.

Bucher et al. (26) were the first to observe that hepatic HMG-CoA reductase activity is reduced in fasted rats. The effects of fasting might be mediated via cholesterol, since an accumulation of bile acids followed by a rise in liver cholesterol occurred on fasting (42).

Exogenous bile acids depress hepatic cholesterol synthesis and HMG-CoA reductase activity. However, physiological concentrations of bile acids do not inhibit reductase activity. Weis and Dietschy (43) suggested that the effect of bile acids may result from increased absorption and accumulation of liver cholesterol.

Hormones have been implicated in the maintenance of the diurnal rhythm in reductase synthesis and in the over-all regulation of cholesterol synthesis. Glucagon, hydrocortisone and cAMP abolish the diurnal rise in HMG-CoA reductase (44, 45). Insulin and the thyroid hormones cause an increase in reductase activity (46). Oswald and Wiss (47) showed that physiological doses of insulin and glucagon cause an initial stimulation followed by inhibition of cholesterol synthesis. Oswald and Wiss believe that this is evidence against a rate-limiting function of HMG-CoA reductase.

Several authors have reported the occurrence of tissue factors capable of inhibiting HMG-CoA reductase <u>in vitro</u>. Carlson et al. (48)

presented evidence that SCP is capable of modifying HMG-CoA reductase activity *in vitro*. Tormanen et al. (49) reported that the rapid irreversible inactivation of HMG-CoA reductase at 4°C is caused by the presence of a natural inactivating factor(s) in the crude soluble extract. HMG-CoA reductase of rat liver is inactivated *in vitro* by Mg^{+2} and ATP (50) or ADP in a time-dependent process that is mediated by an inactivator present both in liver cytosol and microsomes. The inactivator is a protein and appears to have a molecular weight greater than 150,000 (51).

Recent reports have shown that there is a liver cytosolic protein that stimulates HMG-CoA reductase (52). Inactivation of liver reductase by Mg^{+2} and ATP *in vitro* is completely reversed by the activator protein. Nordstrom et al. (51) suggest that the regulation of HMG-CoA reductase *in vitro* may occur via protein-mediated interconversion of forms of differing catalytic activity.

Inhibitors of HMG-CoA reductase have been intensely sought. One class of compounds is a variety of oxygenated sterols that are potent inhibitors of HMG-CoA reductase activity when added to intact cells in tissue culture (53). Recently, an important new class of true HMG-CoA reductase inhibitors has been isolated from the culture broth of the mold *Penicillium citrinum* (54). When incubated with HMG-CoA reductase from rat liver, the most potent of these compounds, compactin (ML-236B), behaved in a competitive fashion with respect to the reductase (55).

Redd et al. (56) suggested that HMG-CoA reductase is regulated by the amount of cholesterol bound to this enzyme. It was shown that the lower the amount of cholesterol bound to HMG-CoA reductase, the greater the specific activity.

In addition to studies on sterol synthesis regulation by hepatic tissue, several recent reports describe the properties of HMG-CoA reductase from intestinal tissue. The reductase activity is located in both the mitochondrial and microsomal fractions of rat intestinal crypt cells, but not of villi (57). However, Sugano et al. (58) have shown that HMG-CoA reductase in the villi fraction is as active as that in the crypt cell fraction. Addition of a trypsin inhibitor to the homogenizing and incubation media was necessary for measurement of the activity of the villi fraction. Intestinal HMG-CoA reductase also exhibits a diurnal rhythm (59). Bile acid feeding did not reduce the basal activity of the intestinal reductase, and did not reduce the normal diurnal rise in activity. However, cholesterol feeding caused a marked suppression of intestinal HMG-CoA reductase activity (60).

Studies from many laboratories have shown that intact segments of arterial wall can synthesize sterols from acetate and mevalonate (61-63). Slakey et al. (64) showed that subcellular fractions of homogenates of hog aorta are (26) capable of carrying out each of the reactions that occur in the conversion of HMG-CoA to squalene. They suggest that HMG-CoA reductase is the rate-limiting enzyme in the aorta.

The regulation of terpenoid synthesis in plants has not been studied. The control enzyme in hepatic tissues, HMG-CoA reductase, was only recently reported in higher plants (65). Suzuki et al. (65) presented the first evidence for the occurrence of HMG-CoA reductase in sweet potato roots. This enzyme is localized in both the mitochondrial and microsomal fractions. The Km for HMG-CoA was

reported as 6.5-21 μM. Arebalo (66) reported the occurrence of two HMG-CoA reductase activities in the leaf of Nepeta cataria. One of the activities was localized in the chloroplasts. The interested reader is referred to reference (66) for more information. The regulation of HMG-CoA reductase in plants has not been investigated.

3. First Enzymic Phosphorylation of Mevalonate

Mevalonate kinase (ATP:mevalonate 5-phosphotransferase, EC 2.7.1.36) catalyzes the phosphorylation of mevalonate at the five positions as shown in Figure 2. Tchen (60) was the first to isolate and purify mevalonate kinase from yeast autolysate. Since mevalonate kinase may play an important role in the regulation of cholesterol biosynthesis, the purification of this enzyme and an investigation of its mechanism of action has been studied.

Mevalonate kinase has been isolated from yeast (60), hog liver (67), rabbit liver (68), pumpkin seedlings (69), rubber latex (70), chicken and rat liver (71), Neurospora crassa (72), rat ovary (73), Sarcophaga bullata (74), Pinus radiata (75) and Euglena gracilis (76). Properties of mevalonate kinase from various sources are shown in Table I (72).

In hog liver, the phosphorylation of mevalonic acid is sequential, that is, all substrates react with the enzyme before the first product is released. Mevalonate reacts with the enzyme first, followed by MgATP. The order of release is first 5-phosphomevalonate followed by ADP (77). ATP is the nucleotide that best supports phosphorylation of mevalonate in hog liver. High levels of ATP has been reported to inhibit the enzyme activity (60).

TABLE I

PROPERTIES OF MEVALONATE KINASE FROM VARIOUS SOURCES

Source	Purification (fold)	Maximal Specific Activity[b] nmoles/min^{-1} mg^{-1}	Optimal pH	K_m	
				ATP	DL-Mevalonate (mM)
Hog Liver	600	17,000	7.3	0.30	0.019
Neurospora crassa	200	5,000	8.0-8.5	1.8	2.8
Hevea latex	140	1,600	7.5	2.0	0.13
Yeast	50	600	6.4-6.7	—	—
Rat Ovary	—	120[c]	7.0-7.5	0.12	0.0036
Rabbit liver	100	110[d]	7.8	—	5.1
Sarcophaga bullata	110	30	7.1	4.7	0.62
Pinus radiata	—	5	6.0	—	—
Euglena gracilis	8	—	—	6.0	0.06
Cucurbita pepo	3	—	5.5-6.0	—	—

TABLE I (Continued)

[a]After Imblum and Rodwell (1974)

[b]Assayed at 30°C in the presence of Mg^{++} except where otherwise noted.

[c]Assayed at 25°C

[d]Assayed at 35°C

An active - SH group is suggested in mevalonate kinase by its sensitivity to sulfhydryl binding agents and the requirement for sulfhydryls in isolation buffers (78).

Mevalonate kinase activity has been measured in subcellular fractions of livers of rats that were either fasted or fasted and refed a fat-free diet, and at various times of the day (79). Mevalonate kinase did not decline rapidly on fasting nor did it exhibit a diurnal cycle. However, the effect(s) of cholesterol and hormones on MVA kinase has not been studied.

Ramachandran et al. (80) studied mevalonate kinase in the 105,000 x g supernatant fractions from rat brain. The enzyme required ATP and Mg^{++} for optimal activity and was active in the pH range of 6.5 - 8.0.

In higher plants some of the enzymes that converts mevalonate to isopentenyl pyrophosphate have been found in chloroplasts (81, 82). Rodgers et al. (83) suggested that there are two pools of mevalonate kinase in plants. The pools are: 1.) the chloroplastidic pool with a pH optimum of 7.5 and 2.) the extra-chloroplastic pool with a pH optimum of 5.5. Arebalo (66) suggested that the mevalonate activating enzymes exist as an enzyme complex in Nepeta cataria. These enzymes were found to co-purify through gel-filtration, sucrose density gradient and ammonium sulfate fractionation. Mevalonate kinase and phosphomevalonate kinase were found in the microsomal membrane fraction and in the chloroplasts.

4. Second Enzymic Phosphorylation of Mevalonate

5-Phosphomevalonate kinase (ATP:5-phosphomevalonate phosphotrans-

ferase, EC 2.7.4.2) catalyzes the second phosphorylation by ATP of 5-phosphomevalonate at the five position (Figure 2). ATP is the only nucleotide that supports phosphorylation (84, 85). A divalent cation such as Mg^{++}, Fe^{++} and Zn^{++} (84, 85) is required for phosphorylation.

This enzyme has been partially purified from a number of sources including rubber latex (70), pine (86), yeast (81) and hog liver (67). Phosphomevalonate kinase does not decline rapidly on fasting and it does not exhibit a diurnal cycle (79).

Ramachandran et al. (80) studied phosphomevalonate kinase in rat brain. It had a specific requirement for ATP and Mg^{++} for optimal activity and was active in a pH range of 6.5-8.0.

5. Enzymic Synthesis of Active Isoprene Units

5-Pyrophosphomevalonate decarboxylase (ATP:5-pyrophosphomevalonate carboxy-lyase, EC 4.1.1.33) catalyzes the synthesis of active isoprene units (Figure 2). ATP and a cation such as Mg^{++}, Mn^{++} or Co^{++} are required for catalysis to occur. Pyrophosphomevalonate decarboxylase activity declined rapidly on fasting and returned to the normal level of a fat-free diet (79). Pyrophosphomevalonate decarboxylase does not exhibit a diurnal cycle.

Ramachandran et al. (80) studied pyrophosphomevalonate decarboxylase in rat brain. It had a specific requirement for ATP. However, Mg^{++} and Mn^{++} were equally effective for optimal activity. Pyrophosphomevalonate decarboxylase was active at pH 6.0.

6. Trans-Methylglutaconate Shunt

In 1966, Gould and Siuyryd (87) offered evidence for secondary

sites of regulation following mevalonic acid, that is, sites of cholesterol biosynthesis regulation in addition to HMG-CoA reductase.

Edmond and Popják (88) presented evidence for the existence of a pathway (trans-methylglutaconate shunt), that leads intermediates arising from mevalonate back to HMG-CoA, ketone bodies, and acetyl-CoA, rather than to squalene and cholesterol.

Results supporting the occurrence of this pathway are the detection of label from mevalonate [2-^{14}C] in fatty acids. Furthermore, [3',4-$^{13}C_2$]-, [5-^{14}C]-, and [4R-4-^{3}H] mevalonate, but not [4S-4-^{3}H] mevalonate, yielded labeled fatty acids (88). Popják and Edmond (89) estimated, based on their experimental observations in young and adult rats, that at least 20% of mevalonate could be shunted through the new pathway. Fogleman et al. (90) showed that [5-^{14}C] mevalonate administered in vivo is metabolized to $^{14}CO_2$.

Righetti et al. (91) observed that the kidney is the most active tissue for mevalonate metabolism by the shunt pathway. The shunt pathway accounts for a significant amount of the mevalonate metabolized in the ileum, spleen, lung and testes, but is undetectable in the liver, brain, skin and adipose tissue.

The discovery of the trans-methylglutaconate shunt revealed another process capable of regulating steroid biosynthesis.

7. Sterol and/or Squalene Carrier Protein

The sterol and/or squalene carrier protein (SCP) is a strong candidate for a regulatory role in steroid biosynthesis. This protein is essential for the conversion of water-insoluble precursors to cholesterol by microsomal enzymes (92). SCP is a ubiquitous protein

in mammalian tissues.

Scallen et al. (93) demonstrated that both microsomal membranes and the 105,000 x g soluble supernatant from rat liver are required for the enzymatic conversion of squalene to cholesterol. It was postulated that the 105,000 x g supernatant contained a noncatalytic carrier protein which was required for this enzymatic process (94). It was later demonstrated that the 105,000 x g supernatant contains at least two proteins which are required for the microsomal conversion of squalene to cholesterol (95). These carrier proteins are designated sterol carrier protein$_1$ (SCP_1) and sterol carrier protein$_2$ (SCP_2). The two proteins differ markedly in molecular size, heat stability, electrophoretic mobility, tissue distribution and substrate specificity. SCP_1 specifically activates the conversion of squalene to lanosterol. SCP_2 specifically activates the conversion of 7-dehydrocholesterol to cholesterol. Thus, SCP_1 is an essential component for sterol biosynthesis *in vivo* and *in vitro*.

Ritter and Dempsey (96) isolated a protein which has sterol carrier protein activity in the conversion of Δ^7-cholesterol to 7-dehydrocholesterol and of 7-dehydrocholesterol to cholesterol. The protein isolated by Ritter and Dempsey has been named "squalene and sterol carrier protein."

For a detailed discussion and comparison of SCP_1 isolated by Scallen et al. with the squalene and sterol carrier protein isolated by Ritter and Dempsey, the reader is referred to reference (95). Scallen et al. concluded that it is unlikely that the protein isolated by Ritter and Dempsey play a role in the conversion of squalene to sterol.

Certain properties of SCP_1 resemble the properties of a partially

purified protein preparation described by Tai and Bloch (97).

SCP_1 and SCP_2 are particle bound. The water-insoluble substrate is held to SCP_1 or SCP_2 by hydrophobic forces. It is possible that SCP_1 and SCP_2 may not be regenerated after the formation of cholesterol, but instead cholesterol may remain bound to SCP_1 and/or SCP_2 (in the form of a lipoprotein) for transport from the liver (95).

8. Lipoproteins

The plasma lipoproteins provide the body with a transport system for insoluble lipids. All lipids except for the free fatty acids and lysolecithin circulate in the plasma from their site of origin to their site of utilization in association with these lipid-protein complexes. Lipoproteins were first described by Macheboeuf in 1929, and are divided into four major families on the basis of their physical characteristics and chemical composition. These four major families are: 1.) Chylomicrons, 2.) very low density lipoprotein (VLDL), 3.) low density lipoprotein (LDL) and 4.) high density lipoprotein (HDL).

Chylomicrons are lipoproteins of density less than 0.95 g/ml and are synthesized in the intestine during active fat absorption (98). They represent a spectrum of particles varying in diameter between 0.1 to 1.0 micron and of molecular weight 100 to 1000 million daltons.

Very low density lipoproteins occupy a density range of 0.95 to 1.006 g/ml and are present normally in fasting plasma. Their diameter varies between 300 to 800 Å and the molecular weight from 5 to 130 million daltons (99).

Low density lipoproteins occupy a density range of 1.006 to

1.063 g/ml. The diameter varies between 200 to 250 Å and the molecular weight from 2.12 to 2.36 x 10^6 daltons (99).

High density lipoproteins occupy a density interval of 1.063 to 1.21 g/ml. HDL represents the smallest lipoprotein particle, of diameter 50 to 100 Å and molecular weight range of 150,000 to 400,000 (100).

Chylomicrons and VLDL are the two major triglyceride transport lipoproteins carrying respectively triglycerides of either exogenous (dietary) or endogenous origin. Triglycerides constitute more than 90% of chylomicron lipids and more than 70% of VLDL lipids (101).

About one-half of the HDL mass is protein; however, phospholipids and cholesterol (mainly in the esterified form) constitute 30% and 20% of the HDL, respectively. HDL may play an important role in the generation of cholesteryl esters in plasma (101).

In man, LDL is the lipoprotein that carries most of the cholesterol in plasma. About three-fourths of the cholesterol in LDL is esterified to long chain fatty acids and these cholesteryl esters are believed to be located in an apolar core of neutral lipid that is surrounded by phospholipid, unesterified cholesterol and a protein called apoprotein B. Of the various cholesteryl esters found in human LDL, cholesteryl linoleate accounts for the largest fraction (102).

The studies of Glomset (103) suggested that HDL is the source of most of the free cholesterol that becomes the esterified cholesterol of LDL. Studies showed that VLDL is converted through a series of reactions to an LDL particle (104).

Brown and Goldstein (102) proposed a mechanism by which certain

mammalian cells (cultured human fibroblasts) are capable of utilizing a specific cell surface receptor in order to protect themselves against an over-accumulation of cholesterol. This cell surface receptor, designated LDL receptor, binds LDL and thereby regulates the rate at which LDL transfers cholesterol into the cell.

Analysis of the events mediated by the LDL receptor has been facilitated by studying the fibroblasts derived from patients with the receptor-negative form of homozygous familial hypercholesterolemia. These mutant cells which lacked functional LDL receptors failed to bind and take up the lipoprotein and therefore failed to hydrolyze its protein or cholesteryl ester components. As a consequence, LDL did not suppress HMG-CoA reductase activity (cholesterol synthesis). Thus, it has been suggested by Brown et al. (102) that the primary defect in homozygous familial hypercholesterolemic cells is a defect in the cell surface LDL binding.

C. Relationship of Cholesterol and Other Risk Factors in the Development of Coronary Heart Disease

Coronary heart disease is a primary health problem of our time. Reliable estimates indicate that the disability and death rates attributable to coronary heart disease in the western culture exceed those of any other illness (6). Epidemiological studies have shown a significant relationship between the incidence of coronary heart disease and various prospective characteristics commonly called "risk factors." These factors are: 1.) elevated levels of cholesterol and other lipids, 2.) hypertension, 3.) diabetes mellitus, 4.) obesity,

5.) sex (a pronounced predilection for the male sex), 6.) heavy cigarette smoking and 7.) family history (105).

It is now an established fact that coronary atherosclerosis is the underlying pathological process in 90% of patients with coronary insufficiency of acute myocardial infarction. Considerable evidence supports the role of lipids in the development of atherosclerosis. Cholesterol is a major constituent of the atherosclerotic plaque. Cholesterol and other lipids apparently are derived by diffusion from the blood plasma. One hypothesis for the mechanism of cholesterol and other lipids deposition in the arterial walls is that plasma lipoproteins enter the arterial wall from the lumen and because of localized relative inadequacy of metabolic removal of the constituent lipids, these lipids accumulate in selected areas of the arterial intima, giving rise to the lesions of atherosclerosis (6).

Twenty-four million Americans suffer from hypertension (high blood pressure). Hypertension is the primary cause of 60,000 American deaths each year and is a significant causative factor in the more than 1,500,000 heart attacks and strokes suffered annually by Americans (106). Hypertension afflicts not only the elderly, but also young and middle-aged adults. Hypertension is more racially identifiable than the other risk factors. The incidence of hypertension is higher for blacks than for whites for all ages up to 80, the ratio being about 2:1 (6). Numerous reports proclaim the importance of hypertension as a predisposing condition in the development of atherosclerosis. The coincidence of hypertension and atherosclerosis is statistically considerable, and in the hypertensive population, coronary heart disease is currently the most common cause of death.

A widely held opinion is that diabetes mellitus is a disorder characterized by an excessive incidence of atherosclerosis. It has been suggested that atheromas develop much more frequently, at an earlier age, and in a more severe form in diabetic persons than in the general population. The likelihood of death due to myocardial infarction is stated to be more than twice as great for the diabetic as for the non-diabetic (107). Although diabetes mellitus facilitates capillary dysfunction, there is no substantial evidence that diabetes per se contributes to atherogenesis other than via lipid metabolic dysfunction and accompanying hypertension, obesity and aging.

Obesity is considered by many to play a significant role in the development of atherosclerosis. Evaluation of the role played by obesity is complicated by the high incidence of accompanying hypertension. It has, however, been shown that blood lipid abnormalities are more common in those who are overweight as compared with the general population (108).

Men in the United States have a higher frequency of manifestations of coronary heart disease, clinical and fatal, than do women. Early in adult life the incidence of clinical coronary heart disease and fatality is 4 to 5 times greater in men than women. There is however, a progressive decrease in this ratio of attacks, fatalities and incidence of clinical coronary heart disease between men and women with age, such that in approximately the eighth decade the incidence rate approaches equality. The reason for the large difference in coronary heart disease mortality rate among young women and young men is presently unknown (108).

The impression has been widespread that through some mechanism

cigarette smoking accelerates the rate of development of coronary heart disease. The statistical calculations of Hammond and Horn shows that regular smokers of 40 cigarettes per day show approximately 2.2 times as high an incidence rate of clinical coronary heart disease as do non-smokers (108).

One of the most widespread impressions today concerning coronary heart disease is that it occurs prematurely in certain families. Families do exist with extreme elevation of one or another lipoprotein class, and in these families it is well documented by evidence reported throughout the world that premature clinical coronary heart disease is rampant (108).

Human atherosclerosis is a complex, progressive process of multiple etiology. The extent of atherosclerotic involvement in any specific individual is not easily assessed and is essentially masked until it precipitates a clinical event. Thus, research in this field must be carried out using appropriate animal models.

The ideal laboratory model is an animal that reacts as does man to all the risk factors such as diet, hypertension, age and sex. The lesion produced should resemble the human lesion in chronology and pathology.

Since Ignatowski's (109) original experiments in rabbits, a number of animal models have been used. Wissler and Vesselinovitch (110) have recently reviewed some of the differences among experimental species; these are summarized in Table II. It is virtually impossible to cause atherosclerosis in animals unless their serum β-lipoproteins are elevated. Hypercholesteremia without hyperbetalipoproteinemia will not result in atherosclerosis (111, 112, 113).

TABLE II

SOME CHARACTERISTICS OF ATHEROSCLEROSIS IN SPECIES COMMONLY USED IN EXPERIMENTAL ATHEROSCLEROSIS RESEARCH[a] (0-4 SCALE)

Species	Spontaneous Disease	Sensitivity to Diet	Distribution of Lesions[b]	Small Artery Involvement
Rabbit	0.5	4	1	4
Chicken	2	4	0.5	4
Rat	0	1	0.5	3
Pig	1	2	2.0	2
Squirrel Monkey	2	4	3.5	2
Rhesus Monkey	0	4	3.5	1

[a]After Wissler and Vesselinovitch (1974)

[b]Compared to Man

The animal most frequently used for induction of atherosclerosis has been the rabbit. However, the animal of choice for atherosclerosis research is some type of non-human primate. Primates are omnivorous, similar to man in many aspects of cardiovascular and pulmonary physiology and develop lesions resembling human lesions both in distribution and histology.

CHAPTER III

MATERIALS AND METHODS

A. Materials

1. Animals

Nicholas White poults, tom and hen turkeys were fed controlled diets by the Oklahoma State University Poultry Department. Poults (1-25 days post-hatched) were fed a control, turkey starter (tallow) diet (Table III). Mature turkey hens (12-16 weeks of age) were fed a control, turkey breeder ration (tallow) diet (Table III). However, turkey toms (12-15 weeks of age) were fed a turkey grower, 1% cholesterol diet (Table IV).

The turkeys were kept in a room with lights on from 6:00 a.m. to 6:00 p.m. and lights off from 6:00 p.m. to 6:00 a.m. The turkeys were sacrificed at various times of the day. The liver and kidneys were removed immediately, and either quick-frozen in liquid nitrogen and stored at -20°C until ready for use, or the liver and kidneys were used immediately.

2. Radioactive Compounds

DL-[Mevalonic-2-^{14}C] Acid, DL-3-[Glutaryl-3-^{14}C]-hydroxy-3-methylglutaryl Coenzyme A (HMG-CoA) and DL-5-^{3}H(N)-Mevalonic Acid were purchased from New England Nuclear, Boston, Massachusetts. The 2-^{14}C-

TABLE III

TURKEY STARTER AND TURKEY BREEDER RATION (TALLOW) DIETS FOR POULTS AND TURKEY HENS

Ingredients	Poults	Turkey Hens
	Percent/100 lbs.	Percent/100 lbs.
Tallow, feed grade	1.40	5.12
Corn, ground yellow	35.92	52.59
Soybean oil meal (44%)	37.30	28.20
Meat and bone scrap (50%)	14.50	1.67
Blood meal (80%)	—	1.71
Live yeast culture (14%)	2.50	2.50
Dried whey (12%)	2.80	0.68
Alfalfa meal (17%)	4.50	0.68
Dicalcium phosphate (Ca 27-P18)	—	1.30
Calcium carbonate	—	4.63
Salt	0.50	0.50
Turkey Breeder Vitamin Mix	0.30[1]	0.25[1]
CCC Trace Mineral[2]	0.10[3]	0.06[4]
dl-Methionine	0.18	0.11

[1]Vitamin A - 8.0×10^6 I.U./ton of feed; Vitamin D_3 - 3.0×10^6 I.U./ton of feed; Vitamin E - 2.7×10^4 I.U./ton of feed; Menadione Sodium Bisulfite Complex - 2.0×10^3 mg/ton of feed; Riboflavin - 5.0×10^3 mg/ton of feed; Niacin - 1.5×10^4 mg/ton of feed; d-Pantothenic acid - 1.6×10^4 mg/ton of feed; Choline - 4.0×10^5 mg/ton of feed; Thiamine - 2.0×10^3 mg/ton of feed; Pyridoxine - 2.0×10^3 mg/ton of feed; Vitamin B_{12} - 8.0 mg/ton of feed; d-Biotin - 1.0×10^2 mg/ton of feed; Folic Acid - 1.0×10^3 mg/ton of feed

[2]CCC - Calcium Carbonate Company

[3]Manganese - 90 ppm; Zinc - 60 ppm; Iron - 45 ppm; Copper - 7.5 ppm; Iodine - 0.75 ppm

TABLE III (Continued)

[4]Manganese - 120 ppm; Zinc - 80 ppm; Iron - 60 ppm; Copper - 10 ppm; Iodine - 1.0 ppm

TABLE IV

TURKEY GROWER, CHOLESTEROL DIET FOR TURKEY TOMS

Ingredients	Turkey Toms
	Percent/100 lbs.
Corn oil or Tallow	3.90
Corn, ground yellow	42.60
Milo, ground yellow	16.60
Soybean oil meal (44%)	17.60
Meat and bone scrap (50%)	9.30
Alfalfa Meal (17%)	2.40
Dried whey	1.50
Distiller's solubles	2.00
Live yeast culture	2.00
dl-Methionine	0.10
Turkey Breeder Vitamin Mix[1]	0.40
CCC Trace Mineral Mix[2]	0.10
Salt	0.50
Phosphorous Supplement (28ea-18P)	1.00
Cholesterol	1.00

[1]Vitamin A - 6.4×10^6 I.U./ton of feed; Vitamin D_3 - 2.4×10^6 I.U./ton of feed; Vitamin E - 2.1×10^4 I.U./ton of feed; Menadione Sodium Bisulfite Complex - 1.6×10^3 mg/ton of feed; Riboflavin - 4.0×10^3 mg/ton of feed; Niacin - 1.2×10^4 mg/ton of feed; d-Pantothenic Acid - 1.3×10^4 mg/ton of feed; Choline - 3.2×10^5 mg/ton of feed; Thiamine - 1.6×10^3 mg/ton of feed; Pyridoxine - 1.6×10^3 mg/ton of feed; Vitamin B_{12} - 6.4 mg/ton of feed; d-Biotin - 80 mg/ton of feed; Folic Acid - 8.0×10^2 mg/ton of feed

[2]Manganese - 120 ppm; Zinc - 80 ppm; Iron - 60 ppm; Copper - 10 ppm; Iodine - 1.0 ppm

Mevalonate was obtained as the dibenzoyl-ethylenediamine salt with a specific activity of 45.9 millicuries per millimole of acid. The HMG-CoA was obtained in an aqueous solution, pH 5.0 with a specific activity of 49.5 millicuries per millimole. The 5-^{3}H-Mevalonate was obtained as the dibenzoyl-ethylenediamine salt with a specific activity of 5.00 curies per millimole of acid.

3. Enzymes, Substrates and Cofactors

Glucose-6-phosphate dehydrogenase was purchased from Sigma Chemical Company, St. Louis, Missouri.

ATP as the sodium salt, glucose-6-phosphate as the disodium salt and $NADP^{+}$ as the sodium salt were purchased from Sigma Chemical Company, St. Louis, Missouri.

4. Chemicals and Reagents

Solvents and reagents were of analytical grade or of the highest quality available unless otherwise specified. Insta-Gel was purchased from Packard Instrument Company, Inc., Downers Grove, Illinois.

B. Methods

1. Preparation of Microsomes

The turkeys were sacrificed, the livers and kidneys immediately removed and were either quick-frozen in liquid nitrogen and stored at -20°C until ready for use or the tissues were used immediately. Approximately 25-35 grams of liver and 15-20 grams of kidneys were used in all experiments.

Turkey liver and kidney microsomal preparations were prepared in Krebs-Ringer phosphate buffer (2 ml/gram of tissue) containing 0.154 M NaCl, 0.154 M KCl, 0.11 M $CaCl_2$, 0.15 M $MgSO_4$, 5 mM dithiothreitol, 1 mM EDTA and 0.1 M potassium phosphate at pH 7.4 according to the procedure described by Slakey et al. (114). After the tissue was minced with scissors, homogenization was performed in a Potter-Elvejhem glass tissue grinder with a motor driven, grooved teflon pestle. The time required to obtain a homogenous mixture was approximately 1-2 minutes.

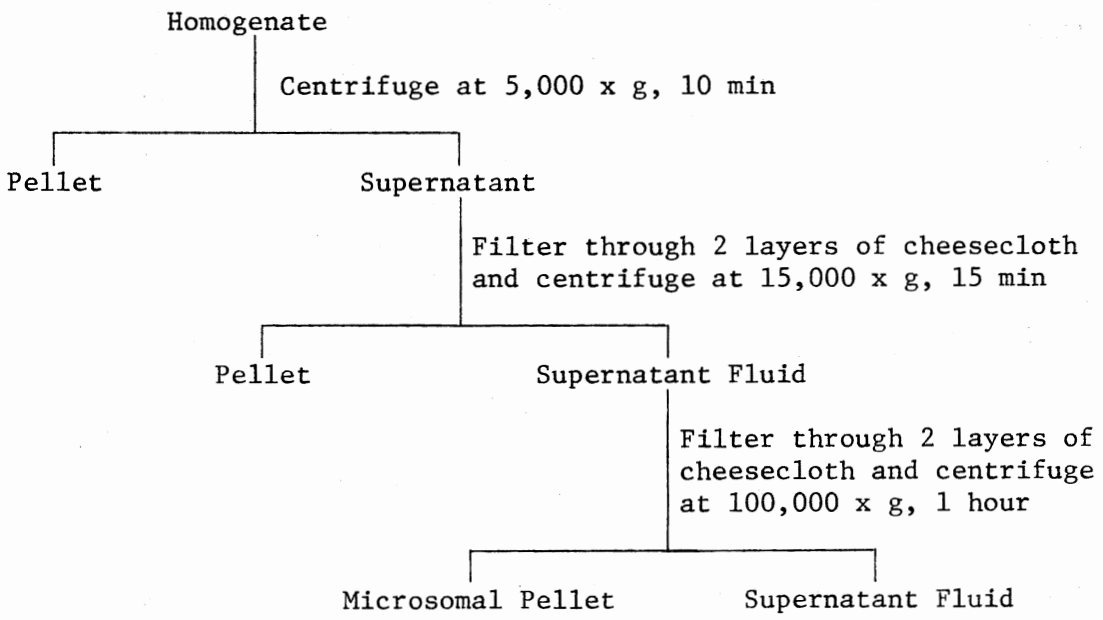
The homogenate obtained in the above procedure was subjected to a series of centrifugations as described in Figure 3. The 5,000 x g pellet contained nuclei and unbroken cells. The 15,000 x g pellet contained the mitochondrial fraction. The 100,000 x g 1 hour pellet contained the microsomes. The microsomal pellet was resuspended in a small volume of buffer with the aid of a homogenizer.

2. Sucrose Density Gradient

A linear sucrose density gradient of 20-45 percent was used in some of the experiments. The sucrose solutions were made up in buffer containing 5 mM $MgCl_2$, 1 mM EDTA, 0.5 mM dithiothreitol and 0.1 M potassium phosphate pH 7.4. The concentration of the sucrose solutions was checked with a refractometer.

Approximately 25 mg of microsomal protein was layered on a sucrose density gradient. The sucrose gradient was centrifuged at 20,000 rpm with a SW 25.1 Spinco Rotor for two hours. Five fractions, each consisting of 6 ml, were collected by using a density gradient fractionator. The five fractions were dialyzed against buffer containing 5 mM $MgCl_2$, 0.5 mM dithiothreitol and 0.1 M potassium phosphate pH 7.4.

Figure 3. Preparation of Microsomes.


Homogenate
Centrifuge at 5,000 x g, 10 min
Pellet
Supernatant
Filter through 2 layers of cheesecloth and centrifuge at 15,000 x g, 15 min
Pellet
Supernatant Fluid
Filter through 2 layers of cheesecloth and centrifuge at 100,000 x g, 1 hour
Microsomal Pellet
Supernatant Fluid

3. Assays

HMG-CoA reductase activity was measured by an adaption of the method of Huber et al. (115). The reaction mixture contained 10 mM glucose-6-phosphate, 1 mM $NADP^+$, glucose-6-phosphate dehydrogenase (2 units), 1 mM EDTA, 1 mM dithiothreitol, 0.1 M potassium phosphate buffer pH 7.4, 100 µg microsomal protein and 3-^{14}C-HMG-CoA (≅ 7.9 X 10^4 dpm). The reactions were performed in a total volume of 1.0 ml. The reaction mixture was incubated at 37°C for 1 hour. ^{3}H-Mevalonic acid (≅ 2.5 X 10^5) was added as an internal standard. The reaction was stopped by quick freezing in a dry ice/isopropanol mixture and 200 µl 2 N H_2SO_4 was added. The reaction mixture was allowed to set at room temperature for 10 minutes in order for lactonization of mevalonate to occur. Anhydrous Na_2SO_4 (6 g) was mixed with the reaction mixture. After standing for 1 hour at room temperature, the samples were extracted by four elutions with diethyl ether (1 X 9 ml, 3 X 5 ml).

The combined extracts were evaporated to dryness in a hot water bath. The residue was dissolved in 1.0 ml of water, placed on a Dowex-1 formate column (0.5 X 5 cm) and eluted with 5.0 ml of water. The eluate, which contained the mevalonolactone was collected in a scintillation vial. Only 2.0 ml of the eluate was added to 5.0 ml of Insta-gel. The scintillation vial was counted in a Packard PL scintillation spectrometer.

