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INTO PROTEIN IN THE NORMAL, AGING AND
CATARACTOGENIC RAT LENS.

The City University of New York, Ph.D., 1973
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SELENOMETHIONINE TRANSPORT AND INCORPORATION
INTO PROTEIN IN THE NORMAL, AGING AND
CATARACTOGENIC RAT LENS

by

NORMAN A. SCHERZER

A dissertation submitted to the Graduate
Faculty in Biology in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy, the City University
of New York.

1973

This manuscript has been read and accepted for the Executive Committee in Biology in satisfaction of the dissertation requirement for the degree of Doctor of Philosophy.

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DEDICATION

This Doctoral thesis is dedicated to M.D.L.

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I wish to acknowledge with appreciation and gratitude the help and guidance given by my major sponsor, Dr. Morris Green. Deepest appreciation to my major committee members, Dr. Richard C. Mawe and Dr. Marcia Brody for their assistance and guidance. My deepest gratitude and appreciation to Mrs. Natalie Toran for her immeasurable encouragement, assistance and stimulating discussions during the entire course of this study. I am grateful for the expert advice of Dr. Erich Heinz and Dr. Harold Kern who have read this manuscript and contributed their suggestions and criticisms.

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I. INTRODUCTION

The studies presented in this manuscript are concerned with the transport of the amino acid Se-75-selenomethionine and its subsequent incorporation into protein in the rat lens. Influx, efflux and incorporation of selenomethionine were studied and related to the current knowledge of the metabolic activity in normal, aging and cataractogenic lenses.

Both amino acid transport and amino acid incorporation into protein are altered during early stages of cataractogenesis and undergo alterations during aging. These alterations may represent the primary factors responsible for the pathogenic changes in the lens. Efflux was extensively studied since it enabled us to observe the changes in membrane permeability (non-mediated diffusion) and changes in the membrane carrier system (exchange diffusion) independent of the energetic parameter present in active transport.

During the course of this study we focused our attentions on several specific topics which included: (1) characterization of selenomethionine transport; (2) the interdependency of amino acid transport and amino acid incorporation into protein; (3) the modes of action, in the lens, of several metabolic inhibitors; (4) alterations in diffusion and exchange diffusion during cataractogenesis and their similarities to changes during aging; (5) changes in protein synthesis during cataractogenesis as related to changes in lens metabolism; (6) dietary factors which alter protein synthesis and/or membrane transport.

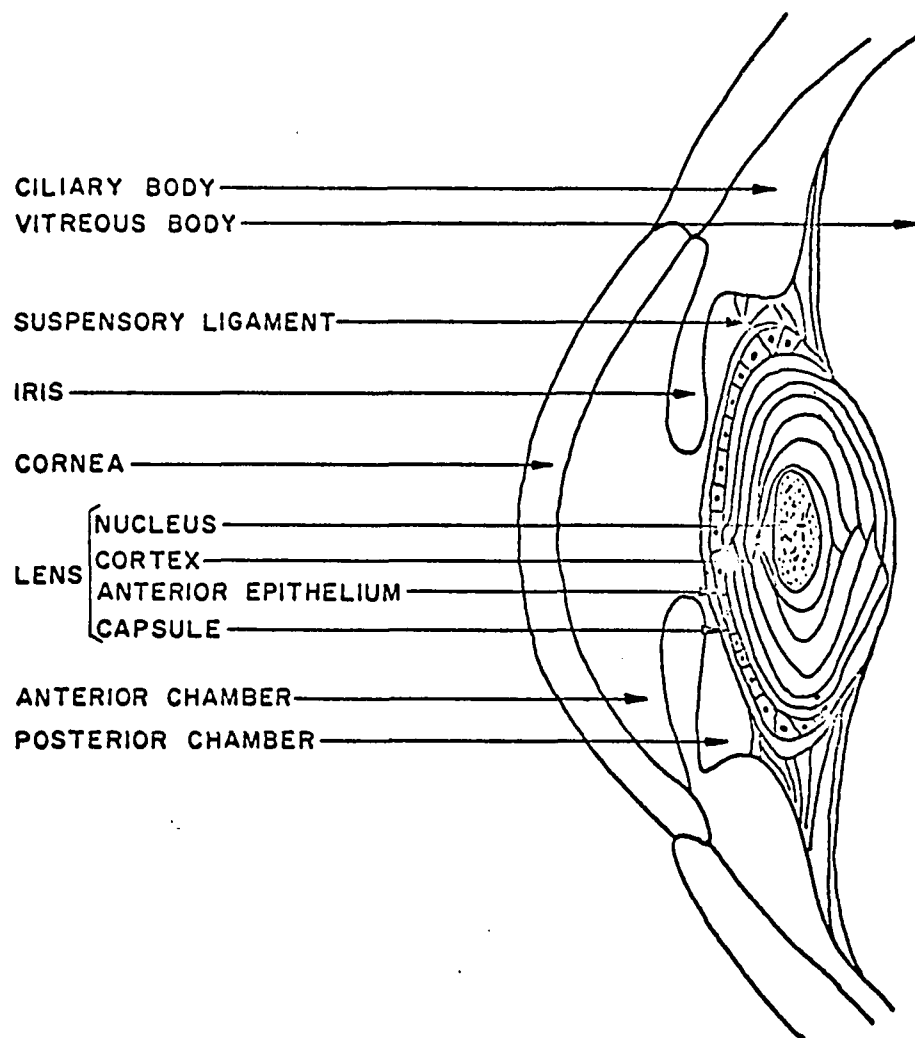


Figure 1. A diagrammatic section of the rat lens and associated structures of the eye. (from Pirie and van Heyningen, 1956).

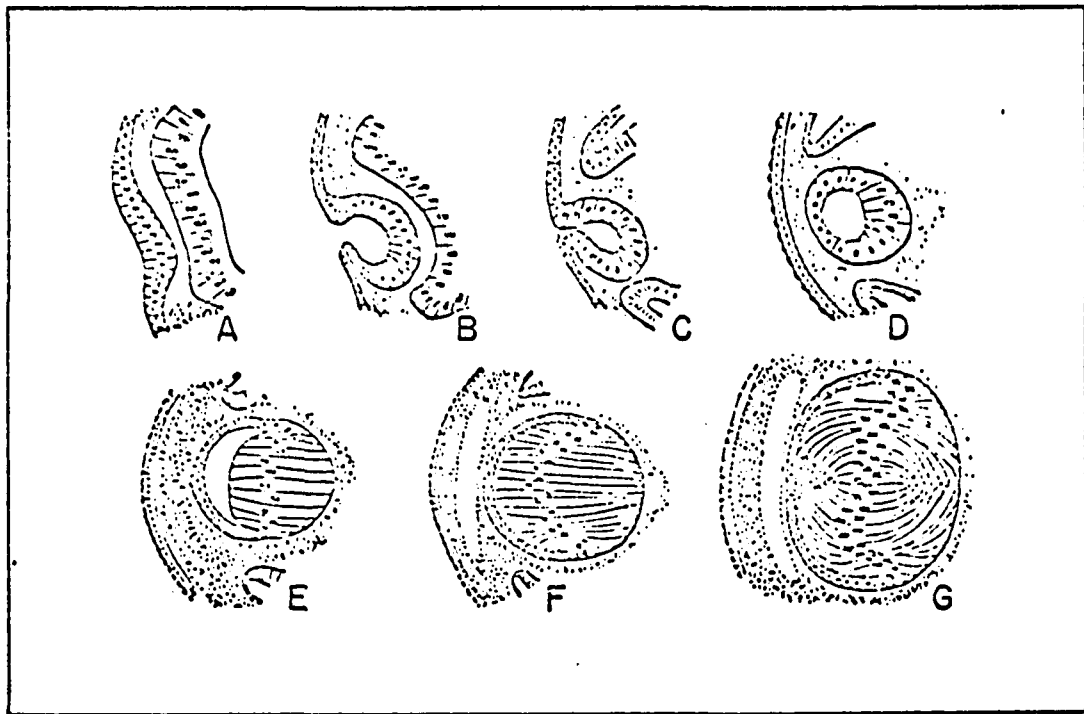


Figure 2. Development of the rat lens. A, ectodermal thickening; B, formation of the lens pit; C, closure of the lens pit; D, the lens vesicle; E, elongation of the posterior cells; F, obliteration of the lens vesicle cavity by the posterior cells; G, meeting of the posterior and anterior fibers. (from Pirie and van Heyningen, 1956).

The necessary background for the understanding of lens structure and function are presented in this introduction.

A. EMBRYOLOGY AND MORPHOLOGY OF THE LENS

The lens is a transparent, avascular spheroid-bi-convex organ bounded anteriorly by the iris and the aqueous humor of the eye, and posteriorly by the vitreous humor. The accommodation and support of the lens is accomplished via suspensory ligaments, the Zonules of Zinn, which attach to the lens equator and ciliary body (Fig. 1). The lens can be divided histologically and metabolically into four distinct regions: the capsule, the anterior epithelium, the cortex and the lens nucleus.

Development of the lens (Fig. 2) is initiated by a thickening of the neural ectoderm (lens plate) which then invaginates and pinches off to form a lens vesicle. The initial growth of the lens vesicle consists of an elongation of posterior cells into the vesicle cavity. Further growth is accomplished by increased mitotic activity (Srinivasan and Harding, 1965) of the germinative epithelium, 5 cells in width (Hanna and Keats, 1968), located at the lens equator. The migrating cells elongate and differentiate into lens fiber cells of the cortex within 6-10 weeks (ibid.). During their migration the cell nuclei enlarge and there is an increase in RNA synthesis (Hanna, 1965) and an increase in rough endoplasmic reticulum (Eguchi, 1964). Mitochondria are present in small number, the majority of which are confined to the anterior epithelium (Kuwabara, et, al., 1969). Within 8-10

weeks the fiber cells lose their nuclei as they become compressed towards the center of the lens where they become lens fibers (Hanna and Keats, 1968). There is a decrease in ribosomes and DNA synthesis (Kuwabara, et. al., 1969; Eguchi, 1964) accompanying the loss of nuclei. Thus, lenticular cells are isolated from surrounding tissue (van Heyningen, 1962b) and their compression towards the lens nucleus provides a continuous record of lens growth. The lens nucleus, in older animals, is very dense, amorphous in structure, and contains a large percentage of insoluble protein. During the course of lens growth the epithelial cells remain constant in number, with the rate of new epithelial cell generation equaling the rate of fiber cell formation (Coulombre, 1965).

The acellular capsule, collagenous in nature, is continually being secreted by the epithelium and posterior fiber cells. Injury to the epithelium results in an increase in mitochondrial cristae, and what appears to be a transfer of cytoplasm between cells (Gorthy, 1968), concomitant with increased mitotic activity (Srinivasan and Harding, 1965). Mechanical injury to the anterior or the posterior surface of the lens results in decreased ability of cation and amino acid transport, increased diffusion and loss of clarity (Harris and Becker, 1965).

B. METABOLISM

The lens derives its energy from the metabolism of glucose (Green and Solomon, 1957). The enzymes necessary for

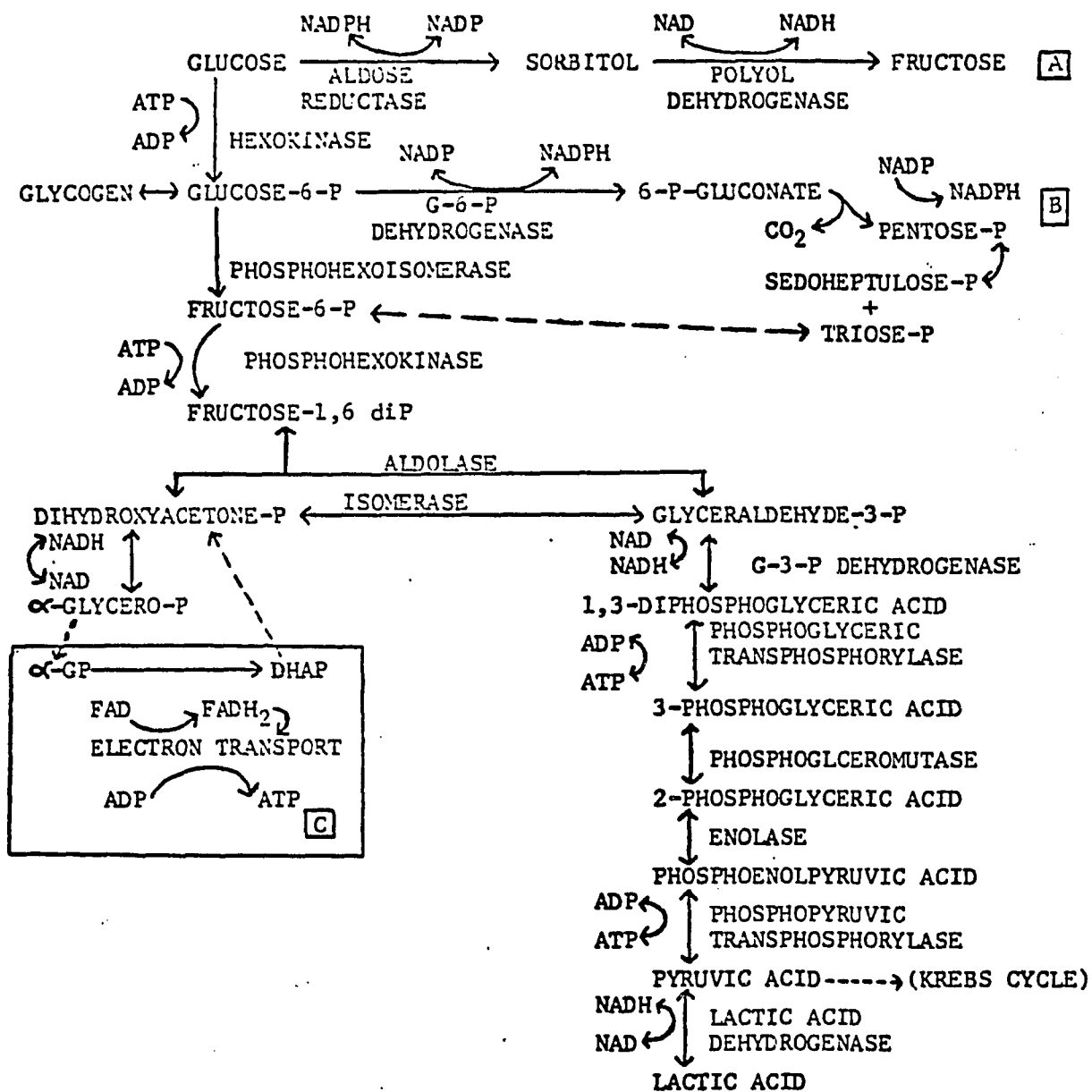


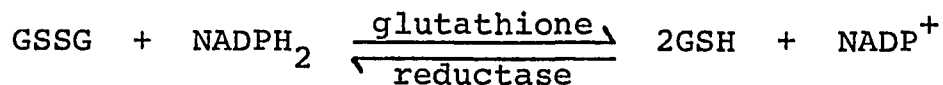
Figure 3. Pathways of glucose metabolism in the lens. A, sorbitol pathway; B, hexosemonophosphate shunt; C, α -glycerophosphate shuttle.

anaerobic and aerobic glucose metabolism have been found in the lenses of various species of mammals (Hockwin, 1965; Lerman, 1965; van Heyningen, 1965). The hexosemonophosphate shunt (Kinoshita, 1955), the sorbitol pathway (van Heyningen, 1962a) and the α -glycerophosphate shuttle (Pirie, 1962; Griffiths, 1966) have been demonstrated in the lens (Fig. 3). Although all the enzymes necessary for the operation of a citric acid cycle have been found in the lens (Wortman and Becker, 1956), there is minimal citric acid cycle activity (Harris and Gruber, 1962; Kinoshita and Merola, 1961) which accounts for no more than 2% of the glucose metabolism.

Glycolysis accounts for 85% of the glucose metabolized, with the remaining glucose being metabolized via the hexosemonophosphate shunt (HMP), (Kinoshita, 1965). The rate limiting steps of glycolysis are hexokinase (ibid.) and phosphofructokinase (Chylack et. al., 1970). Hexokinase exists in two forms in the lens, Type I with a K_m of $7.4 \times 10^{-5}M$ and Type II with a K_m of $10^{-3}M$. The epithelium contains more than 10% of the total lenticular hexokinase, all of which is Type II. The anterior cortex, contains 94% Type II and 6% Type I in rabbit (ibid.). An increase in external glucose concentration results in an increased HMP and sorbitol pathway activity, no change in lactate production (Kinoshita, 1965), an increase in the NADH/NAD ratio (Kuck, 1961) and an increased accumulation of α -glycerophosphate (Pirie, 1962).

The HMP shunt involves the reduction of NADP which under normal glucose levels is reoxidized, in the presence of

glutathione, by glutathione reductase (Kinoshita, 1955).



When excess sugar is present the oxidation of NADPH_2 can be accomplished by the sorbitol pathway (Fig. 3) which acts as a transhydrogenase system. No transhydrogenase has been found in the lens which would catalyze the transfer of hydrogen between NAD and NADP directly (van Heyningen, 1962b). Levvari et. al., (1964) has demonstrated that insulin, at glucose concentrations of 1mg/cc or less, can stimulate the activity of the HMP shunt.

The sorbitol pathway at normal glucose levels is almost inoperative since aldose reductase, which catalyzes the reduction of glucose to sorbitol, has a K_m of 10^{-1}M which is approximately 100 times greater than the K_m of hexokinase (Kinoshita, 1965). Elevated levels of glucose or galactose result in the appearance of polyols in the equatorial and anterior regions of the lens (Kinsey et. al., 1958; Kuck, 1961). The oxidation of sorbitol to fructose is accomplished by polyol dehydrogenase and results in the production of NADH_2 . Polyol dehydrogenase is inhibited by reduced glutathione, EDTA and cysteine (Smith, 1962).

That the α -glycerophosphate shuttle is the principal oxidative metabolic pathway for glucose has been suggested by Pirie (1962) and Griffiths (1966). Pirie had observed increased levels of α -glycerophosphate, primarily in the anterior cortex, after incubating lenses in high concentrations of glucose or fructose diphosphate; while Griffiths

had demonstrated the existence of the components necessary for the operation of the shuttle. The incubation of lenses aerobically in the presence of pyruvic acid does not result in an increase in ATP levels while elevated glucose levels under aerobic conditions result in an elevation of ATP (Kinoshita and Merola, 1961). Although the energy required for the transport of amino acids (Kern, 1962) and cations (Becker, 1962; Harris and Gruber, 1962) is derived from glycolysis, the incorporation of amino acids into protein is inhibited by anaerobiosis in the absence of glucose, and by dinitrophenol (Kinoshita, Kern and Merols, 1961).

Thus, glucose, at normal levels, appears to be metabolized via the Embden-Myerhoff pathway and the hexosemonophosphate shunt concomitant with an operative α -glycerophosphate shuttle. The oxidation of HMP derived NADPH is accomplished by glutathione reductase, and the reoxidation of NADH is accomplished by lactic dehydrogenase and in all probability the α -glycerophosphate shuttle. At elevated glucose levels the sorbitol pathway and the HMP shunt are increased, with the former acting as a transhydrogenase system for the oxidation of increased levels of NADPH and subsequently producing NADH. Increased levels of glucose also increase the concentration of α -glycerophosphate which in turn increases shuttle activity.

C. LENTICULAR PROTEINS AND LIPIDS

The lenticular proteins have been classified by Morner (1894) into three soluble fractions, α , β and γ crystallins, and an insoluble fraction denoted as albuminoid. Each of the soluble fractions have been demonstrated by Spector (1965) to consist of subunits. The α and β crystallins appear to be structural while the γ crystallins are believed to be associated with the differentiation of epithelial cells into fiber cells (Papaconstantinou, 1967).

The N-terminal groups of α -crystallin and albuminoid have been determined by Mok et. al., (1968) to consist of N-acetylmethionine. The methionine content in each of the soluble and albuminoid fractions (expressed as methionine residues per thousand amino acid residues) are:

α - 11 to 13 (Li and Spector, 1972; Lerman et. al., 1968), β - 16, γ - 27 and albumoid - 22 (ibid.) Incorporation of labeled arginine, aspartate and histidine into calf lenses was found to be greatest in the γ crystallin fraction, with the equatorial region and the anterior surface having the highest specific activity (Spector and Kinoshita, 1964). The incorporation of S^{35} -methionine into rabbit lens proteins, after 21 hours incubation, resulted in 90% of the activity being located in

the anterior epithelium, 8% in the cortex and 2% in the nucleus (Lessinger et. al., 1960). The S^{35} label of S^{35} -methionine was detected, in small quantities, upon prolonged incubation in cysteine and glutathione (ibid.) The turnover rate for the total protein pool has been estimated at 3% per month in the rat (Young and Fulhorst, 1966). The lens contains a proteinase that digests α_2 crystallin and which is inhibited by the presence of β crystallin or N-ethylmaleimide (Waley and van Heyningen, 1962).

The fatty acid composition of the lens is similar to that of other neural ectoderm. There is high proportion of saturated (43%) and monounsaturated (43%) fatty acids, with oleic and palmitic acid being present in the greatest quantity (Turchetto et. al., 1963). The greatest concentration of phospholipids is found in the mitochondrion (ibid.). Cholesterol is present in the rat lens (0.6 to 0.8 mg/g of lens), and accounts for 10% of the total lens lipids (van Heyningen, 1969b). Lipoproteins and glycolipoproteins have also been detected in the lens (Dische, 1965).

D. LENTICULAR TRANSPORT

The lens receives its supply of nutrients from the aqueous humor which resembles an ultrafiltrate of the blood

plasma (Reddy, 1967). In rabbits the concentration of amino acids in the aqueous humor is higher than in the plasma or vitreous humor (Reddy, Rosenberg and Kinsey, 1961). Reddy and Kinsey (1962a) have demonstrated in vivo the active transport of amino acids by the ciliary body. The active transport of amino acids into the aqueous humor of rabbit and calf appears to be the exception since rat, cat and several species of monkeys have amino acid levels in the aqueous humor identical to plasma levels (Reddy, 1967). In the latter group of animals the transport of amino acids into the aqueous humor is via a mediated (saturable) diffusion (ibid.).

The vitreous humor contains glucose (Davson and Duke-Elder, 1948), ascorbic acid, sodium (Reddy, 1966) and amino acids (Reddy, Rosenberg and Kinsey, 1961) in low concentrations. Chylack and Kinoshita (1972) have demonstrated that the vitreous is protective in nature, with respect to the lens, in that it is not readily permeable to a variety of smaller molecules (i.e., glucose).

The lens capsule is permeable to small molecules, but not to high molecular weight proteins (Francois and Rabaey, 1958). Non-surgical removal of the capsule (i.e., collagenase) did not alter cation permeability (Kinsey and Reddy, 1965) but did result in a slight increase in α -AIB efflux (Kern, 1970).

The existence of a lenticular active transport system was first demonstrated in vitro for cations by Kinoshita, Kern and Merola (1961) and for amino acids by Kern (1962). The presence of free amino acids in the lens at concentrations higher than that found in the aqueous humor, and the accumulation of α -AIB by the lens, in vivo, against a concentration gradient further substantiated the existence of an active transport system (Reddy and Kinsey, 1962a, 1962b). The active accumulation of potassium and α -AIB, and the extrusion of sodium is determined by the anterior epithelium and not by the fiber walls (Kinsey and Reddy, 1965). The passage of chloride and water through the epithelium is via passive diffusion (ibid.). Thoft and Kinoshita (1965) have demonstrated the necessity of calcium for maintenance of rubidium and α -AIB accumulation. Calcium deficiency increases the passive efflux of rubidium and α -AIB from the lens and increased the sucrose and mannitol space but not the inulin space (ibid.). The estimates of the extracellular space of the lens range from 6 to 10% depending upon the substance utilized.

Cation transport has been studied extensively in the lens by the use of K^{42} , Na^{22} and Rb^{86} . The excretion of sodium and the accumulation of potassium is associated

with a membrane bound Na-K-ATPase located almost exclusively in the anterior epithelium (Bonting et. al., 1961). Rubidium is actively accumulated by the lens (Becker, 1962) and within three hours, in the rat, it reaches a T/M (tissue/medium) ratio of 7, with the bulk of the label located near the epithelium (Cotlier and Becker, 1965). The Na-K pump can be supported entirely by anaerobic glycolysis, and is inhibited by ouabain, iodoacetate and low temperature (Kinoshita, Kern and Merola, 1961; Kern, Roosa and Murray, 1962; Harris and Gruber, 1962). Failure of the pump leads to increased hydration of the lens (ibid.). Sodium exists in a free and bound form, with the capsule-epithelium layer containing only free sodium, and the cortex and nucleus containing 7% and 20% non-exchangeable sodium respectively (Paterson, 1970a). Inhibition of the pump by iodoacetate is complete within two hours, in rabbit lenses, while ouabain inhibition is slower acting (Kinsey and Reddy, 1965). Removal of the surface membranes of the lens produces alteration in lenticular cations similar to those found with ouabain (ibid.). The kinetics of the non-mediated efflux of both Rb^{86} and Na^{22} appears to be a two-component curve, the nature of which has not been determined (Paterson, 1970b).

Glucose is facilitatively transported into the lens and its net uptake at 37°C is more dependent upon its utilization than on the capacity of the carrier mediated facilitation (Farkas and Roberson, 1964). Pentoses do not compete with glucose for entry into the lens, while fructose does compete (Kern, 1965), as does galactose (Kern, personal communication). Glucose transport appears to occur at the lenticular fiber membrane, and is inhibited by phlorizin, N-ethylmaleimide and iodoacetate (Patterson, 1965). Polyols (i.e., sorbitol, dulcitol and xylitol) do not permeate lenticular membranes (van Heyningen, 1962a).

The active transport of amino acids can be supported entirely by glycolysis in the absence of oxygen (Kern, 1962). The incubation of calf lenses, for 20 hours, in the presence of proline, tyrosine or tryptophan results in high T/M ratios, with the greatest concentration of amino acid being located near the epithelium (ibid.). The injection of labeled α -AIB intravenously into rabbits results in high levels of α -AIB in the lens, with respect to the aqueous, 80% of which is attributed to active transport and 20% to passive diffusion (Kinsey and Reddy, 1962). Competitive inhibition by other amino acids has been demonstrated

in calf (Kern, 1962) and rabbit (Kinsey and Reddy, 1963). Amino acid accumulation is not inhibited by dinitrophenol (Kern, 1962; Kinsey, 1965) but is inhibited by iodoacetate (Kinsey and Reddy, 1963). Ouabain inhibits the transport of hydroxyproline (Kern, 1962) and α -AIB (Kinsey and Reddy, 1965) and is associated with the sodium dependent transport of amino acids. Removal of the capsule and epithelium results in minimal accumulation of amino acids which is uniform on all lens surfaces (ibid.). The transport of taurine into the lens is similar to β -alanine transport; it has a high Q_{10} (5); it is rapidly inhibited by ouabain and has peculiar saturation kinetics (Reddy, 1970).

The A, L, and X sites described by Oxender and Christensen, (1963) for the transport of neutral amino acids in Ehrlich Ascites tumor cells, have been identified in lens by Brassil and Kern (1968). The A and X sites are associated with small, low molecular weight amino acids, (i.e., glycine, α -AIB) while the L site is associated with larger amino acids with bulky side chains (i.e., leucine, methionine; Christensen, 1962). There appears to be a deficiency of A sites in the rat lens (Brassil and Kern, 1968) as is the case in mammalian red blood cells (Inui and Christensen, 1966). Sodium is

required for the transport of several amino acids into the lens (Cotlier and Beaty, 1967). Kern and Brassil (1968) have demonstrated that the A and X sites in lens are sodium dependent, but not the L site. The lack of sodium appears to alter only the mediated accumulation of amino acids since no increase in diffusion is observed (ibid.).

The efflux of amino acids from the lens occurs via passive diffusion and exchange diffusion (Kern, 1970). Exchange diffusion of an amino acid can be demonstrated by the presence of the same amino acid or a competitive amino acid on the trans side of the membrane (Heinz and Walsh, 1958). In lens, Kern (1970) has demonstrated that exchange is associated primarily with the L site and is relatively sodium independent.

E. AGING

Aging has been studied primarily with young and mature animals, but rarely with animals that were in the last 20% of their life span. Thus, many of the changes reported that occur with age do not necessarily reflect the conditions at the time of senile cataract development.

Anaerobic glycolysis remains the predominant pathway of glucose utilization throughout life (Lerman et. al., 1962; Lerman, 1965) although lactate production, per mg of lens, decreases with age (Fig. 4, Sippel, 1965). The specific activity of hexokinase decreases (Green and Solomon, 1959) but the total activity increases with age (Chylack et. al., 1970). Lactic dehydrogenase (Bernstein et. al., 1966) and most of the other glycolytic enzymes (Hockwin, 1965) decrease with age. The uptake of glucose decreases with age (Muller et. al., 1956) as a result of its decreased metabolism while the free glucose pool remains unchanged (Kuck, 1963). The level of pyridine nucleotides (NAD, NADP) does not change during lens growth (Sippel, 1962).

Hexosemonophosphate shunt activity as a function of age has not been resolved; Kinoshita and Wachtl (1958) reported decreasing activity while Sippel (1965) infers an increase in activity. Glutathione levels, beyond the first two months of age, remain constant (Sippel, 1962).