Mevalonate kinase activity was measured by an adaption of the method of Green and Levinthal (116). The reaction mixture in a volume of 1.0 ml contained 5 mM $MgCl_2$, 0.5 mM DDT, 1.0 mM EDTA, 5 mM

ATP, 2-^{14}C-MVA (≅ 9.6 X 10^5 dpm), 100 µg microsomal protein and 0.1 M potassium phosphate buffer pH 7.4. The reaction was incubated for 1 hour at 37°C. The reaction was terminated by boiling in water for 5 minutes. The products were separated by applying the supernatant to a Dowex-1 formate column (0.5 X 5 cm).

4. Protein

Protein concentrations were determined by a modification of the Lowry procedure as described by Hartree (117) using bovine serum albumin as a standard.

5. Chromatography

Ion exchange chromatography of mevalonate metabolites was completed as described by Suzue (118). Dowex-1 formate resins were packed in 0.5 X 5.0 cm columns. The stepwise elution of the Dowex-1 formate columns was achieved with the following eluting solvents: a) 25 ml H_2O; b) 25 ml 2 N formic acid; c) 50 ml 4 N formic acid; d) 50 ml 0.4 N ammonium formate in 4 N formic acid and e) 50 ml 0.8 N ammonium formate in 4 N formic acid. Approximately 3.0-3.5 ml fractions were collected.

6. Detection and Measurement of Radioactivity

The MVA-kinase radioactive elution profiles from Dowex-1 formate ion exchange columns were obtained by counting 100 µl of each fraction in a toluene-ethanol scintillation cocktail. The toluene-ethanol scintillation cocktail contained four grams of 2,5-diphenyloxazole (PPO) and 0.2 grams of 1,4-bis (2-(5-phenyloxazole))-benzene; phenyl-

oxazoylphenyl (POPOP) in 400 ml of sulfur free toluene and 600 ml absolute ethanol. All samples were counted in 10 ml of scintillation cocktail.

The isotopic source for the standard quench set was ^{14}C-benzoic acid. Various amounts of reagent grade acetone was used as a quencher. The efficiency of the samples counted in toluene-ethanol cocktail was determined to be between 45 and 65 percent efficiency.

HMG-CoA reductase radioactivity eluted from a Dowex-1 formate ion exchange column was obtained by counting 2.0 ml of the eluant in 5.0 ml of Insta-Gel.

The isotopic source for the standard quench set was ^{14}C-toluene. The efficiency of the samples counted in Insta-Gel cocktail was determined to be between 45 and 60 percent.

CHAPTER IV

RESULTS

A. Diurnal Cycle of Mevalonate Kinase Activity, HMG-CoA Reductase Activity and Protein Concentration in Liver and Kidneys From Normal Turkeys

Mature (sixteen weeks old) female turkeys were fed a control (tallow) diet and were sacrificed at four-hour intervals, commencing at 12:00 a.m., for twenty-four hours. The liver and kidneys were removed and a 100,000 x g pellet was prepared according to the procedure under Materials and Methods. The resuspended 100,000 x g pellet was assayed for HMG-CoA reductase and mevalonate kinase activities, and protein concentration according to the procedures under Materials and Methods.

1. Profile of the Mevalonate Activating Enzymes in Liver and Kidneys at Various Times of the Day

High levels of MVA kinase, MVAP kinase and MVAPP decarboxylase activities were observed in microsomes from the liver of turkeys sacrificed at 12:00 a.m. However, in the kidneys of these turkeys only MVA kinase activity was observed. Figure 4 shows the Dowex-1

Figure 4. Metabolism of ^{14}C-Mevalonic Acid by Liver and Kidney Microsomes from Turkeys Sacrificed at 12:00 a.m.

Dowex-1 formate elution pattern: mevalonate kinase assay of the microsomes from liver and kidneys of turkeys fed a control (tallow) diet. Turkeys were sacrificed at 12:00 a.m. (A) Control turkey liver (⊙——⊙); (B) Control turkey kidneys (●——●).

Elution profiles following assay for mevalonate kinase in liver and kidney microsomes under normal assay conditions. Elution of Dowex-1 formate column:

A. 25 ml water

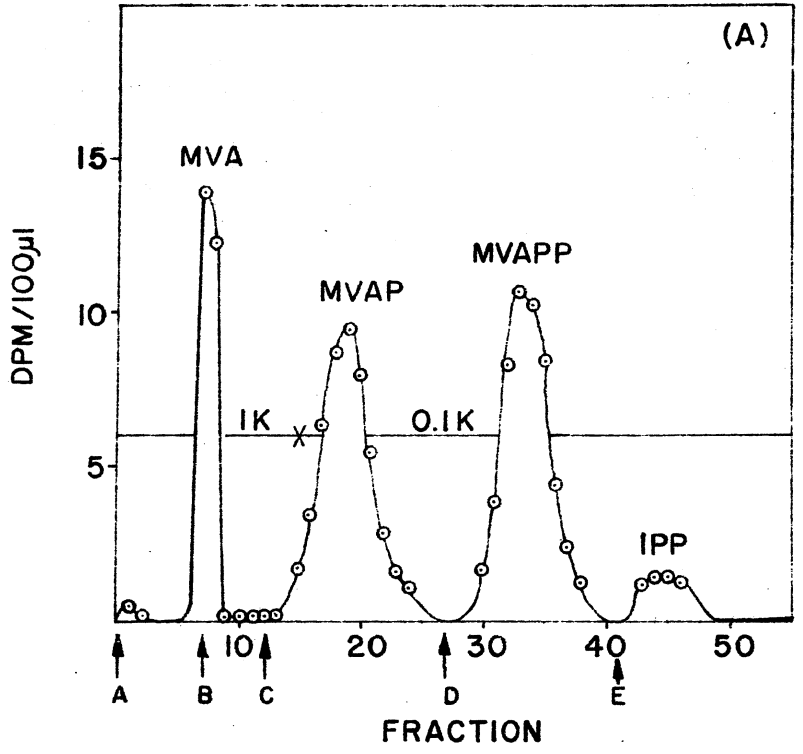
B. 25 ml 2 N formic acid

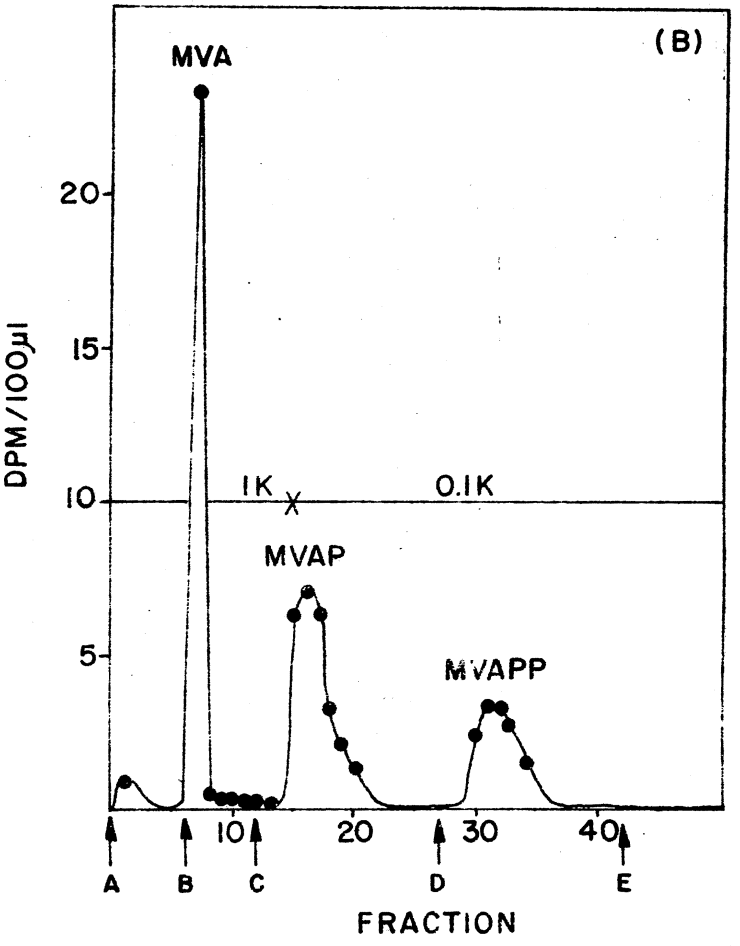
C. 50 ml 4 N formic acid

D. 50 ml 4 N formic acid + 0.4 N ammonium formate

E. 50 ml 4 N formic acid + 0.8 N ammonium formate

Collect approximately 3.0-3.5 ml fractions.


(A)
MVA
MVAP
MVAPP
IPP
1K
0.1K
DPM/100µl
15
10
5
10
20
30
40
50
A
B
C
D
E
FRACTION


(B)
MVA
MVAP
MVAPP
1K
0.1K
DPM/100µl
20
15
10
5
10
20
30
40
A
B
C
D
E
FRACTION

formate column elution profiles of the products from mevalonate metabolism by liver and kidney microsomes.

Low levels of MVA kinase and MVAP kinase activities were observed in microsomes from the liver and kidneys of turkeys sacrificed at 4:00 a.m. The Dowex-1 formate column elution profiles of the products from mevalonate metabolism by liver and kidney microsomes are shown in Figure 5.

High levels of MVA kinase, MVAP kinase and MVAPP decarboxylase activities were observed in microsomes from the liver of turkeys sacrificed at 8:00 a.m., but only a low level of MVA kinase activity was observed in microsomes from the kidneys of turkeys sacrificed at 8:00 a.m. Figure 6 shows the Dowex-1 formate column elution profiles of the products from mevalonate metabolism by liver and kidney microsomes.

High levels of MVA kinase, MVAP kinase and MVAPP decarboxylase activities were observed in microsomes from the liver of turkeys sacrificed at 12:00 p.m. Again a much lower level of MVA kinase and MVAP kinase activities were observed in the kidneys of turkeys sacrificed at 12:00 p.m. The Dowex-1 formate column elution profiles of the products from mevalonate metabolism by liver and kidney microsomes are shown in Figure 7.

Extremely low levels of MVA kinase and MVAP kinase activities were observed in microsomes from the liver of turkeys sacrificed at 4:00 p.m. However, a high level of MVA kinase activity was observed in microsomes from the kidneys of turkeys sacrificed at 4:00 p.m. Figure 8 shows the Dowex-1 formate column elution profiles of the products from mevalonate metabolism by liver and kidney microsomes.

Figure 5. Metabolism of ^{14}C-Mevalonic Acid by Liver and Kidney Micro-Microsomes from Turkeys Sacrificed at 4:00 a.m.

Dowex-1 formate elution pattern: mevalonate kinase of the microsomes from liver and kidneys of turkeys fed a control (tallow) diet. Turkeys were sacrificed at 4:00 a.m. (A) Control turkey liver (⊙——⊙); (B) Control turkey kidneys (●——●).

Elution profiles following assay for mevalonate kinase in liver and kidney microsomes under normal assay conditions. Elution of Dowex-1 formate column:

A. 25 ml water

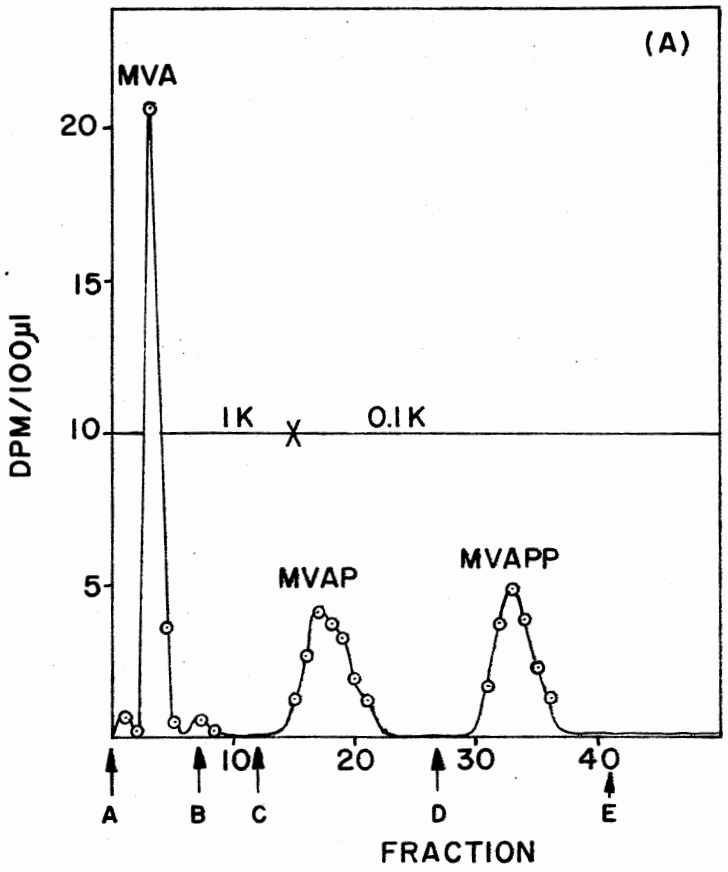
B. 25 ml 2 N formic acid

C. 50 ml 4 N formic acid

D. 50 ml 4 N formic acid + 0.4 N ammonium formate

E. 50 ml 4 N formic acid + 0.8 N ammonium formate

Collect approximately 3.0-3.5 ml fractions.


(A)
MVA
MVAP
MVAPP
1K
0.1K
DPM/100µl
20
15
10
5
10
20
30
40
A
B
C
D
E
FRACTION

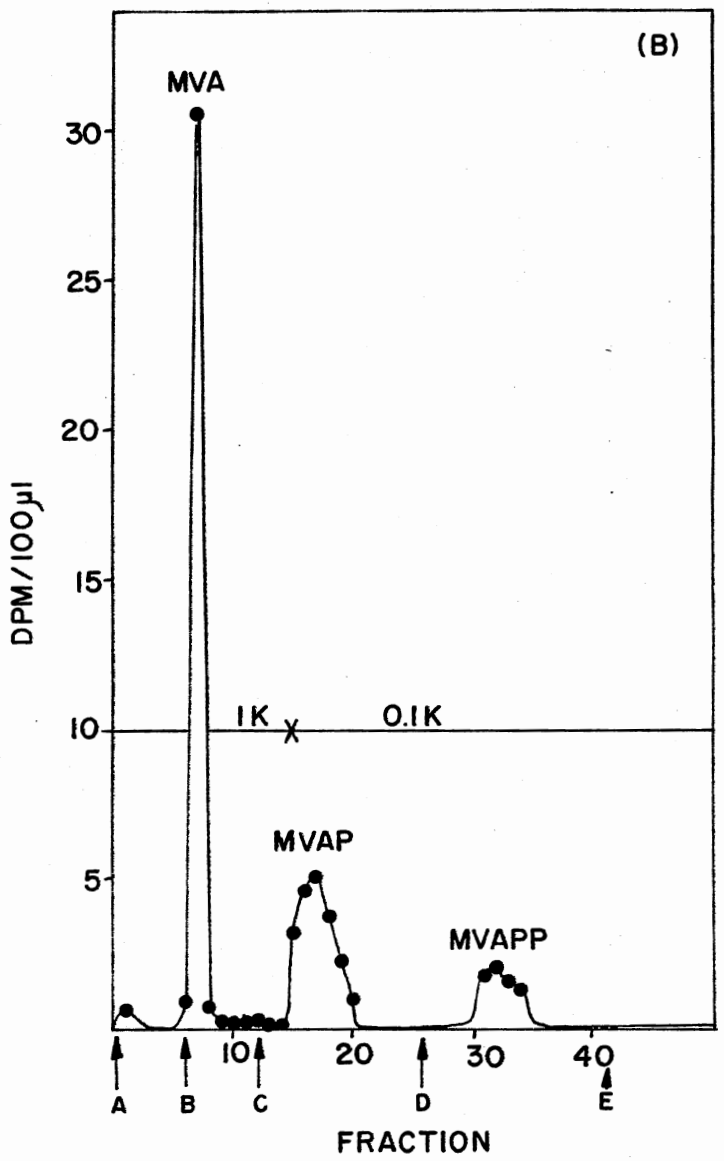

(B)
MVA
MVAP
MVAPP
1K
0.1K
DPM/100µl
30
25
20
15
10
5
10
20
30
40
A
B
C
D
E
FRACTION

Figure 6. Metabolism of ^{14}C-Mevalonic Acid by Liver and Kidney Microsomes from Turkeys Sacrificed at 8:00 a.m.

Dowex-1 formate elution pattern: mevalonate kinase assay of the microsomes from liver and kidneys of turkeys fed a control (tallow) diet. Turkeys were sacrificed at 8:00 a.m. (A) Control turkey liver (⊙———⊙); (B) Control turkey kidneys (●———●).

Elution profiles following assay for mevalonate kinase in liver and kidney microsomes under normal assay conditions. Elution of Dowex-1 formate column:

A. 25 ml water

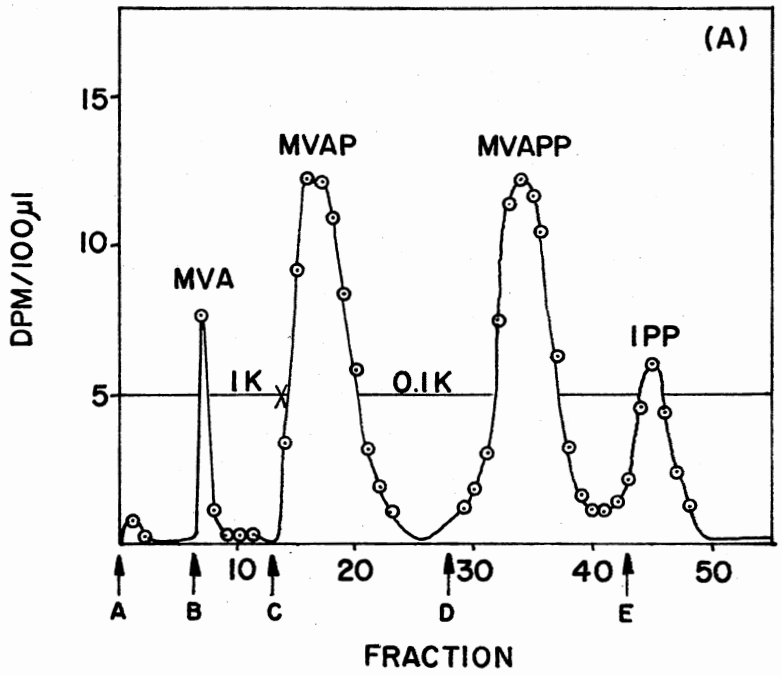
B. 25 ml 2 N formic acid

C. 50 ml 4 N formic acid

D. 50 ml 4 N formic acid + 0.4 N ammonium formate

E. 50 ml 4 N formic acid + 0.8 N ammonium formate

Collect approximately 3.0-3.5 ml fractions.


(A)
DPM/100µl
15
10
5
MVA
MVAP
MVAPP
IPP
1K
0.1K
10
20
30
40
50
A
B
C
D
E
FRACTION

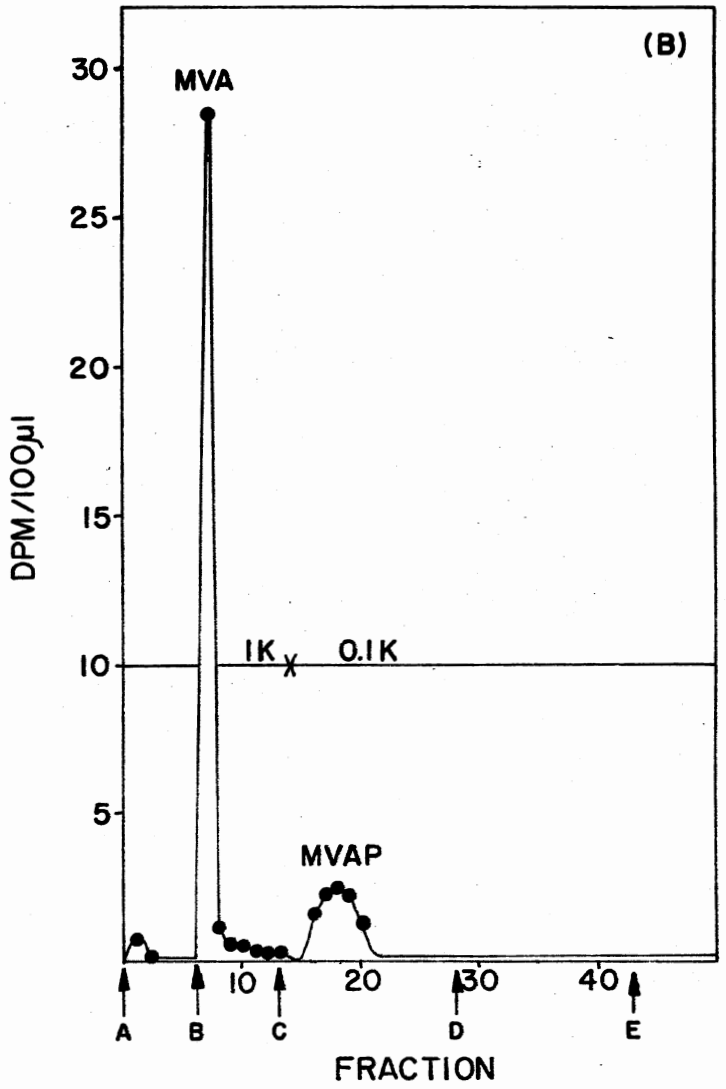

(B)
DPM/100µl
30
25
20
15
10
5
MVA
1K
0.1K
MVAP
10
20
30
40
A
B
C
D
E
FRACTION

Figure 7. Metabolism of ^{14}C-Mevalonic Acid by Liver and Kidney Microsomes from Turkeys Sacrificed at 12:00 p.m.

Dowex-1 formate elution pattern: mevalonate kinase assay of the microsomes from liver and kidneys of turkeys fed a control (tallow) diet. Turkeys were sacrificed at 12:00 p.m. (A) Control turkey liver (⊙———⊙); (B) Control turkey kidneys (●———●).

Elution profiles following assay for mevalonate kinase in liver and kidney microsomes under normal assay conditions. Elution of Dowex-1 formate column:

A. 25 ml water

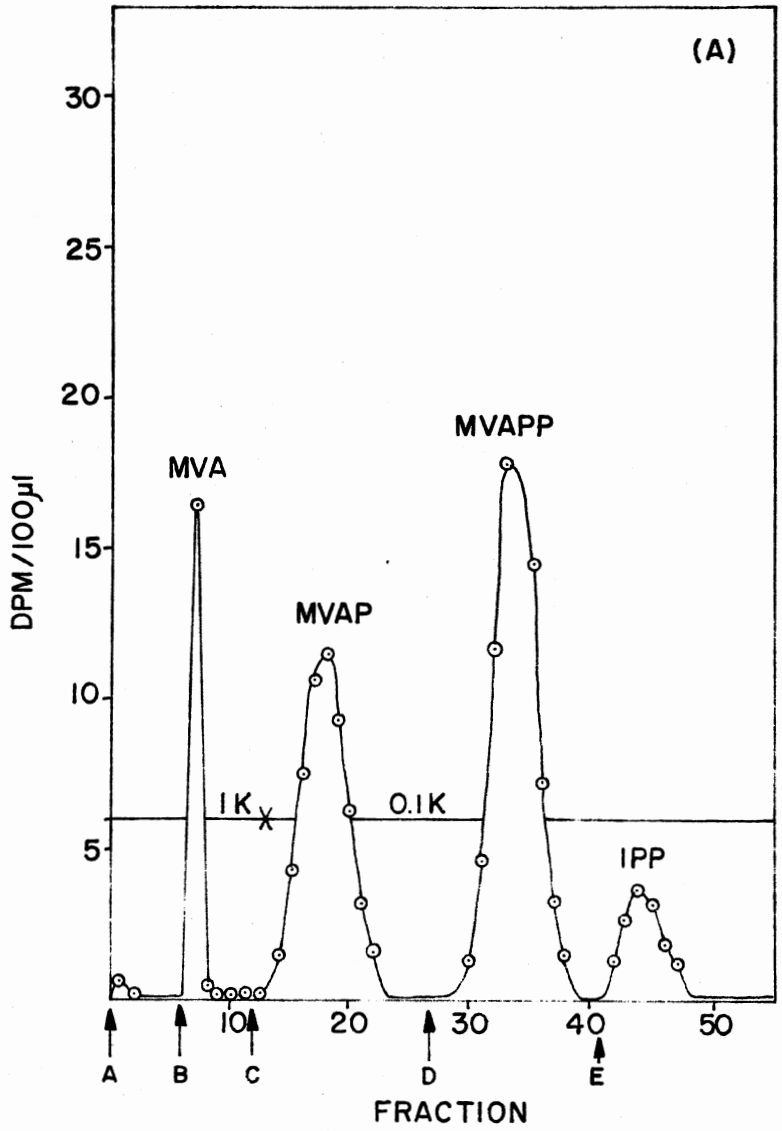
B. 25 ml 2 N formic acid

C. 50 ml 4 N formic acid

D. 50 ml 4 N formic acid + 0.4 N ammonium formate

E. 50 ml 4 N formic acid + 0.8 N ammonium formate

Collect approximately 3.0-3.5 ml fractions.


(A)
DPM/100µl
30
25
20
15
10
5
MVA
MVAP
MVAPP
IPP
1K
0.1K
10
20
30
40
50
A
B
C
D
E
FRACTION

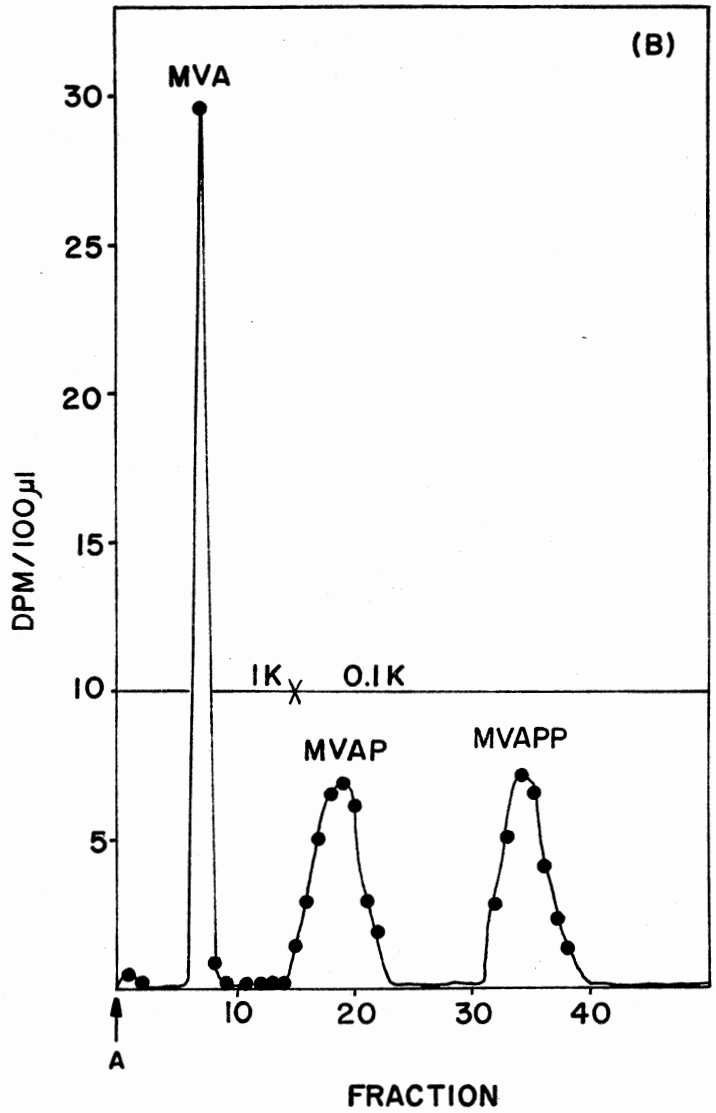

(B)
DPM/100µl
30
25
20
15
10
5
MVA
1K
0.1K
MVAP
MVAPP
10
20
30
40
A
FRACTION

Figure 8. Metabolism of ^{14}C-Mevalonic Acid by Liver and Kidney Microsomes from Turkeys Sacrificed at 4:00 p.m.

Dowex-1 formate elution pattern: mevalonate kinase assay of the microsomes from liver and kidneys of turkeys fed a control (tallow) diet. Turkeys were sacrificed at 4:00 p.m. (A) Control turkey liver (⊙———⊙); (B) Control turkey kidneys (●———●).

Elution profiles following assay for mevalonate kinase in liver and kidney microsomes under normal assay conditions. Elution of Dowex-1 formate column:

A. 25 ml water

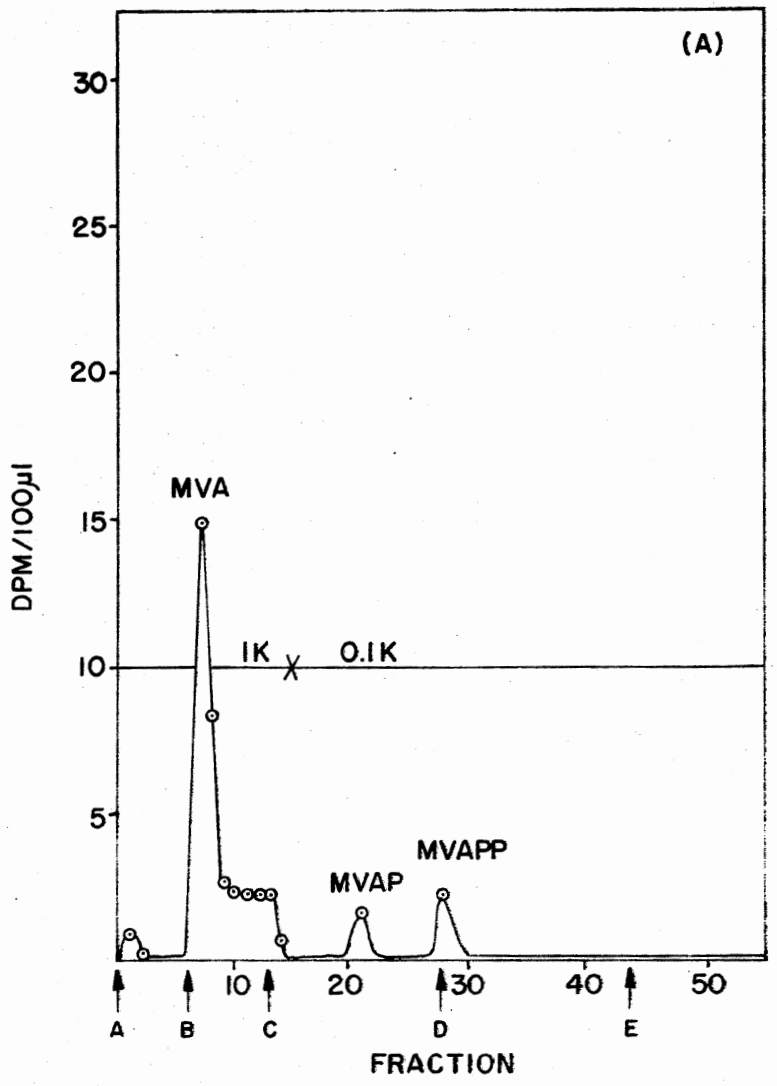
B. 25 ml 2 N formic acid

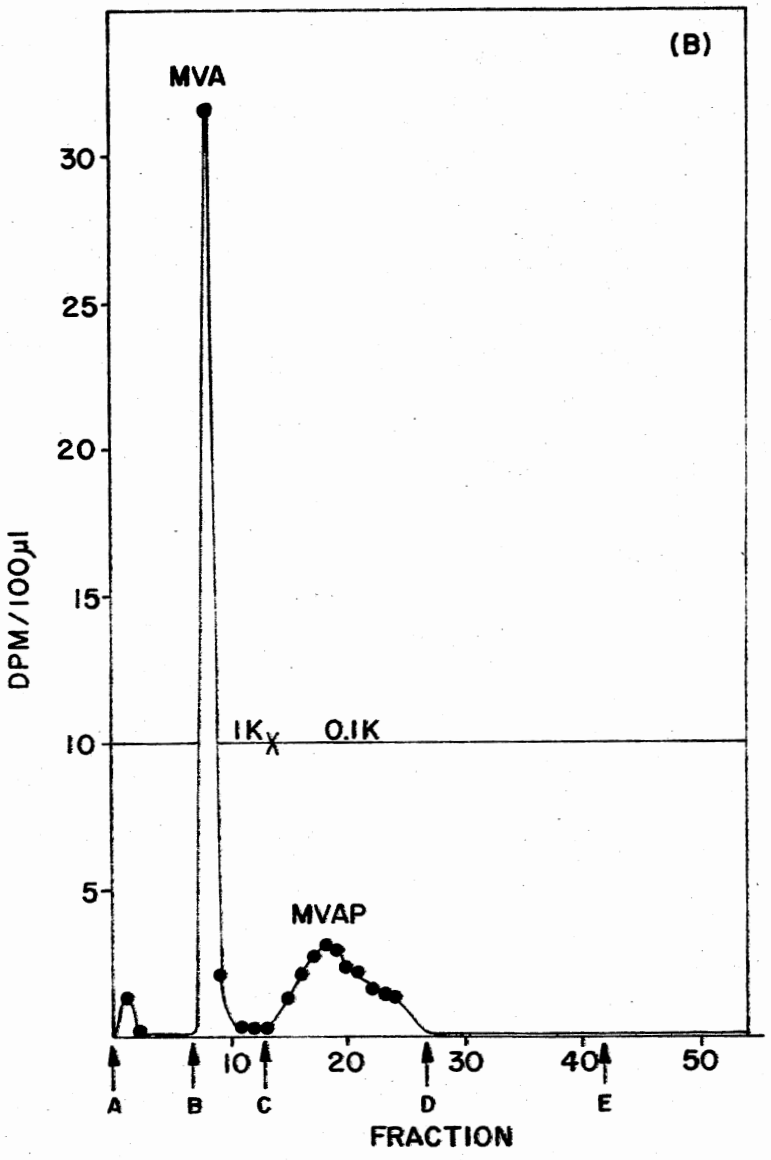
C. 50 ml 4 N formic acid

D. 50 ml 4 N formic acid + 0.4 N ammonium formate

E. 50 ml 4 N formic acid + 0.8 N ammonium formate

Collect approximately 3.0-3.5 ml fractions.