Increases in the concentration of sorbitol, fructose (Kuck, 1965) aldose reductase (Hayman and Kinoshita, 1965) and polyol dehydrogenase (Hockwin et. al., 1966) indicate increased sorbitol pathway activity with age. The increase in fructose may be due in part to the decreased

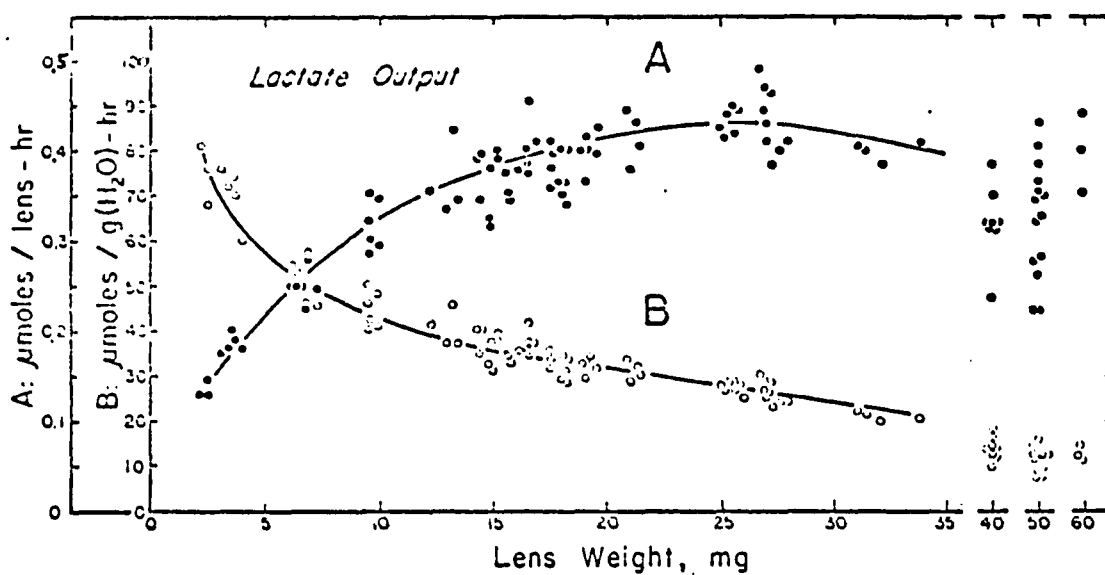


Figure 4. Lactate production by the rat lens as a function of lens weight (Sippel,1965).

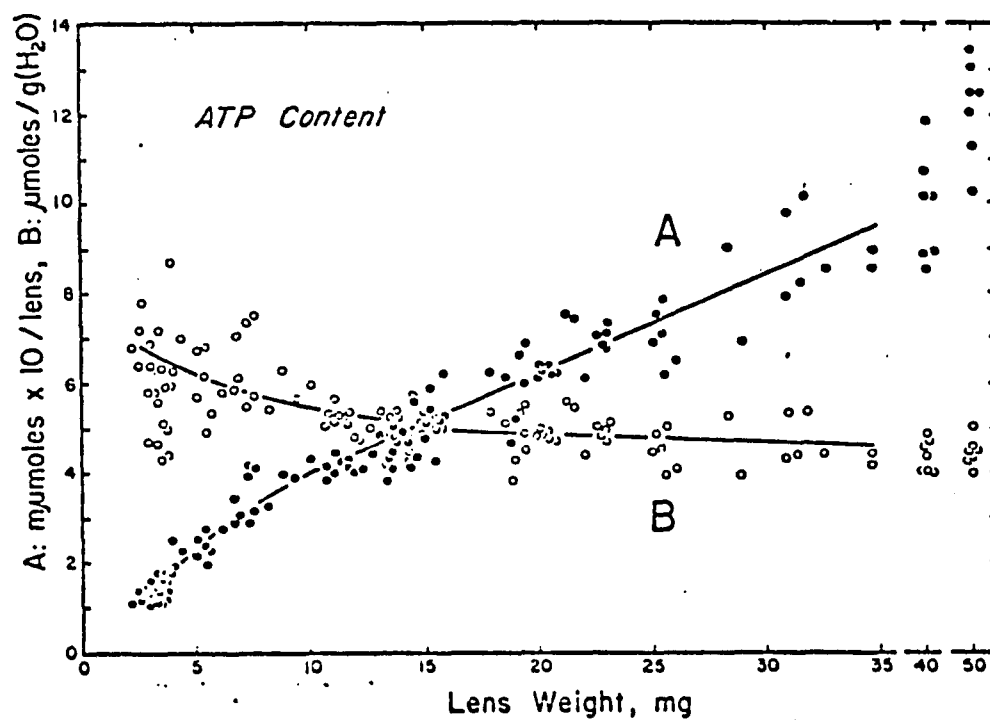


Figure 5. The ATP content of the rat lens as a function of lens weight (Sippel,1965).

permeability of lenticular membranes to fructose (Kuck, 1965). Adenosine triphosphate increases almost linearly with lens growth (Fig. 5, Sippel, 1965) with little change in concentration. The concentration of α -glycerophosphate (Pirie, 1962) decreases with age. Total oxygen consumption is relatively constant beyond the first few weeks of life (Fig. 6, Sippel, 1965). Sippel (1965) postulates a shift from anaerobic to aerobic glycolysis with age. In general, metabolic activity when expressed as specific activity decreases. This is a reflection, in part, of regions of the lens which are metabolically inactive.

Lens growth (Fig. 7) is an exponential function of age (Hockwin et. al., 1963; Hockwin, 1965), with the lens exhibiting a rapid decline in mitotic activity (von Sallman, 1966) and cell migration (Srinivasan and Harding, 1965) on maturation. Incorporation of amino acids into protein decreases with age (Lessinger and Mandel, 1960) although methionyl-tRNA synthetase activity increases with age (Weller and Green, 1969). Satoh (1972) found a sharp increase in the insoluble protein and α crystallin in human lenses beyond the age of 40, no changes in β crystallin and a drop in γ crystallin early in life. Amino acid levels decrease with age, with a drop in methionine which parallels an increase in taurine (Dardenne and Kirsten, 1962). The ability

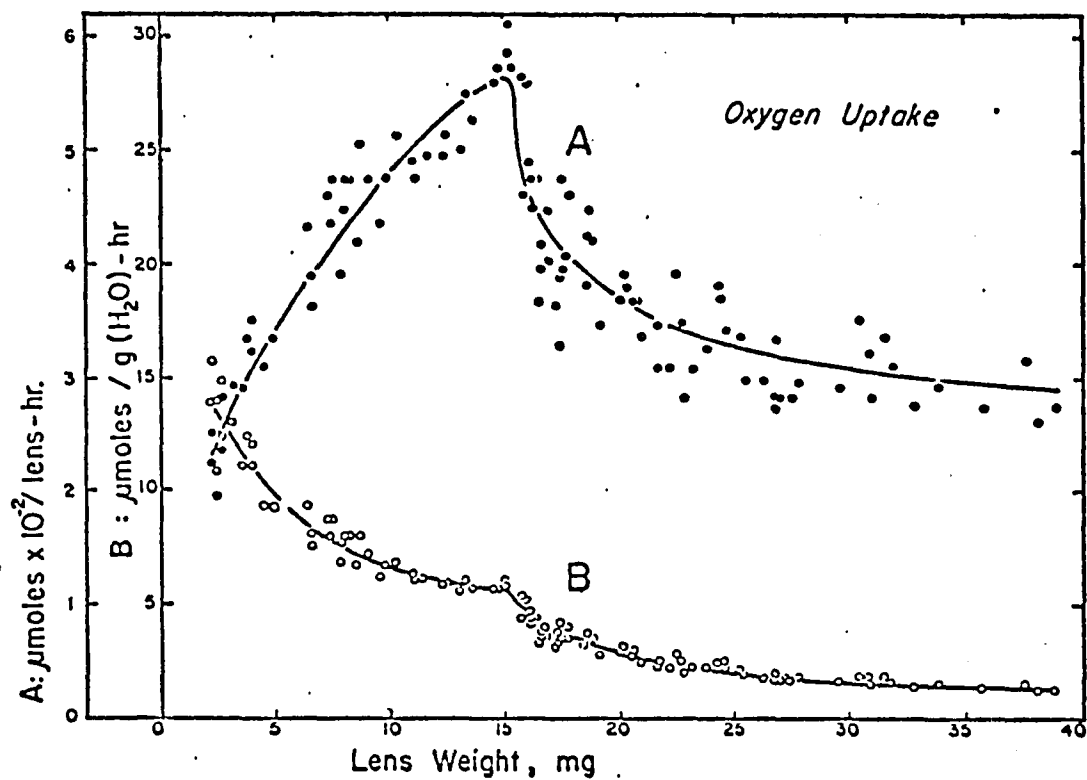


Figure 6. Oxygen consumption by the rat lens as a function of lens weight (Sippel, 1965).

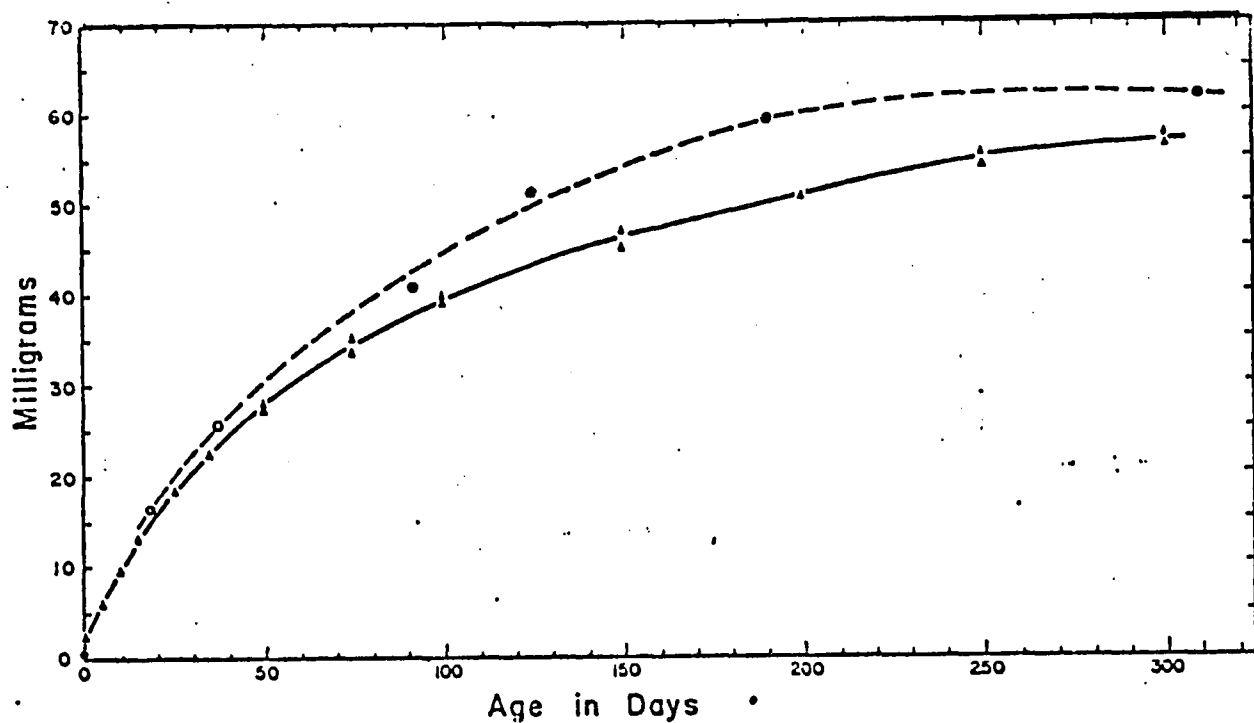


Figure 7. Lens weight as a function of age, in the rat; solid line refers to the Wistar strain (Sippel, 1965), dashed line refers to Sprague-Dawley strain used in this study.

of the rat lens to metabolize methionine to other substances is markedly decreased in old lenses (Lessinger and Mandel, 1960).

F. CATARACTOGENESIS

Cataracts can be induced in animals by feeding high levels of galactose (Patterson, 1953) or xylose (Lerman and Heggeness, 1961) or by the induction of diabetes (Pirie, 1965). The production of cataracts by chemical agents, x-irradiation, microwaves, dietary deficiencies, viruses, hypoparathyroidism and osmotic changes is reviewed thoroughly by van Heyningen (1962b). This discussion will emphasize the two experimental cataracts utilized in this paper, galactose and xylose cataracts.

The induction of dietary cataracts is more rapid in younger animals than old (Lerman, 1965) and the time of appearance is dependent upon the concentration of the cataractogenic sugar in the plasma (Patterson, 1954, 1955; Keiding and Mellemaard, 1972). Ligation of the carotid artery or the presence of phlorizin (lowers blood sugar) result in the delay of opacification (Patterson, 1955). The presence of high concentrations of protein and/or lipid in a galactose diet will delay the appearance of the opacity (Patterson, 1955, 1965;

Patterson et. al., 1962). Feeding galactose or xylose results in the appearance of high concentrations of corresponding sugar alcohols (dulcitol and xylitol, van Heyningen, 1959) while high concentrations of glucose result in the accumulation of sorbitol (van Heyningen, 1962a). Kinoshita, Merola and Dikmak 1962a, b, c) proposed that the formation of sugar cataracts was related to osmotic changes, a theory now generally accepted.

Accumulation of certain sugars (glucose, galactose, xylose and arabinose) result in their conversion into their corresponding sugar alcohols (sorbitol, dulcitol, xylitol and arabitol, respectively) via the sorbitol pathway. The first step of this pathway is mediated by aldose reductase, which requires NADPH as a cofactor and which has a higher K_m than hexokinase. The nucleotide NADPH is furnished by increased HMP shunt activity. The polyols do not permeate lenticular fibers, and hence accumulate. The enzyme polyol dehydrogenase does not utilize dulcitol as a substrate and its affinity is low for sorbitol and xylitol. As a result of the elevation of polyols in the lens, water is drawn in to maintain osmotic equilibrium. Osmotic swelling first results in the appearance of hydropic

lenticular fibers and then vacuolation of the cortical fibers which become filled with precipitated proteins (Kuwabara et. al., 1969).

Evidence supporting the theory of osmotic swelling includes: accumulation of polyols in high concentrations in lenses from rats fed a high cataractogenic sugar diet (van Heyningen, 1962a); increased HMP shunt activity with no further increased lactate production when lenses are incubated in high levels of galactose (Kinoshita, et. al., 1963); the inhibition of cataract formation and permeability changes by osmotic compensation in the media during the incubation of lenses in the presence of galactose (Kinoshita et. al., 1969), or by co-incubation with tetramethylene glutaric acid (TMG, inhibitor of aldose reductase, ibid.); and by the parallelism between hydration and dulcitol accumulation in lenses of galactose fed rats (Kinoshita et. al., 1962b). In 1964, Kinoshita and Merola attributed the formation of nuclear cataracts, accompanied by further hydration, to the limited operation of the sodium-potassium pump concomitant with sodium leakage into the lens, which in turn draws chloride into the lens. The high concentrations of sodium chloride result in further imbibition of water.

As sugar cataracts progress there are gross changes in the biochemistry and physiology of the lens. The free amino acid pool is decreased within two days (Reddy, 1965; Barber, 1968; Kinoshita et. al., 1969), followed by a decrease in the net synthesis of protein (Dische et. al., 1957) and a decline of total soluble protein in the lens (Sippel, 1966a). Charlton and van Heyningen (1968) have shown that the loss of low molecular weight proteins parallels the loss of endogenous inositol. A new protein forms, derived from beta crystallin, which precipitates upon oxidation (Dische et. al., 1957). The synthesis of insoluble proteins appears to be unaffected during the course of cataract development (ibid.). Sippel (1966a, b) has reported that galactose feeding results in a decrease in: oxygen uptake (Fig. 8), lactate production (Fig. 9), ATP content (Fig. 10) and glutathione content (Fig. 11). The HMP shunt decreases after one week of galactose feeding (Lerman, 1960) while ADP and AMP levels do not change for at least nine days (Keiding et. al., 1972).

Most enzymes retain their normal activity during the first two weeks of galactose diet and then begin to decrease (i.e., lactic dehydrogenase, glucose-6- PO_4 dehydrogenase, Sippel, 1967; creatine phosphatase, Cotlier,

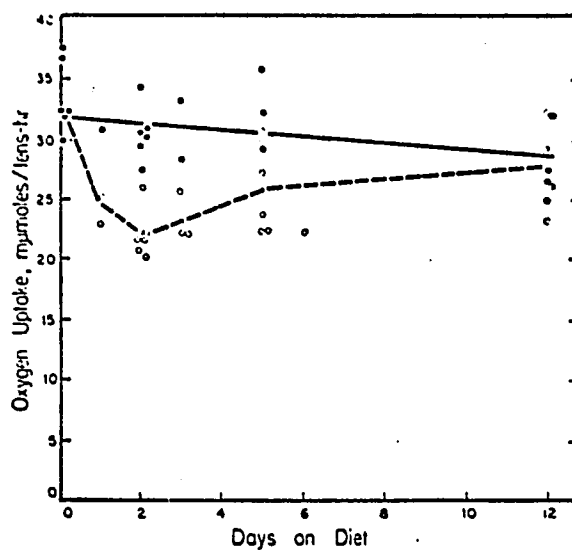


Figure 8. The effect of galactose feeding on oxygen uptake by the rat lens; closed circles- controls, open circles- galactose fed, (Sippel, 1966a).

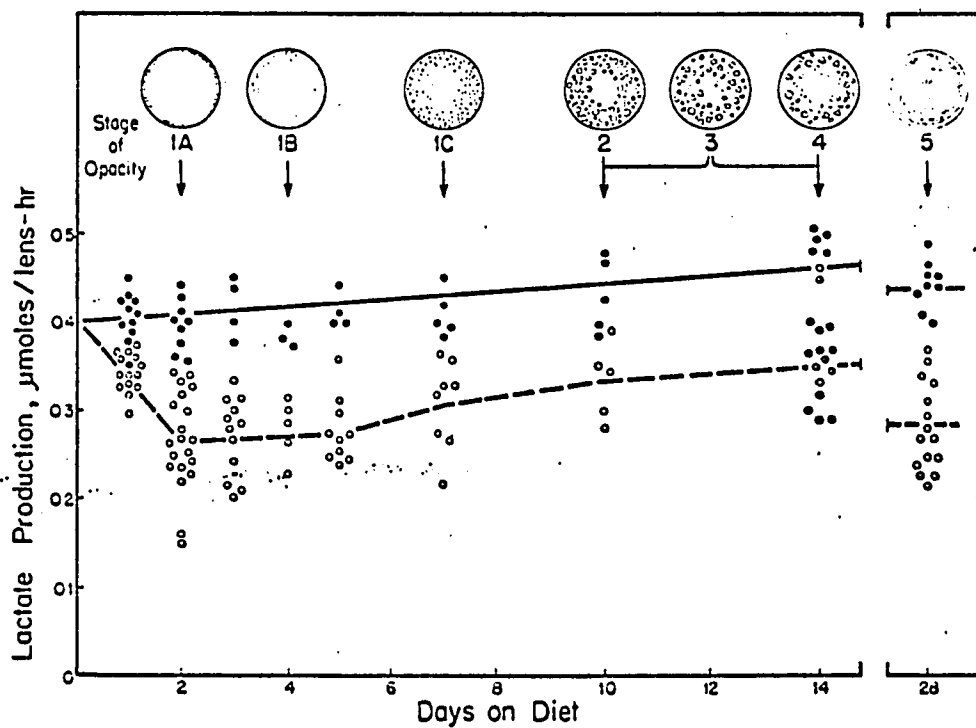


Figure 9. The effect of galactose feeding on lactate production by the rat lens; closed circles- controls, open circles- galactose fed, (Sippel, 1966a).

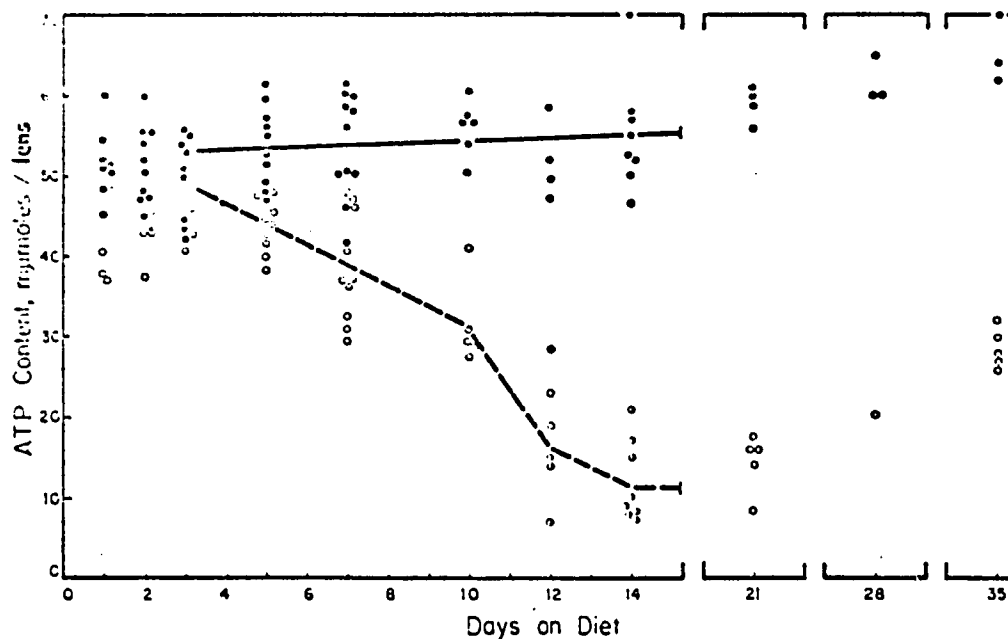


Figure 10. The ATP content of lenses from rats fed a galactose diet; closed circles- controls, open circles- galactose fed, (Sippel,1966a).

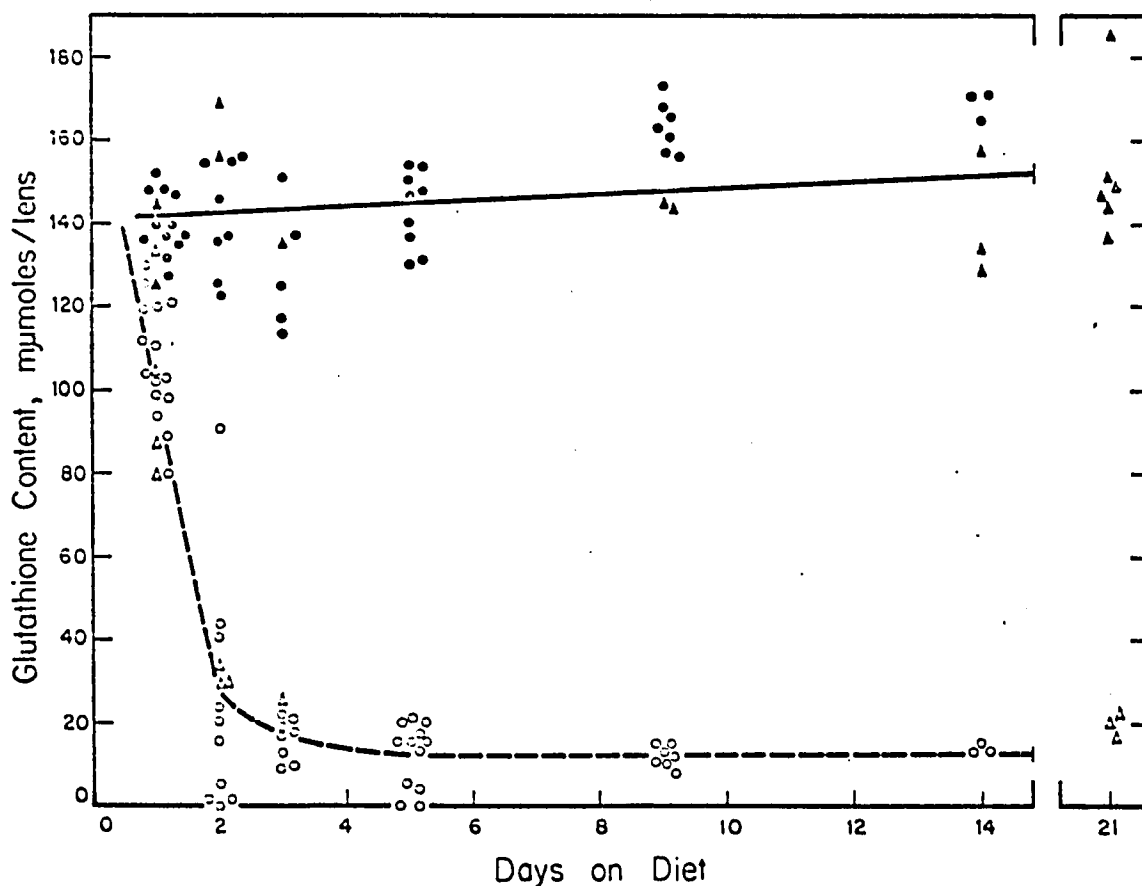


Figure 11. Glutathione content of lenses from rats fed a galactose diet; closed circles- controls, open symbols- galactose fed, (Sippel,1966b),

1964). Aldolase was found to decrease at the same rate as glutathione during galactose feeding (Sippel, 1967).

Cation transport is impaired within two days of galactose feeding (Cotlier and Becker, 1965). Incubation of lenses in the presence of 30mM galactose cause an efflux of potassium accompanied by osmotic swelling (Kinoshita, Lorenzo et. al., 1968). Increasing the tonicity of the media prevented both the loss of potassium and hydration, although dulcitol levels were high (ibid.). In vivo there is no change in cations during the early stages of vacuolization, but only after osmotic swelling (ibid.). Accumulation of cycloleucine into the aqueous humor and lens, in rabbits, was inhibited 50% within two days of galactose feeding (Reddy and Kinsey, 1963). Kinoshita, Merola and Hayman (1965) demonstrated that lenses co-incubated with high levels of galactose or glucose accumulate less α -AIB and cycloleucine, but not when co-incubated with fructose. The inhibition of accumulation is due in part to an increase in the passive diffusion of the amino acid out of the lens (ibid.). The amino acid content in lenses of galactose fed rats is decreased within two days (Reddy, 1965). This may explain in part the

decreased amino acid incorporation into protein. Lerman and Devi (1961) attribute the early decrease in amino acid incorporation in galactose fed rats, to unavailability of ATP; addition of ATP, in vitro, resulted in normal incorporation values. Grimes and von Sallman (1968) report an increase in the mitotic activity in lenses of rats fed galactose, but not xylose, during the first few days of the diet.

The free methionine content in lenses from galactose fed rats decreases 90% within one week, while glucose diets (7-14 days) result in a 56% decrease (Kinoshita et. al., 1969).

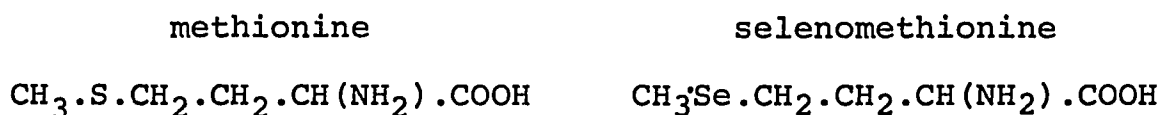
Proteolipids are decreased in the lenses of galactose fed rats, and there is a predominance of unbound lipid (Feldman and Feldman, 1965). Hatcher and Andrews (1970) report changes in the composition of long chain unsaturated fatty acids, concomitant with a decrease in the fatty acid content during the first two weeks of a galactose diet. An unidentified fatty acid is present on the 15th day of the diet.

Xylose cataracts are not progressive, nor are they permanent with respect to opacification (Lerman and Heggeness, 1961). The cataract progresses initially in proportion to the xylitol content of the lens, followed by a regression by 14 days (van Heyningen, 1969a). At

the time of regression the content of xylose in the plasma is decreased indicating a compensatory response at the animal (ibid.).

G. SELENOMETHIONINE

Selenomethionine is an analogue of methionine in which the sulfur has been replaced with selenium.



Selenomethionine was first produced by Painter (1947) in the racemic form, and later isolated as L-selenomethionine from yeast grown on methionine-free medium containing sodium selenide (Blau, 1961). Selenomethionine was intensely investigated in the early 1960's when Blau and Bender (1962) found that human pancreas could be "visualized" after administration of the compound. Proteins containing selenomethionine function similarly to the corresponding methionine containing proteins (Shrift et. al., 1961; Cowie et. al., 1957; Cohen et. al., 1957). Both analogues mimic one another in transmethylation reactions (Bremer et. al., 1960; Mudd et. al., 1957). Spencer and Blau (1962) were the first to use the radioactive analogue, Se^{75} -selenomethionine for the study of amino acid transport. The

transport system of Se^{75} -selenomethionine and S^{35} -methionine, in the presence of unlabeled methionine, had the same apparent K_m , as observed in the transport through the everted intestinal sacs of hamsters (ibid.).

Se^{75} -selenomethionine has a half life of 120 days; and is produced by neutron bombardment of Se^{74} . Decay is by K capture with the emission of gamma rays only. The energies of the resulting gamma rays (electron capture) range from 0.024 MeV to 0.401 MeV, with two major peaks of 0.136 and 0.265 MeV.

II. MATERIALS AND METHODS

A. ACCUMULATION AND PROTEIN INCORPORATION

After decapitation, lenses from Sprague-Dawley (Charles River, C.D.) rats were removed from the enucleated eye via a posterior incision. Incubation was usually in 0.5cc pH 7.3 Krebs-Ringers phosphate buffer (Table I) containing 100mg% d(+)dextrose, 0.5 μ Ci L-Se-75-selenomethionine (Amersham-Searle) and 0.034 micromoles D.L-selenomethionine (Sigma). After incubation, at 35-37°C, lenses were removed, washed with Krebs-Ringers phosphate buffer, placed into 0.50cc 5% trichloroacetic acid (TCA) containing 1mg/cc L-methionine, and counted to determine total accumulation. Lenses were then homogenized and washed three times with 5% TCA containing methionine. The precipitate was counted to determine incorporation of the label into protein. In some experiments the precipitate was further washed with hot 5% TCA (90°C) and with a mixture of ethanol: ether: chloroform (2:1:1: v/v/v) to determine the incorporation of the label into RNA and lipid TCA precipitable fractions respectively. All counting was carried out with a Nucleonic Corp. of America RCR-3 or RCG-3A scaler in conjunction with a one half inch or one inch scintillation crystal.

Table I. Krebs-Ringer-Phosphate Buffer (pH 7.3).

50.0 ml	0.77M	Sodium Chloride
2.0 ml	0.77M	Potassium Chloride
0.5 ml	0.55M	Calcium Chloride
0.5 ml	0.77M	Magnesium Sulfate
50.0 ml	0.10M	Phosphate Buffer *
212.0 ml		Water

* Phosphate Buffer (pH 7.3): 17.8 g $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$
and 20 ml 1.0 N HCl, dilute to 1 liter.

B. EFFLUX

A new procedure was developed to facilitate the handling of the lens with minimum trauma during efflux studies. A paraffin cup (Fig. 12) was constructed to contain the lens during the pulse and efflux phases of the experiments. The cup, approximately 8mm in diameter and 7.5mm deep, was perforated to allow fluid exchange between the cup and the surrounding media. The cup was held submerged by a nichrome wire which maintained the cup base 3 to 4 mm from the vial base.

Lenses were pulsed for one hour in 1.5cc of dose. Each cup containing a lens was removed from the dose vial containing buffer. Lenses with low counts were assumed to be surgically traumatized and were discarded. Thus all lenses used in the experiment had about the same radioactivity. Each cup, containing a lens, was transferred through its own series of efflux vials. The efflux time intervals were 5, 5, 5, 15, and 30 minutes for a total efflux time of 90 minutes. This system minimized trauma during transfer. Short efflux periods and relatively large ratio of medium to lens water minimized reabsorption of the labeled amino acid. Efflux was determined by counting the Se-75-selenomethionine present in each of the efflux vials and by counting the radioactivity in the lens.



Figure 12. Preparation of the Lens Loop. Nichrome wire was formed into a circular loop on one end and trimmed to eliminate sharp ends. The loop, which was bent at a 90° angle to the stem, was then dipped into melted paraffin so that a liquid skin formed over the loop. The loop was quickly placed into warm water so that the paraffin solidified to a pliable state. A glass rod was pushed gently into the loop, depressing the paraffin and thus forming a wax cup. This cup is approximately 8 mm in diameter and 7 mm deep. After allowing the wax to harden fully a hot pasteur pipet was used to perforate the cup by gently pushing the pipet through the wax from the inside towards the outside, thus eliminating any protrusions within the cup. The upper part of the stem was bent to facilitate hanging on the sides of vials.

Lenses were pulsed for 90 minutes with a high dose (1×10^6 c/m) of Se-75-selenomethionine followed by a two hour period of efflux under exchange diffusion conditions. This effluxed radioactivity (presumably unaltered selenomethionine) was then subjected to amino acid analysis, (see section IIE).

In most efflux experiments two conditions were investigated - labeled amino acid effluxing into media with or without unlabeled selenomethionine. The former represents total efflux (T) while the latter represents diffusion (D). Exchange diffusion (E) for any time period can thus be expressed as: $E = T - D$.

C. CALCULATIONS

Accumulation is expressed as:

$$\% \text{ Uptake} = \frac{\text{total c/m in lens}}{\text{c/m in dose}} \times 100$$

Protein incorporation is expressed as:

$$\% \text{ Protein Incorporation} = \frac{\text{c/m in lens protein}}{\text{total c/m in lens}} \times 100$$

In all of the efflux data c/m in lens is equal to the total c/m in the lens minus the c/m incorporated into protein. Percent cumulative efflux is expressed as:

$$\% E_{\text{cum}} = \frac{\text{cumulative c/m effluxed at time t}}{\text{total c/m accumulated by the lens}} \times 100$$

where t = any efflux time interval from 0-90 min.

The apparent efflux rate per minute for any time period is given as follows:

$$K \text{ min}^{-1} = \frac{\text{c/m effluxed for any t} / \text{c/m in lens prior to t}}{\text{number of minutes in time period}}$$

D. DIETARY EXPERIMENTS

Cataracts were induced by feeding rats either a 50% galactose - 50% Purina Chow diet (Table II), or a 66% galactose-casein diet (Table III). The former diet was used in most studies since it was both convenient to prepare and produce changes in the lens comparable to those reported in the literature. In all studies the control groups were fed Purina Chow. A 50% glucose fed group was included for most experiments.

E. CHEMICAL PURITY OF SELENOMETHIONINE

Chemical purities of L-Se-75-selenomethionine and D L-selenomethionine were determined by use of paper and column chromatography. Amino acids were visualized by ninhydrin and/or autoradiography using Osray double emulsion film. Spots placed on paper were dried under a stream of nitrogen. The solvent systems used in paper chromatography and the respective R_f values obtained are given in Table IV.

Table II. Sugar-Chow diet.

Diet prepared with 50% d(+) glucose or galactose and 50% Purina laboratory chow (Ralston Purina Co.).

----- Purina Chow -----

Crude protein not less than	23%
Crude fat not less than	4.5%
Crude fiber not less than	6%

----- Ingredients of Purina Chow -----

Meat and bone meal	Vitamin B-12 supplement
Dried skimmed milk	Calcium pantothenate
Wheat germ meal	Choline chloride
Fish meal	Folic acid
Animal liver meal	Riboflavin supplement
Dried beet pulp	Brewers dried yeast
Ground extruded corn	Vitamin A supplement
Oat middlings	D activated plant sterol
Soybean meal	Vitamin E supplement
Dehydrated alfalfa meal	Thiamin
Cane molasses	Niacin
Animal fat preserved with BHA	Trace of zinc oxide
0.075% Ferric ammonium citrate	0.5% defluorinated PO ₄
0.02% Manganese sulphate	0.5% iodized salt

Table III. Sugar-Casein diet.

Ingredients	Grams
d(+) Glucose or Galactose	340.0
Vitamin-free Casein	90.0
Mazola Corn Oil	40.0
Brewers Yeast	10.0
Salt Mixture W *	20.0
Vitamin Mixture **	10.0

All ingredients purchased from Nutritional Biochemicals Corp., Cleveland.

* Salt Mixture W - Formulated per Wesson modification of Osborne and Mendel's salt mixture.

** Vitamin Mixture Ingredients:

Vitamin A (200,000 U/g)	45.0 mg
Vitamin D (400,000 U/g)	2.5 mg
α -Tocopherol	50.0 mg
Ascorbic Acid	450.0 mg
Inositol	50.0 mg
Choline Chloride	750.0 mg
Menadione	22.5 mg
Para Aminobenzoic acid	50.0 mg
Niacin	45.0 mg
Riboflavin	10.0 mg
Pyridoxine-HCl	10.0 mg
Thiamine-Hcl	10.0 mg
Calcium Pantothenate	30.0 mg
Biotin	0.2 mg
Folic Acid	0.9 mg
Vitamin B ₁₂	13.5 μ g

Table IV. Solvent systems for paper chromatography of Methionine (M) and Selenomethionine (SeM).

System	Solvent V/V/V	M Rf	SeM Rf	Rf of SeM Degradation Products
1	Butanol:Acetic Acid: Water, 25/6/25	.43	.49	.18 .10
2	Pyridine:Water: Methanol, 4/20/80	.60	.62	-
3	n-Butanol:Pyridine: Water, 1/1/1	.56	.56	.11
4	n-Butanol:Acetic Acid: Water, 60/15/25	.49	.54	.24 .14
5	Phenol:Water, 80/20	.74	.74	.41
6.	Ethanol:Water: 14.8N Ammonium Hydroxide, 90/5/5	.28	.28	.13
7.	Ethanol:Water: 14.8N Ammonium Hydroxide, 80/14/6	-	.35	-

Solvent front- 14 cm; Whatman No.1 paper; sample size 0.34 μ moles/10 μ l. All Rf values are $\pm$ 0.05.

Quantitative studies were performed by means of a Technicon Amino Acid Analyzer. A 0.25cc sample containing 0.25 μ moles D L-selenomethionine, 0.25 μ moles L-nor-leucine and 0.5 μ Ci L-Se-75-selenomethionine in 12.5% sucrose was applied to the column. A flow rate of 0.5cc/min was maintained.

III. RESULTS

A. CHEMICAL AND RADIOCHEMICAL PURITY OF SELENOMETHIONINE

Chemical and radiochemical purity of selenomethionine was determined by the use of paper and column chromatography. Table IV gives the R_f values determined in several solvent systems. Only systems 1 and 4 were capable of distinguishing between methionine and selenomethionine. Radioactive contaminants were present in significant amounts when samples were air dried under mild heating. When samples were dried under a stream of nitrogen almost no breakdown was discernable. Solutions of selenomethionine incubated at temperatures greater than 45°C showed a slight increase in breakdown.

Column chromatography of selenomethionine was carried out on a Technicon Amino Acid Analyzer. Figure 13 shows that at least 93% of the radioactivity is the selenomethionine peak. This peak gave a positive ninhydrin reaction and appears in the region of isoleucine. Some of the activity found at other regions may have been produced by the technique, e.g., thermal degradation on the column (65°C) over a ten hour period.

In order to prove that selenomethionine was taken up and remained unaltered in the lens the following experiment was performed. Six lenses were pulsed for one

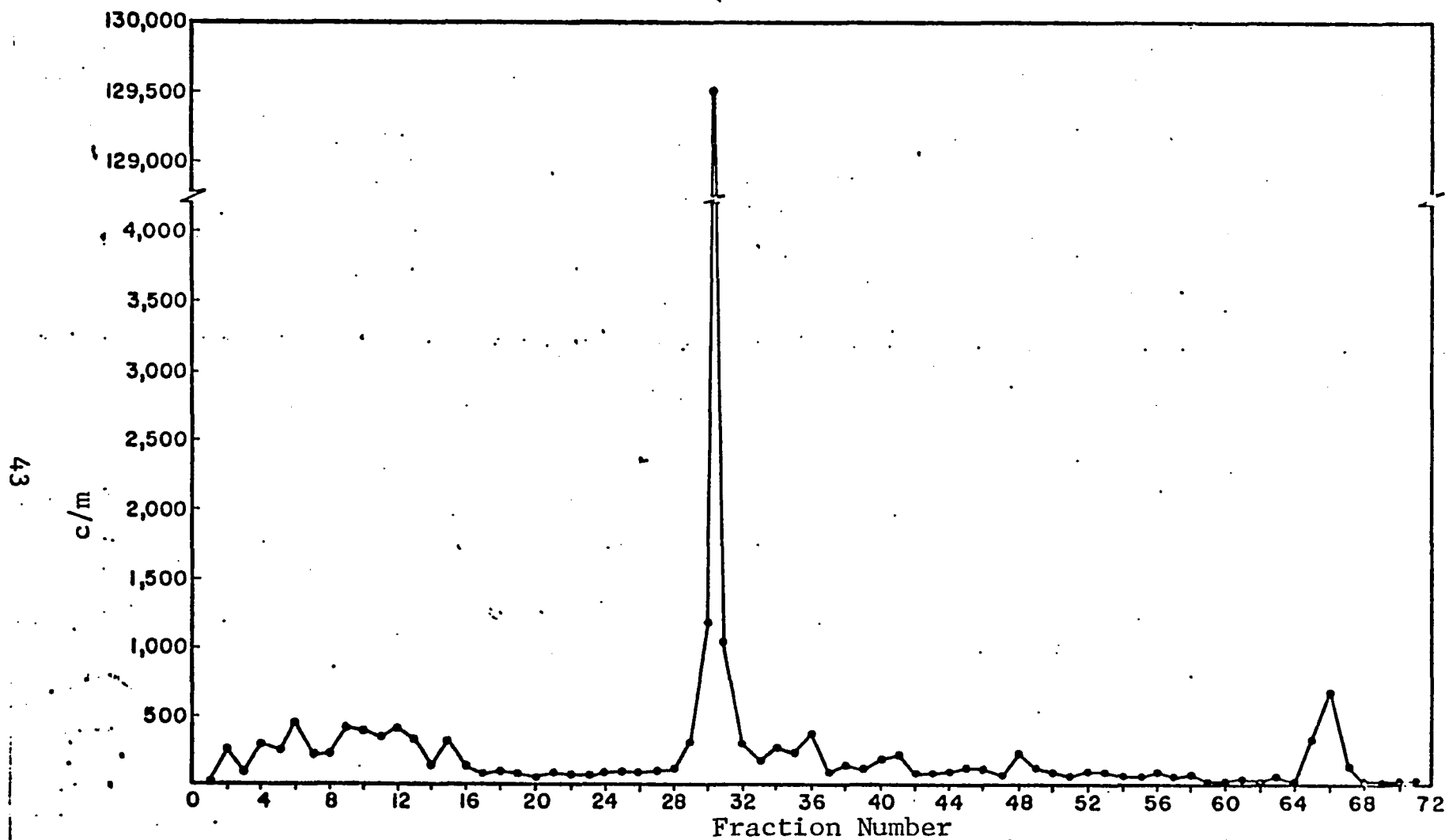


Figure 13. Column chromatography of Se-75-selenomethionine. Technicon Amino Acid Analyzer. Each fraction represents 10 ml of eluent.

and one half hours in 0.75cc Krebs-Ringers phosphate buffer (pH 7.2) containing 0.33 μ Ci L-Se-75-selenomethionine, 190 μ g D L-selenomethionine and 0.75mg D(+) glucose. The accumulation value of selenomethionine by the lenses was approximately 10% of dose. The lenses were then washed and allowed to efflux into a 2.5cc buffer containing 2.5 mg glucose and 150 μ g D, L-selenomethionine for two hours. Selenomethionine was added to the efflux media to facilitate the efflux of Se-75-selenomethionine out of the lens via exchange diffusion. A 0.5cc aliquot of the effluent was placed on the column for analysis (Fig. 14). A quantitative ninhydrin analysis was performed on each fraction. The only region giving a positive ninhydrin reaction corresponded to selenomethionine and more than 95% of the total radioactivity was found to be present in this fraction. Minor peaks in this run corresponded to those present when analyzing the stock solution.

B. INCORPORATION OF SELENOMETHIONINE INTO PROTEIN

Washing of the protein precipitate with hot trichloroacetic acid (90°C) or with an ethanol, ether, chloroform mixture did not significantly reduce the c/m of the precipitate. Non-specific binding of Se⁷⁵-selenomethionine to protein occurs at higher incubation temperatures (65°C).

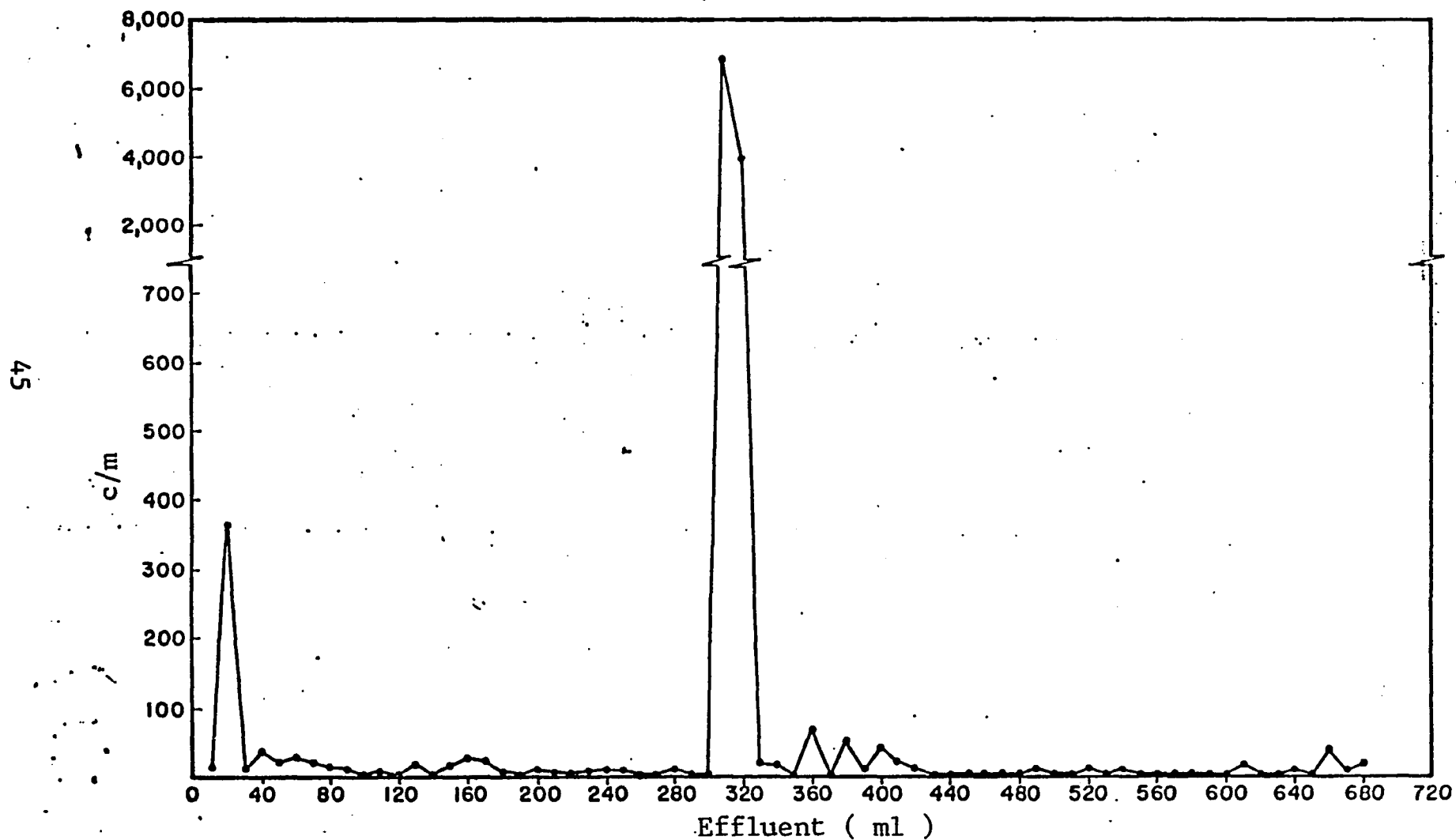


Figure 14. Column chromatography of Se-75-selenomethionine effluxed from rat lenses. Efflux was in the presence of unlabeled selenomethionine . Technicon Amino Acid Analyzer.

By redissolving the protein precipitate in one ml of 0.1% NaOH for 15 minutes and then reprecipitating with TCA (0.2cc 50% TCA = 5cc 5% TCA - methionine), most of the non-specific binding can be eliminated. For lenses incubated at 65°C there was a 37% loss of activity after the NaOH wash while the 37°C group lost less than 10% of the counts in protein.

As the following experimental results clearly indicate, a "sticky" radioactive derivative was produced at 65°C. Lenses were placed into a dose that was pre-incubated at 65°C for 10 minutes; a second set of lenses were placed into dose pre-incubated at room temperature. Both sets were then allowed to incubate for 10 minutes at 65°C. The accumulation values for the two groups were 4.13% and 4.35% respectively. However, the % incorporation value in the 65°C group was 14.6% as compared to the control value of 6.0%. Hence, the longer the incubation of the dose at 65°C, the higher the % "sticking" to protein.

Pre-incubation of lenses in puromycin (0.25mg/0.5cc) prior to pulsing with Se-75-selenomethionine, at 37°C, reduces protein incorporation to 1% or less of the total accumulation in the lens.

C. CHARACTERIZATION OF SELENOMETHIONINE ACCUMULATION AND PROTEIN INCORPORATION

The accumulation of selenomethionine by the lens for the first two hours of incubation is shown in Figure 15. Based on simple diffusion, the expected accumulation would be approximately 2.7 to 4.6% of the total dose for lenses weighing 24 to 40 mg respectively. Lens water has been calculated as 60% of the total lens weight. The tissue/medium (T/M) ratio exceeded the expected diffusion value after 10 minutes of incubation and reached a value of 4 to 8 after two hours. Per cent amino acid incorporation into protein increases linearly (disregarding the initial uptake) for the first hour of incubation and then begins to level off (Fig. 16).

Uptake and amino acid incorporation into protein as a function of temperature is shown in Figure 17. The temperature optimum for a 40 minute incubation period was 46°C for uptake and 37°C for per cent incorporation into protein. The increase in incorporation between 45°C and 65°C is due to non-specific binding of selenomethionine to the protein, as discussed on page 46. The Q_{10} (26° -36°C) for uptake and protein incorporation were 1.7 and 3.0 respectively. The enzymes and/or carrier systems involved in transport appear to be less thermolabile than the enzymes involved in protein incorporation. All subsequent experiments were carried out at 37°C.

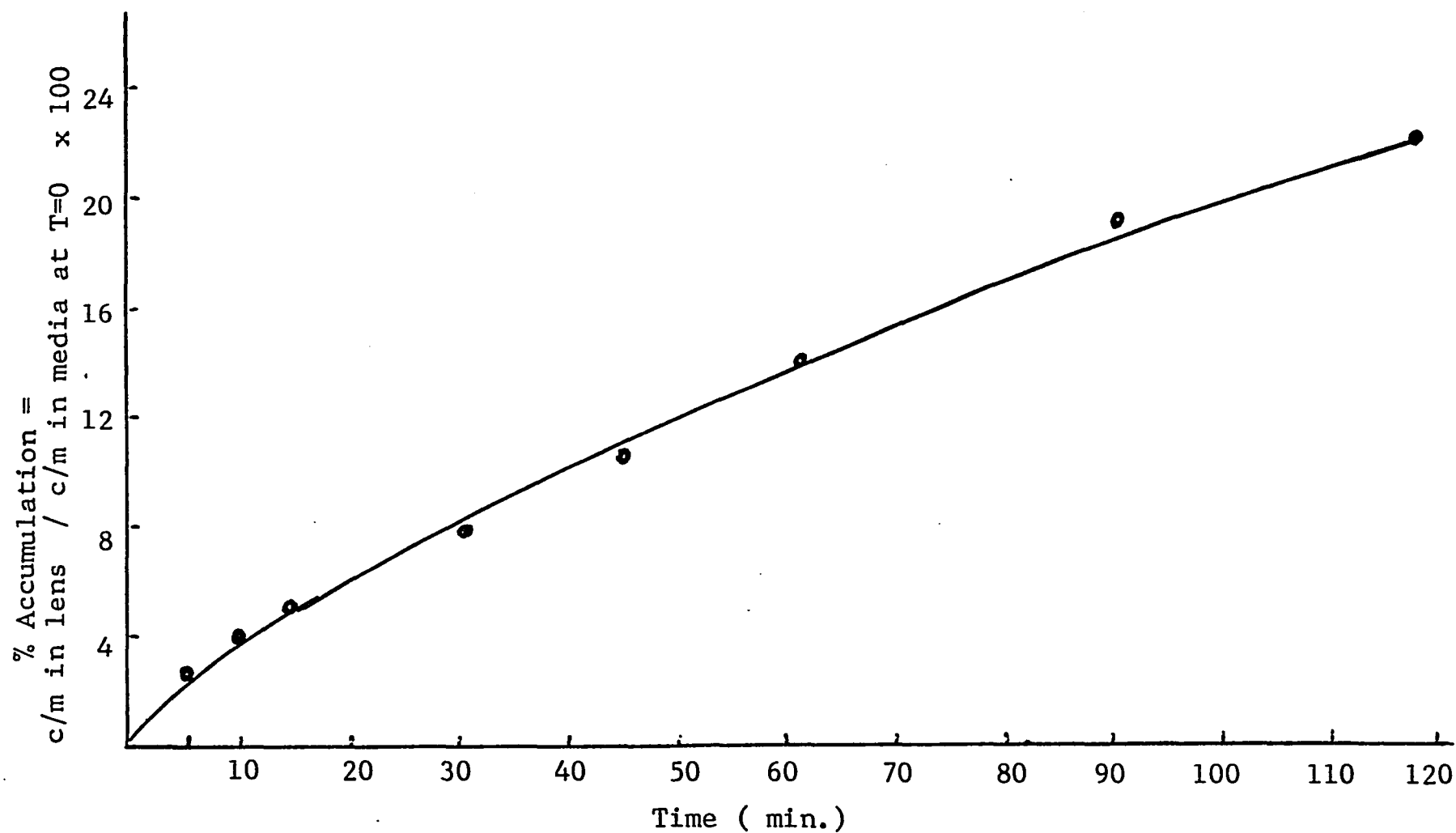


Figure 15. The % accumulation of selenomethionine as a function of time.
N= 7 to 12 lenses per point.

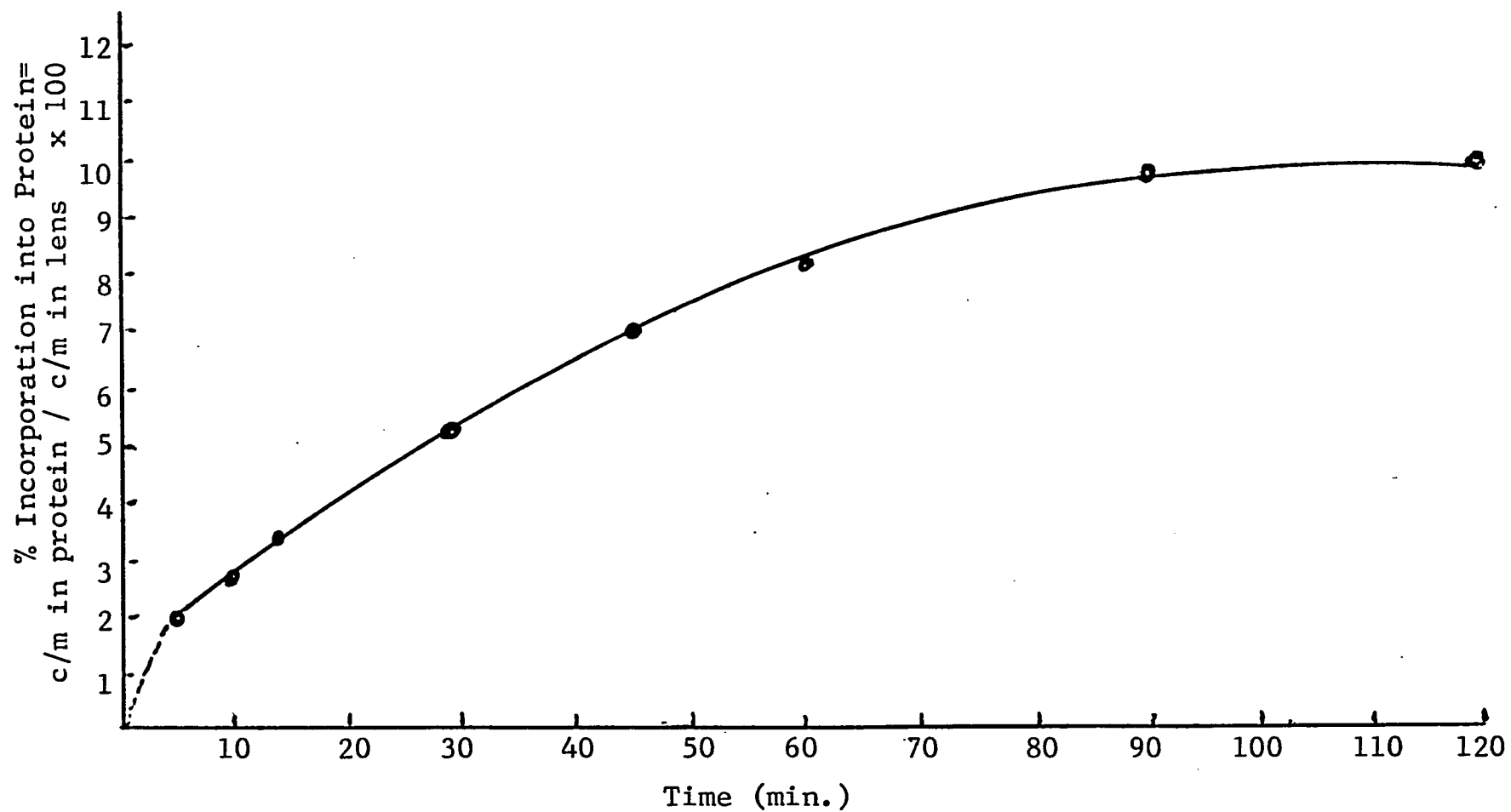


Figure 16. The % incorporation of selenomethionine into protein as a function of time. N= 7 to 12 lenses per point.