(A)
DPM/100µl
30
25
20
15
10
5
MVA
1K
0.1K
MVAP
MVAPP
10
20
30
40
50
A
B
C
D
E
FRACTION


(B)
MVA
DPM/100µl
30
25
20
15
10
5
1K
0.1K
MVAP
10
20
30
40
50
A
B
C
D
E
FRACTION

A low level of MVA kinase activity was observed in microsomes from liver and kidneys of turkeys sacrificed at 8:00 p.m. The Dowex-1 formate column elution profiles of the products from mevalonate metabolism by liver and kidney microsomes are shown in Figure 9.

2. Activity of the Mevalonate Activating Enzymes in Liver and Kidneys at Various Times of the Day

MVA kinase, MVAP kinase and MVAPP decarboxylase specific activities in microsomes from liver of turkeys sacrificed at 12:00 a.m. were 22.80, 12.00 and 1.12 nmoles MVA metabolized mg protein in $assay^{-1}$ hr^{-1}, respectively (Table V). MVA kinase and MVAP kinase specific activities in microsomes from kidneys of turkeys sacrificed at 12:00 a.m., were 6.10 and 2.80 nmoles MVA metabolized mg protein in $assay^{-1}$ hr^{-1}, respectively (Table VI). There was no MVAPP decarboxylase activity in the kidneys.

Table V also shows that MVA kinase and MVAP kinase specific activities in microsomes from liver of turkeys sacrificed at 4:00 a.m., decreased to 4.13 and 3.60 nmoles MVA metabolized mg protein in $assay^{-1}$ hr^{-1}, respectively. MVA kinase and MVAP kinase specific activities in microsomes of kidneys from turkeys sacrificed at 4:00 a.m., decreased to 4.50 and 1.42 nmoles MVA metabolized mg protein in $assay^{-1}$ hr^{-1}, respectively (Table VI). At 4:00 a.m. there was no measurable MVAPP decarboxylase activity in the liver or kidneys.

MVA kinase, MVAP kinase and MVAPP decarboxylase specific activities in microsomes from liver of turkeys sacrificed at 8:00 a.m., increased to 28.00, 12.50 and 2.70 nmoles MVA metabolized mg

Figure 9. Metabolism of ^{14}C-Mevalonic Acid by Liver and Kidney Microsomes from Turkeys Sacrificed at 8:00 p.m.

Dowex-1 formate elution pattern: mevalonate kinase assay of the microsomes from liver and kidneys of turkeys fed a control (tallow) diet. Turkeys were sacrificed at 8:00 p.m. (A) Control turkey liver (⊙———⊙); (B) Control turkey kidneys (●———●).

Elution profiles following assay for mevalonate kinase in liver and kidney microsomes under normal assay conditions. Elution of Dowex-1 formate column:

A. 25 ml water

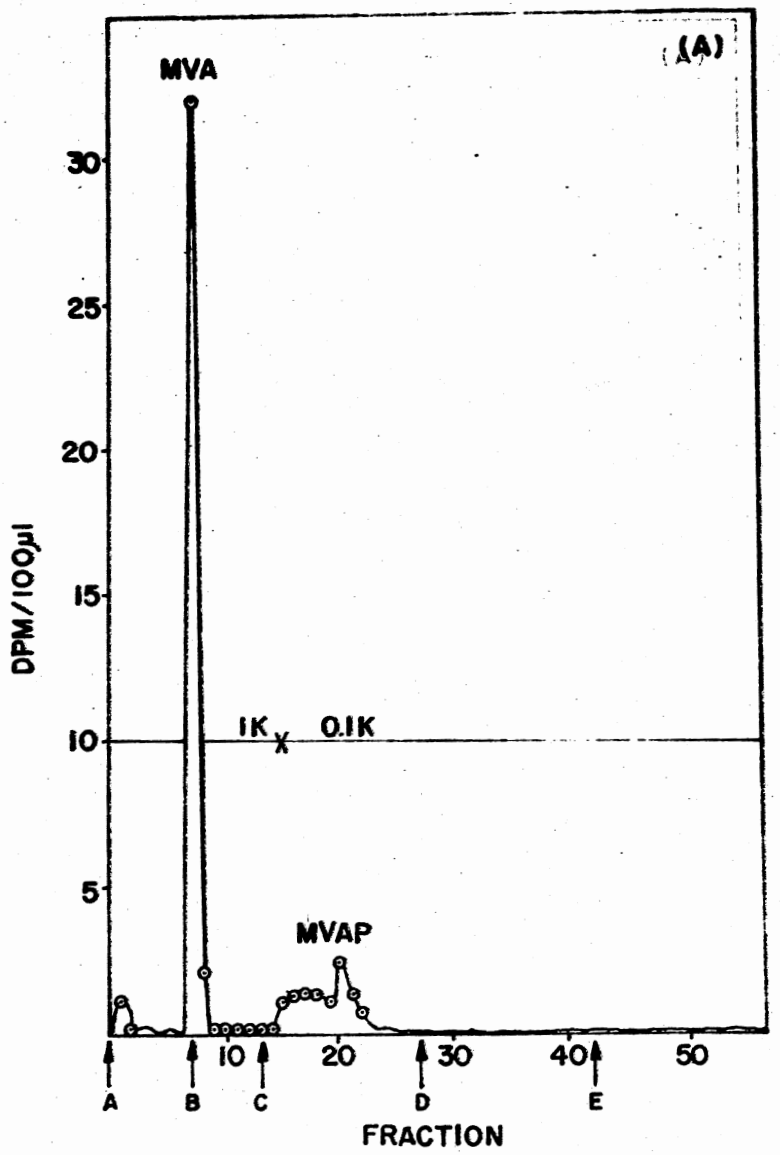
B. 25 ml 2 N formic acid

C. 50 ml 4 N formic acid

D. 50 ml 4 N formic acid + 0.4 N ammonium formate

E. 50 ml 4 N formic acid + 0.8 N ammonium formate

Collect approximately 3.0-3.5 ml fractions.


(A)
MVA
30
25
20
15
10
5
DPM/100µl
IK
X
0.1K
MVAP
10
20
30
40
50
A
B
C
D
E
FRACTION

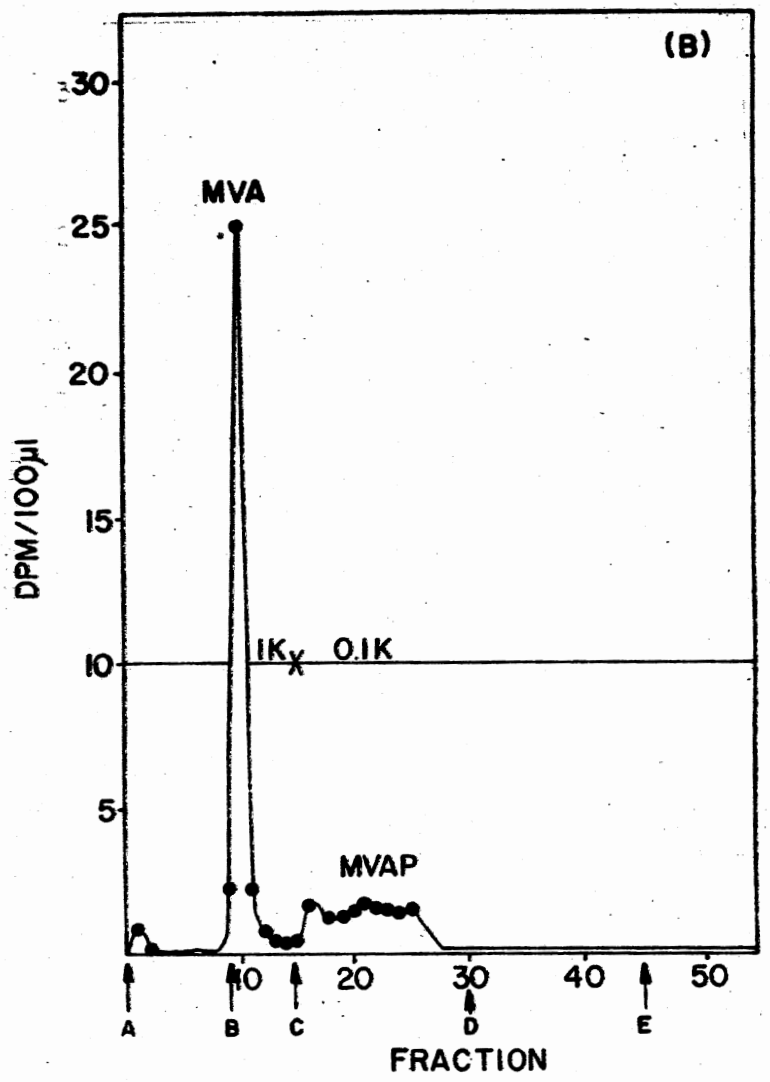

(B)
30
25
20
15
10
5
MVA
DPM/100µl
IK
X
0.1K
MVAP
10
20
30
40
50
A
B
C
D
E
FRACTION

TABLE V

EFFECT OF TIME ON HMG-CoA REDUCTASE, MEVALONATE KINASE, PHOSPHOMEVALONATE KINASE, AND PYROPHOSPHOMEVALONATE DECARBOXYLASE ACTIVITY FROM LIVER OF CONTROL FED TURKEYS

Time of Day	100,000 X g Pellet (Microsomes)							
	HMG-CoA Reductase[1]		Mevalonate Kinase[2]		Phosphomevalonate Kinase[2]		Pyrophosphomevalonate Decarboxylase[2]	
		Average		Average		Average		Average
12:00 a.m.	0.28 0.28	0.28	21.30 24.30	22.80	13.00 11.00	12.00	1.31 0.93	1.12
4:00 a.m.	1.80 1.05	1.40	4.90 3.36	4.13	3.71 3.50	3.60	— —	—
8:00 a.m.	1.12 0.89	1.00	28.00 28.10	28.00	13.50 11.50	12.50	2.81 2.60	2.70
12:00 p.m.	1.40 1.61	1.50	30.00 22.00	26.00	12.40 10.00	11.20	1.50 1.41	1.45
4:00 p.m.	0.31 0.09	0.20	0.75 0.76	0.75	0.44 0.41	0.43	— —	—
8:00 p.m.	0.23 0.22	0.22	1.72 1.71	1.72	— —	—	— —	—

[1]HMG-CoA Reductase - nmoles of mevalonate formed, mg protein in assay^{-1} hr^{-1}

[2]Mevalonate Kinase, Phosphomevalonate Kinase and Pyrophosphomevalonate Decarboxylase - nmoles mevalonate metabolized, mg protein in assay^{-1} hr^{-1}

TABLE V (Continued)

[3]A blank value (—) indicates that the enzyme activity was undetectable according to assays

TABLE VI

EFFECT OF TIME ON HMG-CoA REDUCTASE, MEVALONATE KINASE, PHOSPHOMEVALONATE KINASE AND PYROPHOSPHOMEVALONATE DECARBOXYLASE ACTIVITY FROM KIDNEYS OF CONTROL FED TURKEYS

Time of Day	100,000 X g Pellet (Microsomes)							
	HMG-CoA Reductase[1]		Mevalonate Kinase[2]		Phosphomevalonate Kinase[2]		Pyrophosphomevalonate Decarboxylase[2]	
		Average		Average		Average		Average
12:00 a.m.	0.65 2.20	1.43	8.20 3.91	6.10	2.91 2.70	2.80	— —	—
4:00 a.m.	0.09 0.05	0.07	5.60 3.00	4.50	1.50 1.34	1.45	— —	—
8:00 a.m.	0.33 0.34	0.33	2.60 2.20	2.40	— —	—	— —	—
12:00 p.m.	0.25 0.37	0.31	7.50 7.31	7.40	3.72 3.50	3.60	— —	—
4:00 p.m.	0.08 0.07	0.07	6.90 4.50	5.70	— —	—	— —	—
8:00 p.m.	0.13 0.14	0.13	2.50 2.51	2.50	— —	—	— —	—

[1]HMG-CoA Reductase - nmoles of mevalonate formed, mg protein in assay^{-1} hr^{-1}

[2]Mevalonate Kinase, Phosphomevalonate Kinase and Pyrophosphomevalonate Decarboxylase - nmoles mevalonate metabolized, mg protein in assay^{-1} hr^{-1}

TABLE VI (Continued)

[3]A blank value (—) indicates that the enzyme activity was undetectable according to assays

protein in assay^{-1} hr^{-1}, respectively (Table V). In contrast, MVA kinase specific activity in microsomes from kidneys of turkeys sacrificed at 8:00 a.m., decreased further to 2.40 nmoles MVA metabolized mg protein in assay^{-1} hr^{-1} (Table VI).

Again Table V shows that MVA kinase, MVAP kinase and MVAPP decarboxylase specific activities in microsomes from liver of turkeys sacrificed at 12:00 p.m., decreased slightly to 26.00, 11.20 and 1.45 nmoles MVA metabolized mg protein in assay^{-1} hr^{-1}, respectively. MVA kinase and MVAP kinase specific activities in microsomes from kidneys of turkeys sacrificed at 12:00 p.m., increased to 7.40 and 3.60 nmoles MVA metabolized mg protein in assay^{-1} hr^{-1}, respectively (Table VI).

MVA kinase and MVAP kinase specific activities in microsomes from liver of turkeys sacrificed at 4:00 p.m., decreased to 0.75 and 0.43 nmoles MVA metabolized mg protein in assay^{-1} hr^{-1}, respectively (Table V). MVA kinase specific activity in microsomes from kidneys of turkeys sacrificed at 4:00 p.m., decreased slightly to 5.70 nmoles MVA metabolized mg protein in assay^{-1} hr^{-1} (Table VI).

Table V shows that MVA kinase specific activity in microsomes from liver of turkeys sacrificed at 8:00 p.m., increased slightly to 1.72 nmoles MVA metabolized mg protein in assay^{-1} hr^{-1}. MVA kinase specific activity in microsomes from kidneys of turkeys sacrificed at 8:00 p.m., decreased to 2.50 nmoles MVA metabolized mg protein in assay^{-1} hr^{-1} (Table VI).

3. Activity of HMG-CoA Reductase in Liver and Kidneys at Various Times of the Day

HMG-CoA reductase activity in microsomes from liver and kidneys of

turkeys sacrificed at 12:00 a.m. were 0.28 and 1.43 nmoles MVA formed mg protein in assay^{-1} hr^{-1}, respectively (Table V and Table VI).

Table V shows that HMG-CoA reductase activity in microsomes from liver of turkeys sacrificed at 4:00 a.m. increased to 1.40 nmoles MVA formed mg protein in assay^{-1} hr^{-1}. However, HMG-CoA reductase activity in microsomes from kidneys of turkeys sacrificed at 4:00 a.m. decreased to 0.07 nmoles MVA formed mg protein in assay^{-1} hr^{-1} (Table VI).

HMG-CoA reductase activity in microsomes from liver of turkeys sacrificed at 8:00 a.m. decreased to 1.00 nmoles MVA formed mg protein in assay^{-1} hr^{-1}, and HMG-CoA reductase activity in microsomes from kidneys of turkeys sacrificed at 8:00 a.m. increased to 0.33 nmoles MVA formed mg protein in assay^{-1} hr^{-1} (Table V and Table VI).

HMG-CoA reductase achieved its highest specific activity in microsomes from liver of turkeys sacrificed at 12:00 p.m. (1.50 nmoles MVA formed mg protein in assay^{-1} hr^{-1}). However, HMG-CoA reductase activity in microsomes from kidneys of turkey sacrificed at 12:00 p.m. decreased slightly to 0.31 nmoles MVA formed mg protein in assay^{-1} hr^{-1} (Table VI).

HMG-CoA reductase activity in microsomes from liver and kidneys of turkeys sacrificed at 4:00 p.m. decreased to 0.20 and 0.07 nmoles MVA formed mg protein in assay^{-1} hr^{-1}, respectively (Table V and Table VI).

Table V and Table VI shows that HMG-CoA reductase activity in microsomes from liver and kidneys of turkeys sacrificed at 8:00 p.m., increased slightly to 0.22 and 0.13 nmoles MVA formed mg protein in assay^{-1} hr^{-1}, respectively.

4. Protein Concentration in Liver and Kidneys at Various Times of the Day

The microsomal protein concentration in the extract from liver and kidneys of turkeys sacrificed at 12:00 a.m. were 31.0 and 26.0 mg protein ml^{-1}, respectively; at 4:00 a.m. the values for liver increased to 71.0 mg protein ml^{-1}, whereas the value for kidneys dropped to 15.0 mg protein ml^{-1}; at 8:00 a.m. the value for liver dropped to 60.0 mg protein ml^{-1} and the value for the kidneys increased slightly to 15.3 mg protein ml^{-1}; at 12:00 p.m. the value for liver increased to 67.0 mg protein ml^{-1} and the value for kidneys decreased to 11.0 mg protein ml^{-1}; at 4:00 p.m. the values dropped to 24.0 and 9.0 mg protein ml^{-1}, respectively; and finally, at 8:00 p.m. the value for liver dropped to 6.0 mg protein ml^{-1}, whereas the kidney value remained at 9.0 mg protein ml^{-1} (Table VII).

5. Profile of Mevalonate Kinase Activity, HMG-CoA Reductase Activity and Protein Concentration in Liver and Kidneys at Various Times of the Day

A comparison was made of the diurnal cycle for HMG-CoA reductase from liver and the diurnal cycle for HMG-CoA reductase from kidneys of turkeys. Figure 10 shows that when HMG-CoA reductase from liver reach its maximum activity at 12:00 p.m., the reductase activity in kidneys is very low, and when the reductase in kidneys reach its maximum activity at 12:00 a.m., the liver reductase activity is very low. In the liver and kidneys, HMG-CoA reductase reach its lowest activity at

TABLE VII

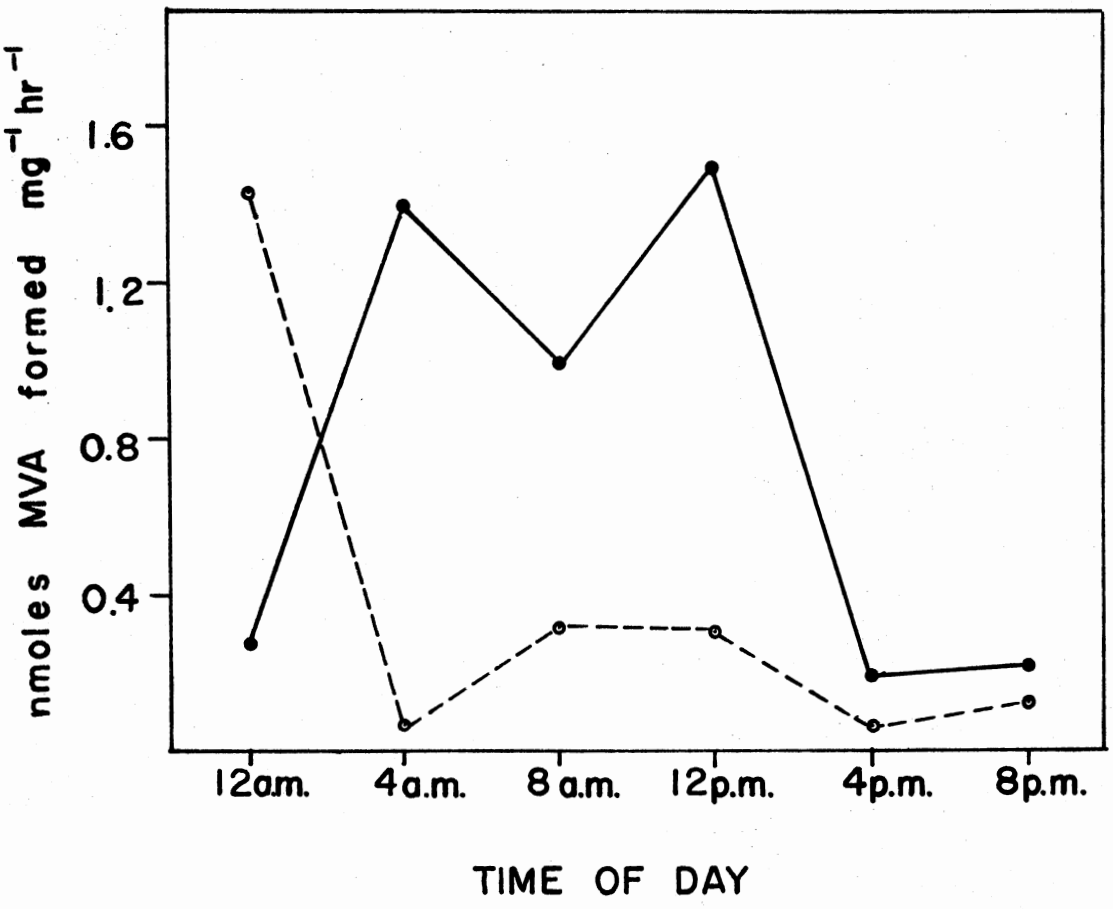
EFFECT OF TIME ON PROTEIN CONCENTRATION FROM LIVER AND KIDNEYS OF CONTROL FED TURKEYS

Time of Day	100,000 x g Pellet (Microsomes)			
	Turkey Liver Protein Concentration[1]	Average	Turkey Kidney Protein Concentration[1]	Average
12:00 a.m.	30.0 32.0	31.0	23.0 28.0	26.0
4:00 a.m.	93.0 50.0	71.0	17.0 12.0	15.0
8:00 a.m.	61.0 60.0	60.0	21.6 4.0	15.3
12:00 p.m.	67.0 66.0	67.0	10.0 12.0	11.0
4:00 p.m.	32.0 16.0	24.0	9.0 8.9	9.0
8:00 p.m.	5.9 6.0	6.0	9.0 9.1	9.0

[1]Protein Concentration - mg protein in the microsomal extract•ml^{-1}

Figure 10. Cyclic Rhythm of HMG-CoA Reductase Activity in Microsomes from Liver and Kidneys of Turkeys Sacrificed at Various Times of the Day.

Turkeys were fed a control (tallow) diet. Turkeys were kept in a room with lights on from 6:00 a.m. to 6:00 p.m. and lights off from 6:00 p.m. to 6:00 a.m. Turkeys were sacrificed at four hour intervals, commencing at 12:00 a.m., for 24 hours. Liver HMG-CoA reductase activity (●——●); Kidney HMG-CoA reductase activity (⊙——⊙).


nmoles MVA formed mg⁻¹ hr⁻¹
1.6
1.2
0.8
0.4
12a.m.
4a.m.
8a.m.
12p.m.
4p.m.
8p.m.
TIME OF DAY

4:00 p.m. and 4:00 a.m., respectively. Figure 10 also shows that the liver, and not the kidneys, is the organ that is most actively involved in cholesterol biosynthesis.

Studies have shown that hepatic mevalonate kinase in rats do not exhibit a diurnal cycle. However, data presented in Table V and Table VI shows that a diurnal cycle does exist for mevalonate kinase in microsomes from liver and kidneys of turkeys. It was of interest to determine if a relationship exist between hepatic mevalonate kinase and mevalonate kinase from kidneys. Figure 11 shows that when hepatic mevalonate kinase reach its maximum activity at 8:00 a.m., mevalonate kinase from kidneys simultaneously reach its lowest activity, and when mevalonate kinase from kidneys reach its maximum activity at 12:00 p.m., mevalonate kinase from liver is very high. In the liver and kidneys, mevalonate kinase reach its lowest activity at 4:00 p.m. and 8:00 a.m., respectively.

The profiles of protein concentration in microsomes from liver and kidneys of turkeys sacrificed at various times of the day are shown in Figure 12. The microsomal protein concentration in the extract from liver of turkeys sacrificed at 4:00 a.m. had the highest protein content. However, the extract from kidneys of turkeys sacrificed at 12:00 a.m. had the highest protein content.

Figure 13 is a plot of the following: HMG-CoA reductase activity, mevalonate kinase activity and protein concentration in microsomes from liver of turkeys sacrificed at various times of the day.

Figure 14 is a plot of the following: HMG-CoA reductase activity, mevalonate kinase activity and protein concentration in microsomes from kidneys of turkeys sacrificed at various times of the day.

Figure 11. Cyclic Rhythm of Mevalonate Kinase Activity in Microsomes from Liver and Kidneys of Turkeys Sacrificed at Various Times of the Day.

Turkeys were fed a control (tallow) diet. Turkeys were kept in a room with lights on from 6:00 a.m. to 6:00 p.m. and lights off from 6:00 p.m. to 6:00 a.m. Turkeys were sacrificed at four hour intervals, commencing at 12:00 a.m., for 24 hours. Liver mevalonate kinase activity (●——●); Kidneys mevalonate kinase activity (⊙——⊙).

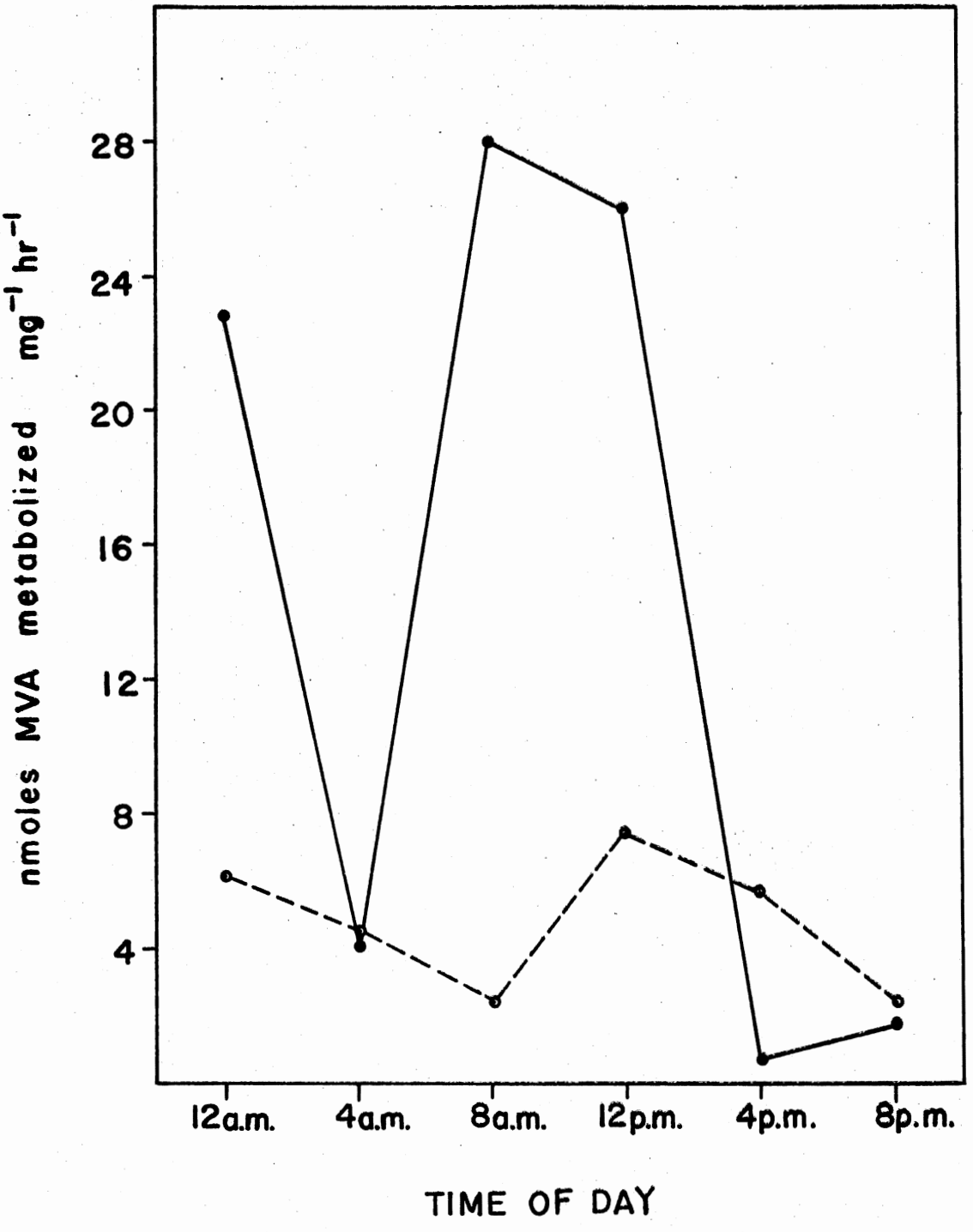

nmoles MVA metabolized mg⁻¹ hr⁻¹
28
24
20
16
12
8
4
12a.m.
4a.m.
8a.m.
12p.m.
4p.m.
8p.m.
TIME OF DAY

Figure 12. Effect of Time on Protein Concentration in the Microsomal Extract from Liver and Kidneys of Turkeys Sacrificed at Various Times of the Day.

Turkeys were fed a control (tallow) diet. Turkeys were kept in a room with lights on from 6:00 a.m. to 6:00 p.m. and lights off from 6:00 p.m. to 6:00 a.m. Turkeys were sacrificed at four hour intervals, commencing at 12:00 a.m., for 24 hours. Liver protein (●——●); Kidney protein (⊙——⊙).

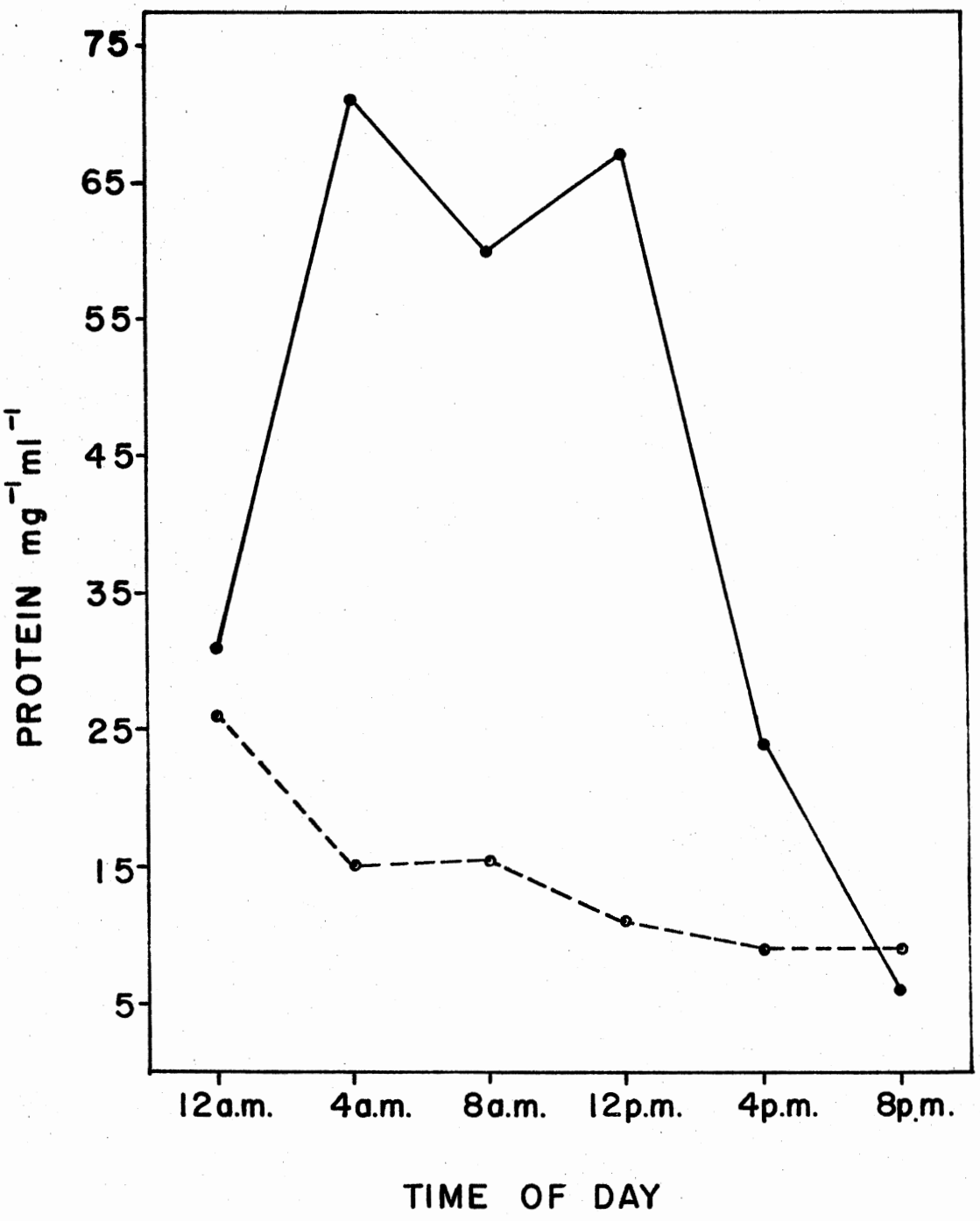

75
65
55
45
35
25
15
5
PROTEIN mg⁻¹ml⁻¹
12a.m.
4a.m.
8a.m.
12p.m.
4p.m.
8p.m.
TIME OF DAY

Figure 13. Cyclic Rhythm of HMG-CoA Reductase Activity, Mevalonate Kinase Activity, and Protein Concentration in Microsomes from Liver of Turkeys Sacrificed at Various Times of the Day.

Turkeys were fed a control (tallow) diet. Turkeys were kept in a room with lights on from 6:00 a.m. to 6:00 p.m. and lights off from 6:00 p.m. to 6:00 a.m. Turkeys were sacrificed at four hour intervals, commencing at 12:00 a.m., for 24 hours. HMG-CoA reductase activity (●——●); Mevalonate kinase activity (⊙-----⊙); Protein (○·····○).

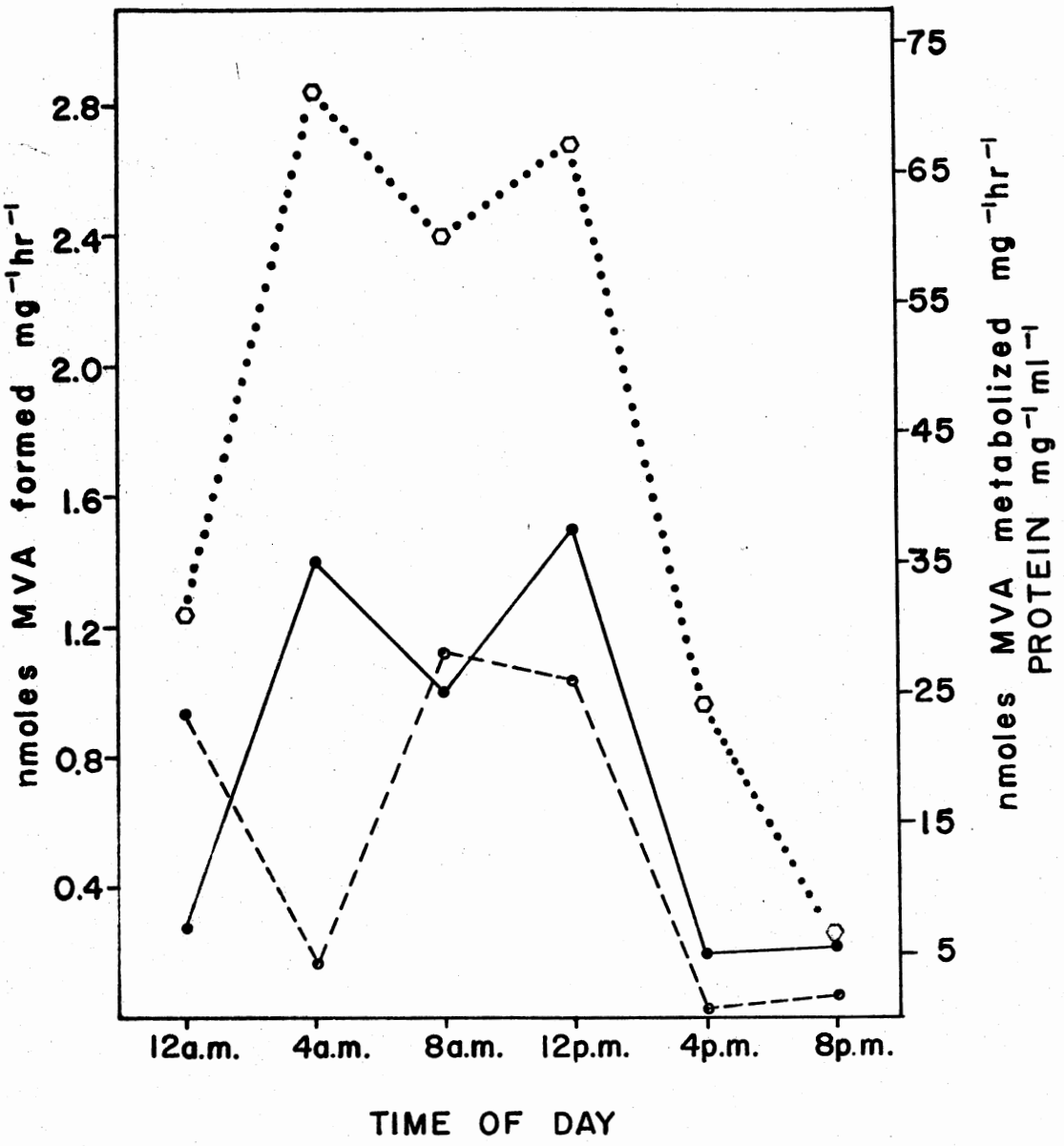
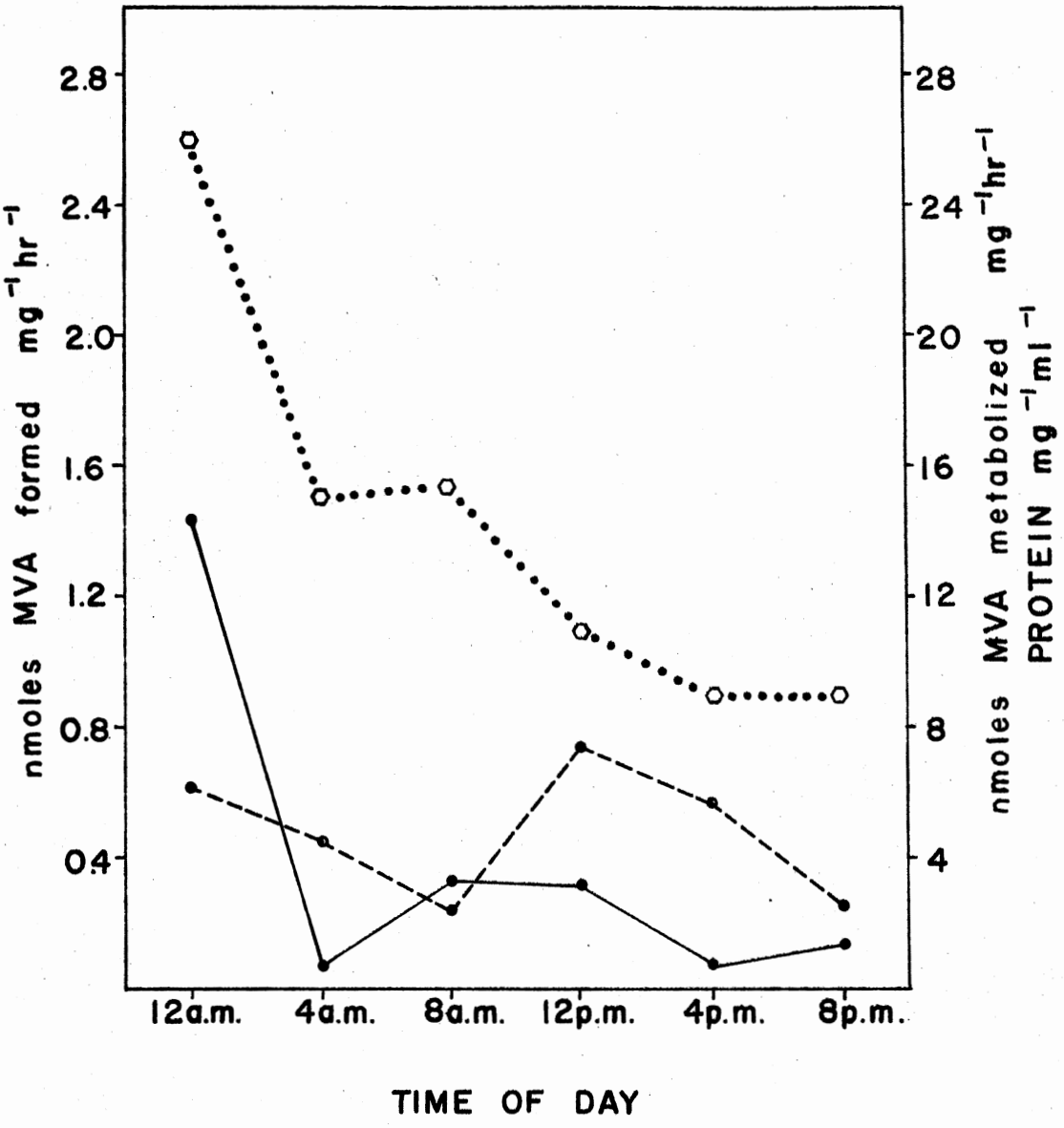

nmoles MVA formed mg⁻¹hr⁻¹
2.8
2.4
2.0
1.6
1.2
0.8
0.4
nmoles MVA metabolized mg⁻¹hr⁻¹
PROTEIN mg⁻¹ml⁻¹
75
65
55
45
35
25
15
5
12a.m.
4a.m.
8a.m.
12p.m.
4p.m.
8p.m.
TIME OF DAY

Figure 14. Cyclic Rhythm of HMG-CoA Reductase Activity, Mevalonate Kinase Activity and Protein Concentration in Microsomes from Kidneys of Turkeys Sacrificed at Various Times of the Day.

Turkeys were fed a control (tallow) diet. Turkeys were kept in a room with lights on from 6:00 a.m. to 6:00 p.m. and lights off from 6:00 p.m. to 6:00 a.m. Turkeys were sacrificed at four hour intervals, commencing at 12:00 a.m., for 24 hours. HMG-CoA reductase activity (●——●); Mevalonate kinase activity (⊙- - - -⊙); Protein (○·····○).


2.8
2.4
2.0
1.6
1.2
0.8
0.4
28
24
20
16
12
8
4
nmoles MVA formed mg⁻¹hr⁻¹
nmoles MVA metabolized mg⁻¹hr⁻¹
PROTEIN mg⁻¹ml⁻¹
12a.m.
4a.m.
8a.m.
12p.m.
4p.m.
8p.m.
TIME OF DAY

B. Fractionation of HMG-CoA Reductase and Mevalonate Kinase Activity by a Linear Sucrose Density Gradient

Turkeys were fed a control (tallow) diet and sacrificed at 12:00 a.m., 8:00 a.m. and 12:00 p.m. The liver and kidneys were removed and a 100,000 x g pellet was prepared and resuspended in buffer. Twenty-five milligrams of microsomal protein was layered on a 20% to 45% linear sucrose density gradient. Five fractions, each consisting of 6 mls, were collected. Each fraction was assayed for mevalonate kinase and HMG-CoA reductase activity according to the procedure under Materials and Methods.

1. Profiles of HMG-CoA Reductase and Mevalonate Kinase Activity in Liver and Kidneys from Turkeys Sacrificed at 8:00 a.m., 12:00 p.m., and 12:00 a.m.

The profiles of HMG-CoA reductase and mevalonate kinase activity in fraction 1 from the sucrose density gradient of liver and kidney microsomes from turkeys which were sacrificed at 8:00 a.m. are shown in Figure 15-A and Figure 15-B.

The profiles of HMG-CoA reductase and mevalonate kinase activity in fraction 1 from the sucrose density gradient of liver and kidney microsomes from turkeys which were sacrificed at 12:00 a.m. are shown in Figure 16-A and Figure 16-B.

The profiles of HMG-CoA reductase and mevalonate kinase activity in fraction 1 from the sucrose density gradient of liver and kidney

Figure 15. HMG-CoA Reductase and Mevalonate Kinase Activity Profile of a 20% → 45% Linear Sucrose Density Gradient from the Liver and Kidneys of Control and Cholesterol Fed Turkeys which were sacrificed at 8:00 a.m. HMG-CoA Reductase Activity (—●—); Mevalonate Kinase Activity (—⊙—).

(A) Liver of control fed turkeys which were sacrificed at 8:00 a.m.

(B) Kidneys of control fed turkeys which were sacrificed at 8:00 a.m.

(C) Liver of cholesterol fed turkeys which were sacrificed at 8:00 a.m.

(D) Kidneys of cholesterol fed turkeys which were sacrificed at 8:00 a.m.

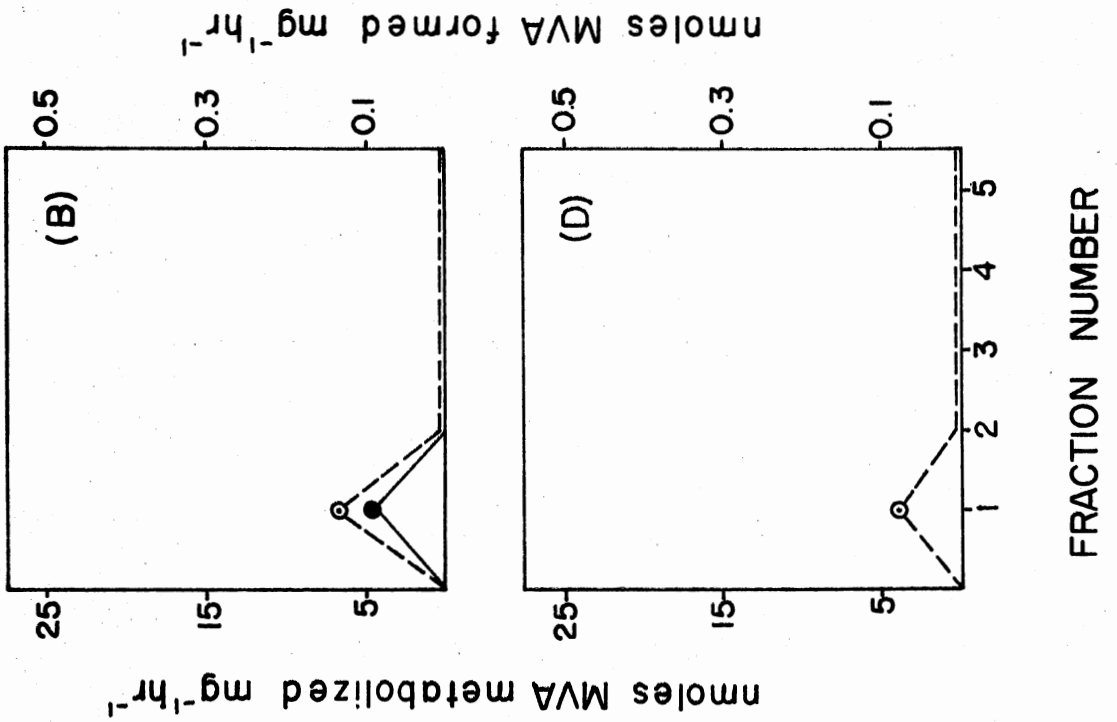

nmoles MVA metabolized mg⁻¹hr⁻¹
nmoles MVA formed mg⁻¹hr⁻¹
(A)
(B)
(C)
(D)
25
15
5
0.5
0.3
0.1
1
2
3
4
5
FRACTION NUMBER

Figure 16. HMG-CoA Reductase and Mevalonate Kinase Activity Profile of a 20% → 45% Linear Sucrose Density Gradient from the Liver and Kidneys of Control Fed Turkeys which were Sacrificed at 12:00 a.m. and 12:00 p.m. HMG-CoA Reductase Activity (—●—); Mevalonate Kinase Activity (—⊙—).

(A) Liver of control fed turkeys which were sacrificed at 12:00 a.m.

(B) Kidneys of control fed turkeys which were sacrificed at 12:00 a.m.

(C) Liver of control fed turkeys which were sacrificed at 12:00 p.m.

(D) Kidneys of control fed turkeys which were sacrificed at 12:00 p.m.

(A)
(B)
(C)
(D)
nmoles MVA metabolized mg⁻¹hr⁻¹
nmoles MVA formed mg⁻¹ hr⁻¹
FRACTION NUMBER
FRACTION NUMBER

microsomes from turkeys which were sacrificed at 12:00 p.m. are shown in Figure 16-C and Figure 16-D.

2. Activity of HMG-CoA Reductase in Liver and Kidneys from Turkeys Sacrificed at 8:00 a.m., 12:00 p.m., and 12:00 a.m.

HMG-CoA reductase specific activity in fraction 1 from the sucrose density gradient of liver microsomes from turkeys which were sacrificed at 12:00 a.m., increased to 0.61 nmoles MVA formed mg protein in assay^{-1} hr^{-1} (Table VIII). Fractions 2-5 from liver and fractions 1-5 from kidneys did not have measurable HMG-CoA reductase activity.

HMG-CoA reductase activity in fraction 1 from the sucrose density gradient of liver and kidney microsomes from turkeys which were sacrificed at 8:00 a.m., decreased to 0.15 and 0.09 nmoles MVA formed mg protein in assay^{-1} hr^{-1}, respectively (Table VIII). Fractions 2-5 did not have measurable HMG-CoA reductase activity.

HMG-CoA reductase specific activity in fraction 1 from the sucrose density gradient of liver microsomes from turkeys which were sacrificed at 12:00 p.m., increased to 2.00 nmoles MVA formed mg protein in assay^{-1} hr^{-1}, and HMG-CoA reductase activity in fraction 1 from the sucrose density gradient of kidney microsomes from turkeys decreased to 0.180 nmoles MVA formed mg protein in assay^{-1} hr^{-1} (Table VIII). Fractions 2-5 did not have measurable HMG-CoA reductase activity.

TABLE VIII

SUCROSE DENSITY GRADIENT SEPARATION OF HMG-CoA REDUCTASE AND MVA KINASE ACTIVITY FROM LIVER AND KIDNEYS OF CONTROL FED TURKEYS

Time of Day	Turkey Liver		Turkey Kidneys	
	HMG-CoA[1] Reductase	Mevalonate[2] Kinase	HMG-CoA[1] Reductase	Mevalonate[2] Kinase
12:00 a.m.				
Microsomal Pellet	0.28	22.80	1.43	6.10
Sucrose Gradient				
fraction 1	0.61	6.53	—	1.31
fractions 2-5	—	—	—	—
8:00 a.m.				
Microsomal Pellet	1.00	28.00	0.32	2.40
Sucrose Gradient				
fraction 1	0.15	25.00	0.09	6.40
fractions 2-5	—	—	—	—
12:00 p.m.				
Microsomal Pellet	1.50	26.00	0.31	7.40
Sucrose Gradient				
fraction 1	2.00	31.70	0.18	8.00
fractions 2-5	—	—	—	—

[1]HMG-CoA Reductase - nmoles of mevalonate formed, mg protein in assay^{-1} hr^{-1}

[2]Mevalonate Kinase - nmoles of mevalonate metabolized, mg protein in assay^{-1} hr^{-1}

[3]A blank value (—) indicates that the enzyme activity was undetectable according to assays

3. Activity of Mevalonate Kinase in Liver and Kidneys from Turkeys Sacrificed at 8:00 a.m., 12:00 p.m. and 12:00 a.m.

MVA kinase specific activities in fraction 1 from the sucrose density gradient of liver and kidney microsomes from turkeys which were sacrificed at 12:00 a.m., decreased to 6.53 and 1.31 nmoles MVA metabolized mg protein in assay^{-1} hr^{-1}, respectively (Table VIII). Fractions 2-5 did not have measurable MVA kinase activities.

Fraction 1 from the sucrose density gradient of liver microsomes from turkeys which were sacrificed at 8:00 a.m., showed that mevalonate kinase activity decreased to 25.00 nmoles MVA metabolized mg protein in assay^{-1} hr^{-1} (Table VIII). Fractions 2-5 of the liver and fractions 1-5 of the kidneys did not have measurable MVA kinase activities.

Mevalonate kinase activities in fraction 1 from the sucrose density gradient of kidney microsomes from turkeys which were sacrificed at 12:00 p.m., increased to 31.70 and 8.00 nmoles MVA metabolized mg protein in assay^{-1} hr^{-1}, respectively (Table VIII). Fractions 2-5 did not have measurable MVA kinase activities.

C. Post-Hatched Developmental Studies of HMG-CoA Reductase and Mevalonate Activating Enzymes Activities in Liver From Turkey Poults

Turkey poults were fed a normal diet and sacrificed at 8:00 a.m. at the age of 5 days, 11 days, 18 days and 25 days. The liver was removed and a 100,000 x g pellet was prepared and assayed for HMG-CoA

reductase activity, mevalonate kinase activity and protein concentration according to procedure under Materials and Methods.

1. Profile of Mevalonate Activating Enzymes in Liver From Turkey Poults

Only MVA kinase activity was observed in the 100,000 x g pellet from the liver of poults sacrificed at the age of five days. Figure 17 shows the Dowex-1 formate column elution profiles of the products from mevalonate metabolism by liver microsomes.

Low levels of MVA kinase and MVAP kinase activities were observed in microsomes from the liver of poults sacrificed at the age of 11 days. The Dowex-1 formate column elution profile of the products from mevalonate metabolism is shown in Figure 18.

High levels of MVA kinase, MVAP kinase and MVAPP decarboxylase activities were observed in microsomes from the liver of poults sacrificed at the age of 18 days. Figure 19 shows the Dowex-1 formate column elution profiles of the products from mevalonate metabolism by liver microsomes.

High levels of MVA kinase, MVAP kinase and MVAPP decarboxylase activities were observed in microsomes from the liver of poults sacrificed at the age of 25 days. The Dowex-1 formate column elution profile of the products from mevalonate metabolism by liver microsomes is shown in Figure 20.

2. Activity of the Mevalonate Activating Enzymes in Liver From Poults

MVA kinase specific activity in the microsomes of liver from

Figure 17. Metabolism of ^{14}C-Mevalonic Acid by Liver Microsomes From Five Days Old Poults.

Dowex-1 formate elution pattern: mevalonate kinase assay of the microsomes from the liver of poults sacrificed at the age of five days (8:00 a.m.). Turkeys were fed a control (tallow) diet.

Elution profile following assay for mevalonate kinase in liver microsomes under normal assay conditions. Elution of Dowex-1 formate column:

A. 25 ml water

B. 25 ml 2 N formic acid

C. 50 ml 4 N formic acid

D. 50 ml 4 N formic acid + 0.4 N ammonium formate

E. 50 ml 4 N formic acid + 0.8 N ammonium formate

Collect approximately 3.0-3.5 ml fractions.

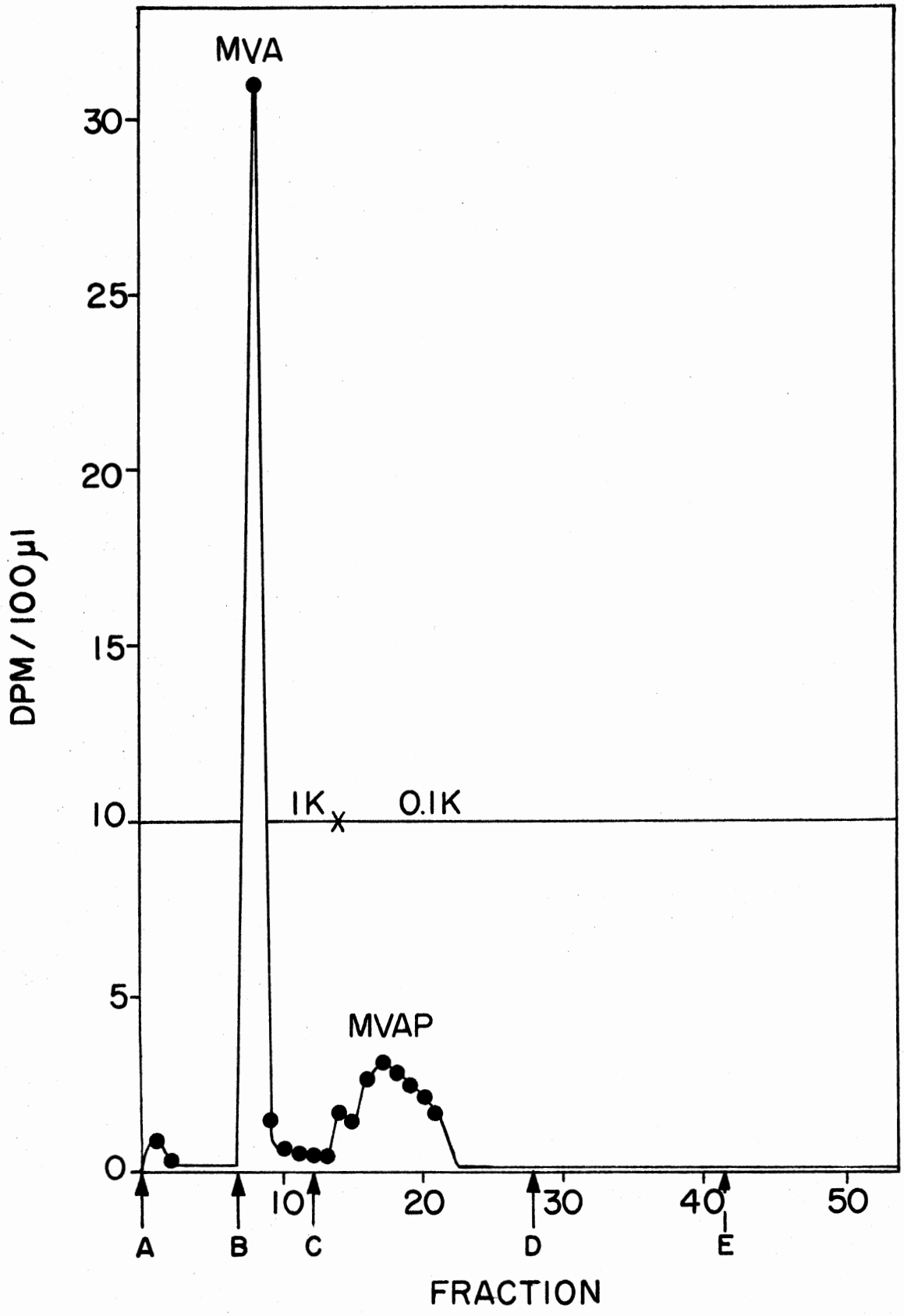

MVA
30
25
20
15
10
5
0
DPM / 100µl
1K
0.1K
MVAP
10
20
30
40
50
A
B
C
D
E
FRACTION

Figure 18. Metabolism of ^{14}C-Mevalonic Acid by Liver Microsomes From Eleven Days Old Poults.

Dowex-1 formate elution pattern: mevalonate kinase assay of the microsomes from the liver of poults sacrificed at the age of 11 days (8:00 a.m.). Turkeys were fed a control (tallow) diet.

Elution profile following assay for mevalonate kinase in liver microsomes under normal assay conditions. Elution of Dowex-1 formate column:

A. 25 ml water

B. 25 ml 2 N formic acid

C. 50 ml 4 N formic acid

D. 50 ml 4 N formic acid + 0.4 N ammonium formate

E. 50 ml 4 N formic acid + 0.8 N ammonium formate

Collect approximately 3.0-3.5 ml fractions.

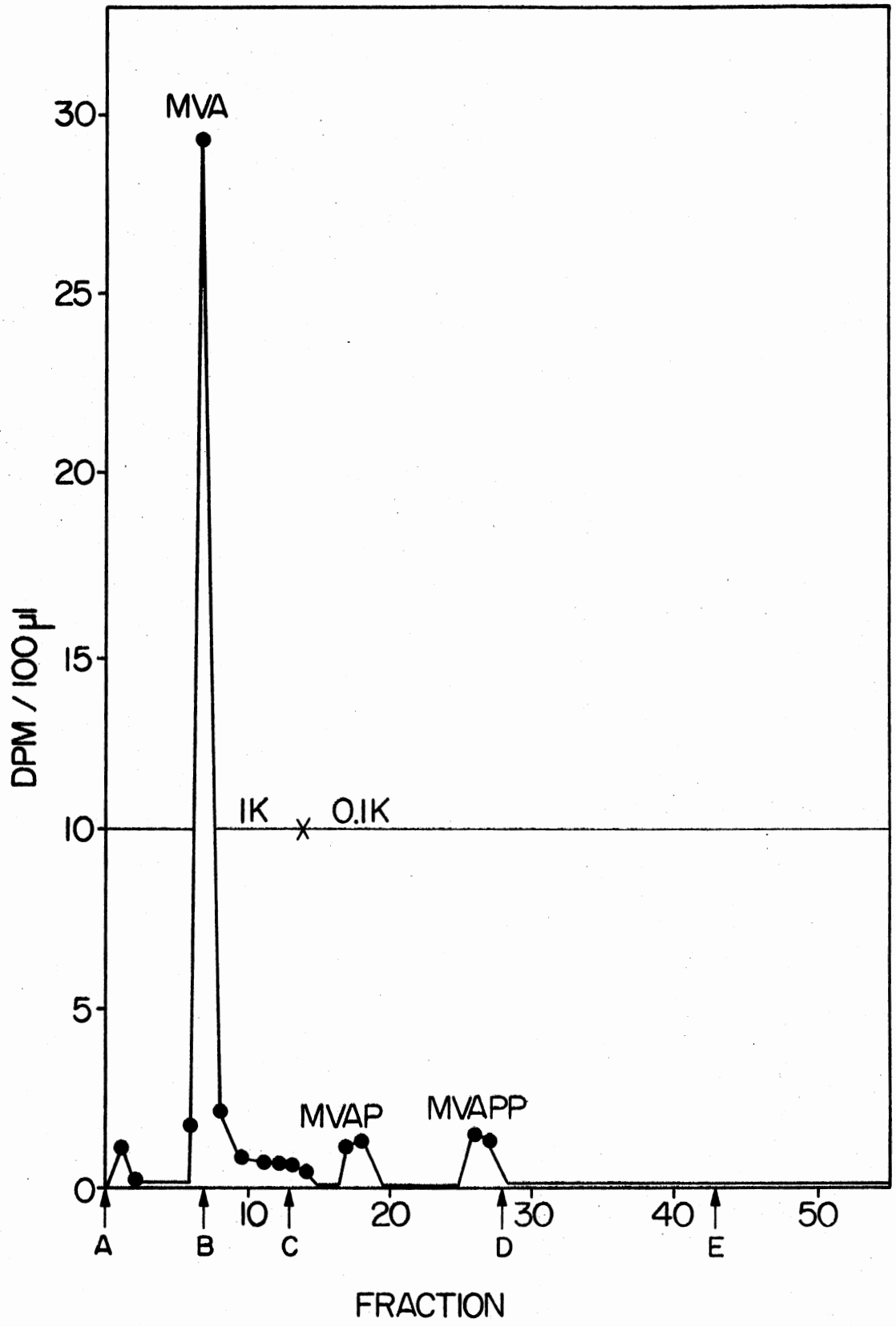

MVA
30
25
20
15
10
5
0
DPM / 100 µl
1K
0.1K
MVAP
MVAPP
10
20
30
40
50
A
B
C
D
E
FRACTION

Figure 19. Metabolism of ^{14}C-Mevalonic Acid by Liver Microsomes From Eighteen Days Old Poults.

Dowex-1 formate elution pattern: mevalonate kinase assay of the microsomes from the liver of poults sacrificed at the age of 18 days (8:00 a.m.). Turkeys were fed a control (tallow) diet.

Elution profile following assay for mevalonate kinase in liver microsomes under normal assay conditions. Elution of Dowex-1 formate column:

A. 25 ml water

B. 25 ml 2 N formic acid

C. 50 ml 4 N formic acid

D. 50 ml 4 N formic acid + 0.4 N ammonium formate

E. 50 ml 4 N formic acid + 0.8 N ammonium formate

Collect approximately 3.0-3.5 ml fractions.

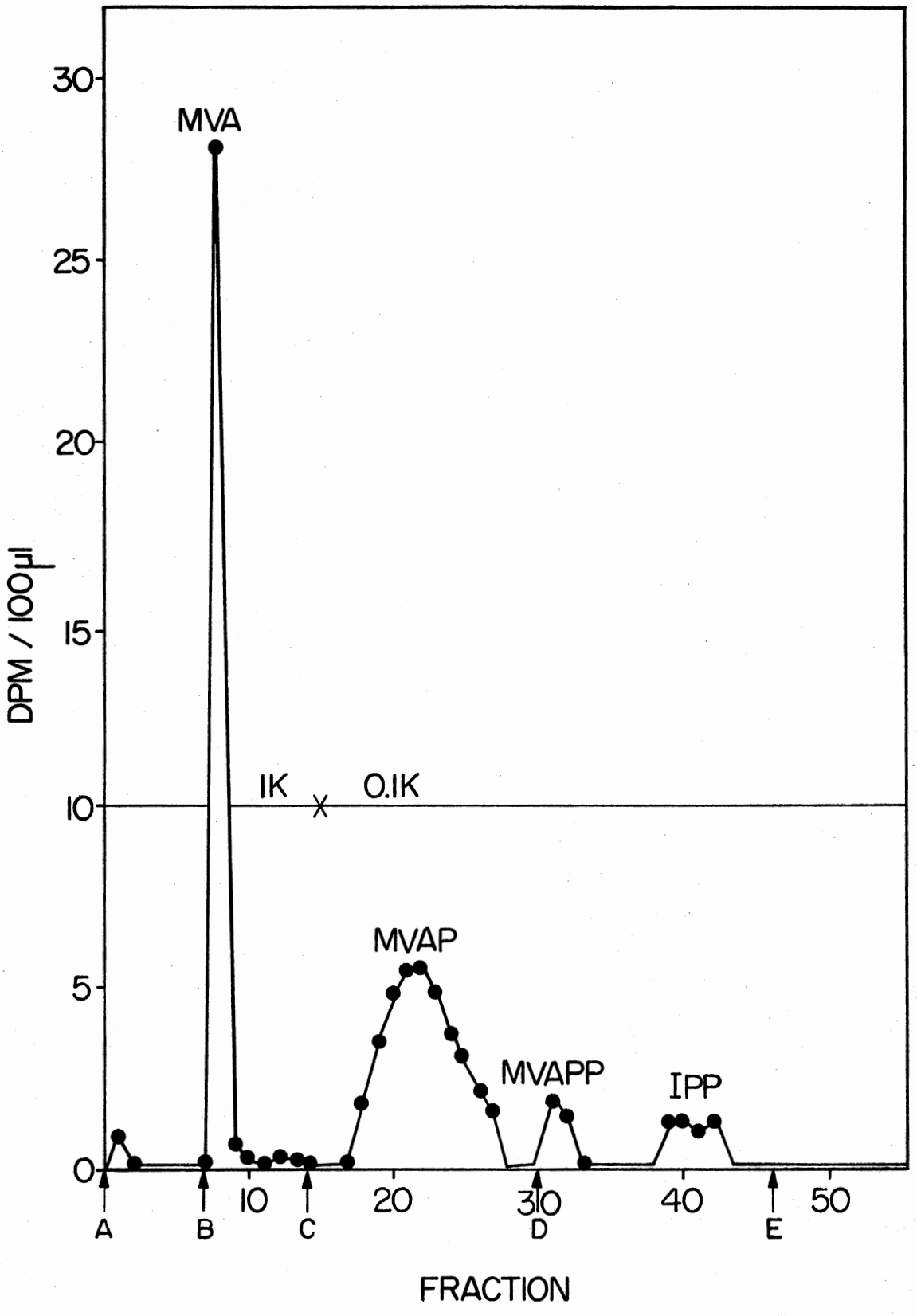

30
25
20
15
10
5
0
DPM / 100µl
MVA
1K
0.1K
MVAP
MVAPP
IPP
A
B
C
D
E
10
20
30
40
50
FRACTION

Figure 20. Metabolism of ^{14}C-Mevalonic Acid by Liver Microsomes From Twenty-five Days Old Poults.

Dowex-1 formate elution pattern: mevalonate kinase assay of the microsomes from the liver of poults sacrificed at the age of 25 days (8:00 a.m.). Turkeys were fed a control (tallow) diet.