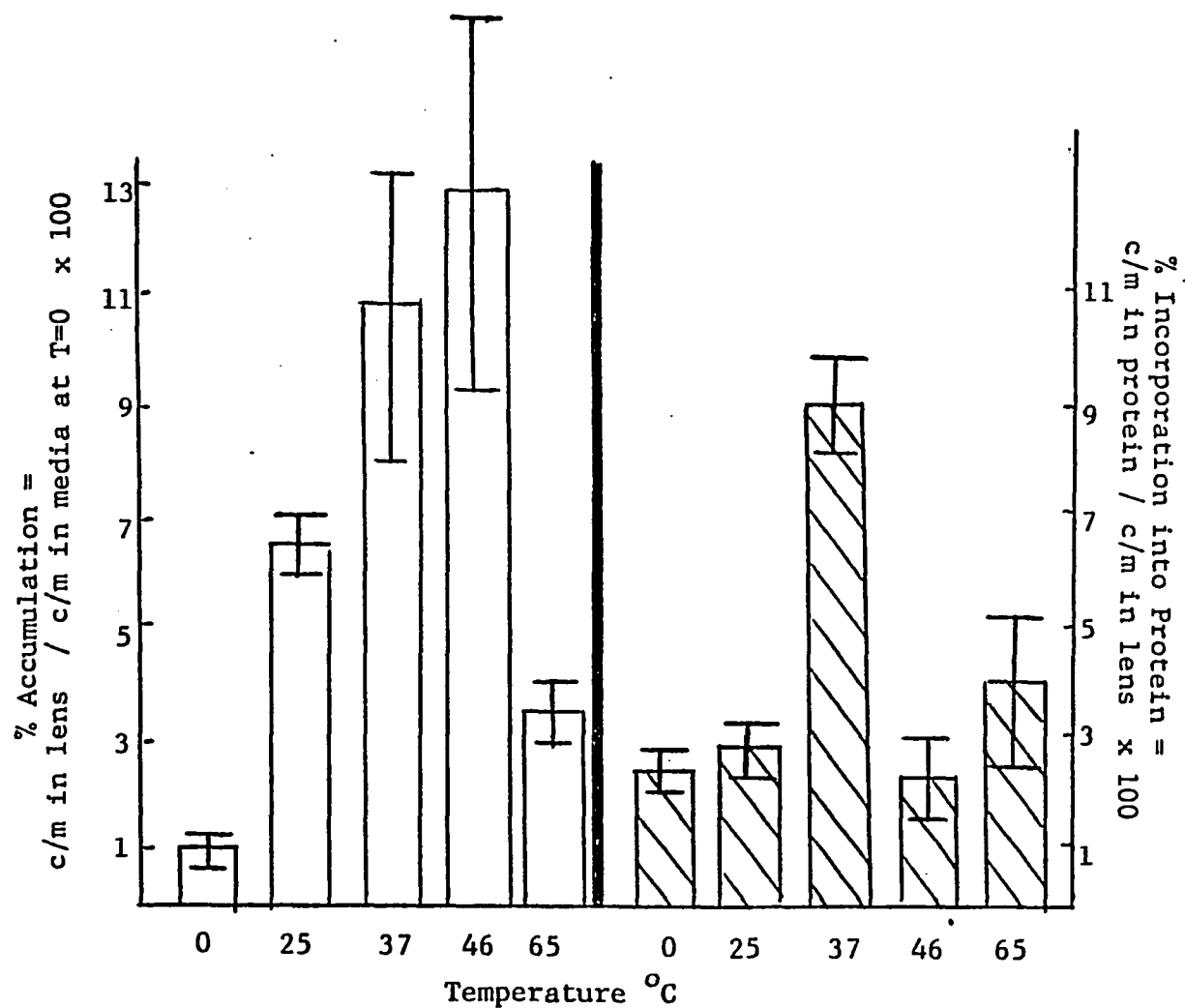


Figure 17. Selenomethionine accumulation and % incorporation into protein as a function of temperature. **I** = S.D.

Selenomethionine accumulation increases with increasing amount of substrate in the media in accordance with Michaelis-Menten kinetics. An apparent K_m and V_{max} were calculated by plotting the reciprocal of selenomethionine concentration in the lens ($1/V$) against the reciprocal of media concentration ($1/S$) in accordance with a method described by Lineweaver and Burk (Fig. 18). The values obtained do not account for errors due to non-mediated transport nor exchange diffusion of selenomethionine into and out of the lens. The apparent K_m was calculated to be 2.04m moles per liter with a V_{max} value of 0.116 μ moles per 40 minutes per 27mg of lens.

Incorporation into protein was found to increase with increasing accumulation and showed signs of saturation beyond 7 μ g accumulation. When the Lineweaver-Burk method was applied by plotting the reciprocals of incorporation into protein ($1/V$) against the reciprocals of accumulation ($1/S$), an apparent K_m and V_{max} for protein incorporation was obtained (Fig. 19). The values obtained were a K_m of 0.0714 μ moles/27mg lens and a V_{max} of 0.00364 μ moles/40 minutes/27mg lens. Although the values obtained do not specifically relate to any particular phase of protein incorporation, they do provide a series of values which reflect a normal state of protein incorporation.

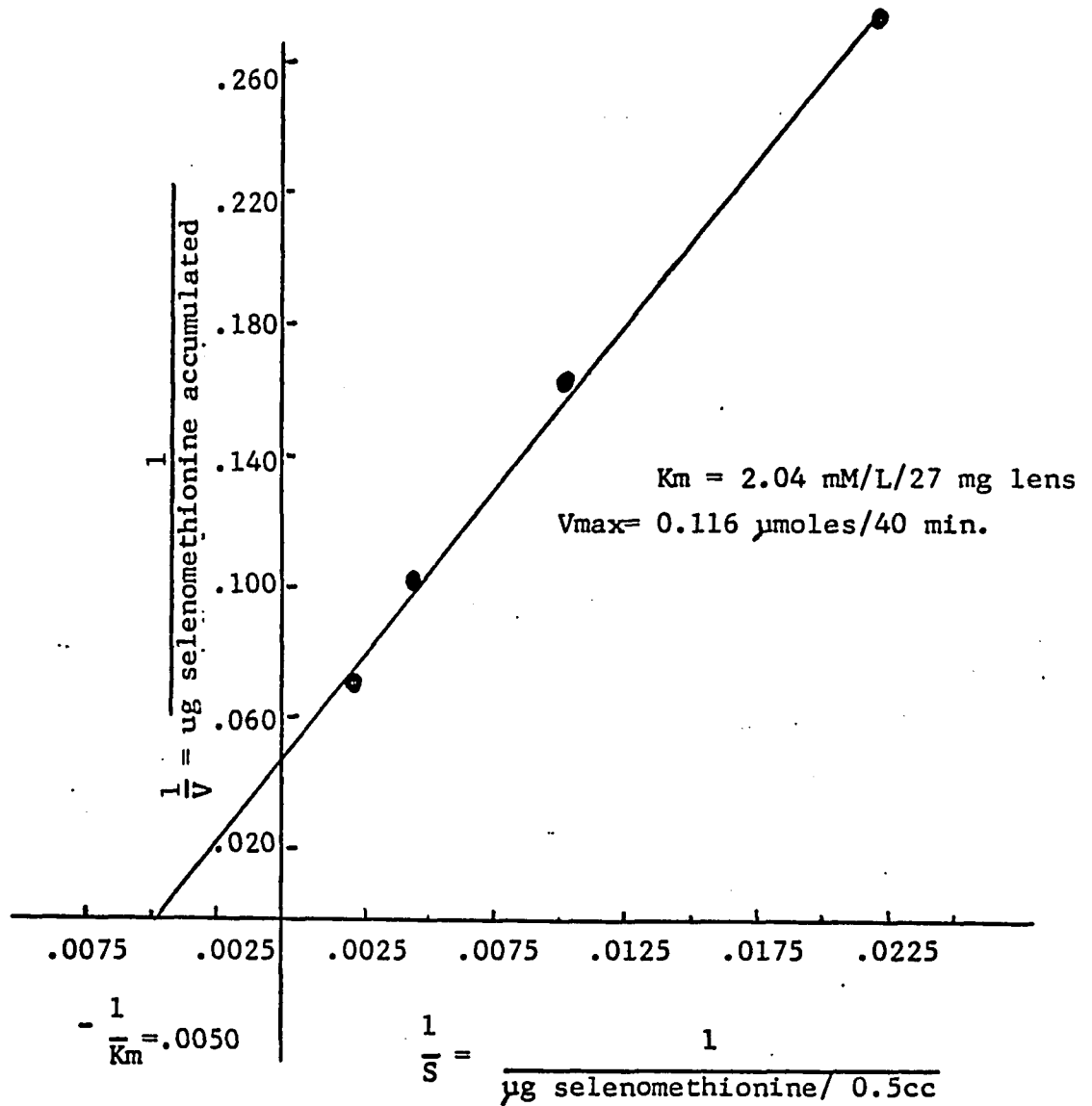


Figure 18. A Lineweaver-Burk plot of selenomethionine accumulation ($1/V$) as a function of media concentration ($1/S$). $N = 5$ to 6 lenses/ point/

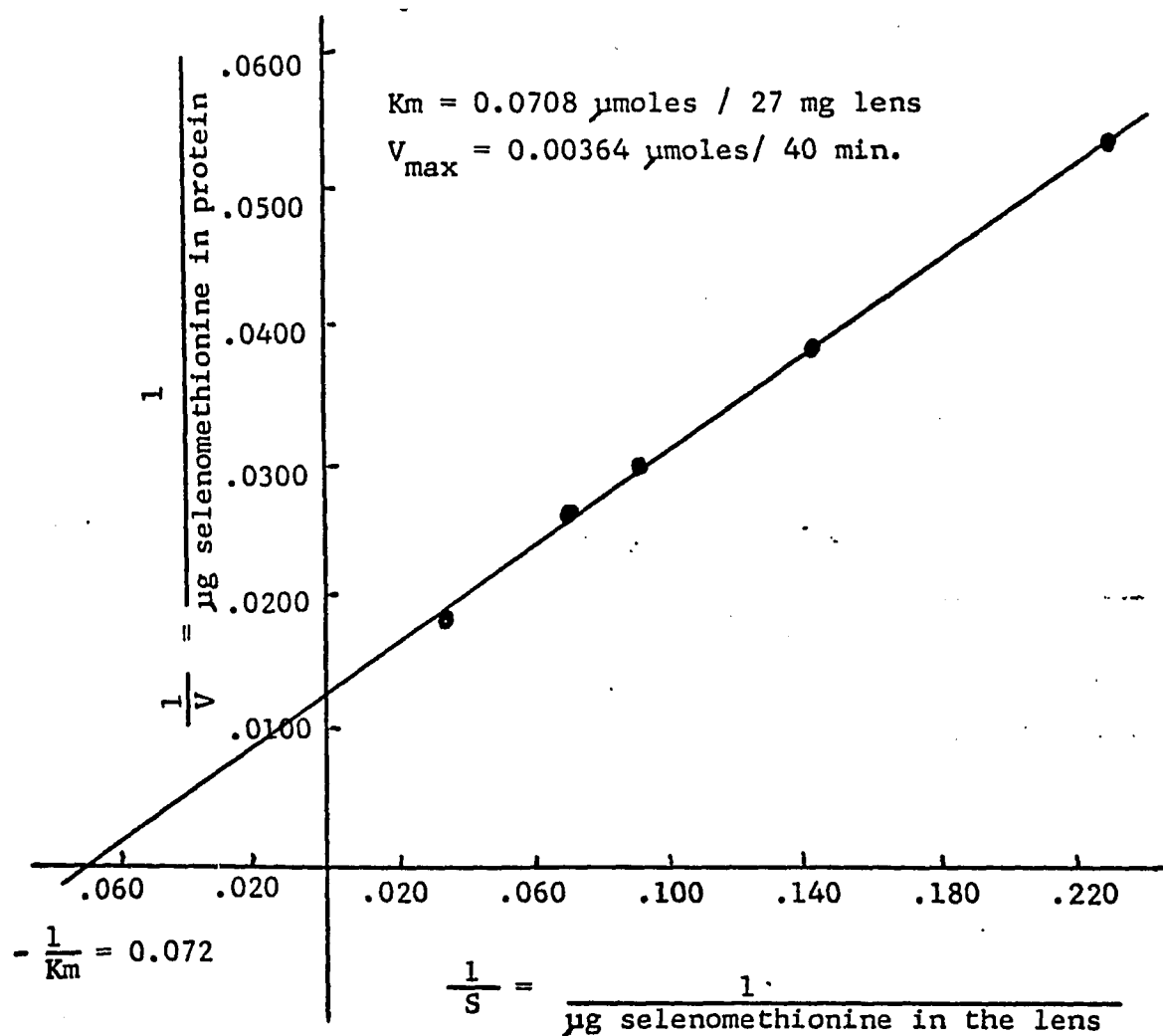


Figure 19. A Lineweaver-Burk plot of selenomethionine incorporation into protein ($1/V$) as a function of selenomethionine accumulated by the lens ($1/S$). $N = 5$ to 6 lenses / point.

in the lens under specified conditions (e.g., lens weight, incubation time, K_m and V_{max} of accumulation).

Studies were carried out to determine whether selenomethionine mimics methionine with respect to transport and/or incorporation into protein (Fig. 20). Se-75-selenomethionine (0.2 μ Ci) was co-incubated with non-radioactive L-methionine, D L-methionine or D L-selenomethionine at final concentrations of 1mM. There was no significant difference for the three groups with respect to accumulation. Protein incorporation was not significantly different for D L-methionine, L-methionine or D L-selenomethionine.

D. METABOLIC INHIBITORS

Several classes of inhibitors were tested as to their effect on amino acid accumulation and protein incorporation. Table V contains those inhibitors which interfere with either energy metabolism or protein synthesis. Both iodoacetic acid, a sulfhydryl reagent known to inhibit the glycolytic pathway, and iodacetamide, a sulfhydryl reagent, lower the accumulation and protein incorporation, the former being more potent. Inhibitors of aerobic metabolism (uncouplers, inhibitors of oxidative phosphorylation and metabolism, Estabrook and Pullman, 1967),

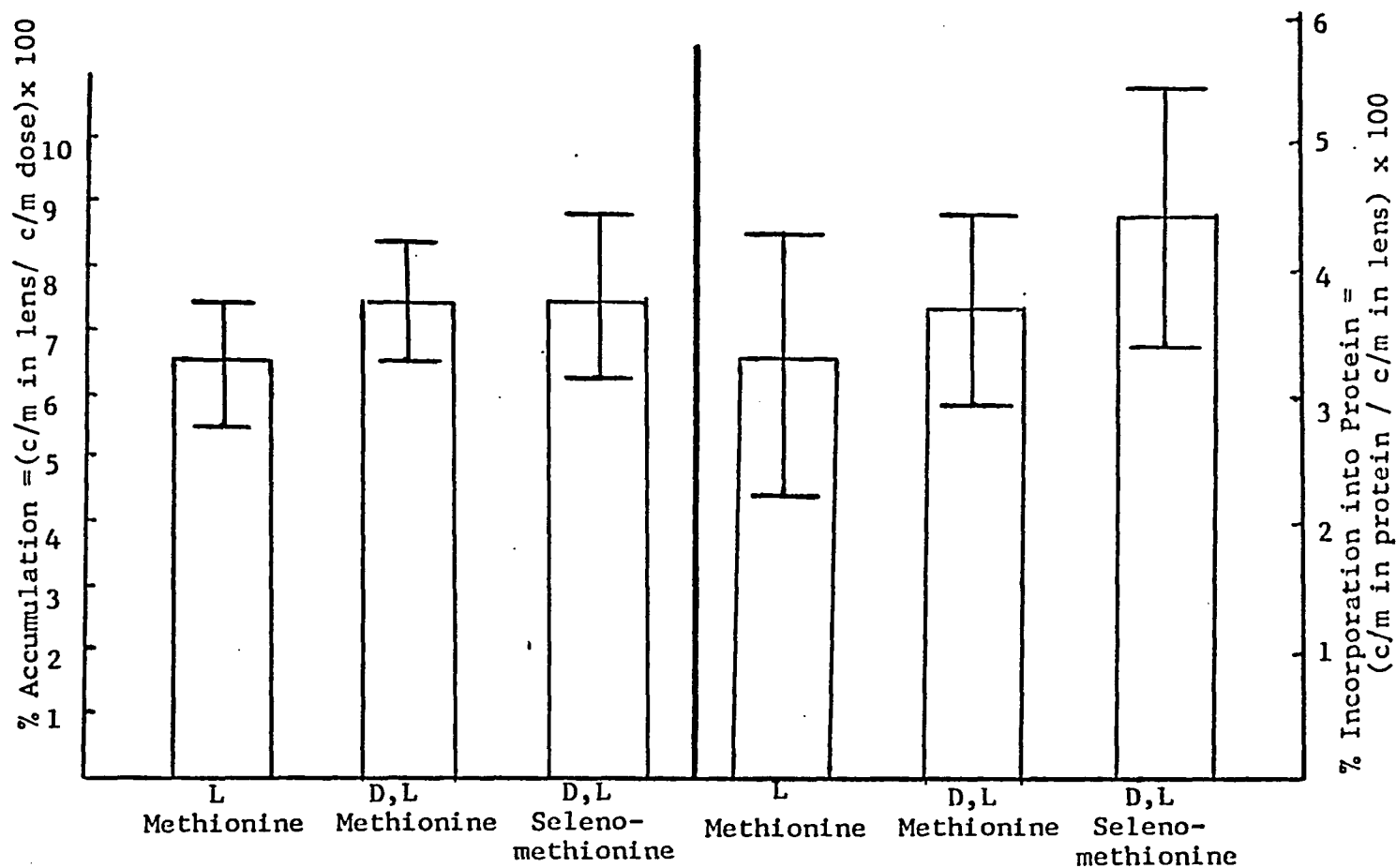


Figure 20. Se-75-selenomethionine accumulation and incorporation into protein in the presence of unlabeled methionine and selenomethionine. Incubation time-one hour. Dose contains 294,400 c/m in 0.5 cc. Carrier amino acids are present in 1 mM concentrations. N=9/group. Brackets denote standard deviation.

Table V. The percent inhibition of selenomethionine accumulation and incorporation into protein in the presence of metabolic inhibitors and inhibitors of protein synthesis. Values are presented as percent of controls. NS= not significant. Incubation time - 1 hr.

Inhibitor	% Uptake	S.D.	T Test	% Incorp. into Protein	S.D.	T Test	N	Age (days)
Control	100.0	-	-	100.0	-	-	62	-
Dinitrophenol (1mM)	92.6	± 12.1	NS	37.6	± 5.6	.001	13	28-40
Iodoacetic Acid (1mM)	54.2	± 9.5	.001	34.0	± 3.6	.001	35	24-40
Iodoacetamide (1mM)	81.1	± 5.6	.001	72.5	± 13.6	.01	7	35
Sodium Azide (1mM)	107.0	± 17.9	NS	87.8	± 16.4	NS	6	28
(10mM)	105.0	± 5.0	NS	43.9	± 4.6	.001	6	35
Sodium Arsenate (1mM)	96.4	± 16.6	NS	102.2	± 10.2	NS	13	28-40
(10mM)	103.7	± 17.3	NS	88.0	± 11.4	NS	5	40
Sodium Fluoride (1mM)	104.4	± 18.1	NS	80.1	± 13.6	.005	7	40
(10mM)	95.8	± 17.6	NS	16.8	± 2.9	.001	14	35-44
Sodium Cyanide (1mM)	112.3	± 37.9	NS	66.7	± 13.0	.001	7	40
(10mM)	41.3	± 11.0	.001	41.2	± 5.6	.001	15	35-40
Puromycin (0.1mM)	88.7	± 17.6	NS	77.0	± 7.3	.001	6	30
(1mM)	102.1	± 13.0	NS	31.9	± 4.0	.001	19	24-44
Chloramphenicol (1mM)	97.8	± 13.6	NS	78.9	± 9.9	.001	5	44

2,4-dinitrophenol, sodium azide and sodium cyanide inhibited incorporation into protein with no effect on accumulation. Azide was found to inhibit only at high concentrations (10mM). No inhibition was found with sodium arsenate for the time period studied. Sodium fluoride did not affect uptake of selenomethionine but was a potent inhibitor of protein incorporation at 10mM.

Inhibitors of protein synthesis, puromycin and chloramphenicol, did not affect accumulation. Puromycin was found to be the more potent inhibitor with a 68% inhibition at 1mM concentration while chloramphenicol inhibited only 21%.

The inhibition of accumulation and protein incorporation by iodoacetate and DNP as a function of time is presented in Table VI. Included in this table are sets of data for lenses preincubated in either iodoacetate or DNP for 30 minutes prior to the pulse.

Accumulation of selenomethionine in control lenses increases for the first two hours and then begins to level off. Lenses coincubated with iodoacetate accumulate 9.4 % of the dose as compared to a control value of 17.33% (45.8% inhibition). Incubation for two hours in iodoacetate results in a maximal uptake of 12.4% and this level of accumulation is maintained for the next two hours of

Table VI. The inhibition of selenomethionine accumulation and incorporation into protein by dinitrophenol and iodoacetic acid (IAA) as a function of time. Inhibitors - 1mM.

Time in Dose	Control % Uptake	IAA % Uptake no preinc. in IAA "A"	IAA % Uptake 30 min. IAA preinc. "B"	Percent Inhibition of "A"	Percent Inhibition of "B"	DNP % Uptake 30 min. DNP preinc.	Percent Inhibition with DNP
30 min.	8.01	-	4.20	-	47.6	7.77	3.0
1 hour	17.33	9.40	7.60	45.8	56.1	15.60	9.9
2 hours	30.40	12.40	-	59.2	-	-	-
3 hours	33.34	12.44	-	63.0	-	-	-
4 hours	31.64	12.00	-	62.0	-	-	-
Time in Dose	Control % Incorp.	IAA % Incorp. no preinc. in IAA "A"	IAA % Incorp. 30 min. IAA preinc. "B"	Percent Inhibition of "A"	Percent Inhibition of "B"	DNP % Incorp. 30 min. DNP preinc.	Percent Inhibition with DNP
30 min.	4.60	-	2.10	-	54.4	1.37	69.1
1 hour	9.59	3.28	2.37	66.0	75.3	1.41	85.3
2 hours	9.50	3.23	-	66.0	-	-	-
3 hours	9.22	3.29	-	64.3	-	-	-
4 hours	-	3.64	-	-	-	-	-

incubation (12.44% and 12.00% uptake at 3 and 4 hours, respectively).

Preincubation of lenses for thirty minutes in the presence of iodoacetate prior to the pulse results in a lower per cent uptake at the end of a 30 minute pulse (4.2% as compared to a control value of 8.0%). This represents a 47.6% inhibition which is the same level of inhibition one finds in the 60 minute pulse without preincubation in iodoacetate (45.8%). The inhibition in the 60 minute pulse lens, which was preincubated in iodoacetate, increases to a level of 56.1%. This level of inhibition is of the same order of magnitude that one finds at the end of two hours in the iodoacetate non-preincubated lens (59.2%).

Dinitrophenol did not inhibit accumulation when co-incubated with the dose for 60 minutes (Table VI) nor did it inhibit in lenses which were preincubated in DNP and subsequently pulsed for 30 and 60 minutes (3% and 9.9% inhibition, respectively).

Per cent incorporation of selenomethionine into protein for control lenses levels off at 60 minutes incubation time at a value of approximately 9.5%. Per cent incorporation into protein in the presence of iodoacetate reaches a maximum value of approximately 3.2% within 60

minutes of incubation. Further incubation (2-4 hours) does not increase that per cent incorporation value.

Preincubation for thirty minutes in iodoacetate gives a per cent incorporation value of 2.1% (54.4% inhibition). If a 60 minute pulse is given, the per cent incorporation value is 2.37% which represents a 75.3% inhibition with respect to the control.

Lenses preincubated in dinitrophenol and subsequently pulsed for 30 and 60 minutes yield per cent incorporation values of 1.37% and 1.41% respectively (69.1% and 85.3% inhibition).

Table VII shows the relative inhibition of accumulation and protein incorporation by British Anti-Lewisite (dimercaprole), antimycin A and oligomycin. In this case the inhibitors are dissolved in 0.4% ethyl alcohol (0.1M). Ethanol, by itself, leads to a 40% reduction in uptake and incorporation into protein. All three compounds had no effect on accumulation beyond the alcohol inhibition but showed a significant inhibition of amino acid incorporation.

The effects of other metabolic inhibitors are given in Table VIII. The sulfhydryl inhibitor N-ethylmaleimide inhibited both transport and incorporation and was a very effective inhibitor at a concentration of 10mM. The

Table VII. The effect of specific inhibitors of oxidative metabolism on selenomethionine accumulation and incorporation into protein. Incubation time - 1 hr.

Inhibitor	% Uptake	S.D.	T Test	% Incorp. into Protein	S.D.	T Test	N	Age (days)
Control	100.0	± 9.0	-	100.0 ± 10.0	-		5	28
British Anti-Lewisite (80 µM)	85.7	±14.0	NS	57.6 ± 5.0	.001		6	28
Antimycin A (36 µM)	85.3	±14.0	NS	63.9 ± 15.0	.001		6	28
Oligomycin (44 µM)	96.9	± 8.0	NS	82.5 ± 11.0	.001		5	28

Table VIII. The effect of N-ethylmaleimide, mercuric ions, ouabain and dimethyl sulfoxide on selenomethionine transport and incorporation into protein.

Inhibitor	% Uptake	S.D.	T Test	% Incorp. into Protein	S.D.	T Test	N	Age (days)
Control	100.0	± 8.0	-	100.0 ± 9.0	-	-	29	35-40
NEM (1mM)*	52.9	± 6.2	.001	83.8 ± 31.3	NS	7	35	
(10mM)	33.6	± 3.9	.001	18.8 ± 5.2	.001	4	35	
(10mM)**	20.6	± 3.1	.001	18.4 ± 2.8	.001	4	35	
Mercuric Chloride								
(1mM)***	14.3	± 2.4	.001	172.3 [‡] ± 49.0	.01	7	34	
(1mM)	30.5	±14.5	.001	71.1 [‡] ± 15.4	.005	6	40	
Ouabain (1mM)	84.4	± 6.8	.005	94.7 ± 9.5	NS	5	44	
(1mM)*	82.3	±10.5	.005	113.7 ± 30.0	NS	8	35	
DMSO (10%)	79.7	+11.0	.01	41.5 + 6.1	.001	7	34	

Incubation time- one hour except: * - 2 hours; ** - 2½ hours; *** - 40 min.
[‡] Technical problem - see text.

cardioglycoside ouabain, which inhibits the membrane Na-K ATPase, had only a minimal effect on accumulation after two hours of incubation and did not influence protein incorporation. A 10% solution of dimethylsulfoxide (DMSO) inhibited uptake by 20% and markedly reduced protein incorporation (48%). A 2% concentration of dimethylsulfoxide showed no signs of inhibition of either accumulation or incorporation. The mercuric ion was a potent inhibitor of accumulation. Accurate measurements of its influence on protein incorporation were not possible since the lens took up very little selenomethionine and there seemed to be non-specific binding of selenomethionine to protein in the presence of mercuric ions.

The effect of divalent cation free media and/or EDTA on accumulation and protein incorporation is given in Table IX. There was no inhibition of either accumulation or protein incorporation when lenses were preincubated in a medium devoid of divalent cations (Ca^{++} and Mg^{++}) with or without the presence of $5 \times 10^{-4}\text{M}$ EDTA. Table X shows the effect of prolonged exposure to 1mM EDTA in a divalent cation free medium. Only after pre-incubation in K-R-PO_4 -divalent cation free buffer with 1mM EDTA was there appreciable inhibition of transport. Protein incorporation was not affected by either the presence of EDTA or the lack of divalent cations in the time period studied.

Table IX. The effects of divalent-cation-free buffer on selenomethionine accumulation and incorporation into protein. Krebs-Ringers-Phosphate buffer (K-R-PO₄) pH 7.3 with or without calcium chloride and magnesium sulfate (divalent-cation-free) was used as the preincubation media. All incubations were in the presence of 100mg% glucose. Preincubation and incubation were 40 min. and 60 min. respectively. Data expressed as % of control.

Preincubation Media	Incubation Media	% Uptake	S.D.	T Test	% Incorp.	S.D.	T Test	N	Age (days)
K-R-PO ₄ Buffer	Standard Dose	100.0 ± 11.5		-	100.0 ± 16.2		-	8	51
K-R-PO ₄ Buffer-Divalent-Cation-Free	Standard Dose	90.7 ± 12.2		NS	98.3 ± 15.7		NS	7	51
K-R-PO ₄ Buffer-Divalent-Cation-Free + 0.5mM EDTA	Standard Dose	97.7 ± 5.6		NS	93.5 ± 13.8		NS	6	51
K-R-PO ₄ Buffer-Divalent-Cation-Free + 0.5mM EDTA	Standard Dose + 0.5mM EDTA	90.4 ± 7.2		NS	90.7 ± 14.0		NS	8	51

Table X. The effects of EDTA and/or divalent-cation-free buffer on selenomethionine accumulation and incorporation into protein. Krebs-Ringers-Phosphate buffer (K-R-PO₄) pH 7.3 with or without calcium chloride and magnesium sulfate (divalent-cation-free) was used as the preincubation and/or incubation media. All incubations were in the presence of 100 mg % glucose. Preincubation and incubation were 40 min. and 60 min. respectively. Data expressed as % of control.

Preincubation Media	Incubation Media	% Uptake	S.D.	T Test	% Incorp.	S.D.	T Test	N	Age (days)
K-R-PO ₄ Buffer	Standard Dose	100.0 ± 5.9		-	100.0 ± 13.4		-	8	40-45
K-R-PO ₄ Buffer	Divalent-Cation-Free Buffer + 1mM EDTA	85.7 ± 12.8		.025	98.0 ± 9.0		NS	8	40-45
K-R-PO ₄ Buffer-Divalent-Cation-Free + 1mM EDTA	Standard Dose	80.9 ± 13.2		.005	103.0 ± 9.1		NS	8	40-45
K-R-PO ₄ Buffer-Divalent-Cation-Free + 1mM EDTA	Divalent-Cation-Free Buffer + 0.75 mM EDTA	65.3 ± 11.0		.001	100.0 ± 8.2		NS	8	40-45

E. COMPETITIVE INHIBITORS

Talbe XI lists the relative accumulation and protein incorporation values of selenomethionine when co-incubated in the presence of other amino acids. The amino acids L-leucine, L-isoleucine, tertiary D L-leucine, L-alanine and L-histidine inhibit the accumulation of selenomethionine (30-50%) and increase its subsequent per cent incorporation into protein. Although L-threonine and α -AIB acted as competitive inhibitors of accumulation (20%), there was no effect on protein incorporation. Methionine, as expected, decreases both uptake and incorporation since it is an analogue of selenomethionine. The amino acids glycine, L-glutamic acid, L-lysine, γ -aminobutyric acid, D L $-\beta$ -aminobutyric acid, diamino-pimelic acid and sarcosine had no effect on either of the two systems under study at 1mM concentration.