Elution profile following assay for mevalonate kinase in liver microsomes under normal assay conditions. Elution of Dowex-1 formate column:

A. 25 ml water

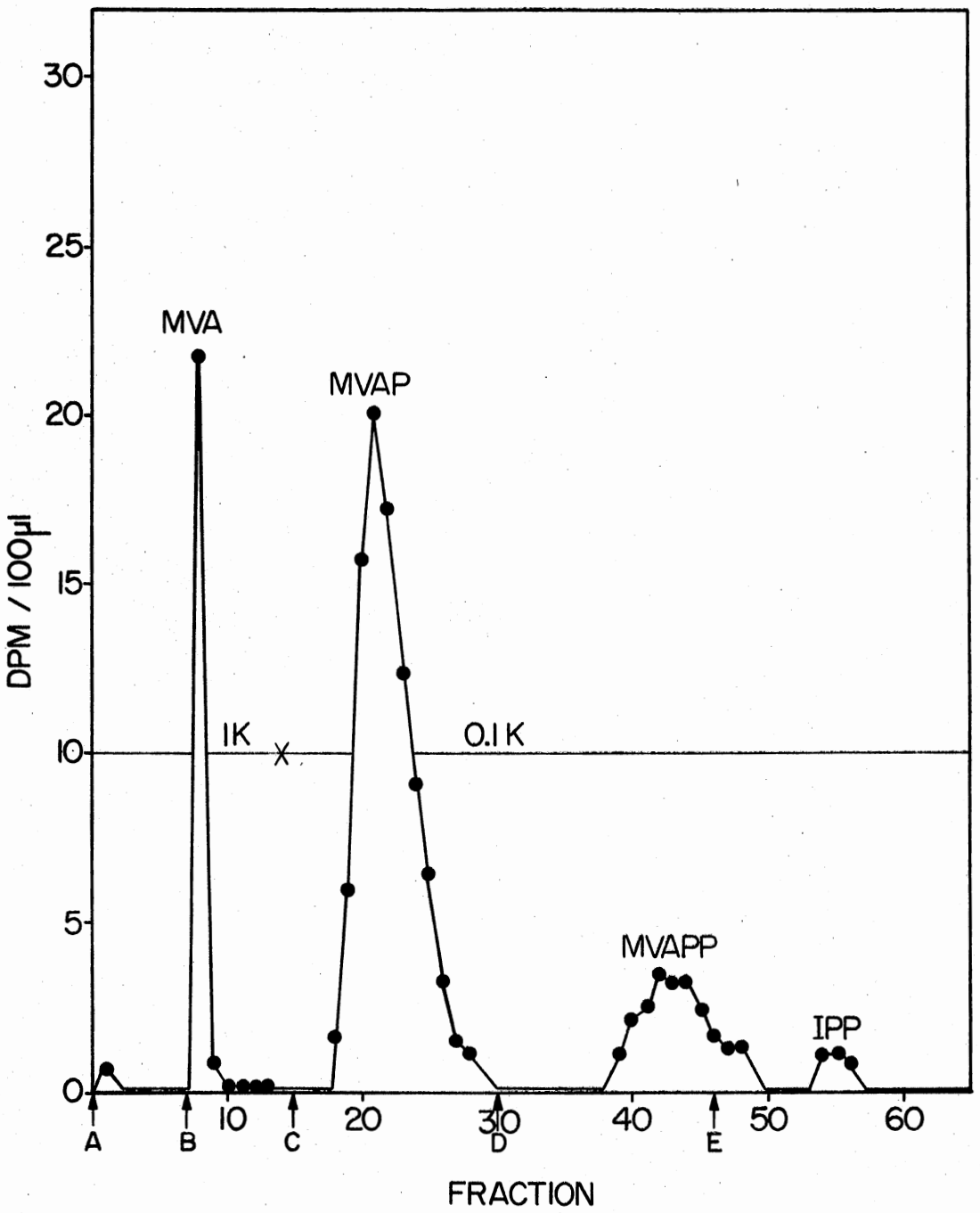
B. 25 ml 2 N formic acid

C. 50 ml 4 N formic acid

D. 50 ml 4 N formic acid + 0.4 N ammonium formate

E. 50 ml 4 N formic acid + 0.8 N ammonium formate

Collect approximately 3.0-3.5 ml fractions.


30
25
20
15
10
5
0
DPM / 100µl
MVA
MVAP
MVAPP
IPP
1K
0.1K
A
B
C
D
E
10
20
30
40
50
60
FRACTION

poults sacrificed at the age of five days, was 3.4 nmoles MVA metabolized mg protein in assay^{-1} hr^{-1} (Table IX).

MVA kinase and MVAP kinase activities in the microsomes from liver of poults sacrificed at the age of 11 days, were 1.12 and 0.520 nmoles MVA metabolized mg protein in assay^{-1} hr^{-1}, respectively (Table IX).

In the microsomes from liver of poults sacrificed at the age 18 days, MVA kinase, MVAP kinase and MVAPP decarboxylase activities were 8.20, 1.50 and 0.930 nmoles MVA metabolized mg protein in assay^{-1} hr^{-1}, respectively (Table IX).

In the microsomes from liver of poults sacrificed at the age of 25 days, MVA kinase, MVAP kinase and MVAPP decarboxylase activities were 21.5, 4.7 and 0.560 nmoles MVA metabolized mg protein in assay^{-1} hr^{-1}, respectively (Table IX).

3. Activity of HMG-CoA Reductase in Liver From Poults

HMG-CoA reductase specific activity in microsomes from the liver of poults sacrificed at the age of five days was 0.031 nmoles MVA formed mg protein in assay^{-1} hr^{-1}; HMG-CoA reductase activity in microsomes from liver of poults sacrificed at the age of 11 days was 2.04 nmoles MVA formed mg protein in assay^{-1} hr^{-1}; HMG-CoA reductase activity in microsomes from liver of poults sacrificed at age of 18 days was 2.92 nmoles MVA formed mg protein in assay^{-1}hr^{-1}; and HMG-CoA reductase activity in liver of poults sacrificed at the age of 25 days was 2.70 nmoles MVA formed mg protein in assay^{-1} hr^{-1} (Table IX).

TABLE IX

EFFECT OF AGE ON HMG-CoA REDUCTASE, MEVALONATE KINASE, PHOSPHOMEVALONATE KINASE AND PYROPHOSPHOMEVALONATE DECARBOXYLASE ACTIVITY FROM LIVER OF CONTROL FED POULTS

Age	100,000 X g Pellet (Microsomes)			
	HMG-CoA Reductase[1]	Mevalonate Kinase[2]	Phosphomevalonate[2] Kinase	Pyrophosphomevalonate[2] Decarboxylase
5 days	0.03	3.40	—	—
11 days	2.04	1.12	0.52	—
18 days	2.92	8.20	1.50	0.93
25 days	2.70	21.50	4.70	0.56

[1]HMG-CoA Reductase - nmoles mevalonate formed, mg protein in assay^{-1} hr^{-1}

[2]Mevalonate Kinase, Phosphomevalonate Kinase and Pyrophosphomevalonate Decarboxylase - nmoles mevalonate metabolized, mg protein in assay^{-1} hr^{-1}

4. Protein Concentration in Liver From Poults

The microsomal protein concentration in the extract from liver of poults sacrificed at the age of 5, 11, 18 and 25 days were 4.60, 13.6, 13.0, and 23.8 mg protein ml^{-1}, respectively (Table X).

5. Profile of Mevalonate Kinase Activity, HMG-CoA Reductase Activity and Protein Concentration in Liver From Poults

Initial studies on the post-hatched developmental pattern of HMG-CoA reductase and mevalonate kinase from liver of poults show that HMG-CoA reaches its maximum activity in poults that are 18 days old, and its lowest activity in poults that are 5 days old (Figure 21). However, mevalonate kinase reaches its maximum activity in poults that are 25 days old and its lowest activity in poults that are 11 days old.

Further studies are needed to determine the age of the poults when these enzyme activities become stabilized.

Initial studies were begun on the post-hatched developmental pattern of hepatic HMG-CoA reductase and mevalonate kinase. HMG-CoA reductase and mevalonate kinase activities were followed in poults from day 5 to day 25 after poults were hatched. HMG-CoA reductase reaches its maximum activity in poults that are 18 days old, and its lowest activity in poults that are five days old (Figure 21). However, mevalonate kinase reaches its maximum activity in poults that are 25 days old and its lowest activity in poults that are 11 days old.

TABLE X

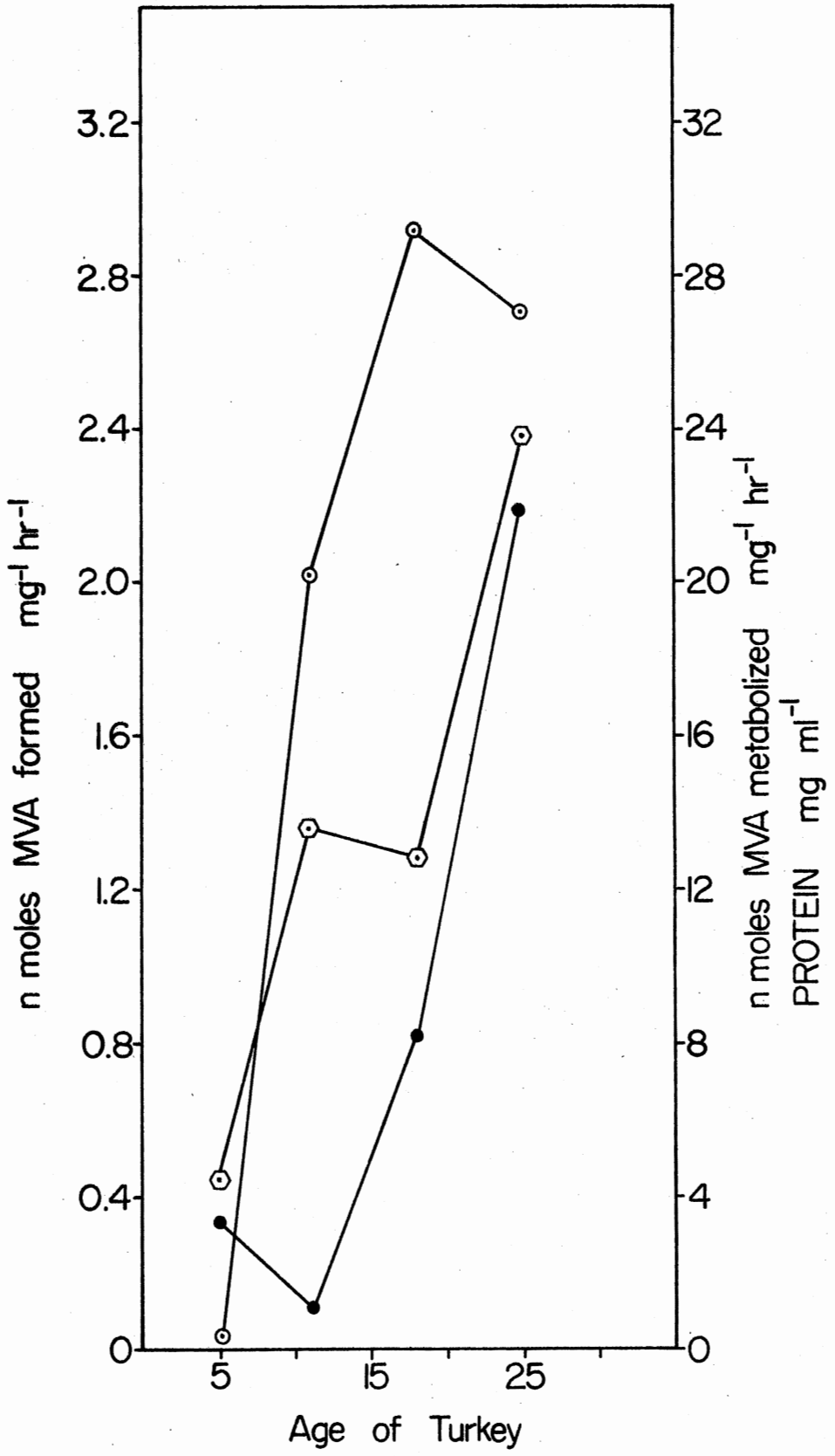
EFFECT OF AGE ON PROTEIN CONCENTRATION IN MICROSOMES FROM LIVER OF CONTROL FED POULTS

Age	Poult Liver Protein Concentration[1]
5 days	4.60
11 days	13.60
18 days	13.00
25 days	23.80

[1]Protein Concentration - mg protein in the microsomal extract•ml^{-1}

Figure 21. Mevalonate Kinase Activity, HMG-CoA Reductase Activity and Protein Concentration in Microsomes from Liver of Poults Sacrificed at the Age of 5, 11, 18 and 25 Days.

Turkeys were fed a control (tallow) diet and sacrificed at 8:00 a.m. HMG-CoA Reductase Activity (⊙———⊙); Mevalonate Kinase Activity (●———●); Protein Concentration (⊙———⊙).


3.2
2.8
2.4
2.0
1.6
1.2
0.8
0.4
0
32
28
24
20
16
12
8
4
0
5
15
25
Age of Turkey
n moles MVA formed mg⁻¹ hr⁻¹
n moles MVA metabolized mg⁻¹ hr⁻¹
PROTEIN mg ml⁻¹

D. HMG-CoA Reductase and Mevalonate Kinase Activity in Liver and Kidneys From Turkeys Fed Cholesterol

Turkeys were fed a 1% cholesterol diet and sacrificed at 8:00 a.m. The liver and kidneys were removed and a 100,000 X g pellet was prepared. HMG-CoA reductase and mevalonate kinase activities were assayed according to the procedures under Materials and Methods. Twenty-five milligrams of the resuspended pellet was layered on a linear sucrose density gradient and five fractions, each consisting of 6 mls, were collected and assayed for HMG-CoA reductase and mevalonate kinase activities.

1. Profile of the Mevalonate Activating Enzymes in Liver and Kidneys From Turkeys Fed Cholesterol

Low levels of MVA kinase and MVAP kinase activities were observed in the microsomes and fraction 1 from the sucrose density gradient of liver microsomes from turkeys fed cholesterol. The Dowex-1 formate column elution profiles of the products from mevalonate metabolism by liver microsomes are shown in Figure 22 and Figure 23, respectively.

Only MVA kinase activity was observed in the microsomes and fraction 1 from the sucrose density gradient of kidney microsomes from turkeys fed cholesterol. The Dowex-1 formate column elution profiles of the products from mevalonate metabolism by kidney microsomes are shown in Figure 24 and Figure 25, respectively.

Figure 22. Metabolism of ^{14}C-Mevalonate Acid by Liver Microsomes From Control and Cholesterol Fed Turkeys.

Dowex-1 formate elution pattern: Mevalonate kinase assay of the microsomes from the liver of turkeys fed a control (tallow) and a cholesterol diet. Turkeys were sacrificed at 8:00 a.m. Control diet (●———⊙); Cholesterol diet (●———●).

Elution profile following assay for mevalonate kinase in liver microsomes under normal assay conditions. Elution of Dowex-1 formate column:

A. 25 ml water

B. 25 ml 2 N formic acid

C. 50 ml 4 N formic acid

D. 50 ml 4 N formic acid + 0.4 N ammonium formate

D. 50 ml 4 N formic acid + 0.8 N ammonium formate

Collect approximately 3.0-3.5 ml fractions.

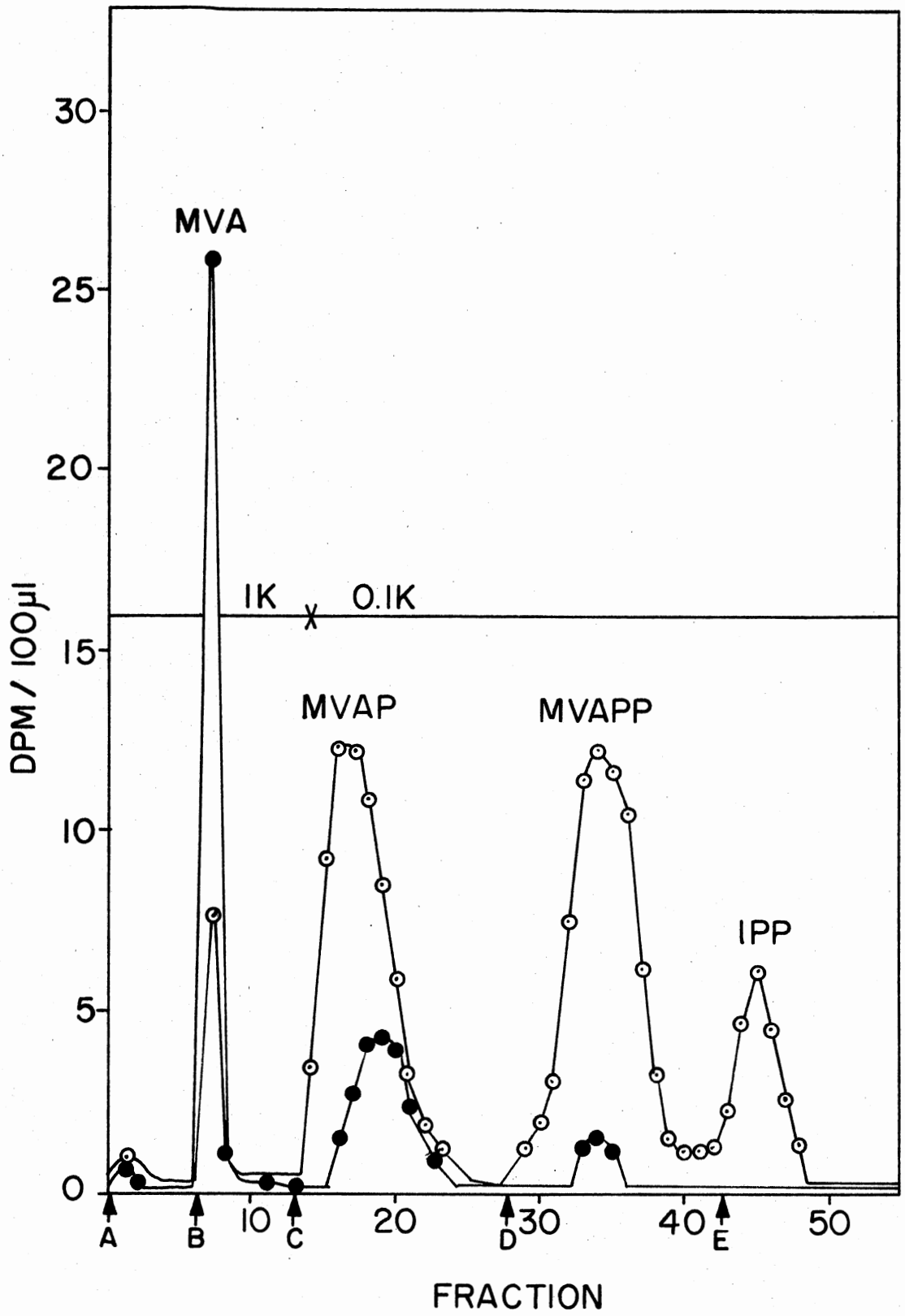

30
25
20
15
10
5
0
DPM / 100µl
MVA
1K
0.1K
MVAP
MVAPP
IPP
A
B
C
D
E
10
20
30
40
50
FRACTION

Figure 23. Metabolism of ^{14}C-Mevalonic Acid by Fraction One From the Sucrose Density Gradient of Liver Microsomes From Turkeys Fed a Control and Cholesterol Diet.

Dowex-1 formate elution pattern: mevalonate kinase assay of fraction one from a 20% → 45% linear sucrose density gradient from turkeys fed a control (tallow) and a cholesterol diet. Turkeys were sacrificed at 8:00 a.m. Control diet (⊙——⊙); Cholesterol diet (●——●).

Elution profile following assay for mevalonate kinase in fraction one from 20% → 45% linear sucrose density gradient under normal assay conditions. Elution of Dowex-1 formate column.

A. 25 ml water

B. 25 ml 2 N formic acid

C. 50 ml 4 N formic acid + 0.4 N ammonium formate

D. 50 ml 4 N formic acid + 0.8 N ammonium formate

E. 50 ml 4 N formic acid + 0.8 N ammonium formate

Collect approximately 3.0-3.5 ml fractions.

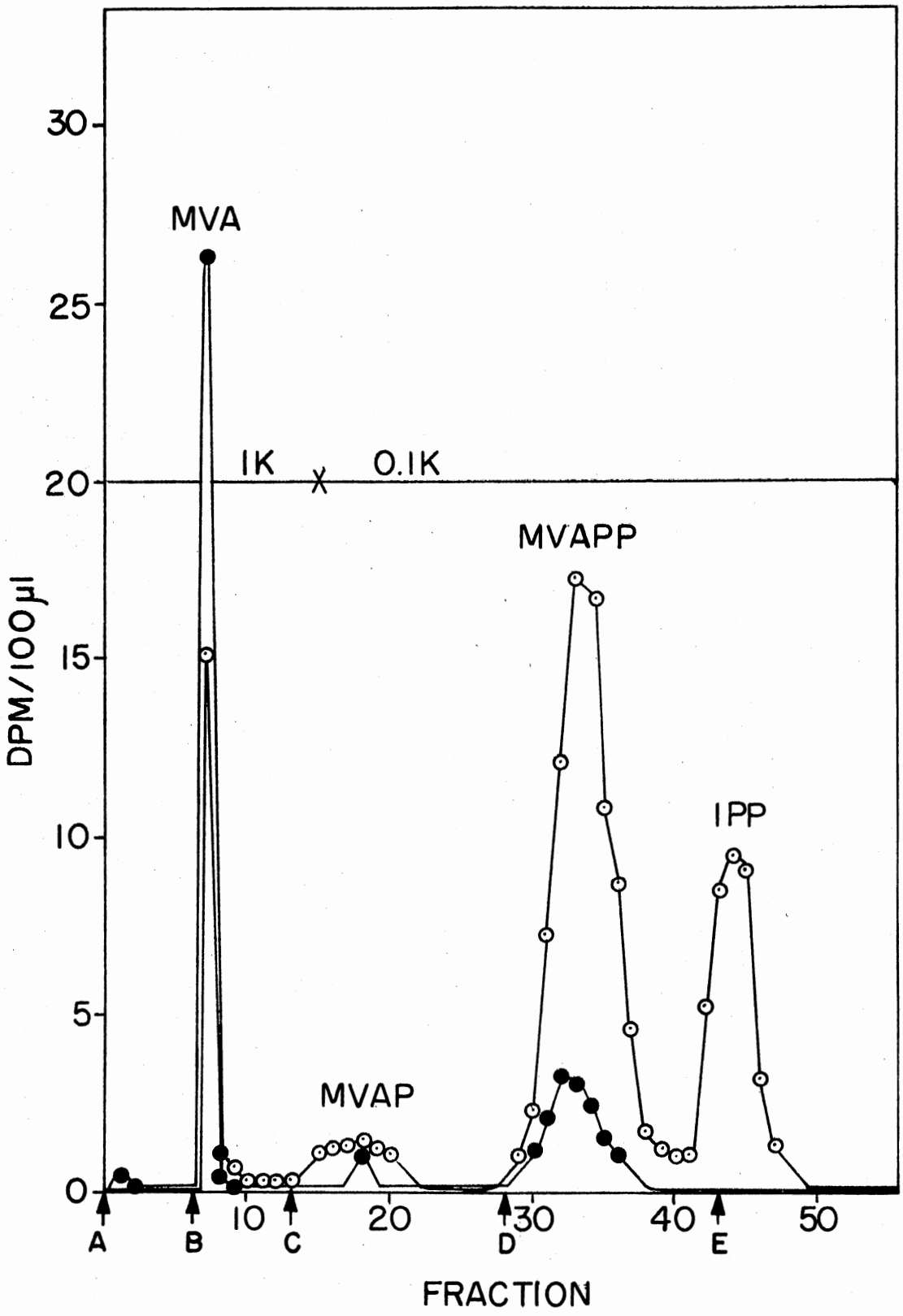

30
25
20
15
10
5
0
DPM/100µl
MVA
1K
0.1K
MVAPP
IPP
MVAP
A
B
C
D
E
10
20
30
40
50
FRACTION

Figure 24. Metabolism of ^{14}C-Mevalonic Acid by Kidney Microsomes From Control and Cholesterol Fed Turkeys.

Dowex-1 formate elution pattern: mevalonate kinase assay of microsomes from the kidneys of turkeys fed a control (tallow) and a cholesterol diet. Turkeys were sacrificed at 8:00 a.m. Control diet (○——○); Cholesterol diet (●——●).

Elution profile following assay for mevalonate kinase in kidney microsomes under normal assay conditions. Elution of Dowex-1 formate column:

A. 25 ml water

B. 25 ml 2 N formic acid

C. 50 ml 4 N formic acid

D. 50 ml 4 N formic acid + 0.4 N ammonium formate

E. 50 ml 4 N formic acid + 0.8 N ammonium formate

Collect approximately 3.0-3.5 ml fractions.

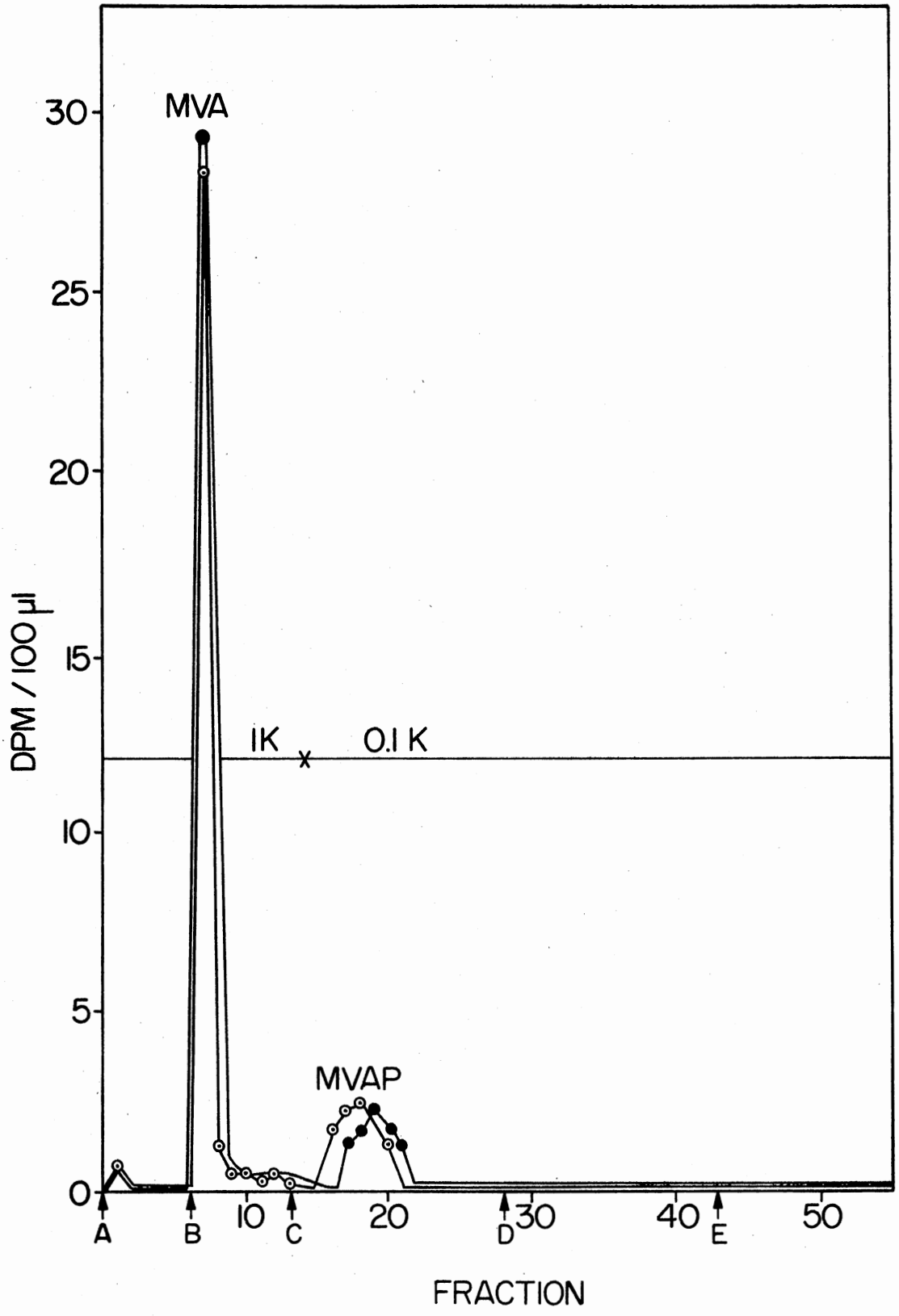

MVA
MVAP
1K
0.1 K
DPM / 100 µl
FRACTION
0
5
10
15
20
25
30
A
B
C
D
E
10
20
30
40
50

Figure 25. Metabolism of ^{14}C-Mevalonic Acid by Fraction One From the Sucrose Density Gradient of Kidney Microsomes From Turkeys Fed a Control and Cholesterol Diet.

Dowex-1 formate elution pattern: mevalonate kinase assay of fraction one from a 20% → 45% linear sucrose density gradient from turkeys fed a control (tallow) and a cholesterol diet. Turkeys were sacrificed at 8:00 a.m. Control diet (⊙———⊙); Cholesterol diet (●———●).

Elution profile following assay for mevalonate kinase in fraction one from 20% → 45% linear sucrose density gradient under normal assay conditions. Elution of Dowex-1 formate column:

A. 25 ml water

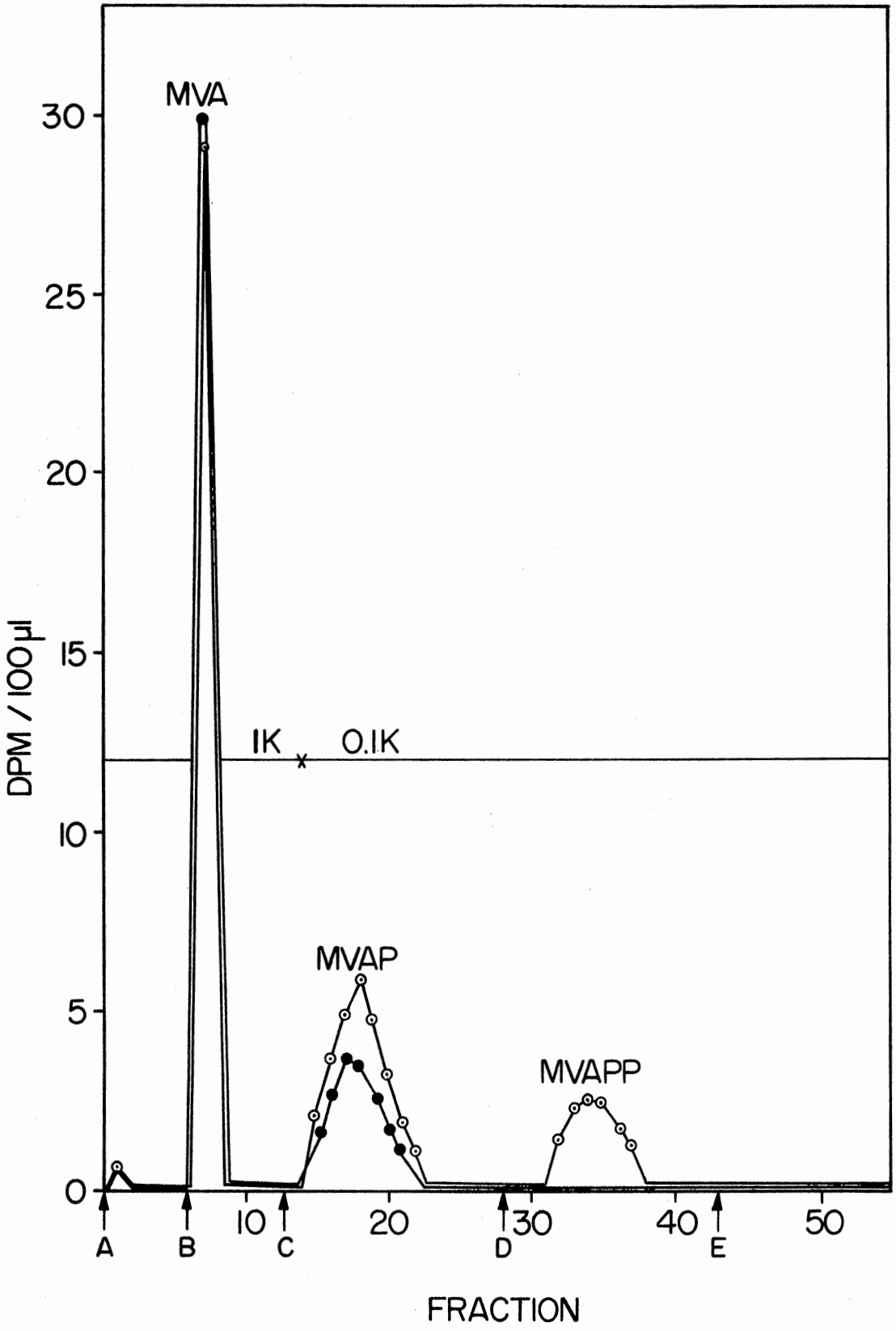
B. 25 ml 2 N formic acid

C. 50 ml 4 N formic acid

D. 50 ml 4 N formic acid + 0.4 N ammonium formate

E. 50 ml 4 N formic acid + 0.8 N ammonium formate

Collect approximately 3.0-3.5 ml fractions.


MVA
MVAP
MVAPP
1K
0.1K
DPM / 100µl
FRACTION
A
B
C
D
E
0
5
10
15
20
25
30
10
20
30
40
50

2. Activity of the Mevalonate Activating Enzymes in Liver and Kidneys From Turkeys Fed Cholesterol

MVA kinase and MVAP kinase activities in microsomes from liver of turkeys fed cholesterol, decreased six-fold to 4.80 and 0.80 nmoles MVA metabolized mg protein in assay^{-1} hr^{-1}, respectively, compared to the same enzyme activities in microsomes from liver of control-fed turkeys. MVA kinase and MVAP kinase activities in fraction 1 from the sucrose density gradient of liver microsomes from turkeys fed cholesterol, decreased seven-fold to 3.52 and 3.26 nmoles MVA metabolized mg protein in assay^{-1} hr^{-1}, respectively (Table XI). Fractions 2-5 did not have measurable enzyme activities.

MVA kinase and MVAP kinase in microsomes and fraction 1 from the sucrose density gradient of kidney microsomes from turkeys fed cholesterol decreased slightly to a specific activity of 2.02 and 3.72 nmoles MVA metabolized mg protein in assay^{-1} hr^{-1}, respectively (Table XII). Fractions 2-5 did not have measurable enzyme activities.

3. Activity of HMG-CoA Reductase in Liver and Kidneys From Turkeys Fed Cholesterol

HMG-CoA reductase activity was completely suppressed in microsomes from liver and kidneys of turkeys by dietary cholesterol (Table XI and Table XII).

TABLE XI

HMG-CoA REDUCTASE, MEVALONATE KINASE, PHOSPHOMEVALONATE KINASE AND PYROPHOSPHOMEVALONATE DECARBOXYLASE ACTIVITY FROM LIVER OF TURKEYS FED A CONTROL AND CHOLESTEROL DIET

STEP	HMG-CoA Reductase[1]	Mevalonate Kinase[2]	Phosphomevalonate Kinase[2]	Pyrophosphomevalonate Decarboxylase[2]
NORMAL DIET				
100,000 X g Pellet	1.00	28.00	12.50	2.70
Sucrose Gradient				
fraction 1	0.15	25.00	24.00	7.10
fractions 2-5	—	—	—	—
CHOLESTEROL DIET				
100,000 X g Pellet	—	4.80	0.80	—
Sucrose Gradient				
fraction 1	—	3.52	3.26	—
fractions 2-5	—	—	—	—

[1]HMG-CoA Reductase - nmoles mevalonate formed, mg protein in an assay^{-1} hr^{-1}

[2]Mevalonate Kinase, Phosphomevalonate Kinase and Pyrophosphomevalonate Decarboxylase - nmoles mevalonate metabolized mg protein in an assay^{-1} hr^{-1}

[3]A blank value (—) indicates that the enzyme activity was undetectable according to the assays.

TABLE XII

HMG-CoA REDUCTASE, MEVALONATE KINASE, PHOSPHOMEVALONATE KINASE AND PYROPHOSPHOMEVALONATE DECARBOXYLASE ACTIVITY FROM KIDNEYS OF TURKEYS FED A CONTROL AND CHOLESTEROL DIET

STEP	HMG-CoA Reductase[1]	Mevalonate Kinase[2]	Phosphomevalonate Kinase[2]	Pyrophosphomevalonate Decarboxylase[2]
NORMAL DIET				
100,000 X g Pellet	0.33	2.40	—	—
Sucrose Gradient				
fraction 1	0.09	6.42	2.40	—
fractions 2-5	—	—	—	—
CHOLESTEROL DIET				
100,000 X g Pellet	—	2.02	—	—
Sucrose Gradient				
fraction 1	—	3.72	—	—
fractions 2-5	—	—	—	—

[1]HMG-CoA Reductase - nmoles mevalonate formed, mg protein in an assay^{-1} hr^{-1}

[2]Mevalonate Kinase, Phosphomevalonate Kinase and Pyrophosphomevalonate Decarboxylase - nmoles mevalonate metabolized, mg protein in an assay^{-1} hr^{-1}

[3]A blank value (—) indicates that the enzyme activity was undetectable according to the assays.

4. Profile of HMG-CoA Reductase and Mevalonate Kinase Activity in Liver and Kidneys From Turkeys Fed Cholesterol

The profiles of HMG-CoA reductase and MVA kinase activities in fraction 1 from a sucrose density gradient of liver and kidney microsomes from turkeys fed cholesterol are shown in Figure 15-C and Figure 15-D.

E. HMG-CoA Reductase and Mevalonate Activating Enzymes in Liver and Kidneys From Turkeys Taken Off Cholesterol and Fed a Normal Diet for Six Weeks

Turkeys were fed a 1% cholesterol diet, commencing at the time poults were hatched. The cholesterol fed turkeys were taken off cholesterol and fed a control (tallow) diet for six weeks. All the turkeys were sacrificed at 8:00 a.m. The liver and kidneys were removed, and a 100,000 X g pellet was prepared. HMG-CoA reductase and mevalonate kinase activities were assayed according to the procedures under Materials and Methods. Twenty-five milligrams of the resuspended pellet was layered on a linear sucrose density gradient and five fractions, each consisting of 6 mls, were collected and assayed for HMG-CoA reductase and mevalonate kinase activities.

1. Profile of the Mevalonate Activating Enzymes in Liver and Kidneys

The fact that cholesterol suppresses HMG-CoA reductase and MVA

kinase activity in turkeys, raised the question as to what effect cholesterol would have on these same enzymes when turkeys were taken off cholesterol and fed a control (tallow) diet.

High levels of MVA kinase, MVAP kinase and MVAPP decarboxylase activities were observed in the microsomes and fraction 1 from the sucrose density gradient of liver microsomes from turkeys taken off cholesterol and fed a control diet for six weeks. The Dowex-1 formate column elution profile of the products from mevalonate metabolism by liver microsomes are shown in Figure 26 and Figure 27, respectively.

A high level of MVA kinase activity was observed in microsomes from kidneys of turkeys taken off cholesterol and fed a control diet for six weeks. Figure 28 shows the Dowex-1 formate column elution profile of the products from mevalonate metabolism by kidney microsomes.

MVA kinase and MVAP kinase activities were observed in fraction 1 from a sucrose density gradient of kidney microsomes from turkeys taken off cholesterol and fed a control diet for six weeks. The Dowex-1 formate column elution profile of the products from mevalonate metabolism by kidney microsomes is shown in Figure 29.

2. Activity of the Mevalonate Activating Enzymes in Liver and Kidneys From Turkeys Taken Off Cholesterol and Fed a Control Diet For Six Weeks

MVA kinase, MVAP kinase and MVAPP decarboxylase activities in microsomes from liver of turkeys taken off cholesterol and fed a control diet for six weeks were 26.2, 10.3 and 1.62 nmoles MVA metabolized

Figure 26. Metabolism of ^{14}C-Mevalonic Acid by Liver Microsomes From Turkeys Taken Off Cholesterol and Fed a Control Diet For Six Weeks.

Dowex-1 formate elution pattern: mevalonate kinase assay of microsomes from the liver of turkeys taken off cholesterol and fed a control (tallow) diet for six weeks. Turkeys were sacrificed at 8:00 a.m.

Elution profile following assay for mevalonate kinase in liver microsomes under normal assay conditions. Elution of Dowex-1 column:

A. 25 ml water

B. 25 ml 2 N formic acid

C. 50 ml 4 N formic acid

D. 50 ml 4 N formic acid + 0.4 N ammonium formate

E. 50 ml 4 N formic acid + 0.8 N ammonium formate

Collect approximately 3.0-3.5 ml fractions.

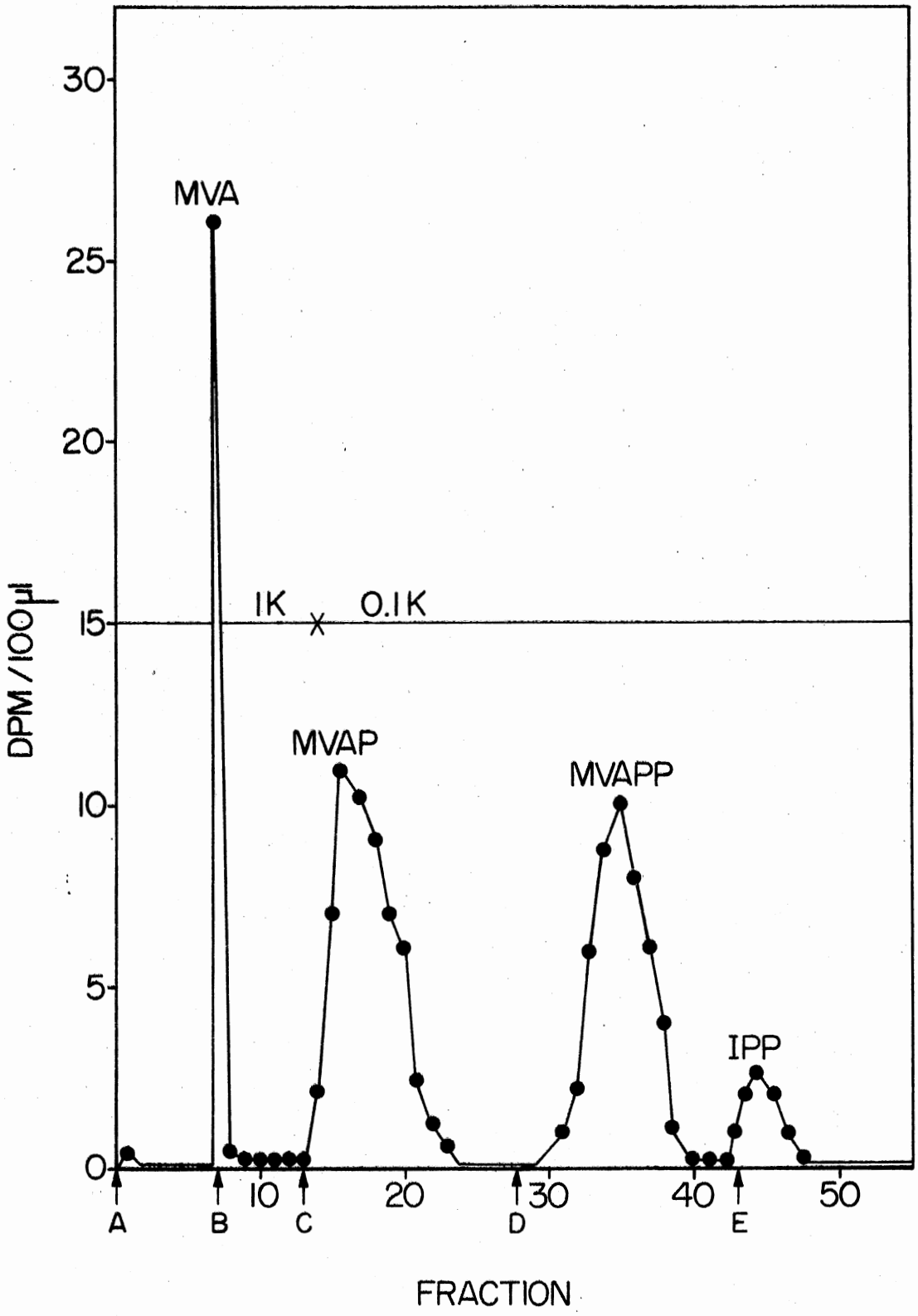

30
25
20
15
10
5
0
DPM / 100µl
MVA
1K
0.1K
MVAP
MVAPP
IPP
10
20
30
40
50
A
B
C
D
E
FRACTION

Figure 27. Metabolism of ^{14}C-Mevalonic Acid by Fraction One From the Sucrose Density Gradient of Liver Microsomes From Turkeys Taken Off Cholesterol and Fed a Control Diet for Six Weeks.

Dowex-1 formate elution pattern: mevalonate kinase assay of fraction one from a 20% → 45% linear sucrose density gradient from turkey liver. Turkeys were taken off cholesterol and fed a control (tallow) diet for six weeks. Turkeys were sacrificed at 8:00 a.m.

Elution profile following assay for mevalonate kinase of fraction one from a 20% → 45% linear sucrose gradient from turkey livers under normal assay conditions. Elution of Dowex-1 column:

A. 25 ml water

B. 25 ml 2 N formic acid

C. 50 ml 4 N formic acid

D. 50 ml 4 N formic acid + 0.4 N ammonium formate

E. 50 ml 4 N formic acid + 0.8 N ammonium formate

Collect approximately 3.0-3.5 ml fractions.

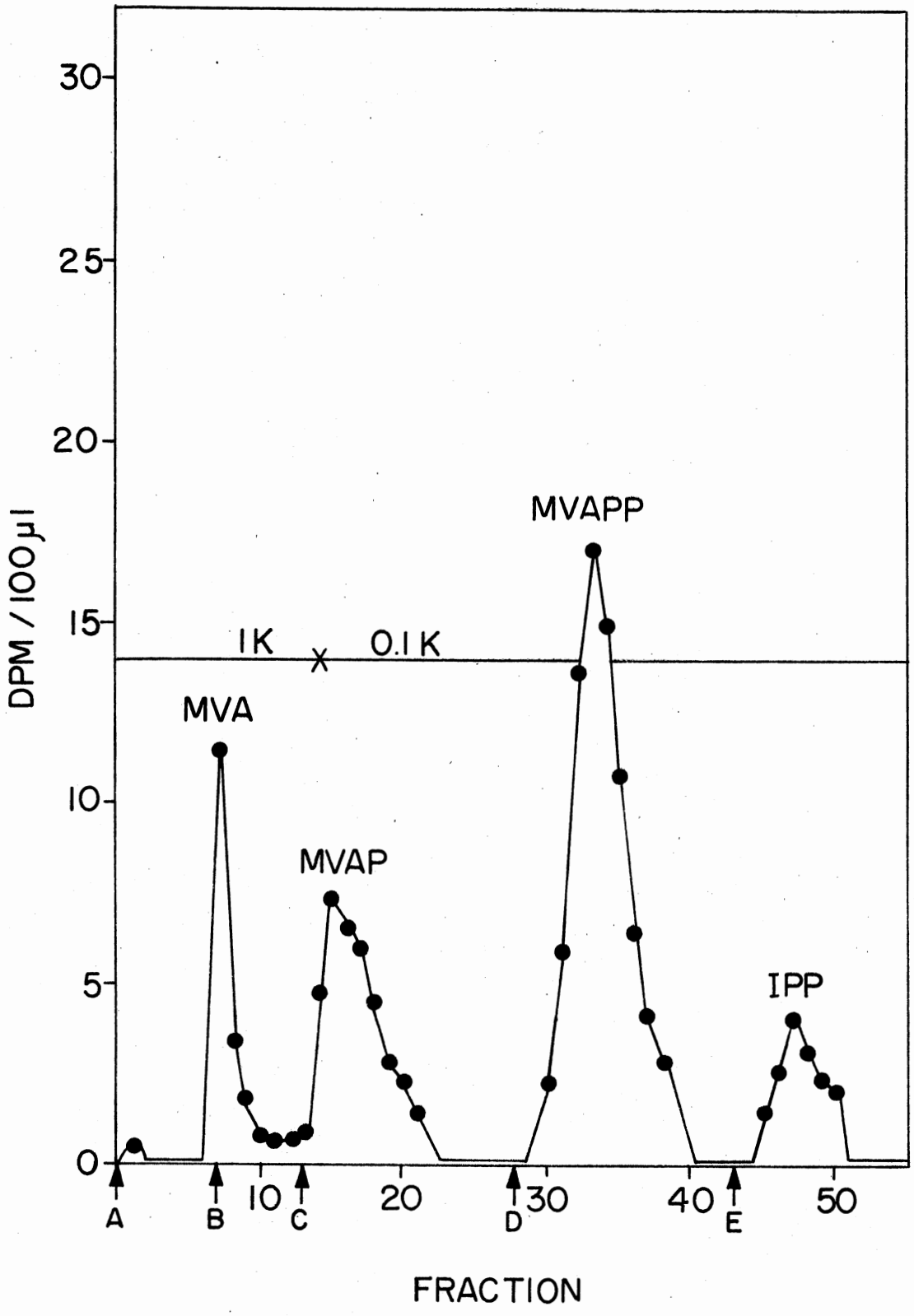

30
25
20
15
10
5
0
DPM / 100µl
1K
0.1K
MVA
MVAP
MVAPP
IPP
A
B
10
C
20
D
30
40
E
50
FRACTION

Figure 28. Metabolism of ^{14}C-Mevalonic Acid by Kidney Microsomes From Turkeys Taken Off Cholesterol and Fed a Control Diet For Six Weeks.

Dowex-1 formate elution pattern: mevalonate kinase assay of microsomes from kidneys of turkeys taken off cholesterol and fed a control (tallow) diet for six weeks. Turkeys were sacrificed at 8:00 a.m.

Elution profile following assay for mevalonate kinase in kidney microsomes under normal assay conditions. Elution of Dowex-1 column:

A. 25 ml water

B. 25 ml 2 N formic acid

C. 50 ml 4 N formic acid

D. 50 ml 4 N formic acid + 0.4 N ammonium formate

E. 50 ml 4 N formic acid + 0.8 N ammonium formate

Collect approximately 3.0-3.5 ml fractions.

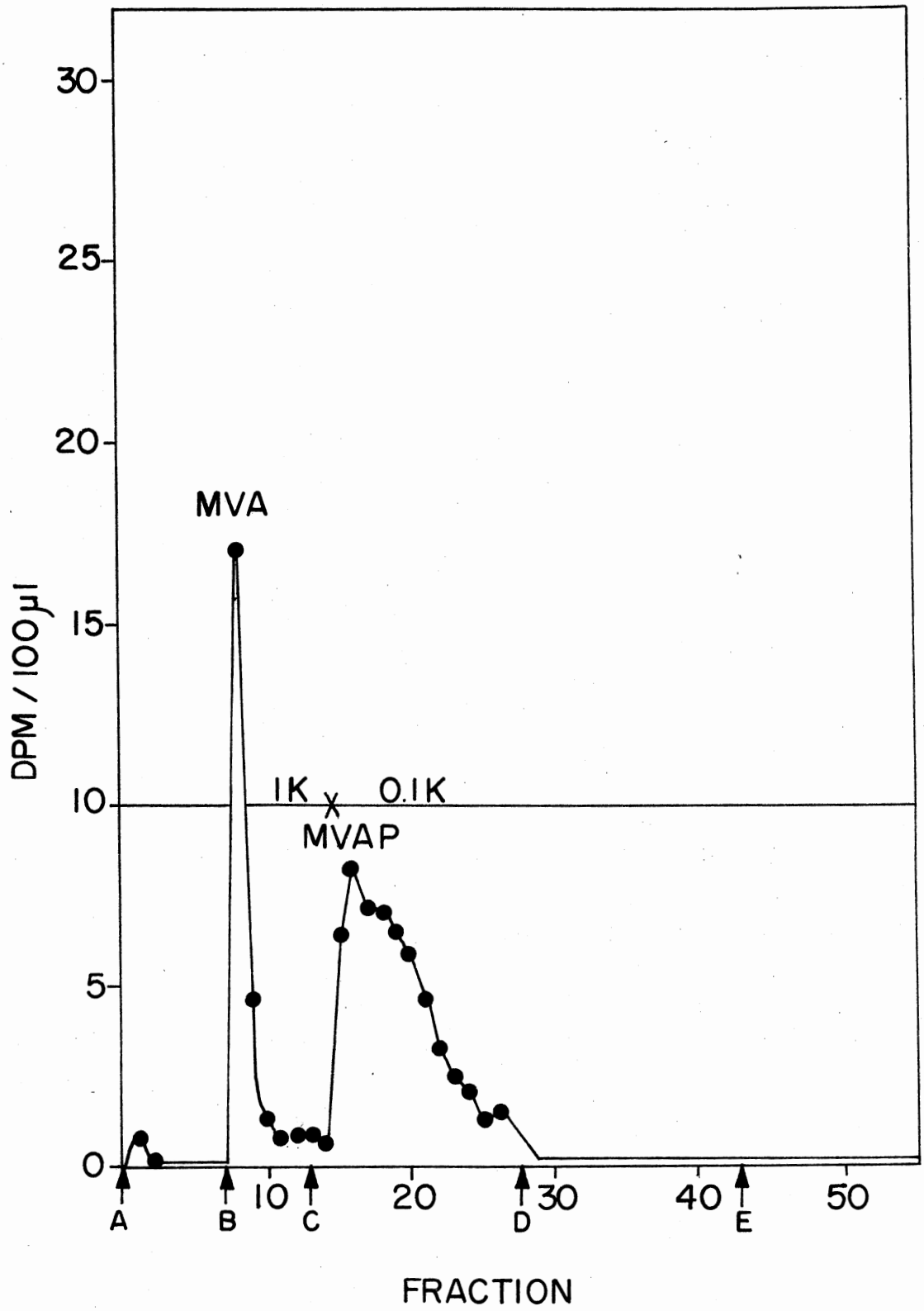

30
25
20
15
10
5
0
DPM / 100µl
MVA
1K
0.1K
MVAP
A
B
10
C
20
D
30
40
E
50
FRACTION

Figure 29. Metabolism of ^{14}C-Mevalonic Acid by Fraction One From the Sucrose Density Gradient of Kidney Microsomes From Turkeys Taken Off Cholesterol and Fed a Control Diet For Six Weeks.

Dowex-1 formate elution pattern: mevalonate kinase assay of fraction one from a 20% → 45% linear sucrose density gradient from turkey kidneys. Turkeys were taken off cholesterol and fed a control (tallow) diet for six weeks. Turkeys were sacrificed at 8:00 a.m.

Elution profile following assay for mevalonate kinase of fraction one from a 20% → 45% linear sucrose gradient from turkey kidneys under normal assay conditions. Elution of Dowex-1 column:

A. 25 ml water

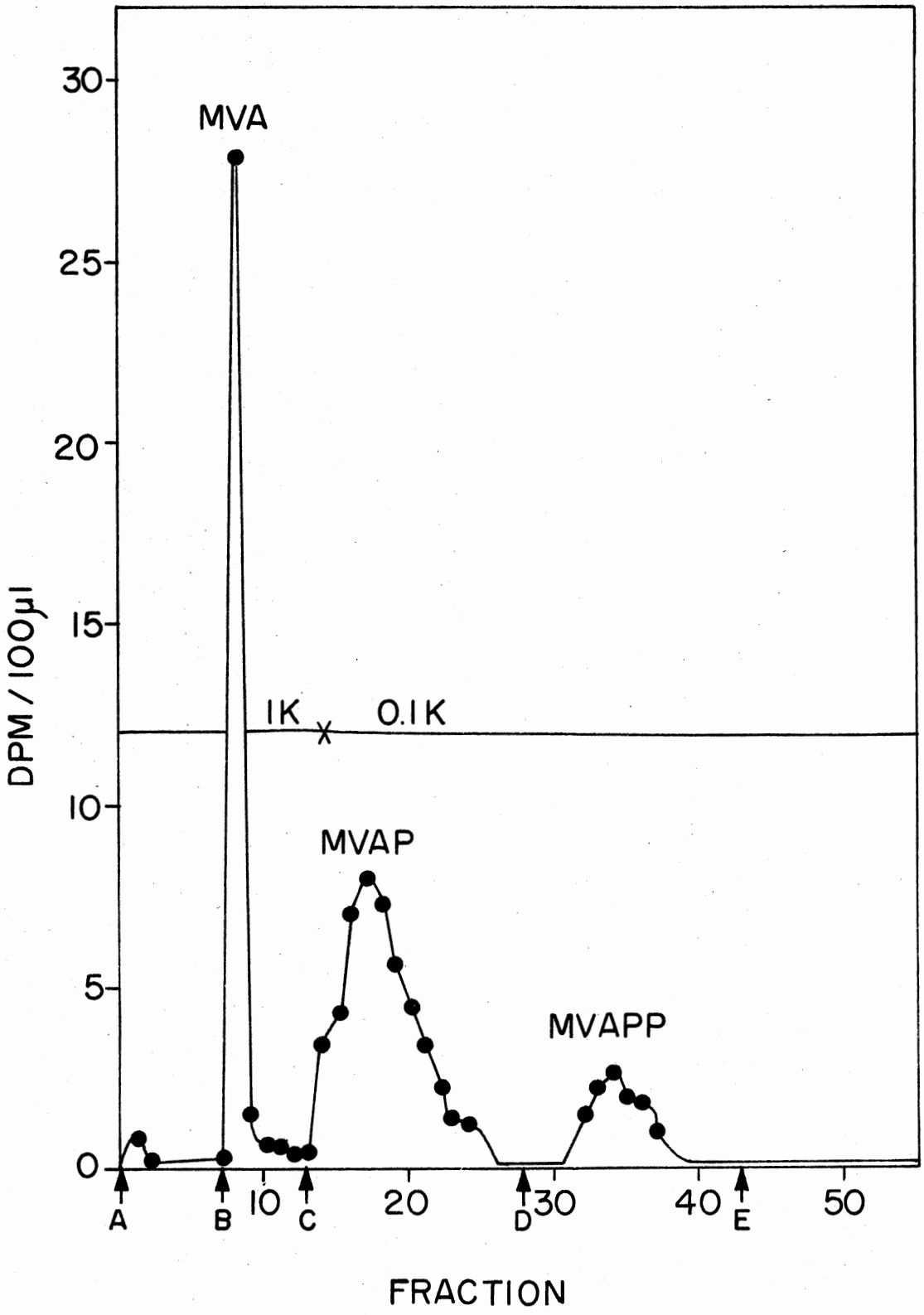
B. 25 ml 2 N formic acid

C. 50 ml 4 N formic acid

D. 50 ml 4 N formic acid + 0.4 N ammonium formate

E. 50 ml 4 N formic acid + 0.8 N ammonium formate

Collect approximately 3.0-3.5 ml fractions.


30
25
20
15
10
5
0
DPM / 100µl
MVA
1K
0.1K
MVAP
MVAPP
A
B
10
C
20
D
30
40
E
50
FRACTION

mg protein in assay^{-1} hr^{-1}, respectively (Table XIII).

MVA kinase, MVAP kinase and MVAPP decarboxylase activities in fraction 1 from a sucrose density gradient of liver microsomes from turkeys taken off cholesterol and fed a control diet were 22.0, 17.0, and 2.90 nmoles MVA metabolized mg protein in assay^{-1} hr^{-1}, respectively (Table XIII). Fractions 2-5 did not have measurable mevalonate kinase activities.

MVA kinase activity in microsomes from kidneys of turkeys taken off cholesterol and fed a control diet was 12.0 nmoles MVA metabolized mg protein in assay^{-1} hr^{-1} (Table XIII). However, MVA kinase and MVAP kinase activities in fraction 1 from a sucrose density gradient of kidney microsomes from turkeys taken off cholesterol and fed a normal diet were 11.6 and 2.20 nmoles MVA metabolized mg protein in assay^{-1} hr^{-1}, respectively (Table XIII).

3. Activity of HMG-CoA Reductase in Liver and Kidneys From Turkeys Taken Off Cholesterol and Fed a Control Diet For Six Weeks

HMG-CoA reductase activity in the microsomes and fraction 1 from a sucrose density gradient of liver microsomes from turkeys taken off cholesterol and fed a control diet were 0.890 and 1.08 nmoles MVA formed mg protein in assay^{-1} hr^{-1}, respectively (Table XIII). Fractions 2-5 did not have measurable HMG-CoA reductase activities.

HMG-CoA reductase activity in the microsomes from the kidneys of turkeys taken off cholesterol and fed a control diet was 0.074 nmoles MVA formed mg protein in assay^{-1} hr^{-1} (Table XIII). Fractions 1-5 did not have measurable HMG-CoA reductase activity.

TABLE XIII

HMG-CoA REDUCTASE, MEVALONATE KINASE, PHOSPHOMEVALONATE KINASE AND PYROPHOSPHOMEVALONATE DECARBOXYLASE IN LIVER AND KIDNEYS FROM TURKEYS TAKEN OFF CHOLESTEROL AND FED A CONTROL DIET FOR SIX WEEKS

STEP	HMG-CoA Reductase[1]	Mevalonate Kinase[2]	Phosphomevalonate Kinase[2]	Pyrophosphomevalonate Decarboxylase[2]
LIVER				
Microsomal Pellet	0.89	26.20	10.30	1.62
Sucrose Gradient				
fraction 1	1.08	22.00	17.00	2.90
fractions 2-5	—	—	—	—
KIDNEYS				
Microsomal Pellet	0.07	12.00	—	—
Sucrose Gradient				
fraction 1	—	11.60	2.20	—
fractions 2-5	—	—	—	—

[1]HMG-CoA Reductase - nmoles mevalonate formed, mg protein in an assay^{-1} hr^{-1}

[2]Mevalonate Kinase, Phosphomevalonate Kinase and Pyrophosphomevalonate Decarboxylase - nmoles mevalonate metabolized, mg protein in an assay^{-1} hr^{-1}

[3]A blank value (—) indicates that the enzyme activity was undetectable according to the assays.

Thus, the liver enzymes involved in cholesterol biosynthesis return to their normal activities after such manipulation, whereas, the kidney enzymes do not return to their normal activities.

F. HMG-CoA Reductase and Mevalonate Kinase Activities in Frozen Liver and Kidneys From Normal and Cholesterol-Fed Turkeys

Turkeys are a relatively large experimental animal and in some instances were not readily available when needed, thus it was of interest to determine the effect quick-freezing would exert on HMG-CoA reductase and mevalonate kinase activities from liver and kidneys.

Control (tallow) and cholesterol-fed turkeys were sacrificed at 8:00 a.m. The liver and kidneys were removed and immediately quick-frozen in liquid nitrogen. A 100,000 X g pellet was prepared and assayed for mevalonate kinase and HMG-CoA reductase activities. Twenty-five milligrams of the resuspended pellet was layered on a linear sucrose density gradient and five fractions, each consisting of 6 mls, were collected and assayed for mevalonate kinase and HMG-CoA reductase activities according to procedure under Materials and Methods.

1. Profile of the Mevalonate Activating Enzymes in Frozen Liver and Kidneys

MVA kinase and MVAP kinase activities were observed in microsomes from frozen liver of control diet turkeys. Figure 30 shows the Dowex-1 formate column elution profiles of the products from mevalonate metabolism by liver microsomes.

Figure 30. Metabolism of ^{14}C-Mevalonic Acid by Fresh and Frozen Liver Microsomes From Turkeys Fed a Control Diet.

Dowex-1 formate elution pattern: mevalonate kinase assay of microsomes from fresh and frozen liver of turkeys fed a control (tallow) diet. Turkeys were sacrificed at 8:00 a.m. Fresh liver (○——○); frozen liver (●——●).

Elution profile following assay for mevalonate kinase in liver microsomes under normal assay conditions. Elution of Dowex-1 formate column.

A. 25 ml water

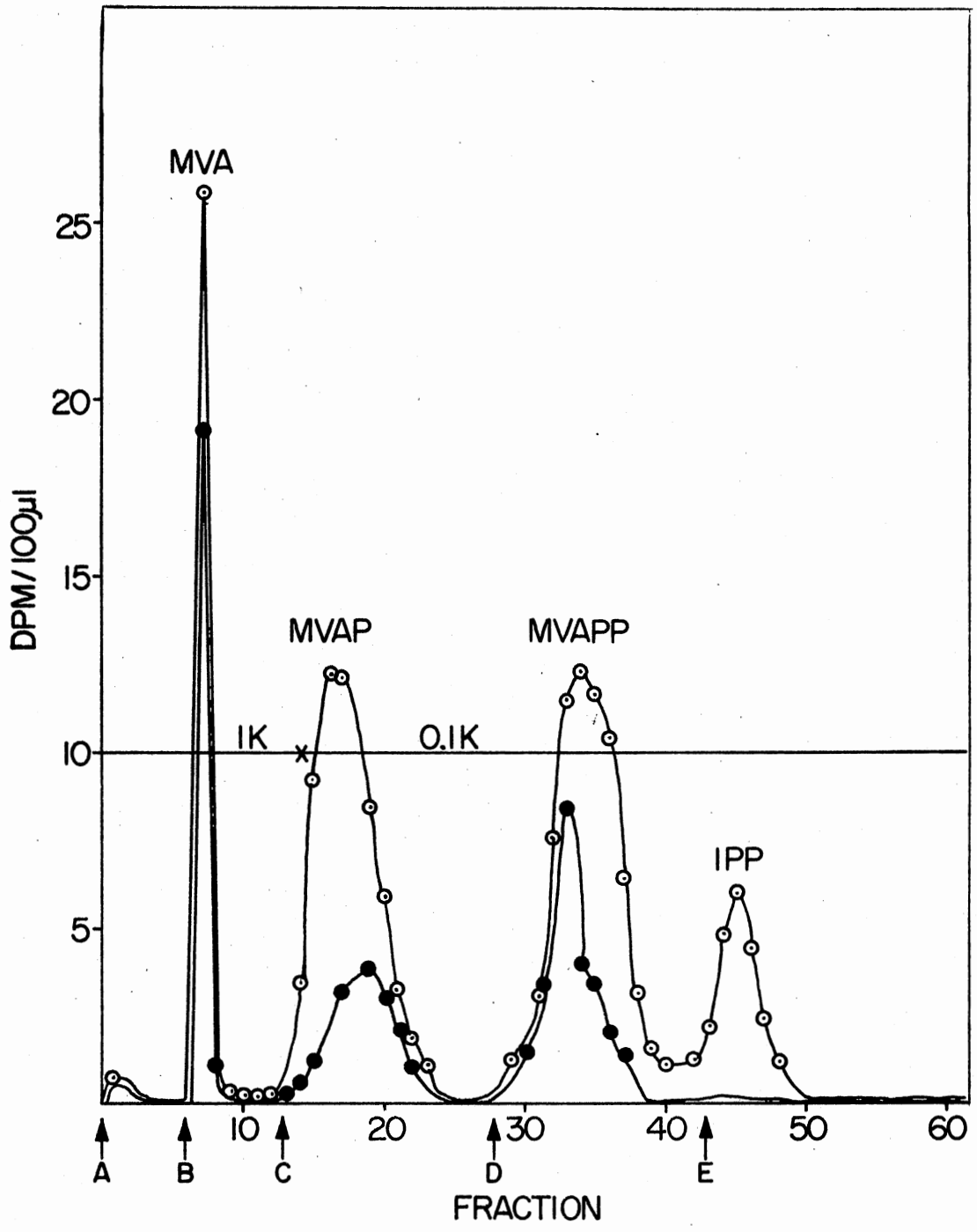
B. 25 ml 2 N formic acid

C. 50 ml 4 N formic acid

D. 50 ml 4 N formic acid + 0.4 N ammonium formate

E. 50 ml 4 N formic acid + 0.8 N ammonium formate

Collect approximately 3.0-3.5 ml fractions.


MVA
MVAP
MVAPP
IPP
1K
0.1K
DPM/100µl
25
20
15
10
5
10
20
30
40
50
60
A
B
C
D
E
FRACTION

MVA kinase and MVAP kinase activities were observed in fraction 1 from a sucrose density gradient of microsomes from frozen liver of control diet turkeys. A Dowex-1 formate column elution profile of the products from mevalonate metabolism by liver microsomes is shown in Figure 31.

Very low MVA kinase and MVAP kinase activities were observed in microsomes from frozen liver of turkeys fed cholesterol. Figure 32 shows the Dowex-1 formate column elution profile of the products from mevalonate metabolism by liver microsomes.

The mevalonate activating enzymes were not measurable in fraction 1 from a sucrose density gradient of microsomes from frozen liver of turkeys fed cholesterol (Figure 33).