Table XII contains the uptake and the incorporation values of several analogues and metabolic derivatives of methionine. The ethylated analogue of methionine, D-ethionine, is a potent inhibitor of transport. Protein incorporation was elevated slightly in the presence of ethionine. The analogue α -methyl methionine and the oxidation product of methionine, L-methionine, D L-sulfoxide, inhibited uptake and had no effect on protein incorporation.

Table XI. The effects of amino acids on selenomethionine accumulation and incorporation into protein. Incubation time:one hour or two hours(*) Selenomethionine concentration- 0.068mM.

Amino Acid in Dose (1mM)	% Uptake	T Test	% Incorp. into Protein	T Test	N	Age (days)
Control	100.0	-	100.0	-	41	-
L-Leucine	60.2	.001	125.6	.01	10	70
L-Isoleucine	59.2	.001	156.3	.001	10	119
Tert.D L-Leucine (5mM)	75.2	.001	130.2	.001	10	119
L-Alanine	71.6	.001	133.6	.01	14	48-77
L-Histidine	53.3	.001	129.2	.01	6	48
L-Methionine	51.7	.001	29.9	.001	6	48
Glycine	97.7	NS	117.8	NS	6	48
L-Glutamic Acid *	95.9	NS	107.9	.01	10	70
L-Lysine *	96.9	NS	104.9	NS	10	70
L-Threonine *	80.1	.001	113.1	NS	8	59
√-Amino Butyric Acid	101.3	NS	114.5	NS	8	77
D L β-Amino Butyric	97.1	NS	110.1	NS	9	77
α-Amino isobutyric	80.7	.001	93.5	NS	8	77
Diaminopimelic Acid*	97.5	NS	103.0	NS	9	56
Sarcosine (5mM)	103.9	NS	98.0	NS	9	.69

Table XII. The effects of methionine derivatives on selenomethionine accumulation and incorporation into protein. Incubation time- 40 min. Selenomethionine concentration- 0.068mM.

Amino Acid in Dose (1mM)	% Uptake	T Test	% Incorp. into Protein	T Test	N	Age (days)
Control	100.0	-	100.0	-	22	-
Taurine	96.8	NS	93.1	NS	9	59
D-Ethionine	60.8	.001	126.2	.025	9	59
α -Methylmethionine	84.6	.01	114.6	NS	7	59
DL allo-Cystathionine	91.7	NS	95.6	NS	8	40
L-Methionine sulfoxide	73.0	.01	116.2	NS	5	40
L-Methionine sulfone	82.7	NS	85.7	NS	6	40
DL-Methionine sulfoxide	85.0	NS	95.1	NS	5	40

F. CHARACTERIZATION OF SELENOMETHIONINE EFFLUX
(DIFFUSION AND EXCHANGE DIFFUSION)

Efflux was studied in order to further elucidate the various components of the transport system in the normal, aging and pathological lens. The efflux system for the amino acid lacks an active transport component and hence it is limited to two basic modes of transport: passive diffusion (non-mediated) and exchange diffusion (mediated). Elucidation of these two systems is accomplished by incubation of pulsed lenses in media which either contain or lack exchangeable amino acids (see materials and methods).

Low temperature, 0-5°C, markedly inhibited the non-mediated diffusion component (D) and the exchange component which is total efflux minus diffusion ($E=T-D$), (Fig. 21). This inhibition may be due to physical changes in the membrane properties.

At higher temperatures (57°C) there is a marked increase in non-mediated efflux (D) when compared to the 37°C group (Fig. 22). The mediated exchange component, E, was higher for the first 30 minutes for the 57°C group as compared to the 37°C group. However, this increased rate does not continue beyond 30 minutes efflux, which may indicate thermal inactivation of the exchange system.

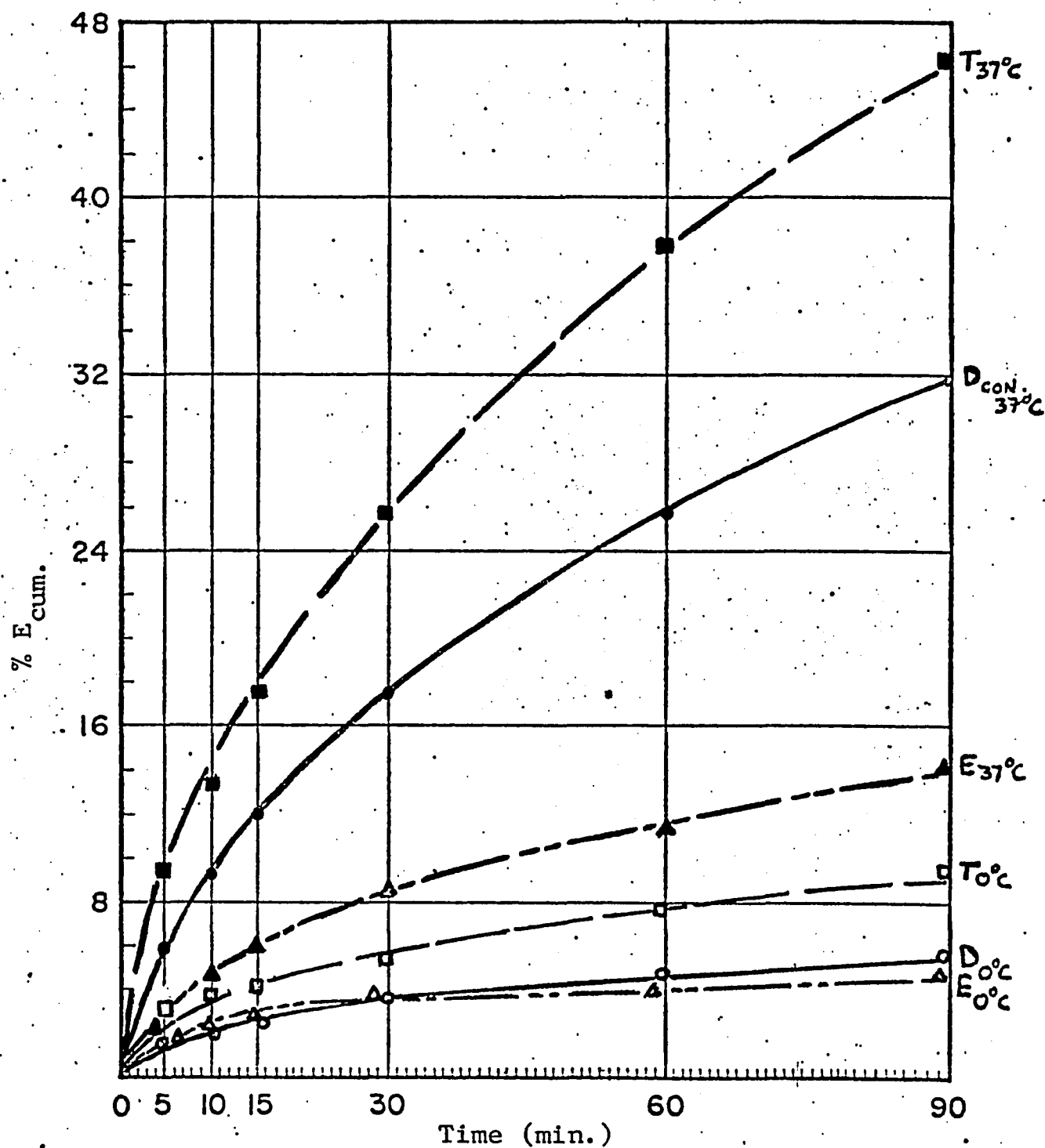


Figure 21. Efflux as a function of temperature; 0°C and 37°C.

37°C	0°C
■ — ■ Total	□ — □
● — ● Diffusion	○ — ○
▲ — ▲ Exchange	△ — △

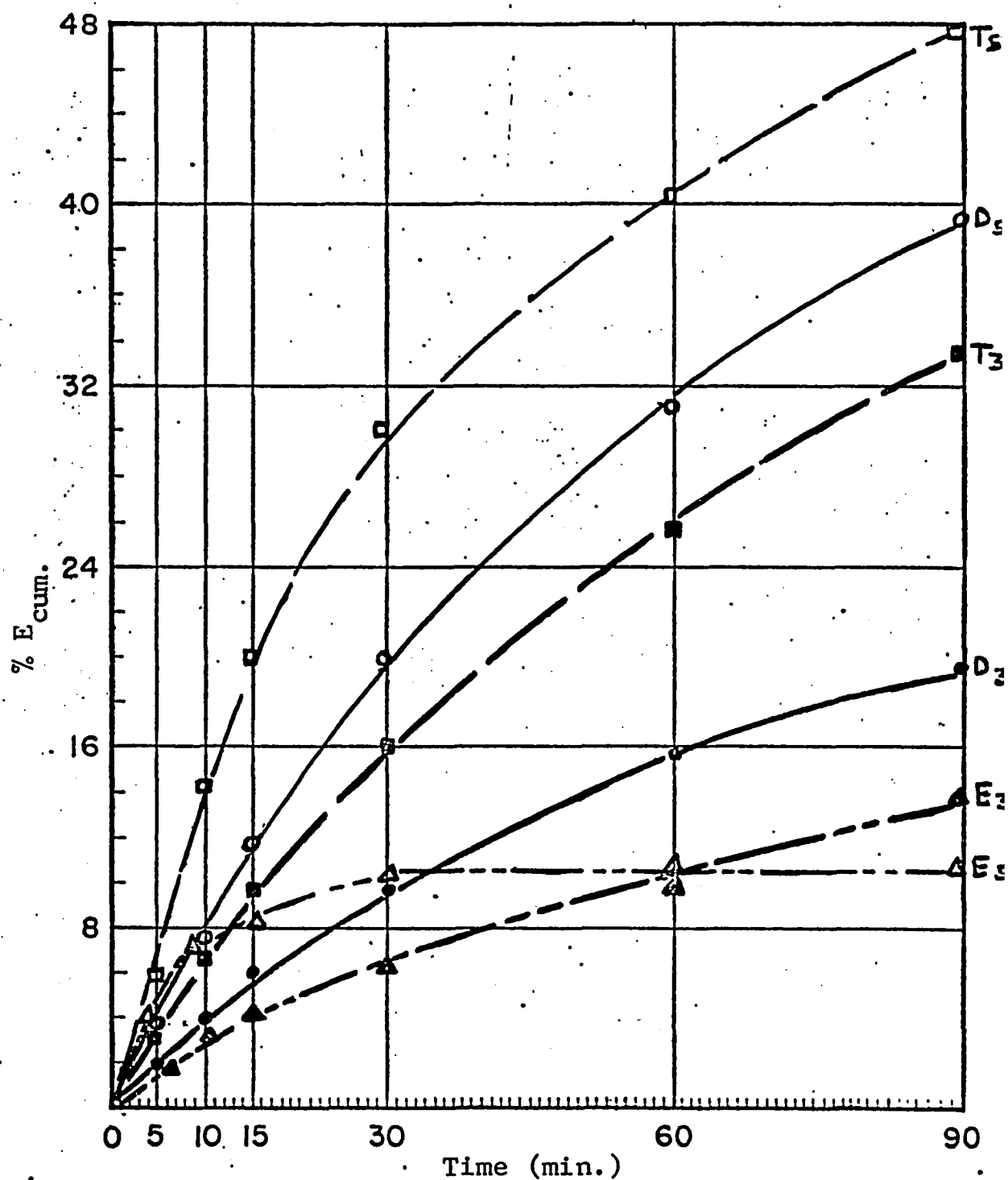


Figure 22. Efflux as a function of temperature; 37°C and 57°C.

37°C 57°C

■ — ■ Total □ — □

● — ● Diffusion ○ — ○

▲ - - ▲ Exchange △ - - △

The relative effectiveness of leucine, glutamic acid and lysine in driving the exchange diffusion system were studied (Fig. 23). Leucine was as effective as selenomethionine in driving the exchange system. Lysine and glutamic acid did not exhibit any ability to drive the exchange system. The values observed for lysine and glutamic acid are similar to the values obtained for the simple diffusion of selenomethionine (see Fig. 26).

Lenses pulsed with Se-75-selenomethionine were allowed to efflux into media containing various quantities of non-labeled selenomethionine. The external selenomethionine concentration varied from 0-30 μg (0-0.15 μmoles) per cc (Fig. 24) and 0-120 μg (0-0.61 μmoles) per cc (Fig. 25). The higher the external concentration of amino acid, the greater the rate of efflux from the lens. The increase in efflux observed was neither linear with external concentration nor did it show signs of saturation.

Lenses were loaded with different concentrations of selenomethionine and allowed to efflux into media containing no exchangeable amino acids. Loading was accomplished by incubating lenses for one hour in media containing Se-75-selenomethionine and non-labeled selenomethionine at concentrations of 0.10, 0.51, 1.02, 2.04 and 4.08 μmoles per cc (20-800 $\mu\text{g/cc}$) and the lenses accumulated 2, 5.8,

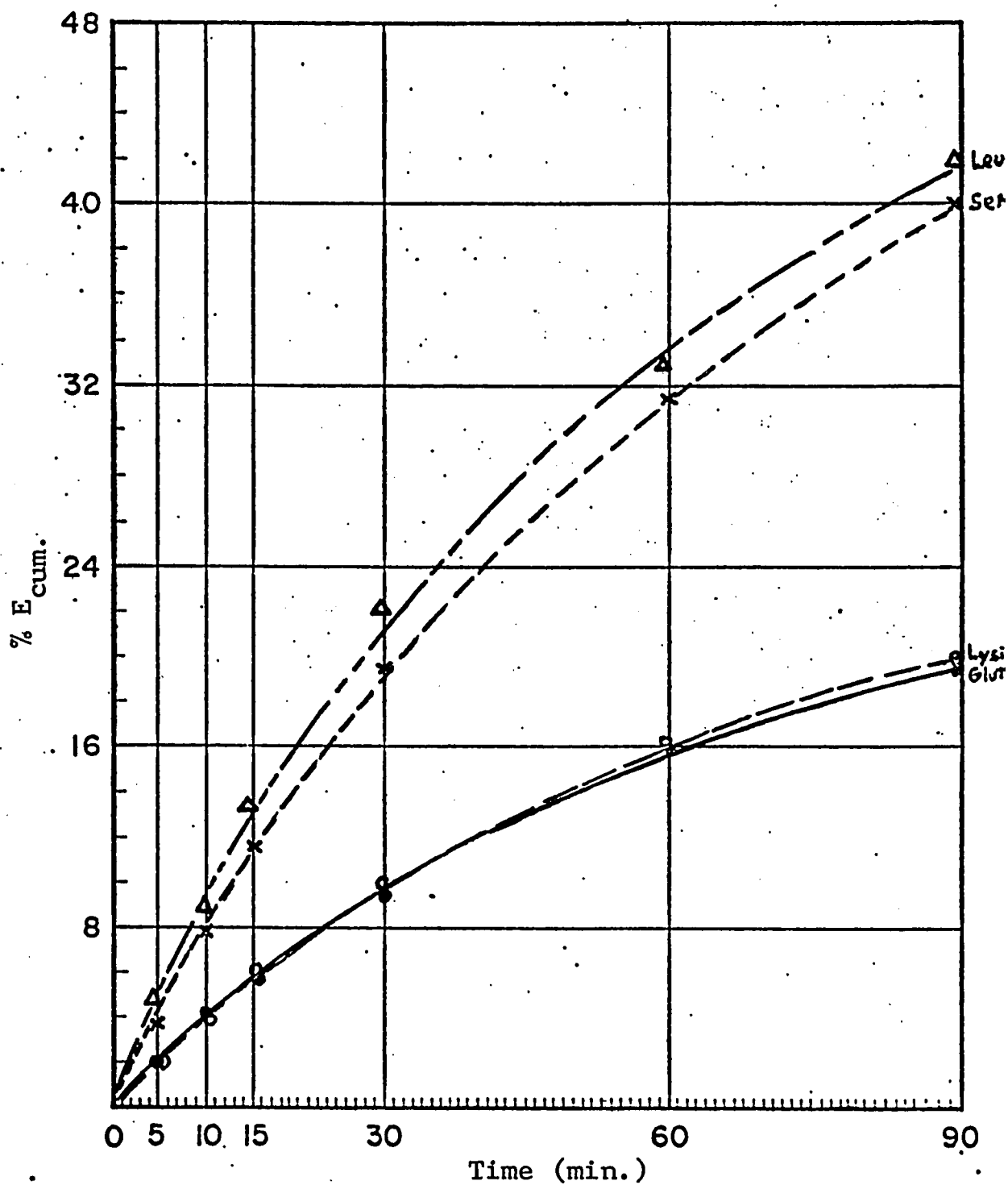


Figure 23. Efflux in the presence of selenomethionine (SeM), leucine, lysine and glutamic acid.

Selenomethionine x — — — — x
 Leucine Δ — — — — Δ

Lysine ● — — — — ●
 Glutamic Acid ○ — — — — ○

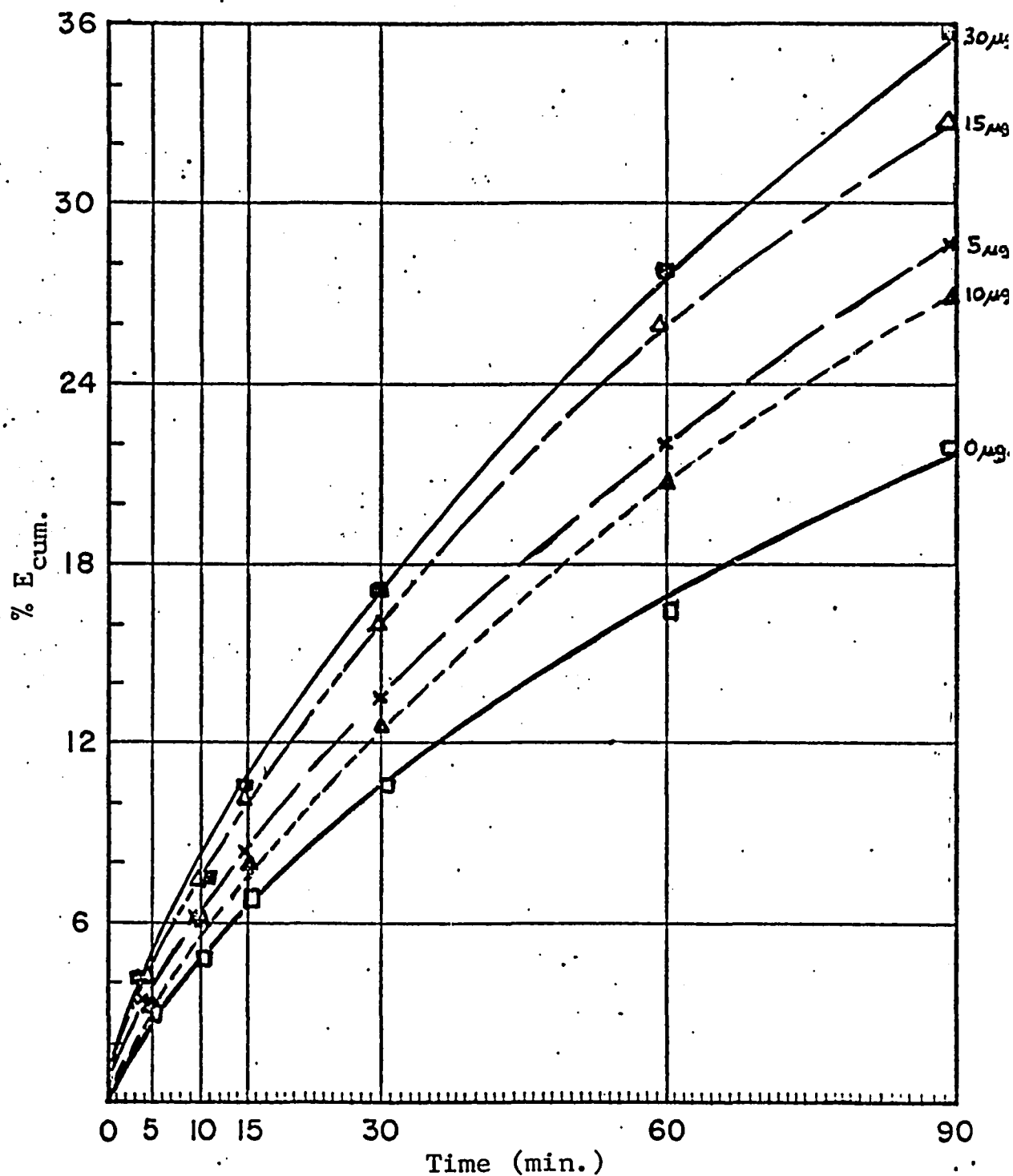


Figure 24. Efflux of Se-75-selenomethionine into media containing unlabeled selenomethionine ; 0, 5, 10, 15 and 30 µg/cc.

0 µg	□ ————— □	15 µg	Δ — — — — — Δ
5 µg	x ————— x	30 µg	■ ————— ■
10 µg	▲ — — — — — ▲		

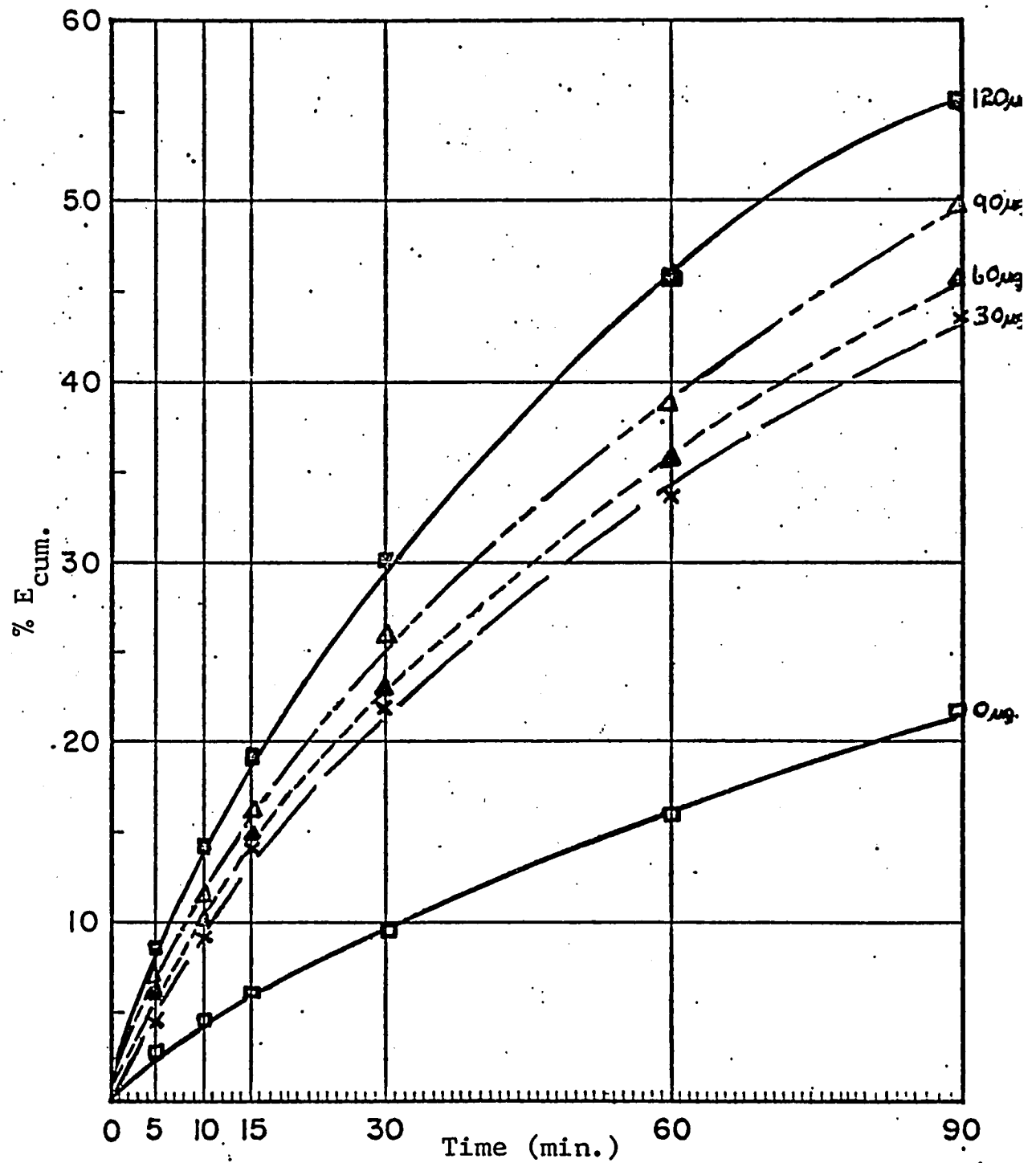


Figure 25. Efflux of Se-75-selenomethionine into media containing unlabeled selenomethionine; 0, 30, 60, 90 and 120 µg/cc.

0 µg	□ ————— □	90 µg	△ ———— △
30 µg	x ————— x	120 µg	■ ————— ■
60 µg	▲ ———— ▲		

10.7, 15 and 21 μg respectively, (0.01-0.10 μmoles). All five lens groups are very similar with respect to their per cent efflux patterns (Fig. 26). In fact, the extreme curves may represent the range of experimental variation. Furthermore, normalization of all time periods (per cent of total counts effluxed per five minutes within each time period), allowed the plotting of a series of Lineweaver-Burk curves (Fig. 27). As expected, all lines approximated an intercept through the origin, thus K_m and $V_{\max}$ are not calculable.

In another series of experiments lenses were preloaded with varying amounts of selenomethionine (1.8, 6.5, 10.7, 13.9 and 19.4 μg per lens) and allowed to efflux into media containing 60 μg selenomethionine per cc. As in the previous set of experiments there was no effect of internal selenomethionine concentration on per cent of selenomethionine efflux with time (Fig. 28). Likewise, the Lineweaver-Burk plots of cumulative time periods yielded a set of lines which intersect the origin thereby indicating no degree of saturation (Fig. 29).

The role of exchange diffusion in accumulation studies was also investigated. Lenses were preincubated, for one hour, in Krebs-Ringers phosphate buffer with

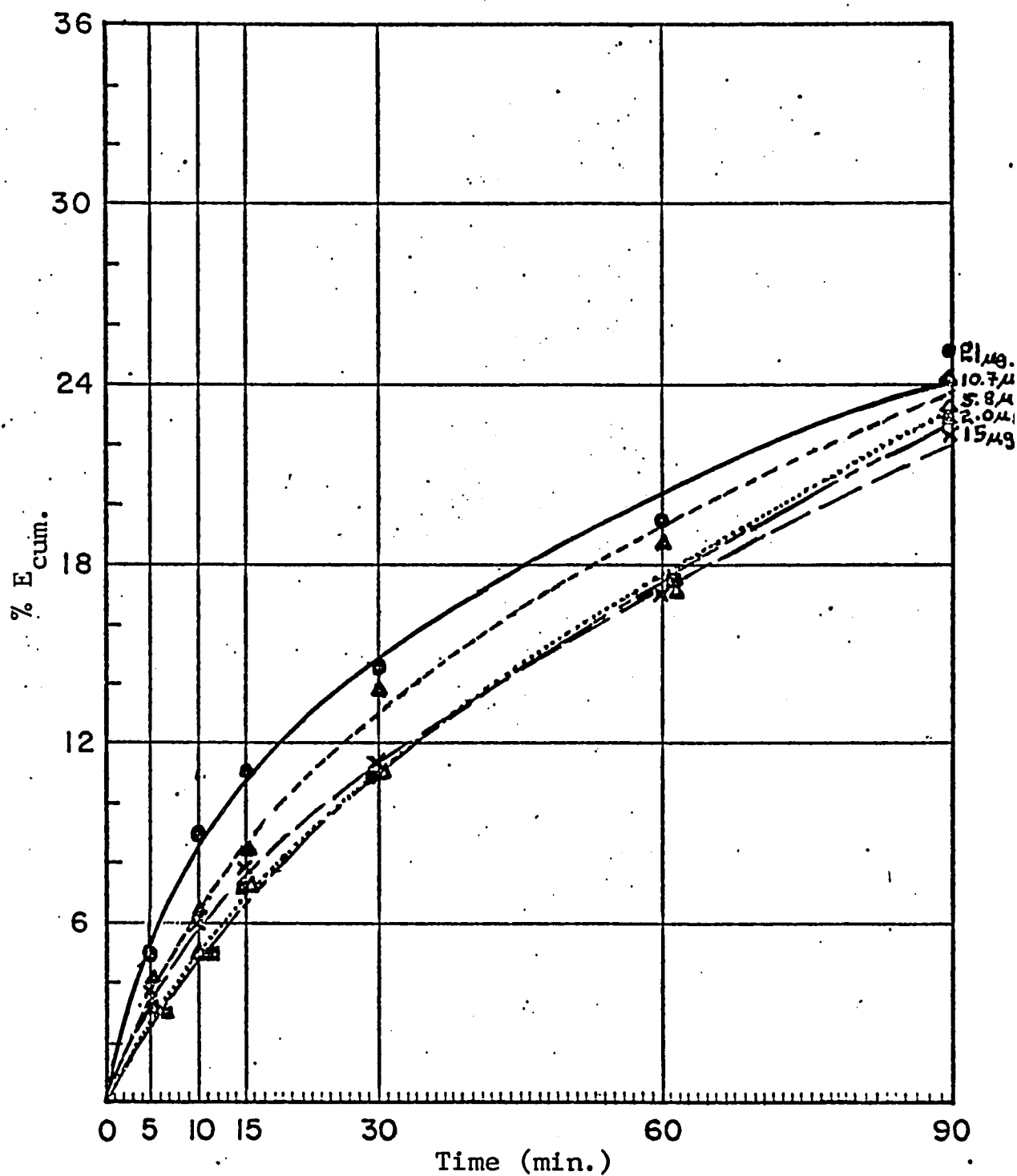


Figure 26. Efflux from lenses loaded with different concentrations of selenomethionine into media contain-
ing no exchangeable amino acid. Accumulations are:

2.0 µg	■	---	■	15.0 µg	x	---	x
5.8 µg	△	...	△	21.0 µg	●	---	●
10.7 µg	▲	---	▲				

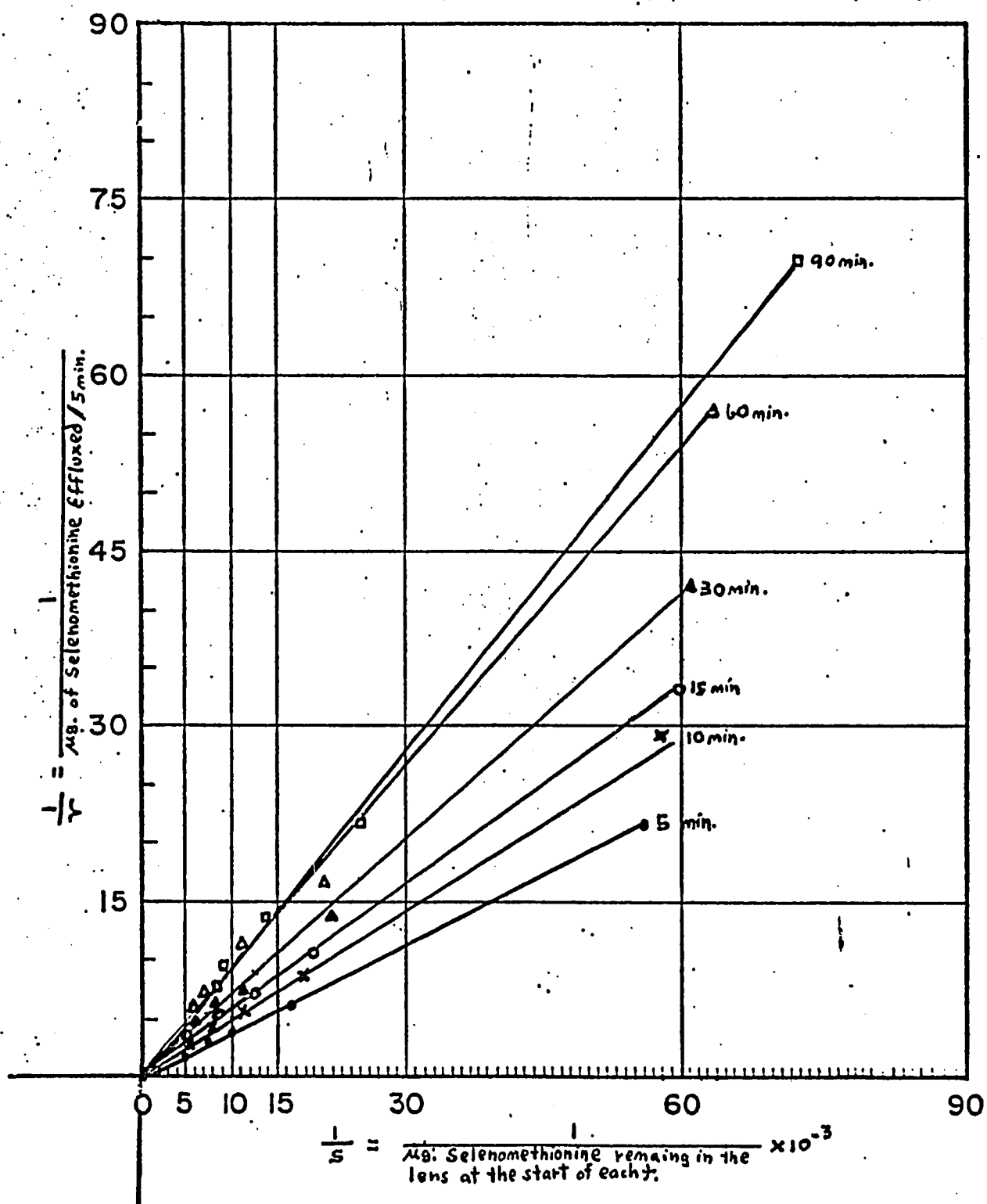


Figure 27. A Lineweaver-Burk plot of non-mediated diffusion for cumulative efflux times ranging from 5 to 90 minutes. Data taken from figure 26.

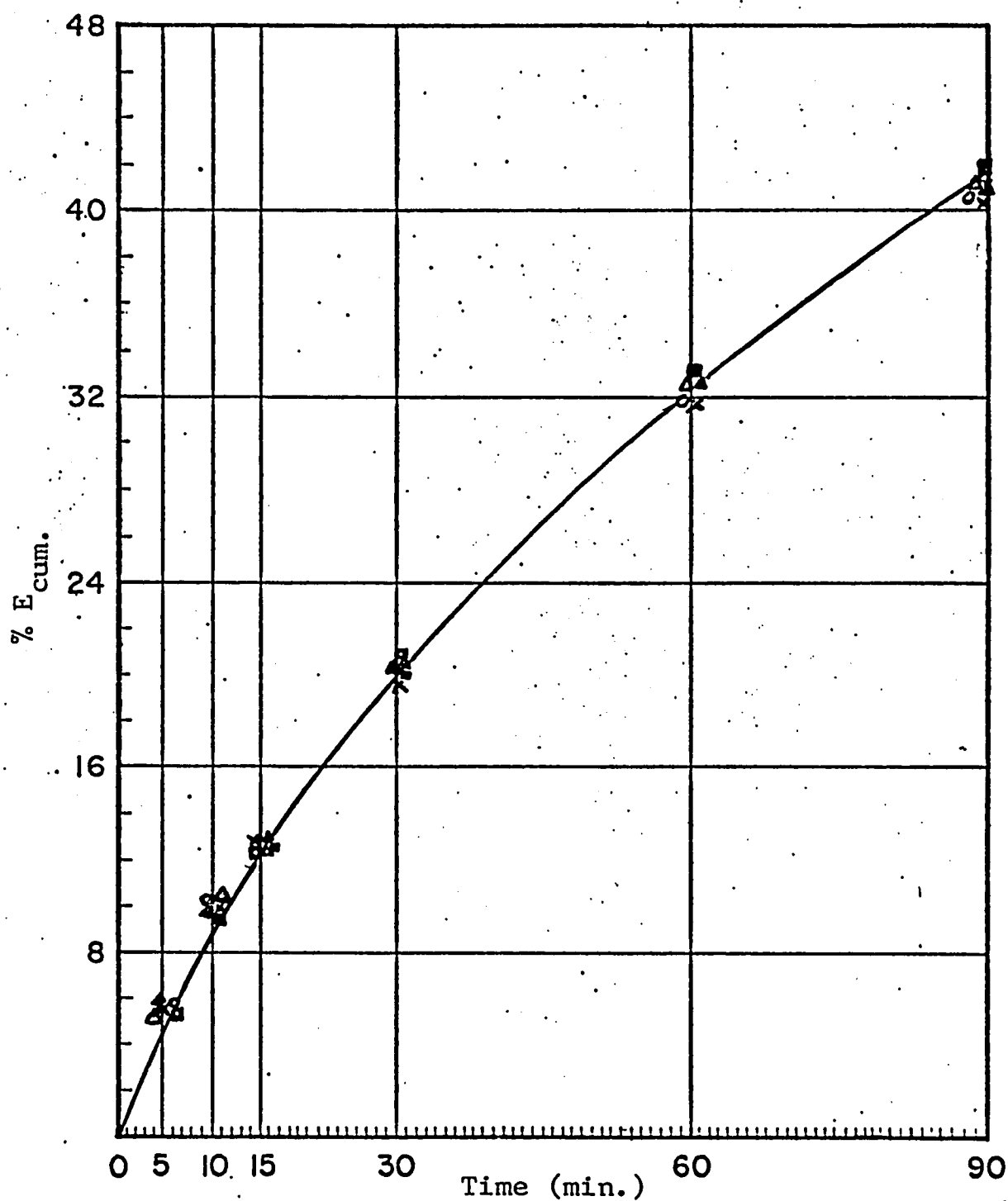


Figure 28. Efflux, from lenses loaded with different concentrations of selenomethionine, into media containing 60 μg selenomethionine. Accumulations are:

1.8 μg ▲	13.9 μg Δ
6.5 μg x	19.4 μg ○
10.7 μg ■	

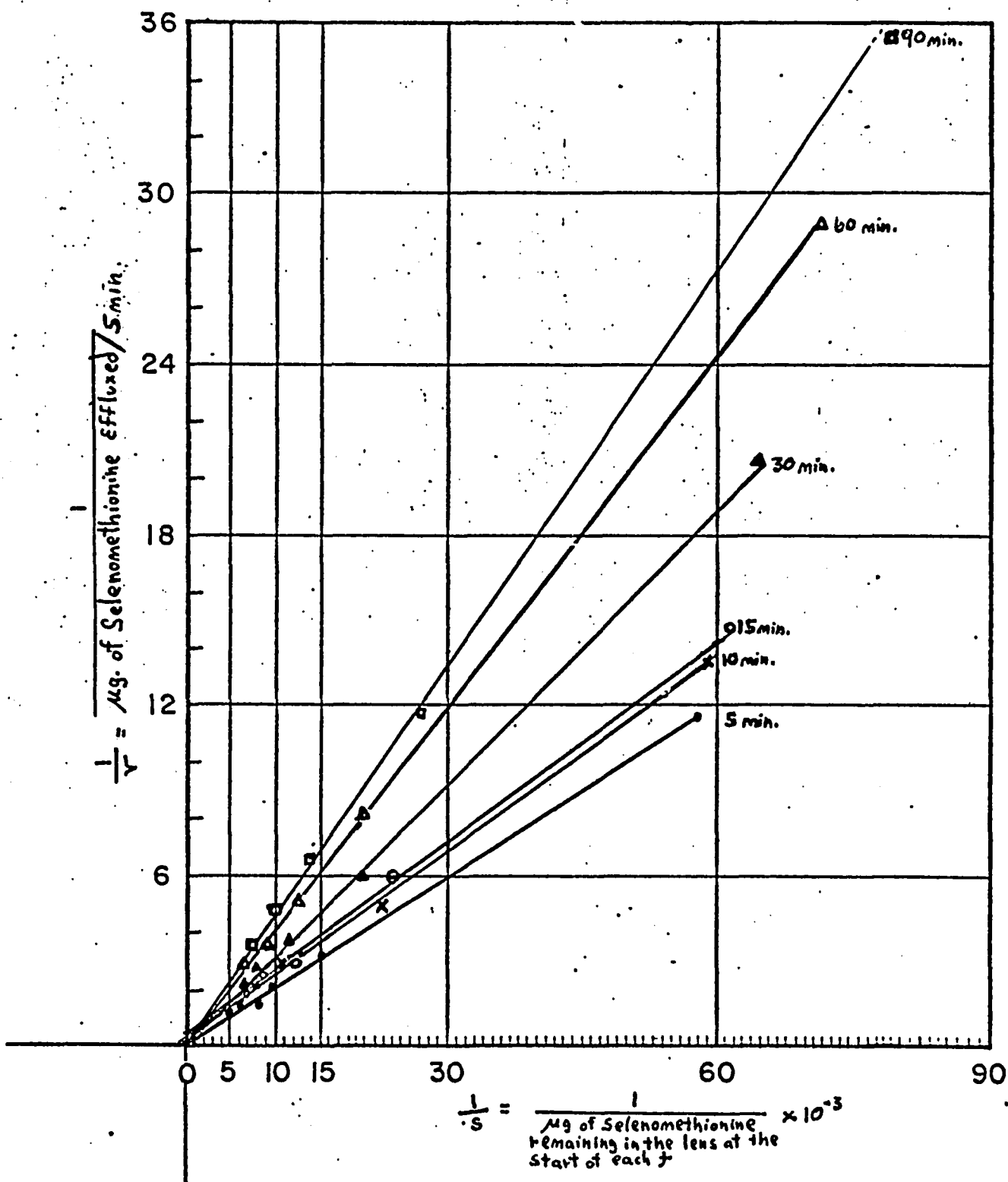


Figure 29. A Lineweaver-Burk plot of total efflux (diffusion + exchange) for cumulative efflux times ranging from 5 to 90 minutes. Data taken from figure 28.

100 mg% glucose with or without carrier selenomethionine at a concentration of 800 μ g per cc. Lense were then placed into the standard radioactive dose containing either 6.75 μ g/0.5cc (Fig. 30) or 30 μ g/0.5cc selenomethionine (Fig. 31). When 19 μ g/cc was used in the dose, there was no discernable difference between the preloaded group and its control with respect to accumulation. A dose containing 60 μ g of selenomethionine per cc did show a more pronounced accumulation (20%) in the preloaded group over the non-preloaded group (Fig. 31). Thus, the greater the outside concentration in the dose, the greater the amount accumulated via exchange diffusion in lenses that have been preloaded with high concentrations of selenomethionine.

Under the conditions used in our accumulation studies (6.75 μ g selenomethionine/0.5cc), there is relatively little accumulation of selenomethionine via exchange diffusion.

G. INHIBITION OF SELENOMETHIONINE EFFLUX

Several inhibitors, which affected accumulation, were investigated as to their effects on the two systems involved in efflux. The mercuric ion (1mM) had no inhibitory effect on simple diffusion, although it did cause

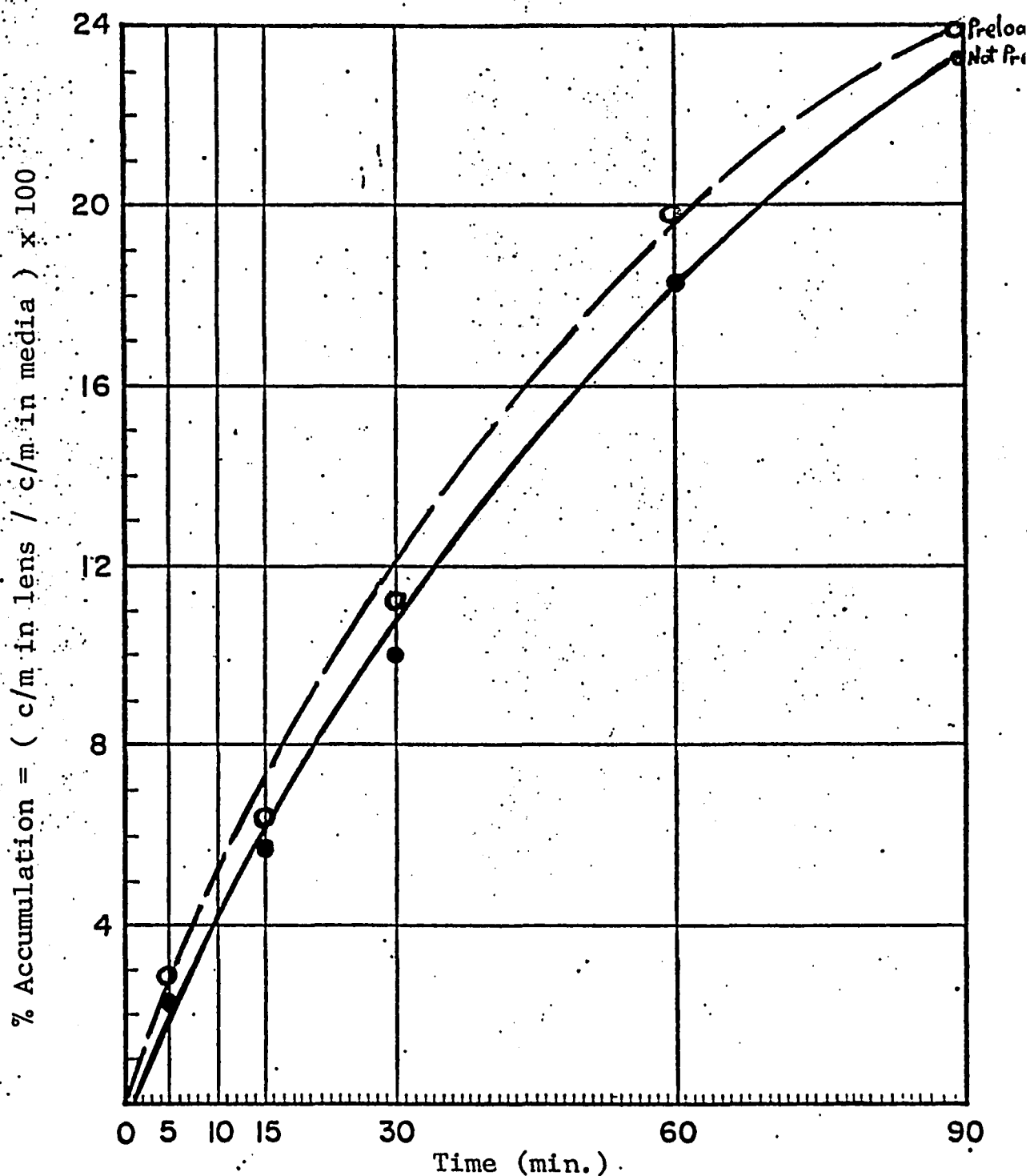


Figure 30. Accumulation of selenomethionine by lenses preloaded with selenomethionine. Lenses were pre-incubated in media containing no exchangeable amino acid ●————● or in media containing 800 µg/cc selenomethionine ○———○. Lenses were pulsed in the presence of 6.75 µg/cc selenomethionine.

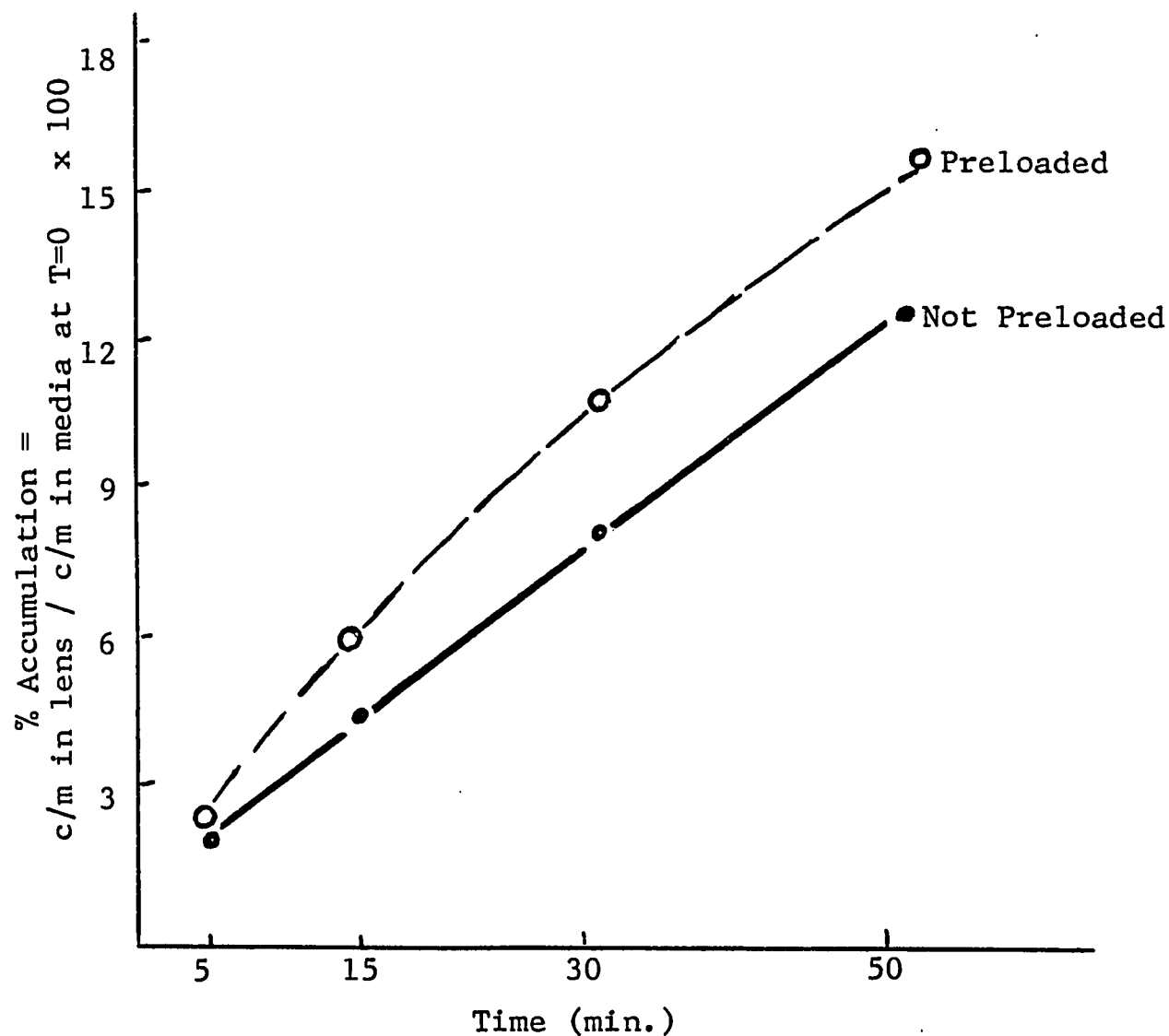


Figure 31. Accumulation of selenomethionine by lenses preloaded with selenomethionine. Lenses were pre-incubated in media containing no exchangeable amino acid ●————● or in media containing 800 $\mu\text{g}/\text{cc}$ selenomethionine ○————○. Lenses were pulsed in the presence of 60.0 $\mu\text{g}/\text{cc}$ selenomethionine.

opacification of the lens. The exchange diffusion system was completely obliterated in the presence of mercuric ions (Fig. 32).

There is no observable change in diffusion (D) in the presence of iodoacetic acid while exchange diffusion (E) is markedly inhibited by iodoacetic acid (Fig 33). This inhibition is apparent after 15 minutes of efflux.

The effect of N-ethylmaleimide (NEM), another sulfhydryl inhibitor, was studied (Fig. 34). Simple diffusion (D_{NEM}) appeared to be slightly increased in the presence of NEM. However, total efflux (T_{NEM}) was markedly lower. After 30 minutes no exchange diffusion (E_{NEM}) was observable. The data of this experiment, when plotted as $K \text{ min}^{-1}$ (rate coefficient) as a function of time (see materials and methods), revealed a diffusion pattern never before observed in any other efflux experiment. As seen in line D_{NEM} in Figure 35, the rate coefficient between 30 and 60 minutes does not change (in most NEM experiments it increased slightly). After 60 minutes the diffusion rate is similar to the control value. The change in rate constant from 30 to 60 minutes has never been observed in any other control or experimental lenses.

In the next set of experiments all lenses were incubated in a dose containing 5mM NEM and subsequently effluxed

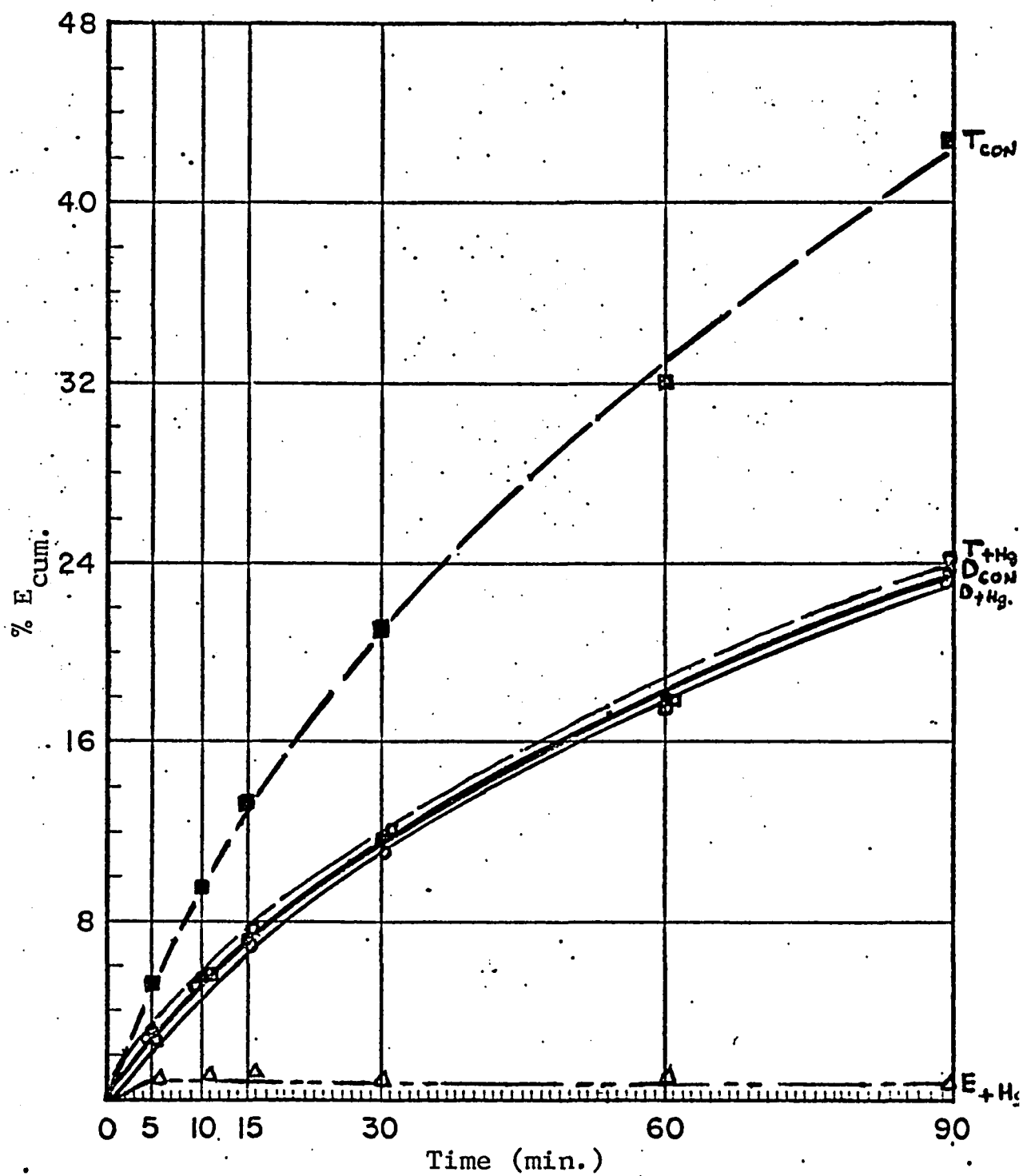


Figure 32. Efflux in the presence of 1mM mercuric chloride.

Control HgCl₂

■ — — — ■ Total □ — — — □

● — — — ● Diffusion ○ — — — ○

Exchange Δ — — — Δ

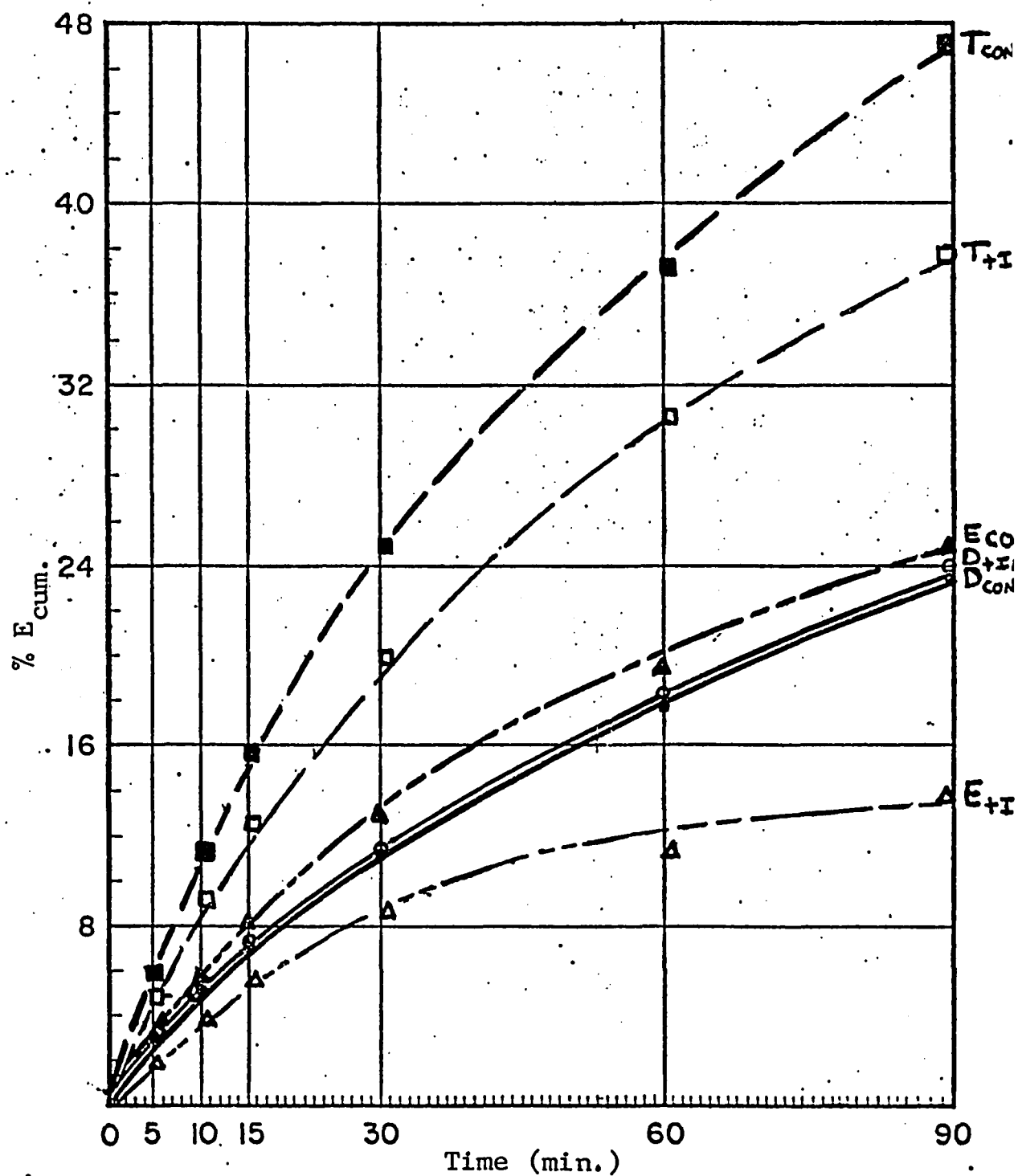


Figure 33. Efflux in the presence of iodoacetic acid.