Low MVA kinase activity was observed in microsomes and fraction 1 from a sucrose density of microsomes from frozen kidneys of control diet turkeys. The Dowex-1 formate column elution profile of the products from mevalonate metabolism are shown in Figure 34 and Figure 35, respectively.

MVA kinase activity was observed in microsomes and fraction 1 from a sucrose density gradient of microsomes from frozen kidneys of turkeys fed cholesterol. The Dowex-1 formate elution profiles of the products from mevalonate metabolism on a Dowex-1 formate column are shown in Figure 36 and Figure 37, respectively.

Figure 31. Metabolism of ^{14}C-Mevalonic Acid by Fraction One from the Sucrose Density Gradient of Liver Microsomes From Turkeys Fed a Control Diet.

Dowex-1 formate elution pattern: mevalonate kinase assay of fraction one from a 20% → 45% linear sucrose density gradient from fresh and frozen liver of turkeys fed a control (tallow) diet. Turkeys were sacrificed at 8:00 a.m. Fresh liver (●——⊙); frozen liver (●——●).

Elution profile following assay for mevalonate kinase in fraction one from a 20% → 45% linear sucrose density gradient under normal assay conditions. Elution of Dowex-1 formate column:

A. 25 ml water

B. 25 ml 2 N formic acid

C. 50 ml 4 N formic acid

D. 50 ml 4 N formic acid + 0.4 N ammonium formate

E. 50 ml 4 N formic acid + 0.8 N ammonium formate

Collect approximately 3.0-3.5 ml fractions.

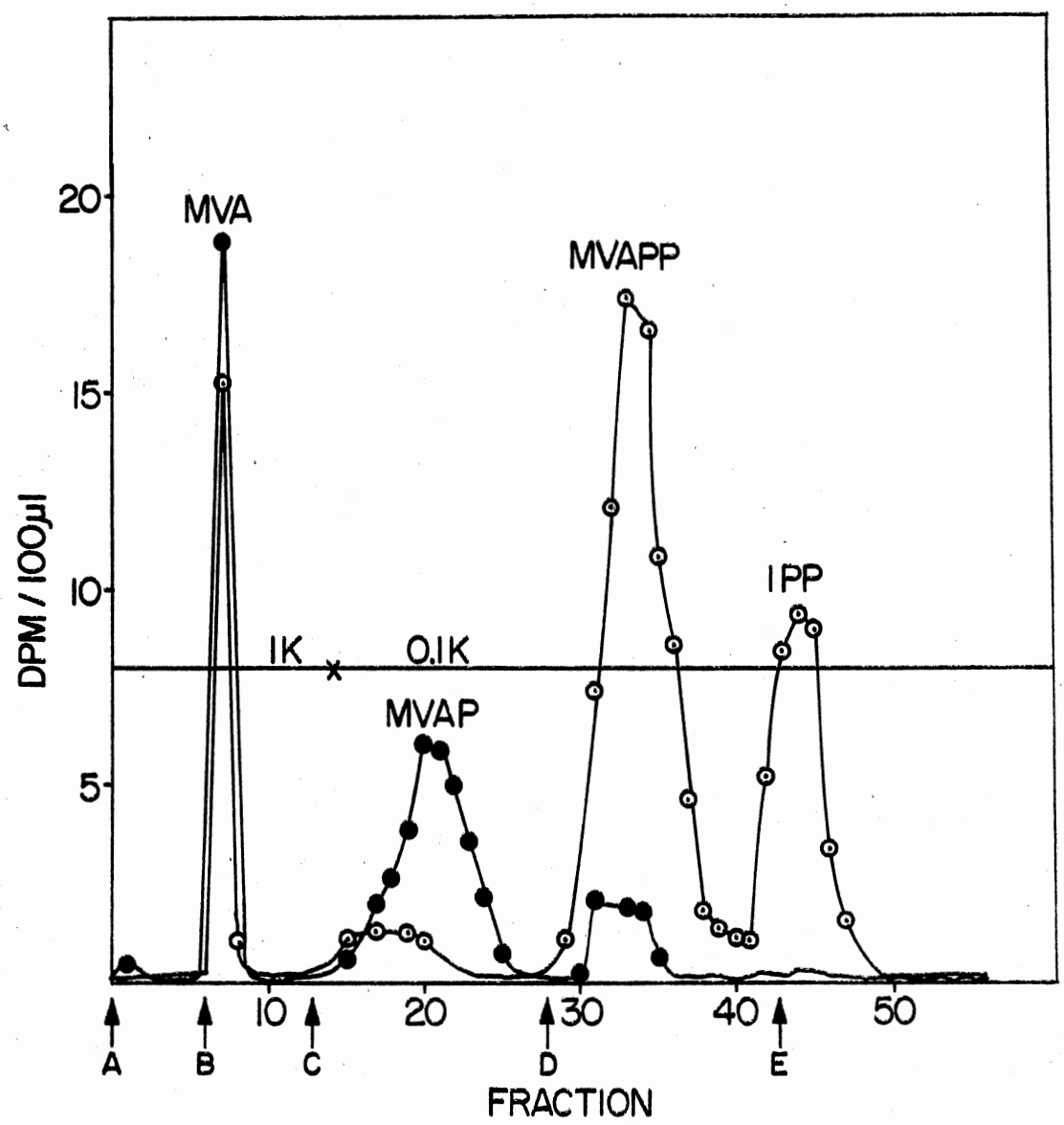

MVA
MVAPP
IPP
MVAP
1K
0.1K
DPM / 100µl
20
15
10
5
10
20
30
40
50
A
B
C
D
E
FRACTION

Figure 32. Metabolism of ^{14}C-Mevalonic Acid by Fresh and Frozen Liver Microsomes From Turkeys Fed a Cholesterol Diet.

Dowex-1 formate elution pattern: mevalonate kinase assay of microsomes from fresh and frozen liver of turkeys fed cholesterol. Turkeys were sacrificed at 8:00 a.m. Fresh liver (⊙———⊙); frozen liver (●———●).

Elution profile following assay for mevalonate kinase in liver microsomes under normal assay conditions. Elution of Dowex-1 formate column:

A. 25 ml water

B. 25 ml 2 N formic acid

C. 50 ml 4 N formic acid

D. 50 ml 4 N formic acid + 0.4 N ammonium formate

E. 50 ml 4 N formic acid + 0.8 N ammonium formate

Collect approximately 3.0-3.5 ml fractions.

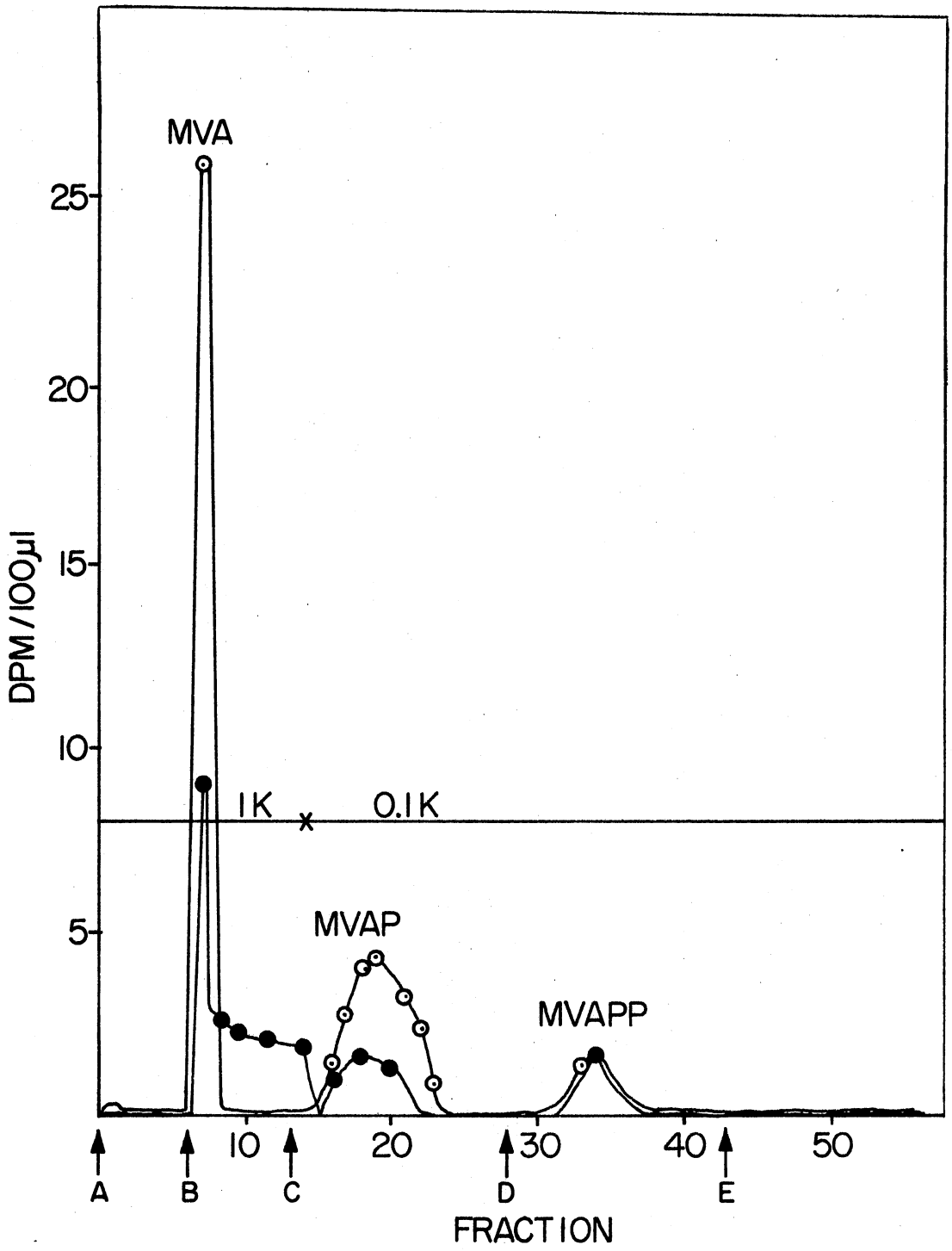

MVA
25
20
15
10
5
DPM/100µl
1K
0.1K
MVAP
MVAPP
10
20
30
40
50
A
B
C
D
E
FRACTION

Figure 33. Metabolism of ^{14}C-Mevalonic Acid by Fraction One From the Sucrose Density Gradient of Liver Microsomes From Turkeys Fed a Cholesterol Diet.

Dowex-1 formate elution pattern: mevalonate kinase assay of fraction one from a 20% → 45% linear sucrose density gradient from fresh and frozen liver of turkeys fed cholesterol. Turkeys were sacrificed at 8:00 a.m. Fresh liver (⊙——⊙); frozen liver (●——●).

Elution profile following assay for mevalonate kinase in fraction one from a 20% → 45% linear sucrose density gradient under normal assay conditions. Elution of Dowex-1 column:

A. 25 ml water

B. 25 ml 2 N formic acid

C. 50 ml 4 N formic acid

D. 50 ml 4 N formic acid + 0.4 N ammonium formate

E. 50 ml 4 N formic acid + 0.8 N ammonium formate

Collect approximately 3.0-3.5 ml fractions.

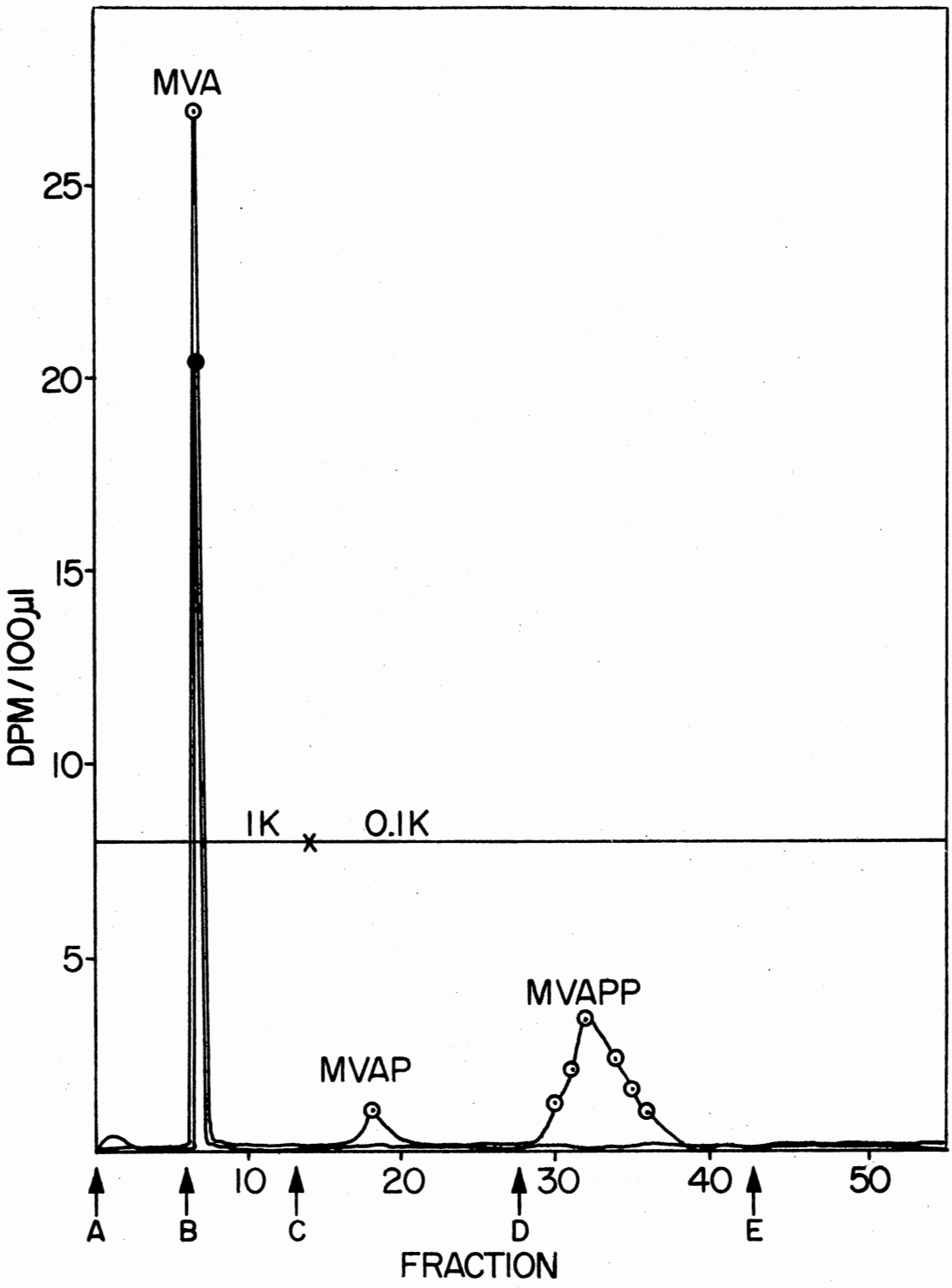

MVA
25
20
15
10
5
DPM/100µl
1K
0.1K
MVAPP
MVAP
10
20
30
40
50
A
B
C
D
E
FRACTION

Figure 34. Metabolism of ^{14}C-Mevalonic Acid by Microsomes From Fresh and Frozen Kidneys of Turkeys Fed a Control Diet.

Dowex-1 formate elution pattern: mevalonate kinase assay of microsomes from fresh and frozen kidneys of turkeys fed a control (tallow) diet. Turkeys were sacrificed at 8:00 a.m. Fresh kidneys (⊙———⊙); frozen kidneys (●———●).

Elution profile following assay for mevalonate kinase in kidney microsomes under normal assay conditions. Elution of Dowex-1 formate column:

A. 25 ml water

B. 25 ml 2 N formic acid

C. 50 ml 4 N formic acid

D. 50 ml 4 N formic acid + 0.4 N ammonium formate

E. 50 ml 4 N formic acid + 0.8 N ammonium formate

Collect approximately 3.0-3.5 ml fractions.

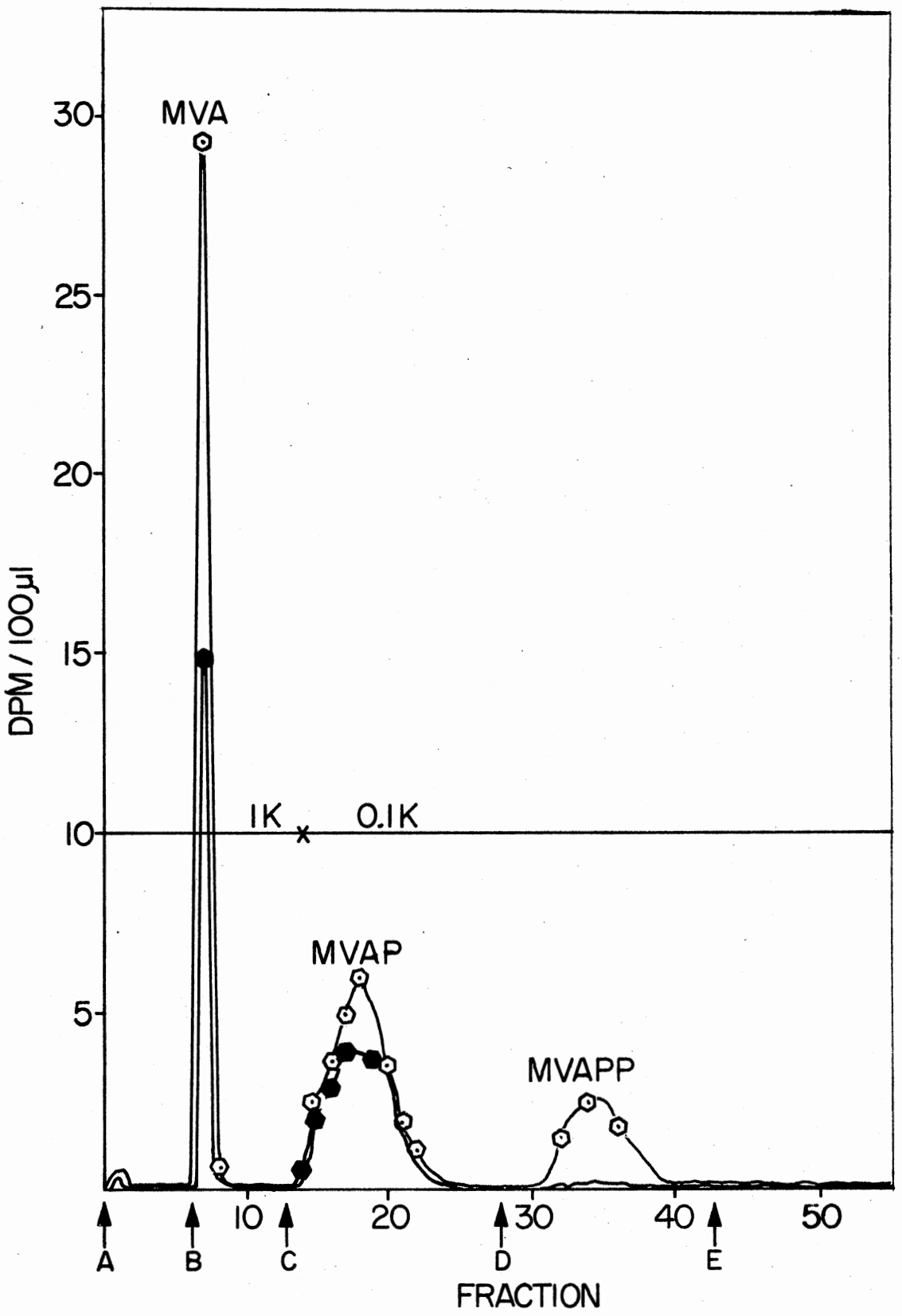

MVA
MVAP
MVAPP
1K
0.1K
DPM / 100µl
30
25
20
15
10
5
10
20
30
40
50
A
B
C
D
E
FRACTION

Figure 35. Metabolism of ^{14}C-Mevalonic Acid by Fraction One From the Sucrose Density Gradient of Microsomes From Fresh and Frozen Kidneys of Turkeys Fed a Control Diet.

Dowex-1 formate elution pattern: mevalonate kinase assay of fraction one from a 20% → 45% linear sucrose density gradient from fresh and frozen kidneys of turkeys that were sacrificed at 8:00 a.m. Fresh kidneys (○———○); frozen kidneys (●———●).

Elution profile following assay for mevalonate kinase in fraction one from a 20% → 45% linear sucrose density gradient under normal assay conditions. Elution of Dowex-1 formate column:

A. 25 ml water

B. 25 ml 2 N formic acid

C. 50 ml 4 N formic acid

D. 50 ml 4 N formic acid + 0.4 N ammonium formate

E. 50 ml 4 N formic acid + 0.8 N ammonium formate

Collect approximately 3.0-3.5 ml fractions.

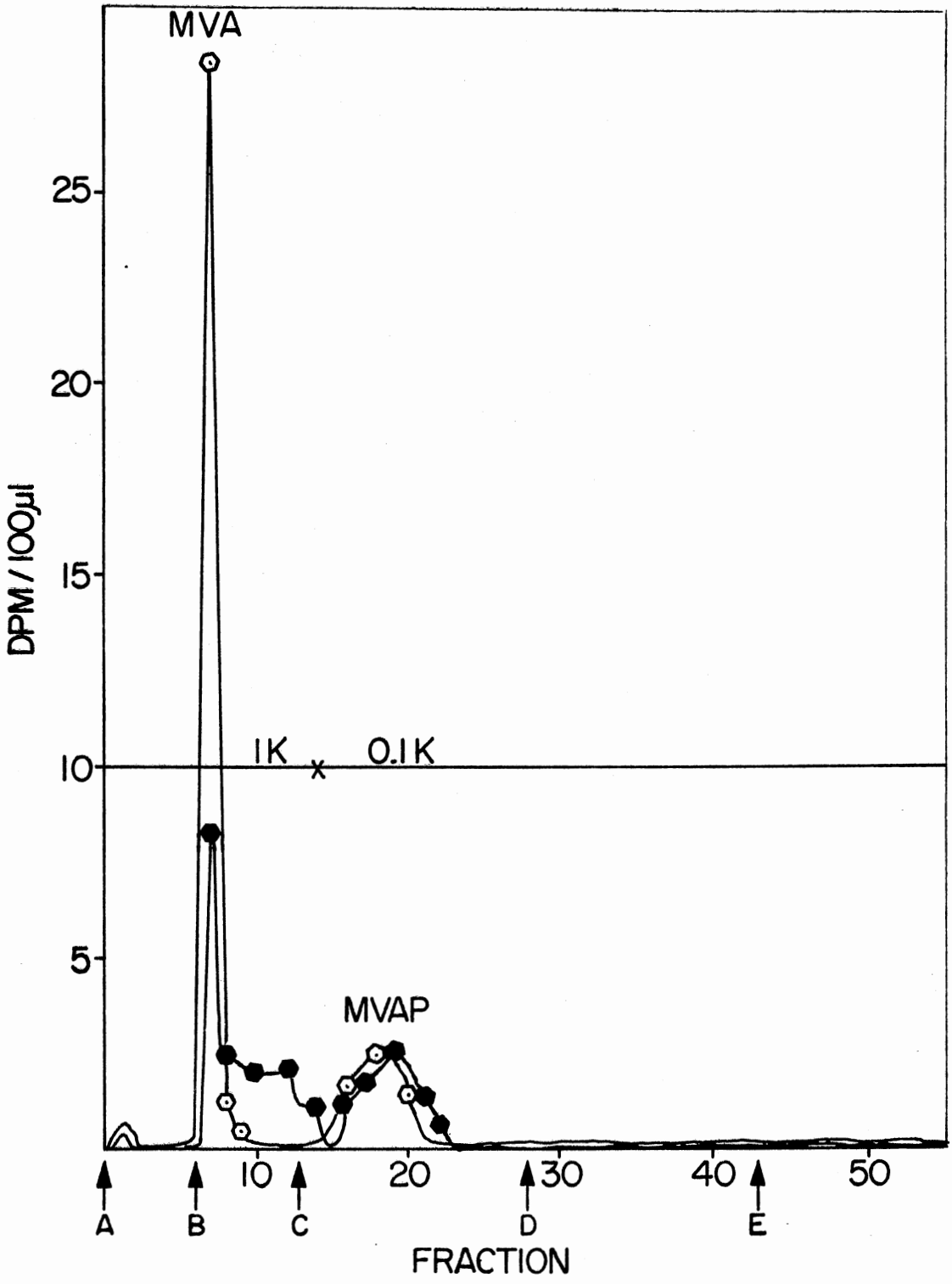

MVA
25
20
15
10
5
DPM/100µl
1K
0.1K
MVAP
10
20
30
40
50
A
B
C
D
E
FRACTION

Figure 36. Metabolism of ^{14}C-Mevalonic Acid by Microsomes From Fresh and Frozen Kidneys of Turkeys Fed Cholesterol.

Dowex-1 formate elution pattern: mevalonate kinase assay of microsomes from fresh and frozen kidneys of turkeys fed cholesterol. Turkeys were sacrificed at 8:00 a.m. Fresh kidneys (⊙——⊙); frozen kidneys (●——●).

Elution profile following assay for mevalonate kinase in kidney microsomes under normal assay conditions. Elution of Dowex-1 formate column:

A. 25 ml water

B. 25 ml 2 N formic acid

C. 50 ml 4 N formic acid

D. 50 ml 4 N formic acid + 0.4 N ammonium formate

E. 50 ml 4 N formic acid + 0.8 N ammonium formate

Collect approximately 3.0-3.5 ml fractions.

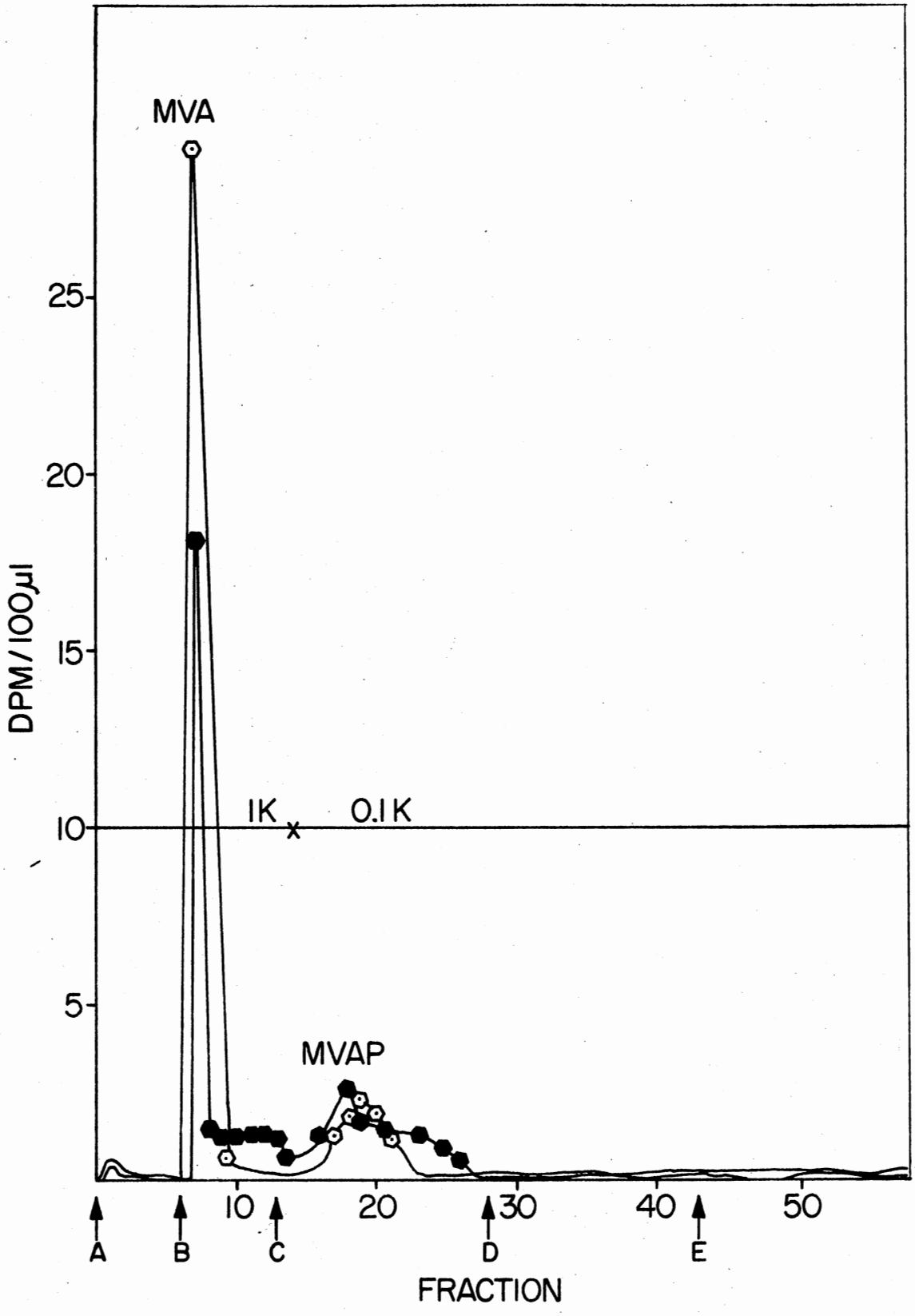

MVA
25
20
15
10
5
DPM/100µl
1K
0.1K
MVAP
10
20
30
40
50
A
B
C
D
E
FRACTION

Figure 37. Metabolism of ^{14}C-Mevalonic Acid by Fraction One From the Sucrose Density Gradient of Microsomes From Fresh and Frozen Kidneys of Turkeys Fed Cholesterol.

Dowex-1 formate elution pattern: mevalonate kinase assay of fraction one from a 20% → 45% linear sucrose density gradient from fresh and frozen kidneys of turkeys fed cholesterol. Turkeys were sacrificed at 8:00 a.m. Fresh kidneys (⬡——⬡); frozen kidneys (⬢——⬢).

Elution profile following assay for mevalonate kinase in fraction one from a 20% → 45% linear sucrose density gradient under normal assay conditions. Elution of Dowex-1 formate column:

A. 25 ml water

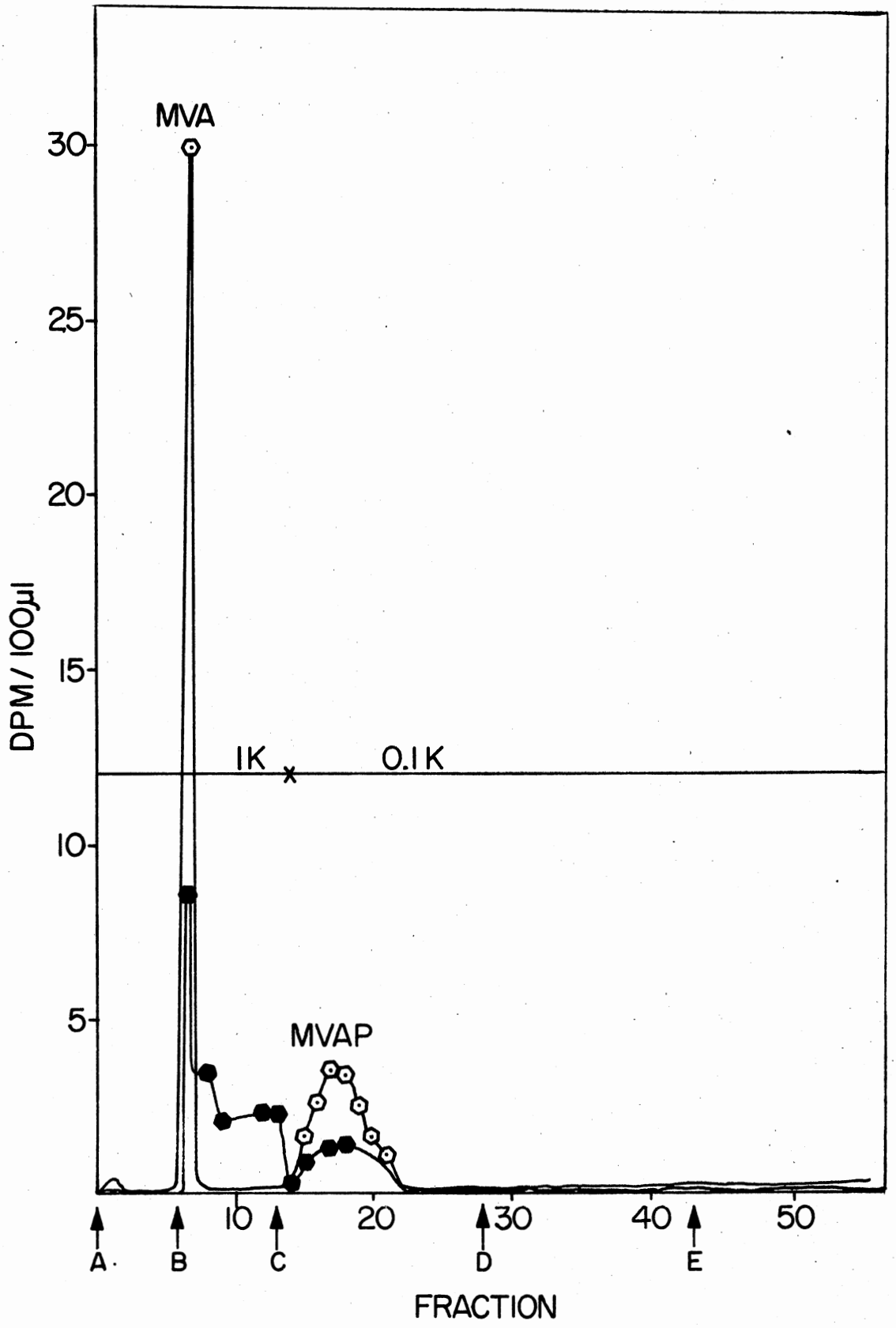
B. 25 ml 2 N formic acid

C. 50 ml 4 N formic acid

D. 50 ml 4 N formic acid + 0.4 N ammonium formate

E. 50 ml 4 N formic acid + 0.8 N ammonium formate

Collect approximately 3.0-3.5 ml fractions.


MVA
30
25
20
15
10
5
DPM/100µl
1K
0.1K
MVAP
10
20
30
40
50
A
B
C
D
E
FRACTION

2. Activity of Mevalonate Kinase in Frozen Liver and Kidneys

MVA kinase activity in microsomes and fraction 1 from a sucrose density gradient of microsomes from frozen liver of control diet turkeys, decreased from 28.20 and 12.20 to 14.60 and 7.32 nmoles MVA metabolized mg protein in assay^{-1} hr^{-1}, respectively (Table XIV).