Control		IAA (2mM)	
■	— Total	□	— Total
●	— Diffusion	○	— Diffusion
▲	— Exchange	△	— Exchange

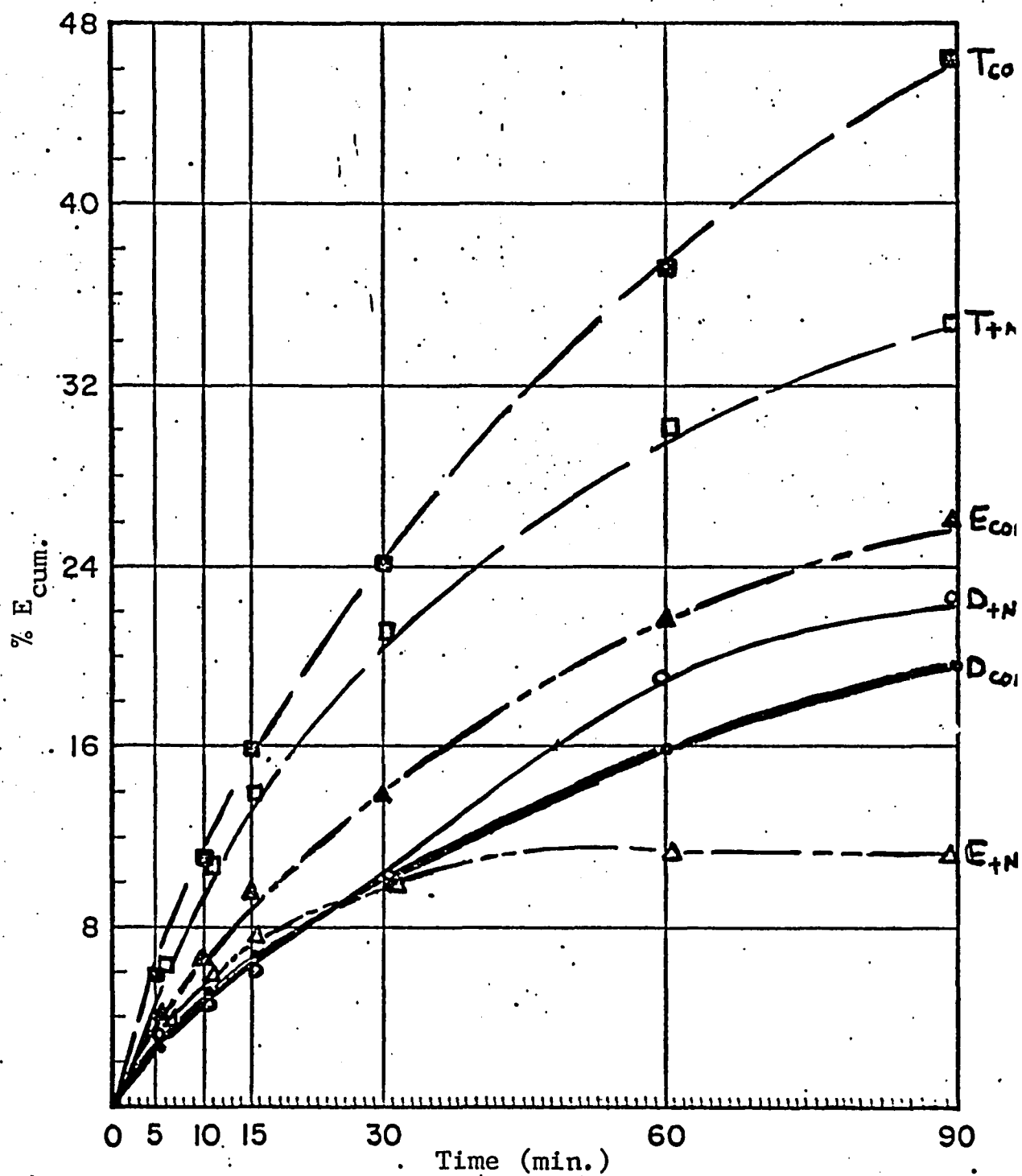
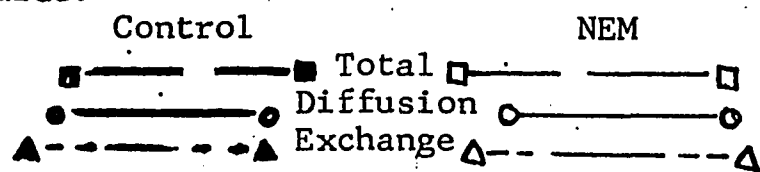


Figure 34. Efflux in the presence of 1mM N-ethylmaleimide.



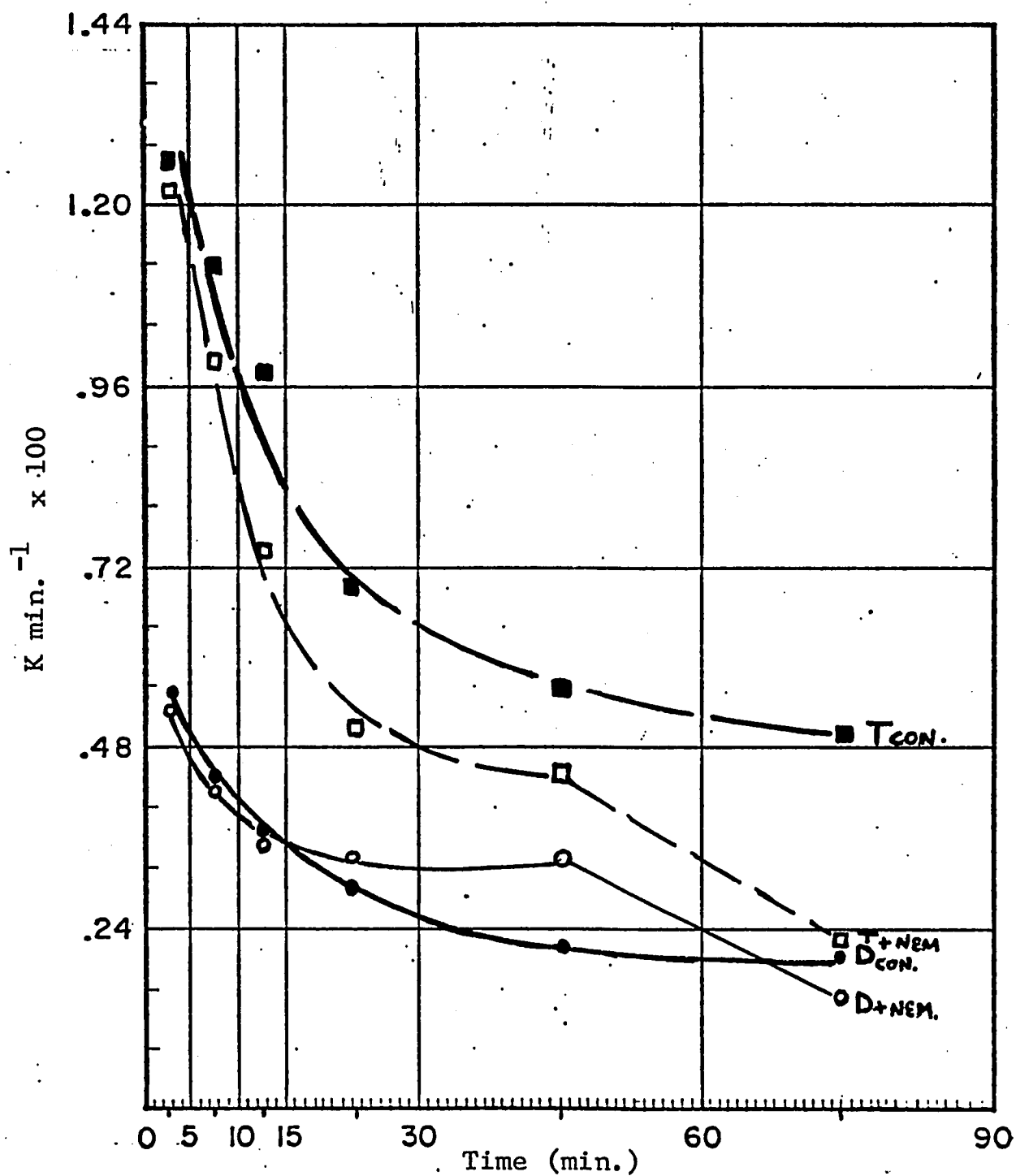


Figure 35. The change in rate coefficients of efflux for diffusion and exchange diffusion in the presence of N-ethylmaleimide.

Control NEM (1mM)

■ — ■ Total □ — □

● — ● Diffusion ○ — ○

in media with or without NEM in order to determine the long term effects of this inhibitor. Simple diffusion from the NEM-pulsed lenses was markedly increased (34%/90 min.) compared to the normal diffusion value, in controls (24%/90 min.). The NEM-pulsed lenses effluxed in the presence of NEM showed an even greater increase in diffusion (38%/90 min.). Those NEM-pulsed lenses allowed to efflux into a media containing NEM showed a complete loss of the exchange system (E_{NEM}) while those NEM-pulsed lenses which were effluxed into a NEM-free media showed a marked decrease in exchange diffusion (Fig. 36).

The role of glucose in maintaining membrane permeability and exchange diffusion was investigated. Lenses were preincubated in the standard dose (containing 1mg/cc glucose) and then allowed to efflux into media with or without glucose (Fig. 37). In the glucose-free media there was a slight increase in diffusion for the 60-90 efflux values. Furthermore, for the 60 and 90 minute values, the exchange diffusion for lenses effluxed in glucose-free media was decreased.

Lenses were incubated in a standard dose and were allowed to efflux into media containing glucose (1mg/cc) with or without D(+) galactose (1mg/cc). No changes in diffusion or exchange diffusion were observed (Fig. 38).

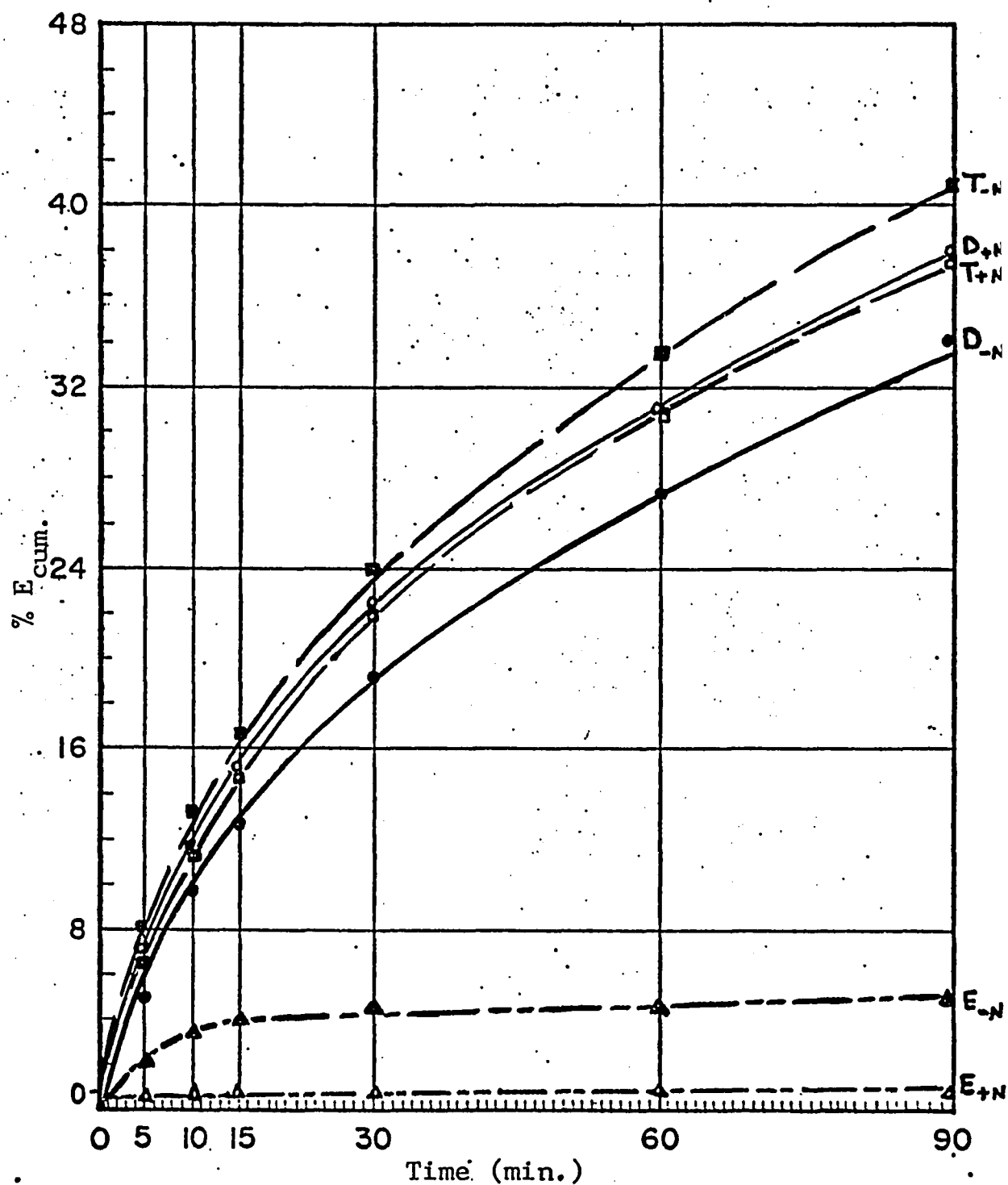


Figure 36. Efflux in the presence or absence of 5mM N-ethylmaleimide from lenses preincubated in the presence of 5mM N-ethylmaleimide.

Efflux with NEM Efflux without NEM

Total Total

Diffusion Diffusion

Exchange Exchange

89

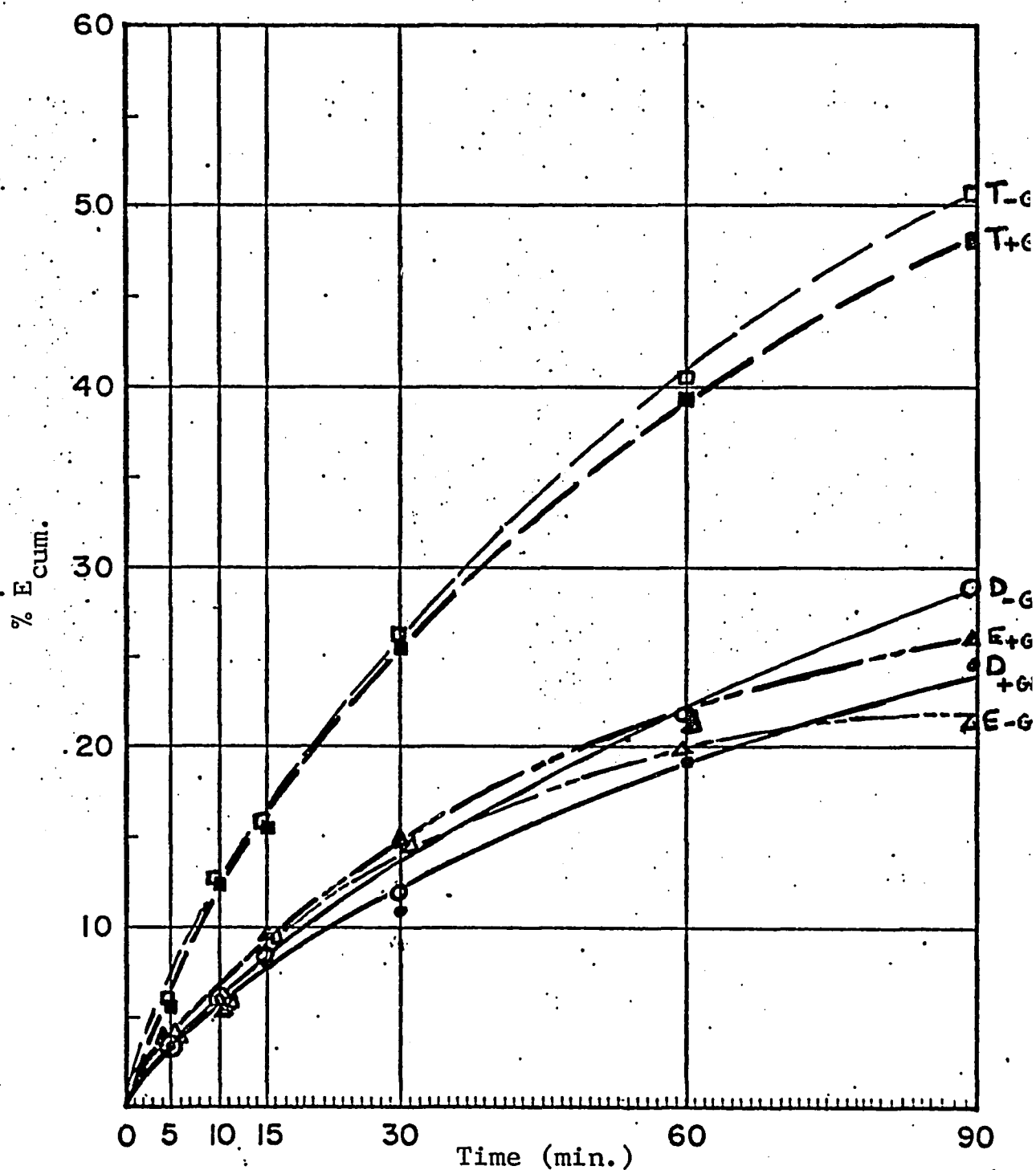


Figure 37. Selenomethionine efflux in the presence or absence of d(+) glucose; 100 mg%.

Glucose Present		Glucose Absent	
■	—	□	—
●	—	○	—
▲	- - -	△	- - -
	Total		Total
	Diffusion		Diffusion
	Exchange		Exchange

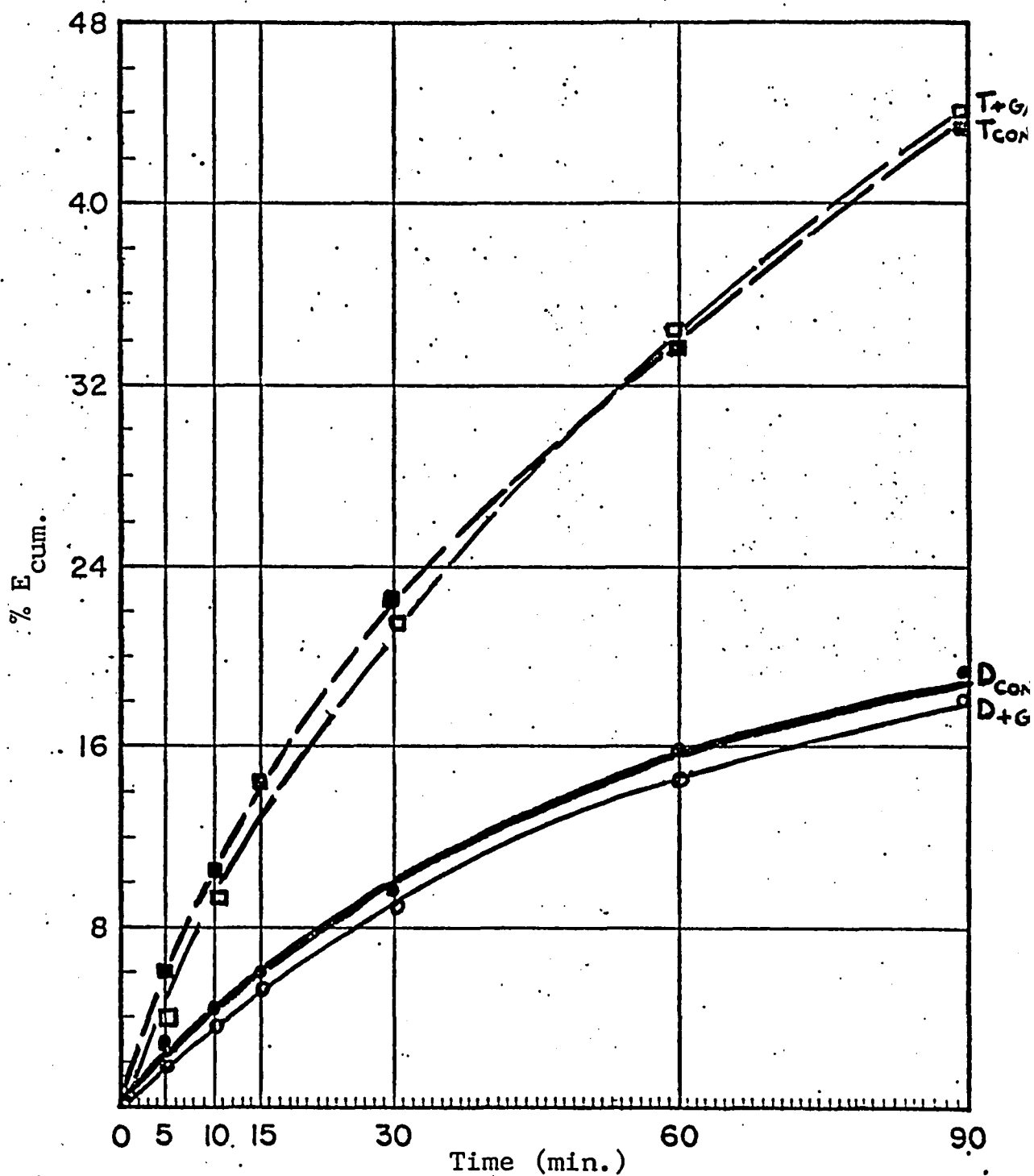


Figure 38. Selenomethionine efflux in the presence or absence of d(+) galactose; 100 mg%.

Galactose Present		Galactose Absent
□ — — — □	Total	■ — — — ■
○ — — — ○	Diffusion	● — — — ●

H. DIETARY STUDIES

1. Selenomethionine Accumulation and Incorporation into Protein in Galactose and Glucose Fed Animals

Rats were fed two types of diet: a sugar-Purina chow diet ("sugar-chow") and a sugar-casein, Mazola corn oil, brewers yeast, salt mixture and vitamin diet ("sugar-casein"). The sugar-chow diet (Table II) was used for most studies since it was convenient to prepare and was widely used by other investigators. In all cases the control group was fed 100% Purina Rat Chow.

The results obtained when feeding rats a 50% sugar-chow diet are presented in Figure 39. The per cent accumulation values for lenses from both control and glucose fed rats remained relatively constant over the 14 day feeding span. Lenses from animals on a 50% galactose diet for one day accumulated 33% less selenomethionine. On day three, accumulation decreased to 1/3 of that of the controls (66% inhibition). This level of inhibition remains constant over the remaining time periods studied (total of 14 days).

The per cent of the accumulated amino acid which was incorporated into protein (TCA-precipitable) is plotted in Figure 40. Lenses from control rats showed a minor decrease in per cent incorporation during the time course of the experiment (on day one the animals were five weeks old and

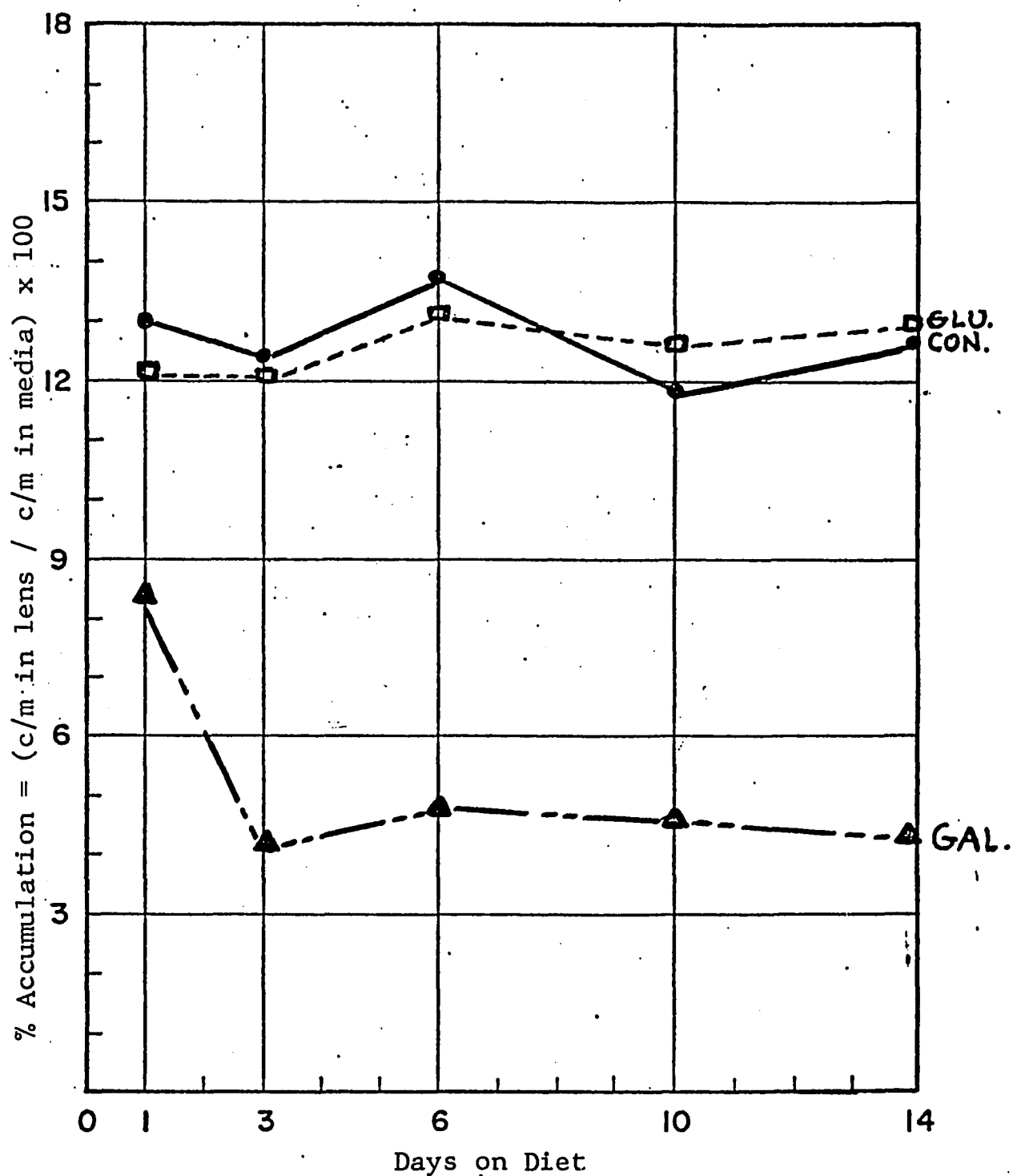


Figure 39. The effects of a glucose or galactose diet on selenomethionine accumulation. Controls were fed a 100% chow diet; sugar diets consisted of a 50% chow-sugar diet. Incubation time- 1 hour. N= 12 lenses/group/ day.

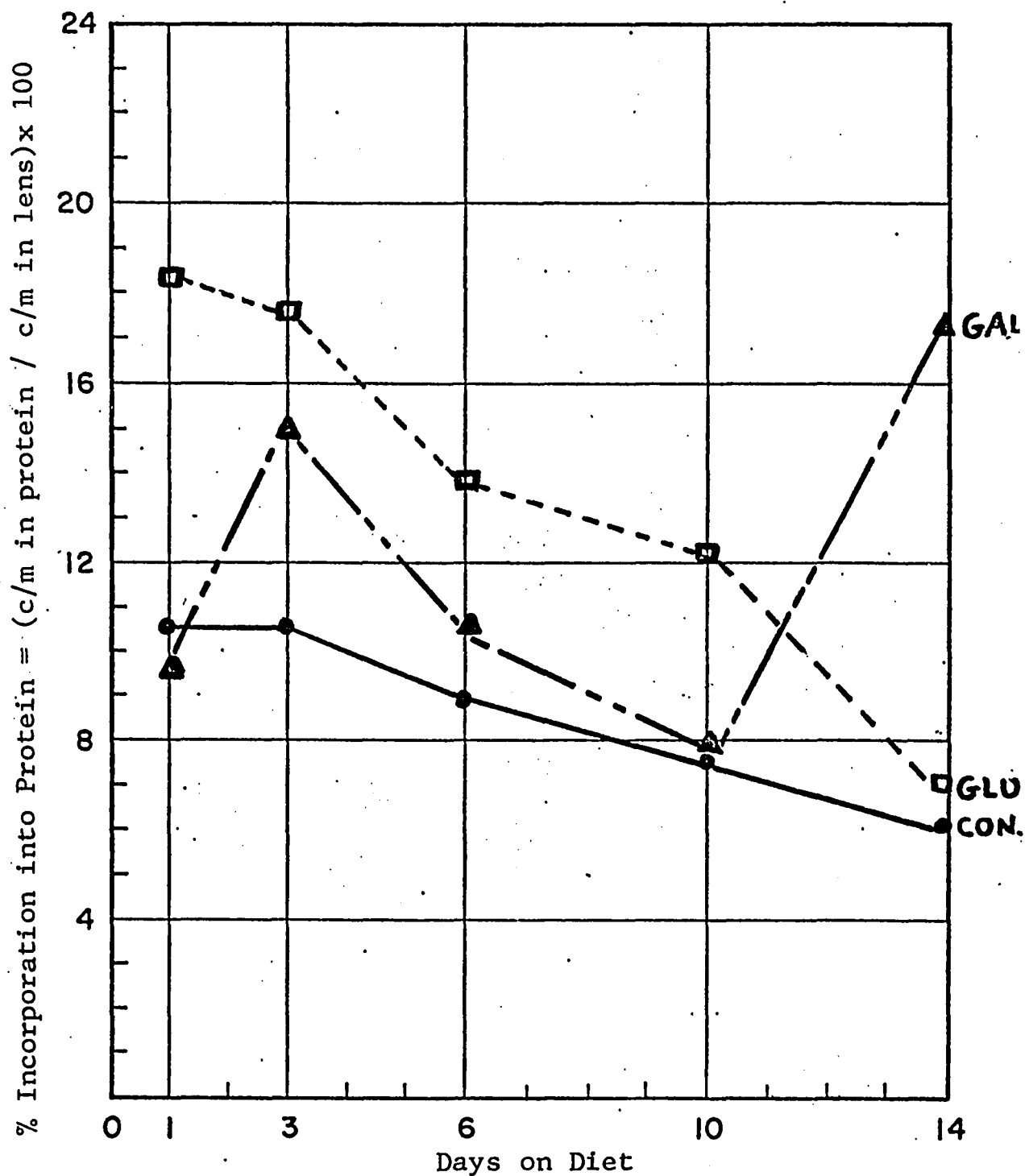


Figure 40. The effects of a glucose or galactose diet on selenomethionine incorporation into protein, A. Diet: 50% sugar-chow. Incubation time - 1 hour. N= 12 lenses/ group/ day.

at the end of the experiment they were seven weeks old). This decrease is attributed to aging and will be discussed later.