MVA kinase activity in microsomes from frozen liver of turkeys fed cholesterol was significantly reduced from 4.80 to 1.72 nmoles MVA metabolized mg protein in assay^{-1} hr^{-1}(Table XIV).

MVA kinase activity in microsomes from frozen kidneys of control diet turkeys increased slightly from 2.40 to 2.51 nmoles MVA metabolized mg protein in assay^{-1} hr^{-1}, and in fraction 1 from a sucrose density gradient, MVA kinase activity decreased from 6.42 to 3.34 nmoles MVA metabolized mg protein in assay^{-1} hr^{-1}(Table XV).

MVA kinase activity in microsomes from frozen kidneys of turkeys fed cholesterol, increased from 2.02 to 2.83 nmoles MVA metabolized mg protein in assay^{-1} hr^{-1}, and in fraction 1 from a sucrose density gradient, MVA kinase activity decreased from 3.72 to 1.70 nmoles MVA metabolized mg protein in assay^{-1} hr^{-1} (Table XV).

3. Activity of HMG-CoA Reductase in Frozen Liver and Kidneys

HMG-CoA reductase activity was not measurable in frozen liver and kidneys (Table XIV and Table XV).

Thus, HMG-CoA reductase and the mevalonate activating enzymes are unstable upon freezing and should be isolated from the liver and kidneys as soon as these tissues are removed from the turkey.

TABLE XIV

EFFECT OF QUICK-FREEZING ON MEVALONATE KINASE AND HMG-CoA REDUCTASE ACTIVITY IN TURKEY LIVER FROM CHOLESTEROL AND CONTROL FED TURKEYS

Step	HMG-CoA Reductase[1]	Mevalonate Kinase[2]
FRESH LIVER[3]		
100,000 X g Pellet	1.00	28.00
Sucrose Gradient		
fraction 1	0.15	25.00
fractions 2-5	—	—
FROZEN LIVER[3]		
100,000 X g Pellet	—	14.60
Sucrose Gradient		
fraction 1	—	7.32
fractions 2-5	—	—
FRESH LIVER[4]		
100,000 X g Pellet	—	4.80
Sucrose Gradient		
fraction 1	—	3.52
fractions 2-5	—	—
FROZEN LIVER[4]		
100,000 X g Pellet	—	1.72
Sucrose Gradient		
fractions 1-5	—	—

[1]HMG-CoA Reductase - nmoles mevalonate formed, mg protein in an assay^{-1} hr^{-1}

[2]Mevalonate Kinase - nmoles mevalonate metabolized, mg protein in an assay^{-1} hr^{-1}

[3]Turkeys were fed a control (tallow) diet.

[4]Turkeys were fed a 1% cholesterol diet.

TABLE XIV (Continued)

[5]A blank value (—) indicates that the enzyme activity was undetectable according to the assays.

TABLE XV

EFFECT OF QUICK-FREEZING ON MEVALONATE KINASE AND HMG-CoA REDUCTASE ACTIVITY IN TURKEY KIDNEYS FROM CHOLESTEROL AND CONTROL FED TURKEYS

Step	HMG-CoA Reductase[1]	Mevalonate Kinase[2]
FRESH KIDNEYS[3]		
100,000 X g Pellet	0.33	2.40
Sucrose Gradient		
fraction 1	0.09	6.42
fractions 2-5	—	—
FROZEN KIDNEYS[3]		
100,000 X g Pellet	—	2.51
Sucrose Gradient		
fraction 1	—	3.34
fractions 2-5	—	—
FRESH KIDNEYS[4]		
100,000 X g Pellet	—	2.02
Sucrose Gradient		
fraction 1	—	3.72
fractions 2-5	—	—
FROZEN KIDNEYS[4]		
100,000 X g Pellet	—	2.83
Sucrose Gradient		
fraction 1	—	1.70
fractions 2-5	—	—

[1]HMG-CoA Reductase - nmoles mevalonate formed, mg protein in an assay^{-1} hr^{-1}

[2]Mevalonate Kinase - nmoles mevalonate metabolized, mg protein in an assay^{-1} hr^{-1}

[3]Turkeys were fed a control diet.

[4]Turkeys were fed a 1% cholesterol diet.

TABLE XV (Continued)

[5]A blank value (—) indicates that the enzyme was undetectable according to the assays.

CHAPTER V

DISCUSSION

The results from studies on HMG-CoA reductase in liver and kidneys of turkeys revealed that this enzyme, like many hepatic enzymes, exhibited a diurnal rhythm. A double-peaked rhythm was observed for hepatic HMG-CoA reductase activity, with a maximum at mid-day (12:00 p.m.) and another peak with a slightly lower activity at 4:00 a.m. This observation differs from the observations made by Shapiro and Rodwell (119), in that hepatic HMG-CoA reductase in rats had a double-peaked rhythm, but the peaks in activity occurred at midnight (12:00 a.m.) and 1:45 a.m., in contrast to turkeys which had the highest activity at midday (12:00 p.m.). A possible explanation is that turkeys consume the bulk of their food during the light phase, thus the peak in hepatic HMG-CoA reductase activity occurs at the midpoint of the light cycle, while adult rats on the other hand, consume the bulk of their food in the dark, and it is likely that the phase relationship of the rhythm represents a response to the cyclic intake of food (40). Interestingly, since rats eat the bulk of their food during the dark cycle and turkeys eat the bulk of their food in the light, and that HMG-CoA reductase activity is highest during the feeding times, then the cyclic nature of the enzyme is due to either induction of enzyme synthesis or enzyme activity. Unfortunately, these experiments cannot make that determination. Experiments on HMG-CoA reductase

in rat liver has shown that protein synthesis is required for control of the enzyme.

A single-peaked rhythm was observed for HMG-CoA reductase in kidneys from turkeys, with a peak in activity occurring at midnight (12:00 a.m.). This observation coincides with hepatic HMG-CoA reductase activity in rats, with the exception that a double-peaked rhythm was observed for hepatic HMG-CoA reductase in rats.

An inverse relationship exists between hepatic HMG-CoA reductase activity and HMG-CoA reductase activity in kidneys from turkeys. That is, hepatic HMG-CoA reductase activity achieved its maximum activity at mid-day (12:00 a.m.) and HMG-CoA reductase activity in kidneys achieved its maximum activity at midnight (12:00 p.m.). It appears that different control mechanisms regulate the activity of HMG-CoA reductase in liver and kidneys of turkeys. However, it is premature to speculate on these control mechanisms.

Mevalonate kinase activity in liver and kidneys from turkeys also exhibited a diurnal rhythm. Maximal mevalonate kinase activity occurred during the light period. It appears that the cyclic rhythm in mevalonate kinase in liver and kidneys of turkeys, as well as HMG-CoA reductase, is related to the cyclic intake of food.

However, reports in the literature state that hepatic mevalonate kinase from rats does not exhibit a diurnal cycle (79). Perhaps this discrepancy is the result of different methods of calculating the specific activity of mevalonate kinase. The specific activity of mevalonate kinase activity in this work was reported as total phosphorylated products (phosphomevalonate + pyrophosphomevalonate + isopentenylpyrophosphate). In the literature (79) however, the specific

activity of mevalonate kinase was reported as the activity in phosphomevalonate only. Since the data from this study on mevalonate kinase contradicted the reports in the literature, the specific activity of mevalonate kinase was also calculated as the amount of phosphomevalonate produced (neglecting the other phosphorylated products). The cyclic rhythm for mevalonate kinase remained even after the specific activity was reported as phosphomevalonate. Thus, a difference in calculation procedures does not explain this discrepancy.

The reports in the literature were for hepatic mevalonate kinase from rats. Thus, a possible explanation for the discrepancy is different experimental animals.

It was observed that HMG-CoA reductase and mevalonate kinase in liver from turkeys had a peak activity during the light phase. However, HMG-CoA reductase in kidneys from turkeys had peak activity during the dark phase whereas mevalonate kinase in kidneys had peak activity during the light phase. These results again suggest that different regulatory mechanisms are involved in controlling these enzymes in liver and kidneys from turkeys.

The post-hatched developmental studies on hepatic HMG-CoA reductase from poults showed that HMG-CoA reductase activity was low 5 days following hatch. However, a sharp increase in activity was observed, with a peak at 18 days, followed by a decline in activity at 25 days. Post-hatched hepatic HMG-CoA reductase activity in poults 11-25 days was higher than hepatic HMG-CoA reductase activity in mature turkeys. McNamara et al. (120) showed that rat hepatic HMG-CoA reductase activity was low the first eight days following birth and remained relatively constant and close to adult values. Then the activity

declined to even lower values and remained relatively constant prior to weaning. By the third day after weaning, the reductase activity overshot adult levels, and returned to adult levels two weeks after weaning.

Only one difference is observed in the post-hatched and post-natal developmental pattern of poults and suckling rats. This difference is probably related to differences in experimental animals. Developing rats consume the bulk of their food during the light phase, as do developing turkeys (40). HMG-CoA reductase activity in both animals exhibits a cyclic rhythm, with peak activity in the light phase. However, developing rats are classified as sucklings and are usually weaned at 20 days of age. During the suckling period of rats, HMG-CoA reductase activity is extremely low. An iniibitor of HMG-CoA reductase has been found in rat milk (120). When the rats were weaned, the reductase activity increased and overshot the adult value. Since the developing turkey is not classified as a suckling and does not have to be weaned, then one would not expect a decline in hepatic HMG-CoA reductase activity in poults that were 11-25 days of age.

Thus, except for the suckling period, HMG-CoA reductase in poults and rats is similar. HMG-CoA reductase activity was low the first 5-8 days after birth, followed by a peak in activity 18-23 days after birth that overshot the adult level of HMG-CoA reductase. The reductase activity then returned to the adult level. The peak in HMG-CoA reductase activity in poults also appears to be related to the cyclic intake of food.

It was reported by Shapiro that Rodwell (121) that dietary cholesterol caused a decrease in reductase activity in liver of rats.

Reductase activity measured in vitro exhibited a decline to about one fifth of control values, ten hours after initiation of cholesterol feeding. However, HMG-CoA reductase activity in the liver and kidneys of turkeys was completely suppressed by dietary cholesterol. The difference in experimental results may be linked to how long the animals are fed cholesterol before they are sacrificed. In the studies of Shapiro and Rodwell, rats were fed cholesterol for 10 hours and sacrificed. In these studies turkeys were fed cholesterol for 1-2 months and sacrificed. Gould et al. (87) reported that prolonged cholesterol feeding depressed cholesterol synthesis at sites beyond mevalonate.

There are currently no published reports on the effect(s) of dietary cholesterol on mevalonate kinase activity. It was observed in these studies that dietary cholesterol exerted a significant effect on mevalonate kinase activity in liver but not kidneys from turkeys. Turkey hepatic mevalonate kinase activity was decreased six-fold by dietary cholesterol.

There are metabolic differences between liver and kidneys. The kidneys are the most active tissue involved in mevalonate metabolism by the transmethylglutaconate shunt (91). By this pathway intermediates arising from mevalonate are converted back to HMG-CoA, ketone bodies and acetyl-CoA, rather than to squalene and cholesterol. The shunt pathway is undetectable in liver.

These results suggest for the first time, that HMG-CoA reductase, mevalonate kinase, phosphomevalonate kinase and pyrophosphomevalonate decarboxylase activities in liver, but not kidneys, from turkeys are coordinately suppressed by dietary cholesterol. In rat liver there

is an inhibition of enzyme synthesis. It remains to be seen whether the inhibition of HMG-CoA reductase by dietary cholesterol in turkey liver is similar to the mechanism in rat liver. Since turkeys show a coordinate suppression of a series of enzymes, the author would suggest that there is a suppression of enzyme (protein) synthesis.

HMG-CoA reductase, mevalonate kinase, phosphomevalonate kinase and pyrophosphomevalonate decarboxylase activity in liver from turkeys taken off a cholesterol diet and fed a control diet for six weeks, returned to the level observed in the liver from control-fed turkeys. However, HMG-CoA reductase activity in kidneys of turkeys taken off a cholesterol diet and fed a control diet was lower than the activity in kidneys from control-fed turkeys and mevalonate kinase activity was higher than the activity in kidneys from control-fed turkeys.

Thus, the suppression of hepatic HMG-CoA reductase, mevalonate kinase, phosphomevalonate kinase and pyrophosphomevalonate decarboxylase activity by dietary cholesterol is a reversible process. However, the suppression of HMG-CoA reductase in kidneys from turkeys taken off a cholesterol diet and fed a control diet for six weeks is an irreversible process and mevalonate kinase activity in kidneys from turkeys taken off cholesterol and fed a normal diet for six weeks is enhanced.

Fractionation of HMG-CoA reductase, mevalonate kinase, phosphomevalonate kinase and pyrophosphomevalonate decarboxylase was achieved on a 20 to 45% linear sucrose density gradient. All of the enzyme activities remained at the top of the density gradient. However, fractionation of the enzymes by sucrose density gradient proved to be of little value in purification. In most instances, all of the above

enzyme activities decreased after fractionation on a sucrose density gradient.

The decrease in activity of HMG-CoA reductase and the mevalonate activating enzymes after fractionation on a sucrose density gradient may result from the loss of an activator(s). Also, an inhibitor of the above enzymes may be released or unmasked due to fractionation and thus, a decrease in enzyme activities is observed.

In most of the studies on HMG-CoA reductase and the mevalonate activating enzymes fresh rat liver was used. However, due to the large size and lack of availability of turkeys, it was of interest to determine the effect(s) of quick-freezing on turkey liver and kidneys. It was observed that freezing fresh liver destroyed the activity of HMG-CoA reductase and lowered the activity of the mevalonate activating enzyme. Frozen kidneys also showed no measurable HMG-CoA reductase activity. However, mevalonate kinase activity was not appreciably affected by quick freezing fresh kidneys.

An interesting observation with respect to this study is the fact that phosphomevalonate (MVAP), pyrophosphomevalonate (MVAPP) and isopentenylpyrophosphate (IPP) were the only products eluted from a Dowex-1 formate column. It appears that cholesterol synthesis occurs in three distinct stages. This study was involved with the first stage. The second stage involves prenyl transferases and the third stage involves the sterol carrier protein.

CHAPTER VI

SUMMARY

HMG-CoA reductase activity in liver and kidneys from turkeys exhibited a diurnal cycle. Hepatic HMG-CoA reductase reached its maximum activity at 12:00 p.m. (midpoint of light phase) and its lowest activity at 4:00 p.m. However, HMG-CoA reductase activity in kidneys reached its maximum activity at 12:00 a.m. (midpoint of dark phase) and its lowest activity at 4:00 a.m. and 4:00 p.m.

Mevalonate kinase in liver and kidneys also showed a diurnal cycle. Hepatic mevalonate kinase reached its maximum activity at 8:00 a.m. and its lowest activity at 4:00 p.m. However, mevalonate kinase in kidneys reached its maximum activity at 12:00 p.m. and its lowest activity at 8:00 a.m.

Initial studies on the post-hatched developmental pattern for hepatic HMG-CoA reductase from poults showed that HMG-CoA reductase activity was low five days after the poults were hatched. However, a sharp increase in activity was observed, with a peak at 18 days, followed by a decline in activity.

The post-hatched developmental pattern for hepatic mevalonate kinase from poults showed that mevalonate kinase activity declined in poults that were 11 days of age, followed by a sharp increase in activity, with a peak at 25 days.

HMG-CoA reductase and mevalonate kinase activities in poults and

adult turkeys appeared to be regulated by the cyclic intake of food.

In the liver from cholesterol-fed turkeys, mevalonate kinase, phosphomevalonate kinase and pyrophosphomevalonate decarboxylase activities were six-fold lower than the activities of the control turkeys. However, mevalonate kinase and phosphomevalonate kinase activities in kidneys from cholesterol-fed turkeys were only slightly lower than the activities of the control turkeys.

HMG-CoA reductase activity was completely suppressed in the liver and kidneys from turkeys fed cholesterol.

These results suggested that HMG-CoA reductase, mevalonate kinase, phosphomevalonate kinase and pyrophosphomevalonate decarboxylase activities in turkey liver were coordinately suppressed by dietary cholesterol.

After cholesterol was removed from the diet and the turkeys were maintained on a control diet for six weeks, HMG-CoA reductase, mevalonate kinase, phosphomevalonate kinase and pyrophosphomevalonate decarboxylase activities in liver returned to the level observed for animals continually maintained on a control (tallow) diet. However, HMG-CoA reductase, mevalonate kinase, phosphomevalonate kinase and pyrophosphomevalonate decarboxylase activities in kidneys from turkeys taken off a cholesterol diet and fed a control diet for six weeks, did not return to the level of activity observed for the control turkeys.

Freezing fresh liver destroyed the activity of HMG-CoA reductase and lowered the activity of mevalonate kinase, phosphomevalonate kinase and pyrophosphomevalonate decarboxylase. Frozen kidneys also showed no measurable HMG-CoA reductase activity; however mevalonate kinase,

phosphomevalonate kinase and pyrophosphomevalonate decarboxylase activities were not appreciably affected.

A SELECTED BIBLIOGRAPHY

(1) Popják, G., and Cornforth, J. W. (1960) Advan. Enzymol., 22, 281-335.

(2) Block, K. (1965) Science, 150, 19-38.

(3) Clayton, R. B. (1965) Quart. Rev. Chem. Soc., 19, 168-200.

(4) Fiecchi, A., Kienle, M. G., Scala, A., Galli, G., Paoletti, E. G., Cattabeni, F., and Paoletti, R. (1972) Proc. Roy. Soc. London B., 180, 147-166.

(5) Frantz, I. D., Jr., and Moore, R. B. (1969) Am. J. Med., 46, 684-690.

(6) Likoff, W., and Moyer, J. H. (1963) in Coronary Heart Disease, W. Likoff and J. H. Moyer, ed., pp. 1-3. Grune and Stratton, New York and London.

(7) Krista, L. M., Waibel, P. E., Burger, R. E. (1965) Poultry Sci., 44, 15-22.

(8) Carnaghan, R. B. A. (1955) Vet. Rec., 67, 568-569.

(9) Gibson, E. A., and de Gruchy, P. H. (1955) Vet. Rec., 67, 650-654.

(10) Ball, R. A. "A Study of Vascular Diseases in the Turkey." (Unpub. Ph.D. Thesis, University of Minnesota, 1964.)

(11) Sturkie, P. D., Weiss, H. S., Ringer, R. K., and Sheahan, M. M. (1959) Poultry Sci., 38, 333-337.

(12) Krista, L. M., Waibel, P. E., Shoffner, R. N., and Sautter, J. H. (1970) Poultry Sci., 49, 405-411.

(13) Siperstein, M. D. (1970) Curr. Top. Cell. Regul., 2, 65-100.

(14) Block, K., and Rittenberg, D. (1942) J. Biol. Chem., 145, 625-636.

(15) Bucher, N. L., and McGarrahan, K. (1956) J. Biol. Chem., 222, 1-15.

(16) Tavormina, P. A., Gibbs, M. H., and Huff, J. W. (1956) J. Am.

Chem. Soc., 78, 4498.

(17) Knauss, H. J., Porter, J. W., and Wasson, G. (1959) J. Biol. Chem., 234, 2835-2840.

(18) Durr, I. F., and Rudney, H. (1960) J. Biol. Chem., 235, 2572-2578.

(19) Steward, R., and Rudney, H. (1966) J. Biol. Chem., 241, 1222-1225.

(20) Bucher, N. L. R., Overath, P., and Lynen, F. (1960) Biochim. Biophys. Acta, 40, 491-501.

(21) Dietschy, J. M., and Wilson, J. D. (1970) N. Eng. J. Med., 282, 1128-1138.

(22) Lane, M. D., Clinkebeard, K. D., Reed, W. D., Sugiyama, T., and Moss, J. (1973) Int. Congr. Biochem. 9th Abstr., 412.

(23) Clinkenbeard, K. D., Sugiyama, T., Moss, J., Reed, W. D., and Lane, M. D. (1973) J. Biol. Chem., 248, 2275-2284.

(24) Sugiyama, T., Clinkenbeard, K., Moss, J., and Lane, M. D. (1972) Biochem. Biophys. Res. Commun., 48, 253-261.

(25) Sullivan, A. C., Hamilton, J. G., Miller, O. N., and Wheatley, V. R. (1972) Arch. Biochem. Biophys., 150, 183-190.

(26) Bucher, N. L. R., Overath, P., and Lynen, F. (1960) Biochim. Biophys. Acta, 40, 491-501.

(27) Guder, W., Nolte, I., and Wieland, O. (1968) Eur. J. Biochem., 4, 273-278.

(28) Shapiro, D. J., and Rodwell, V. W. (1971) J. Biol. Chem., 246, 3210-3216.

(29) Goldfarb, S. (1972) FEBS Lett., 24, 153-155.

(30) Mitropoulos, K. A., Venkatesan, S., Balasubramaniam, S., and Peters, T. J. (1978) Eur. J. Biochem., 82, 419-429.

(31) Shirnizn, I., Nagai, J., Hatanaka, N., and Katsaki, H. (1973) Biochem. Biophys. Acta, 296, 310-320.

(32) Qureshi, N., Dugan, R. E., Nimmannit, S., Wu, W., and Porter, J. W. (1976) Biochem., 15, 4185-4190.

(33) Kawachi, T., Rudney, H. (1970) Biochem., 9, 1700-1705.

(34) Linn, T. C. (1967) J. Biol. Chem., 242, 984-989.

(35) Brown, M. S., Dana, S. E., Dietschy, J. M., and Siperstein, M. D. (1973) J. Biol. Chem., 248, 4731-4738.

(36) Heller, R. A., and Gould, R. G. (1973) Biochem. Biophys. Res. Commun., 50, 859-863.

(37) Tormanen, C. D., Redd, W. L., Srikantaiah, M. V., and Scallen, T. J. (1976) Biochem. Biophys. Res. Commun., 68, 754-762.

(38) Kandutsch, A. A., and Saucier, S. E. (1969) J. Biol. Chem., 244, 2299-2305.

(39) Linn, T. C. (1967) J. Biol. Chem., 242, 990-993.

(40) Rodwell, V. W., McNamara, D. J., and Shapiro, D. J. (1973) Advan. Enzymol., 38, 373-412.

(41) Balasubramaniam, S., Goldstein, J. L., and Brown, M. S. (1977) Proc. Natl. Acad. Sci., 74, 1421-1425.

(42) Cayen, M. N., and Dvornik, D. (1968) Can. J. Biochem., 46, 179-187.

(43) Weis, H. J., and Dietschy, J. M. (1969) J. Clin, Invest., 48, 2398-2408.

(44) Lakshmanan, M. R., Nepokroeff, C. M., Ness, G. C., Dugan, R. E., and Porter, J. W. (1973) Fed. Proc., 32, 1379.

(45) Porter, J. W., Ness, G. C., Dugan, R. E., Nepokroeff, C. M., and Lakshmanan, M. R. (1973) Int. Congr. Biochem., 9th Abstr., 412.

(46) Dugan, R. E., Ness, G. C., Lakshmanan, M. R., Nepokroeff, C. M., and Porter, J. W. (1973) Abstr. Am. Chem. Soc., 46.

(47) Oswald, S., and Wiss, V. (1977) Helvetica Chimica Acta, 60, 1967-1968.

(48) Carlson, J. P., McCoy, K. E., and Dempsey, M. E. (1973) Circulation, 48, 4-70.

(49) Tormanen, C. D., Srikantaiah, M. V., and Hardgrave, J. E. (1977) J. Biol. Chem., 252, 1561-1565.

(50) Beg, Z. H., Allmann, D. W., and Gibson, D. M. (1973) Biochem. Biophys. Res. Commun., 54, 1362-1369.

(51) Nordstrom, J. L., Rodwell, V. W., and Mitschelen, J. J. (1977) J. Biol. Chem., 252, 8924-8934.

(52) Spence, J. T., and Gaylor, J. L. (1977) J. Biol. Chem., 252, 5852-5858.

(53) Bell, J. J., Sargeant, T. E., and Watson, J. A. (1976) J. Biol. Chem., 251, 1745-1758.

(54) Endo, A., Kuroda, M., Tsujita, Y. (1976) J. Antibiot., 29, 1346-1348.

(55) Endo, A., Kuroda, M., and Tanzawa, K. (1976) FEBS Lett., 72, 323-326.

(56) Redd, W. L., Hardgrave, J. E., Srikantaiah, M. V., and Scallen, T. J. (1977) Fed. Proc. Abstr., 36, 2676.

(57) Shefer, S., Hauser, S., Lapar, V., and Mosbach, E. H. (1973) J. Lipid Res., 14, 400-405.

(58) Sugano, M., Ide, T., Osmatsu, H., and Takahara, H. (1977) Biochem. Biophys. Res. Commun., 79, 1092-1097.

(59) Shefer, S., Hauser, S., Lapar, V., and Mosbach, E. H. (1972) J. Lipid Res., 13, 571-573.

(60) Tchen, T. T. (1958) J. Biol. Chem., 233, 1100-1103.

(61) Siperstein, M. D., Chaikoff, I. L., and Chernick, S. S. (1951) Science, 113, 747-749.

(62) Werthessen, N. T., Milch, L. J., Redmond, R. F., Smith, L. L., and Smith, E. C. (1954) Amer. J. Physiol., 178, 23-29.

(63) Azarnoff, D. L. (1958) Proc. Soc. Exp. Biol. Med., 98, 680-683.

(64) Slakey, L. L., Ness, G. C., Qureshi, N., and Porter, J. W. (1973) J. Lipid Res., 14, 485-494.

(65) Suzuko, H., Oba, K., and Vritani, J. (1975) Physiol. Plant Path. Path., 7, 265-276.

(66) Arebalo, R. E. "The Occurrence and Some Properties of 3-Hydroxy-3-Methylglutaryl Coenzyme A Reductase and Mevalonate Kinase in Nepeta Cataria Leaf Tissue." (Unpub. Ph.D. Thesis, Oklahoma State University, 1977.)

(67) Levy, H. R., and Popják, G. (1960) Biochem. J., 75, 417-428.

(68) Markley, K., and Smallman, E. (1961) Biochim. Biophys. Acta, 47, 327-335.

(69) Loomis, W. D., and Battaile, J. (1963) Biochim. Biophys. Acta, 67, 54-63.

(70) Williamson, I. P., and Kekwick, R. G. O. (1965) Biochem. J., 96, 862-871.

(71) Flint, A. P. F. (1970) Biochem. J., 120, 145-150.

(72) Imblum, R. L., and Rodwell, V. W. (1974) J. Lipid Research, 15, 211-222.

(73) Flint, A. P. F. (1970) Biochem. J., 120, 145-150.

(74) Goodfellow, R. D., and Barnes, F. J. (1971) Insect Biochem., 1, 271-282.

(75) Valenuela, P., Cori, O., and Yudelevich, A. (1966) Phytochem., 5, 1005-1011.

(76) Cooper, C. Z., and Benedict, C. R. (1967) Plant Physiol., 42, 515-519.

(77) Beytia, E., Dorsey, J. K., Marr, J., Cleland, W. W., and Porter, J. W. (1970) J. Biol. Chem., 245, 5450-5458.

(78) Downing, M. R., and Mitchell, E. D. (1974) Phytochem., 13, 1419-1421.

(79) Slakey, L. L., Craig, M. C., Beytia, E., Briedis, A., Feldbruegge, D. H., Dugan, R. E., Quershi, A. A., Subbarayan, C., and Porter, J. W. (1972) J. Biol. Chem., 247, 3014-3022.

(80) Ramachandran, C. K., and Shah, S. N. (1977) J. Neurochem., 28, 751-757.

(81) Suzuko, H., Oba, K., and Vritani, J. (1975) Physiol. Plant Path., 7, 265-276.

(82) Rodgers, L. J., Shah, S. P. J., and Goodwin, T. W. (1966) Biochem. J., 99, 381-388.

(83) Rodgers, L. J., Shah, S. P. J., and Goodwin, T. W. (1966) Biochem. J., 100, 14C-17C.

(84) Bloch, K., Chaykin, S., Phillips, A. H., and DeWaard, A. (1959) J. Biol. Chem., 234, 2595-2604.

(85) Skilleter, D. N., and Kekwick, R. G. O. (1971) Biochem. J., 124, 407-417.

(86) Garcia-Peregrin, E., and Mayor, F. (1970) Rev. Espan. Fisiol., 26, 209-216.

(87) Gould, R. G., Swyryd, E. A. (1966) J. Lipid Res., 7, 698-707.

(88) Edmond, J., Popják, G. (1974) J. Biol. Chem., 249, 66-71.

(89) Popják, G., and Edmond, J. (1973) Circulation, 48, IV-69.

(90) Fogelman, A. M., Edmond, J., and Popják, G. (1975) J. Biol. Chem., 250, 1773-1775.

(91) Righetti, M., Wiley, M. H., Murrill, P. A., and Siperstein, M. D. (1976) J. Biol. Chem., 251, 2716-2721.

(92) Ritter, M. C., and Dempsey, M. E. (1973) Proc. Nat. Acad. Sci., 70, 265-269.

(93) Scallen, T. J., Dean, W. J., and Schuster, M. W. (1968) J. Biol. Chem., 243, 5202-5206.

(94) Scallen, T. J., Schuster, M. W., and Dhar, A. K. (1971) J. Biol. Chem., 246, 224-230.

(95) Scallen, T. J., Srikantaiah, M. V., Seetharam, B., Hansbury, E., and Gavey, K. L. (1974) Fed. Proc., 33, 1733-1745.

(96) Ritter, M. C., and Dempsey, M. E. (1971) J. Biol. Chem., 246, PC 1536-1539.

(97) Tai, H. H., and Bloch, K. (1972) J. Biol. Chem., 247, 3767-3773.

(98) Zilversmit, D. B. (1969) in Structural and Functional Aspects of Lipoproteins in Living Systems, p. 329. Academic Press, London and New York.

(99) Lindgren, F. T., Jensen, L. C., Hatch, F. T. (1972) in Blood Lipids and Lipoproteins, pp. 181-272. Interscience, New York.

(100) Forte, G. M., Nichols, A. V., Glaeser, R. M. (1968) Chem. Phys. Lipids, 2, 396-408.

(101) Bencze, W. L., and Dempsey, M. E. (1975) in Hypolipidemic Agents, D. Kritchevsky, ed., pp. 193-195. Springer-Verlag Berlin Heidelberg, New York.

(102) Brown, M. S., and Goldstein, J. L. (1976) Science, 191, 150-154.

(103) Glomset, J. A. (1968) J. Lipid Res., 9, 155-167.

(104) Havel, R. J. (1975) Adv. Exp. Med. Biol., 63, 37-39.

(105) Brand, R. J., Roseman, R. H., Schaltz, R. J., and Friedman, M. (1976) Circulation, 53, 348-355.

(106) Knowles, J. (1974) J. Med. Education, 49, 50-56.

(107) Robbins, S. L., and Tucker, A. W. (1944) New Eng. J. Med., 231, 865-868.

(108) Gofman, J. W. (1959) in Coronary Heart Disease, pp. 121-270.

(109) Ignatowski, A. (1909) Arch. Path. Anat. Physiol., 198, 248-270.

(110) Wissler, R. W., and Vesselinovitch, D. (1974) in Atherosclerosis III, G. Schettler and A. Weizel, eds., pp. 319-325. Springer, Berlin, Heidelberg and New York.

(111) Kellner, A., Correll, J. W., and Ladd, A. T. (1951) J. Exp. Med., 93, 373-383.

(112) Hirsch, R. L., and Kellner, A. (1956) J. Exp. Med., 104, 15-24.

(113) Olson, R. E. (1958) Perspect. Biol. Med., 2, 84-121.

(114) Slakey, L. L., and Craig, M. C. (1972) J. Biol. Chem., 247, 3014-3022.

(115) Huber, J., Latzin, S., Hamprecht, B. (1973) Hoppe-Seyler's Physiol. Chem., 354, 1645-1647.

(116) Green, T. R., and Levinthal, O. S. (1970) Anal. Biochem., 38, 130-138.

(117) Hartree, E. F. (1972) Anal. Biochem., 48, 422-427.

(118) Suzue, G., Orihara, K., Morishima, H., and Tanaka, S. (1964) Radioisotopes, 13, 300-303.

(119) Shapiro, D. J., and Rodwell, V. W. (1972) Biochem., 11, 1042-1045.

(120) McNamara, D. J., Quackenbush, F. W., and Rodwell, V. W. (1972) J. Biol. Chem., 247, 5805-5910.

(121) Shapiro, D. J., and Rodwell, V. W. (1972) Biochem., 11, 1042-1045.

VITA

Carolyn Frances Hunter

Candidate for the Degree of

Doctor of Philosophy

Thesis: REGULATION OF 3-HYDROXY-3-METHYLGLUTARYL COENZYME A REDUCTASE, MEVALONATE KINASE, 5-PHOSPHOMEVALONATE KINASE AND 5-PYROPHOSPHOMEVALONATE DECARBOXYLASE IN TURKEY LIVER AND KIDNEYS

Major Field: Biochemistry

Biographical:

Personal Data: Born in Fairview, Arkansas, August 20, 1952, the daughter of Bert F. and Mary Hunter.

Education: Graduated from Sparkman High School, Sparkman, Arkansas, in 1970; received the Bachelor of Science degree with a major in chemistry from Henderson State University, Arkadelphia, Arkansas, in May, 1974; completed requirements for the Doctor of Philosophy degree at Oklahoma State University in July, 1978.

Professional Experience: Laboratory assistant, Chemistry Department, Henderson State University, September, 1973 to May, 1974; NSF Summer Undergraduate Research Trainee, University of Arkansas, Fayetteville, Arkansas; Chemist -- Camden Paper Company, Camden, Arkansas, July 1, 1974, to July 31, 1974; Graduate Teaching Assistant, Department of Chemistry, Oklahoma State University, Fall Semester 1975, 1976 and 1977; Graduate Teaching Assistant, Department of Biochemistry, Oklahoma State University, Spring, 1977; Biochemist -- Phillips Petroleum Company, Bartlesville, Oklahoma, August 1, 1976, to August 31, 1976; Oklahoma Agricultural Experiment Station Research Assistant, September, 1974, to July, 1978

Professional Organizations: Associate member of the Society of Sigma Xi and a member of Phi Lambda Upsilon Honorary Chemical Society.