Lenses from animals fed galactose for one day had the same per cent incorporation value as did the controls. On day three the per cent incorporation is statistically higher and then returns to the control values on the sixth and tenth day. On the fourteenth day, there is a sharp increase in per cent incorporation into protein (Fig. 40).

Lenses from animals fed glucose for one day showed a significantly higher per cent incorporation into protein as compared to the controls. This increase was maintained over the entire time period studied. However, there is a decline in the magnitude of the increase as time progresses. Figure 41 shows the results of another set of experiments which in essence parallel the results presented in Figure 40 except for the magnitude of the per cent increase at days three and six for the galactose fed group.

In Table XIII, the actual c/m accumulated by the lenses are presented. It is apparent that the activity present in the lenses from galactose fed animals is significantly lower than the controls. The accumulated counts in lenses from glucose and control fed animals are identical for each time period.

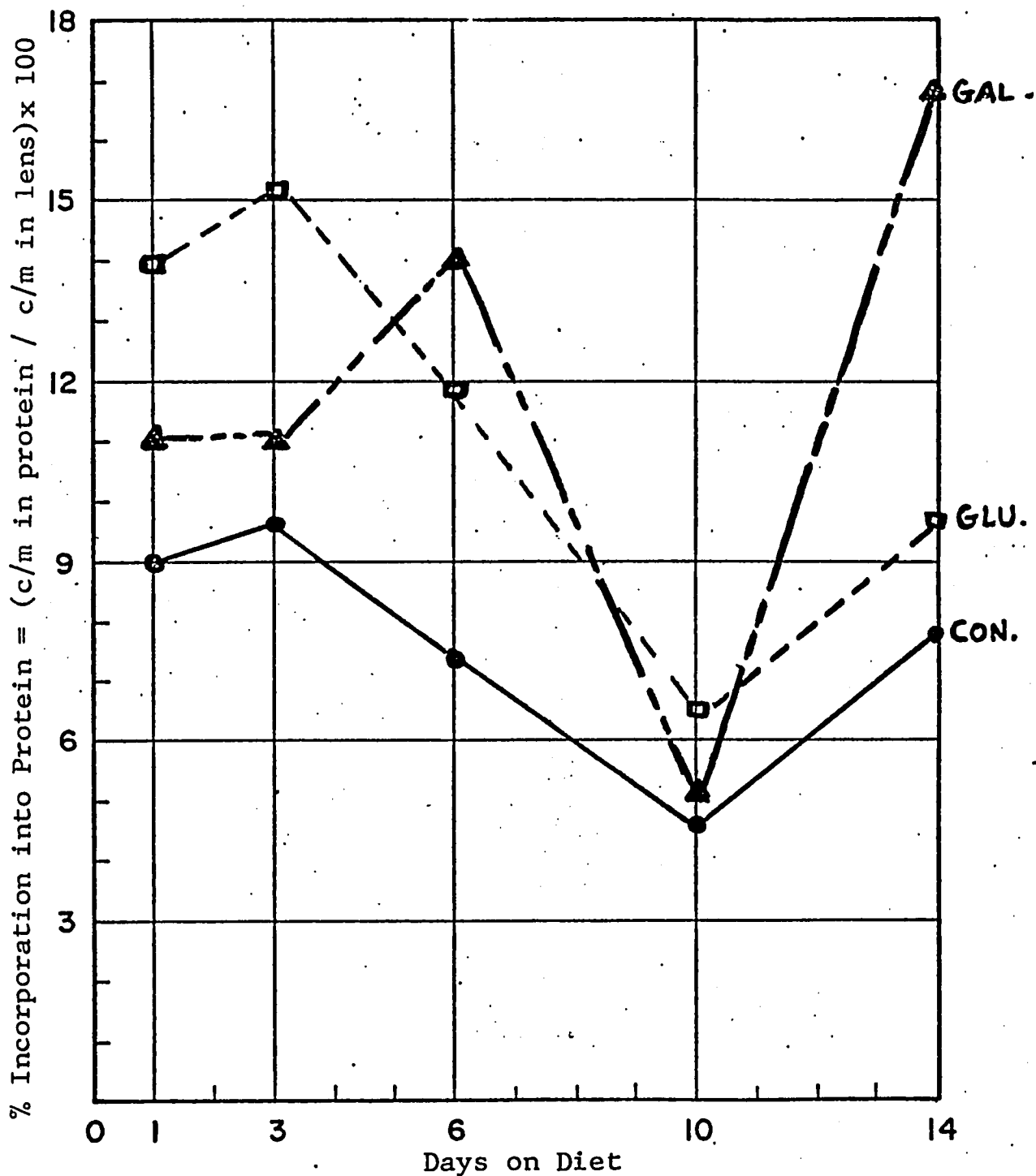


Figure 41. The effects of a glucose or galactose diet on selenomethionine incorporation into protein, B. Diet: 50% sugar-chow. Incubation time- 1 hour. N= 12 lenses/ group/ day.

Table XIII. The effects of glucose and galactose diets on selenomethionine accumulation and incorporation into protein. Control rats were fed purina-rat-chow; experimental rats fed a 50% sugar-casein diet. Incubation time- 40 min.

Diet	c/m Accumulated				
	Days on Diet				
	1	3	6	10	14
Control	25,025	24,173	25,607	23,896	23,400
Glucose	23,465	23,459	24,450	25,180	23,494
Galactose	16,731	8,209	9,044	9,414	8,381

Diet	c/m Incorporated into Protein				
	Days on Diet				
	1	3	6	10	14
Control	2,638	2,507	2,290	1,745	1,547
Glucose	3,979	4,123	3,474	3,223	1,721
Galactose	1,532	1,208	949	916	1,465

The c/m in the protein fractions are also presented in Table XIII. Lenses from galactose fed rats had less counts in the protein on days one, three, six and ten. On day fourteen, the counts in the protein of the galactose lenses were essentially the same as that found in the control lenses. Lenses from glucose fed animals had about 25-40% more counts in protein on days one, three, six and ten. Blood glucose levels were determined by the glucose oxidase method for the three groups on day fourteen of the diet. There was no difference between the control and glucose fed groups which were 88.7mg% and 86.5% respectively. The galactose fed group had a slightly lower blood sugar value of 80.2mg% which was not significantly different from the control.

2. The Effects of Inhibitors on Lenses from Galactose and Glucose Fed Rats

Table XIV shows the effects of 5mM iodoacetic acid and 0.5mM puromycin on lenses from animals fed galactose for two days. IAA inhibited uptake and protein incorporation in normal lenses. The accumulation in galactose fed lenses, which was already much lower than the control value as a result of the diet, was inhibited slightly by IAA. Protein incorporation in lenses from galactose fed rats is

Table XIV. The effects of puromycin and iodoacetic acid (IAA) on selenomethionine accumulation and incorporation into protein in lenses from galactose fed rats. Rats, 29 day old, were fed a 50% galactose-chow diet for 2 days prior to sacrifice. Incubation time - 1 hour. IAA-5mM, Puromycin- 0.5mM. N=8 for each group.

Diet	Inhibitor	c/m in Lens	% Uptake	T Test	c/m in Protein	% Incorp.	T Test
Control	-	54,600	16.25	-	7,080	12.91	-
Control	IAA	31,282	9.31	.001	1,348	4.28	.001
Control	Puromycin	57,490	17.11	NS	2,614	4.55	.001
Galactose	-	26,181	7.78	-	3,412	13.11	NS
Galactose	IAA	21,672	6.45	NS	1,990	9.22	.001
Galactose	Puromycin	28,594	8.51	NS	1,873	6.56	.001

Table XV. The effects of dinitrophenol (DNP) on selenomethionine accumulation and incorporation into protein in lenses from rats fed a high galactose or glucose diet. Rats, 30 to 50 days old, were fed a 50% sugar-chow diet for 2 days prior to sacrifice. Incubation time- 40 min. DNP 1mM; N=6

Diet	Inhibitor	c/m in Lens	% Uptake	c/m in Protein	% Incorp.
Control	-	30,843	13.41	2,685	8.71
Control	DNP	26,220	11.40	785	3.38
Galactose	-	15,525	6.75	1,956	12.62
Galactose	DNP	14,076	6.12	1,149	8.11
Glucose	-	30,802	13.42	3,789	12.27
Glucose	DNP	29,187	12.69	1,237	4.24

inhibited by IAA. This inhibition is not as severe as is found when IAA inhibits the control group, on either a per cent incorporation basis or on the basis on c/m in protein. In the presence of IAA, control value dropped from 7,080c/m to 1,348c/m while the galactose value dropped from 3,412c/m to 1,990c/m.

As expected, puromycin did not inhibit accumulation in the control lenses. Accumulation by lenses from galactose fed rats was also unaffected by puromycin. The per cent incorporation values in both galactose fed and control groups were lowered by puromycin with the former group being more severely affected on a per cent incorporation basis than with respect to actual c/m (Table XIV).

The effects of 1mM dinitrophenol on uptake and protein incorporation for control and galactose fed animals are given in Table XV. Control animals, as in previous experiments, were not affected with respect to uptake but exhibited a 60% inhibition of protein incorporation. Galactose fed animals did not exhibit any significant changes with respect to accumulation in the presence of DNP. Per cent incorporation into protein was reduced in the group fed galactose but not to the same extent as in the control group. As in the case with IAA, dinitrophenol reduced the c/m in the controls (785c/m) to a value lower than that found in the galactose fed group (1,149c/m).

The effects of 1mM dinitrophenol on uptake and protein incorporation for normal and glucose fed animals (a 2-day diet) are presented in Table XV. There were no appreciable effects with respect to accumulation for either control or glucose fed animals. Protein incorporation was higher in the glucose fed group than in the controls. However, both groups are inhibited by DNP to the same level of inhibition with respect to their corresponding controls (61% and 65% inhibition).

Iodoacetic acid decreases accumulation in lenses from rats fed glucose to the same extent that it inhibits normal lenses (Table XVI). Similarly, the per cent incorporation into protein was inhibited in the glucose fed lenses to the same extent as in the controls.

3. Glucose Diets: The Stimulatory Effects on Protein Incorporation

A review of preliminary dietary studies revealed that these initial experiments did not show the stimulatory effect on the per cent protein incorporation in glucose fed rats. Upon comparison of the earlier and later studies, several differences in the methodology were noted. In these very early studies, methionine and not selenomethionine was employed as the non-labeled analogue amino acid in

Table XVI. The effects of iodoacetic acid (IAA) on selenomethionine accumulation and incorporation into protein in lenses from rats fed a 50% glucose-purina -chow diet. Rats, 30 days old, were fed their respective diets for 2 days prior to sacrifice. Incubation time- 1 hour. IAA 5mM.

Diet	Inhibitor	c/m in Lens	% Uptake	c/m in Protein	% Incorp.
Control	-	45,011	19.56	4,038	8.98
Control	IAA	23,910	10.39	1,013	4.23
Glucose	-	42,352	18.40	4,603	10.92
Glucose	IAA	23,925	10.40	1,080	4.53

the dose. The per cent of glucose in the diet varied from 35% to 66% and the composition of the diet also differed (see Table II and III).

Table XVII shows the results of an experiment using glucose diets of three concentrations (35, 50 and 75% glucose) with Purina-chow comprising the remainder of each diet. Animals were maintained on their respective diets for five days. Lenses were incubated in media that contained either methionine or selenomethionine as the non-labeled analogue in the dose. The glucose stimulatory effect on protein incorporation occurred in each of the glucose fed groups to the same extent regardless of the glucose concentration or analogue utilized.

A study was made on the effects of 66% glucose-casein diet, over a two day period, on protein incorporation (Table XVIII). Both the control and glucose groups had the same accumulation values. However, rather than a stimulatory effect of protein incorporation by glucose feeding, there was a slight inhibitory effect (11.53% and 9.77% incorporation for control and glucose fed groups respectively). There was also a noticeable decrease in body weight in those animals placed on the glucose diet.

A study was undertaken to evaluate the effects of starvation on accumulation and incorporation since differences

Table XVII. The effects of feeding diets containing different concentrations of glucose on selenomethionine uptake and incorporation into protein. Rats, 29 days old, were kept on their respective diets for 5 days prior to sacrifice. Incubation time - 1 hour. Dose contained either unlabeled methionine or selenomethionine. N=8/group.

Diet	Analogue	% Uptake	% Incorp.	Avg. weight of animal on day 5. (g)
100% Chow	Selenomethionine	16.67	9.63	60.8
100% Chow	Methionine	17.78	9.51	
35% Glucose	Selenomethionine	16.48	12.62	62.5
35% Glucose	Methionine	17.56	12.54	
50% Glucose	Selenomethionine	15.50	13.49	60.8
50% Glucose	Methionine	15.73	12.85	
75% Glucose	Selenomethionine	15.02	13.18	54.0
75% Glucose	Methionine	15.94	14.18	

Table XVIII. The accumulation and incorporation into protein of selenomethionine in lenses from rats fed a 66% glucose-casein diet. Duration of diet - 2 days. Incubation time - 1 hour. Animal age - 36 days. N=16/group.

Diet	% Uptake	% Incorp. into Protein	Avg. wt. of animal on day 2.
Controls (100% Chow)	17.27	11.53	107.9 g
66% Glucose-Casein Diet	17.37	9.77	88.9 g

Table XIX. The effects of starvation on selenomethionine accumulation and incorporation into protein. Animals were starved for 48 hours. Incubation time - 1 hour. Animal age - 39 days. N=12/group.

Group	% Uptake	% Incorp. into Protein	Avg. wt. of animal on day 2.
Controls	19.34	12.24	108 g
Starved	17.49	9.11	80 g

in body weights were observed for the two groups in the previous experiment. Six animals were starved for 48 hours while six of their litter mates were kept on a normal chow diet. Starvation did not significantly alter accumulation, but it did lead to an inhibition in protein incorporation (Table XIX). The starved groups had an average body weight that was 75% of the controls.

4. Efflux Studies on Lenses from Galactose Fed Rats

Efflux studies were also carried out on lenses from galactose fed rats. Experimental animals were maintained on a 50% galactose chow diet for one, three and seven days. Controls were fed a 100% Purina-chow diet. All animals were starved for 24 hours prior to being fed their respective diets.

Lenses from rats fed galactose for one day showed a marked increase in per cent diffusion (D_{gal}) (Fig. 42). The curves illustrating exchange diffusion will be discussed later. The actual millimicromoles of selenomethionine diffused from lenses of control and one day galactose fed rats is presented in Figure 43. The galactose lenses (which had taken up less selenomethionine than the controls) lost more selenomethionine by simple diffusion over the entire period of efflux. The cumulative efflux values at

90 minutes were $1.32\text{m}\mu$ moles for the controls and $1.94\text{m}\mu$ moles for lenses from galactose fed rats.

On day three, the per cent cumulative diffusion increased to a value of 50% compared to the normal value of the controls of 20% (Fig 44). The same effect was noted in lenses of animals fed galactose for seven days (Fig. 46). Although lenses from galactose fed animals accumulated only $1/3$ as much selenomethionine as the controls, the quantity of selenomethionine effluxed via diffusion by both groups (D_{con} and D_{gal}) for the first thirty minutes was the same (Fig. 45). As Figure 47 illustrates, the results on day seven mimic the results of day three with respect to diffusion.

Per cent exchange diffusion in lenses from galactose fed animals (E_{gal}) on day one was greater than controls (E_{con}) (Fig. 42). The galactose exchange component (E_{gal}) tends to level off beyond 30 minutes while the control continues to increase. On an absolute basis (i.e., in $\text{m}\mu$ moles of selenomethionine), the galactose group exchanges the same quantity of selenomethionine as controls for the first thirty minutes of efflux after which time the control values continue to increase while the galactose values level off. The total number of $\text{m}\mu$ moles effluxed was the same at the end of 90 minutes for both groups, $3.25\text{m}\mu$ moles (T_{con} and T_{gal} , Fig. 43).

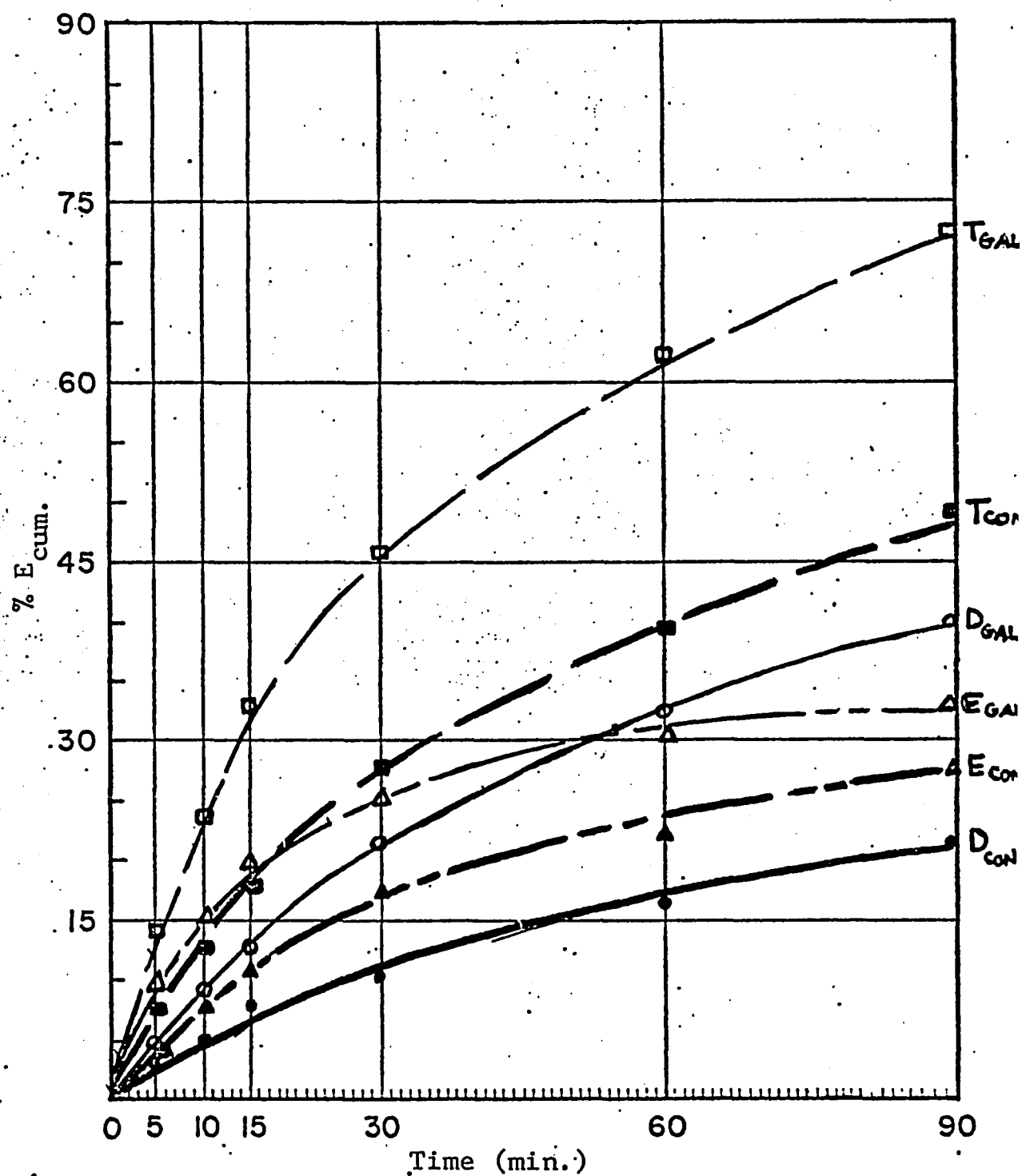


Figure 42. Percent efflux of selenomethionine from lenses taken from rats fed a 50% galactose-chow diet for one day.

	<u>Total</u>	<u>Diffusion</u>	<u>Exchange</u>
Control	●———■	●———●	▲———▲
Galactose	□———□	○———○	△———△

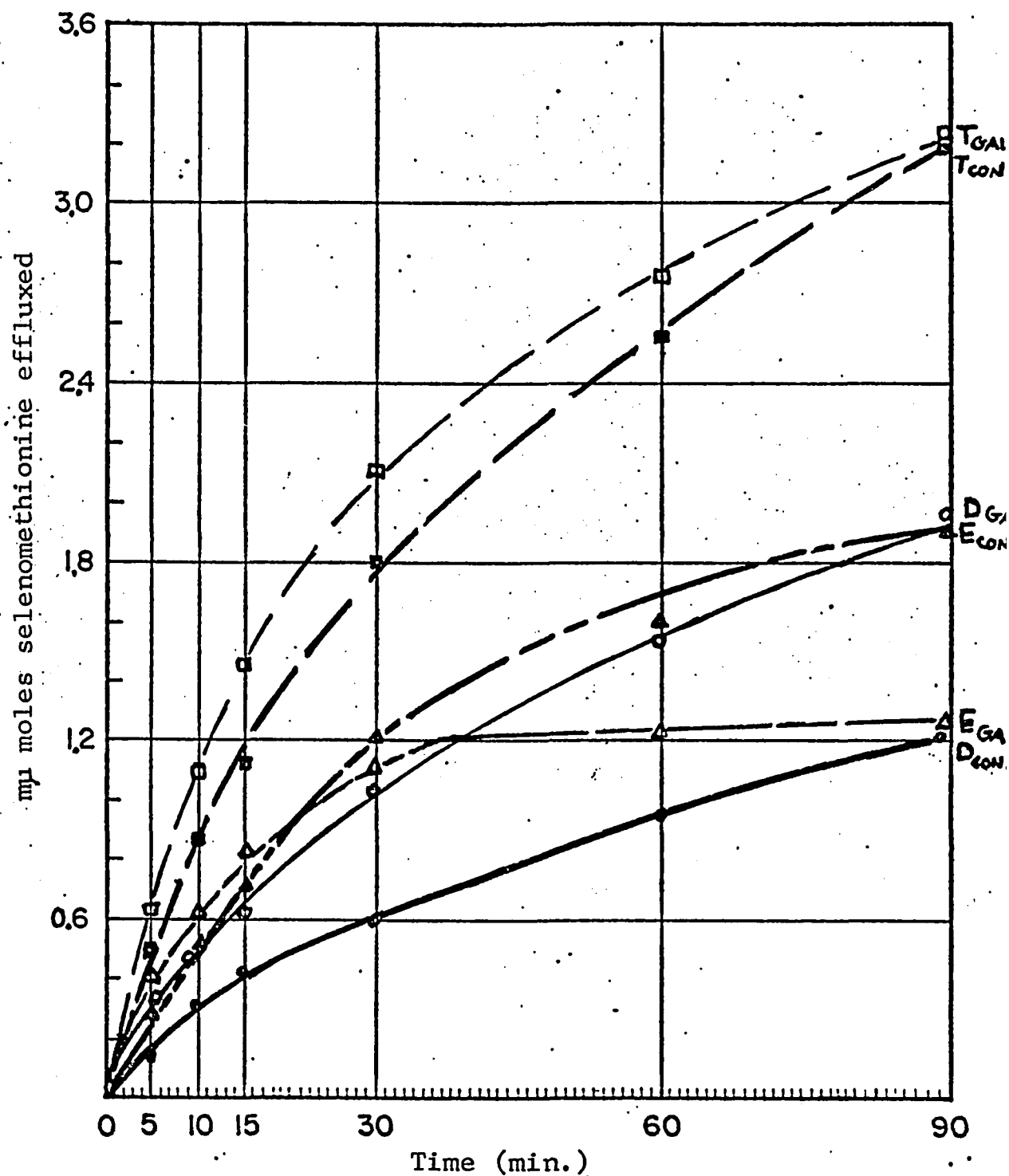


Figure 43. Quantitative efflux of selenomethionine from lenses taken from rats fed a 50% galactose-chow diet for one day.

	<u>Total</u>	<u>Diffusion</u>	<u>Exchange</u>
Control	●—●	●—●	▲—▲
Galactose	□—□	○—○	△—△

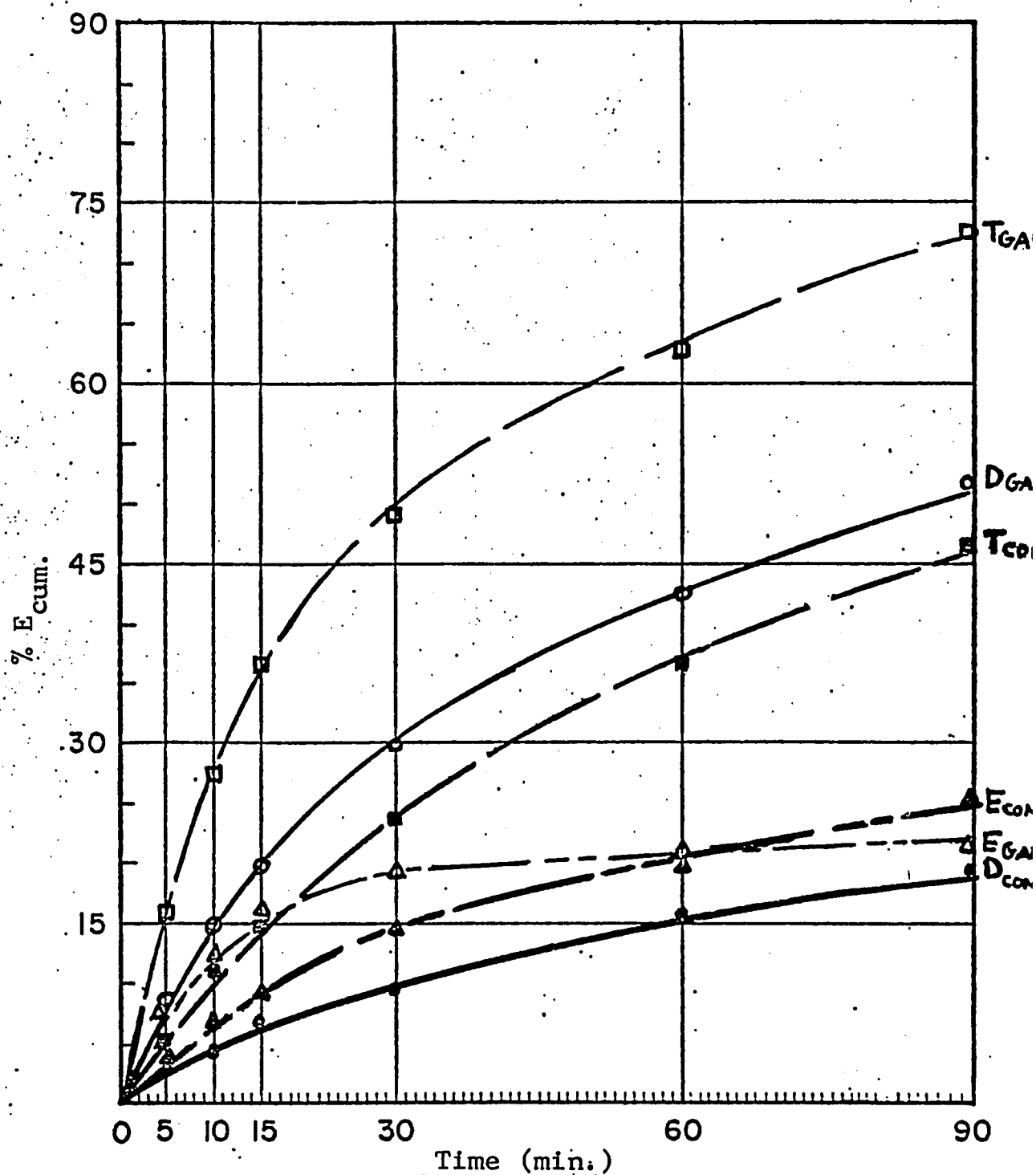


Figure 44. Percent efflux of selenomethionine from lenses taken from rats fed a 50% galactose-chow diet for three days.

	<u>Total</u>	<u>Diffusion</u>	<u>Exchange</u>
Control	—●—●—	—●—●—	—▲—▲—
Galactose	—□—□—	—○—○—	—△—△—

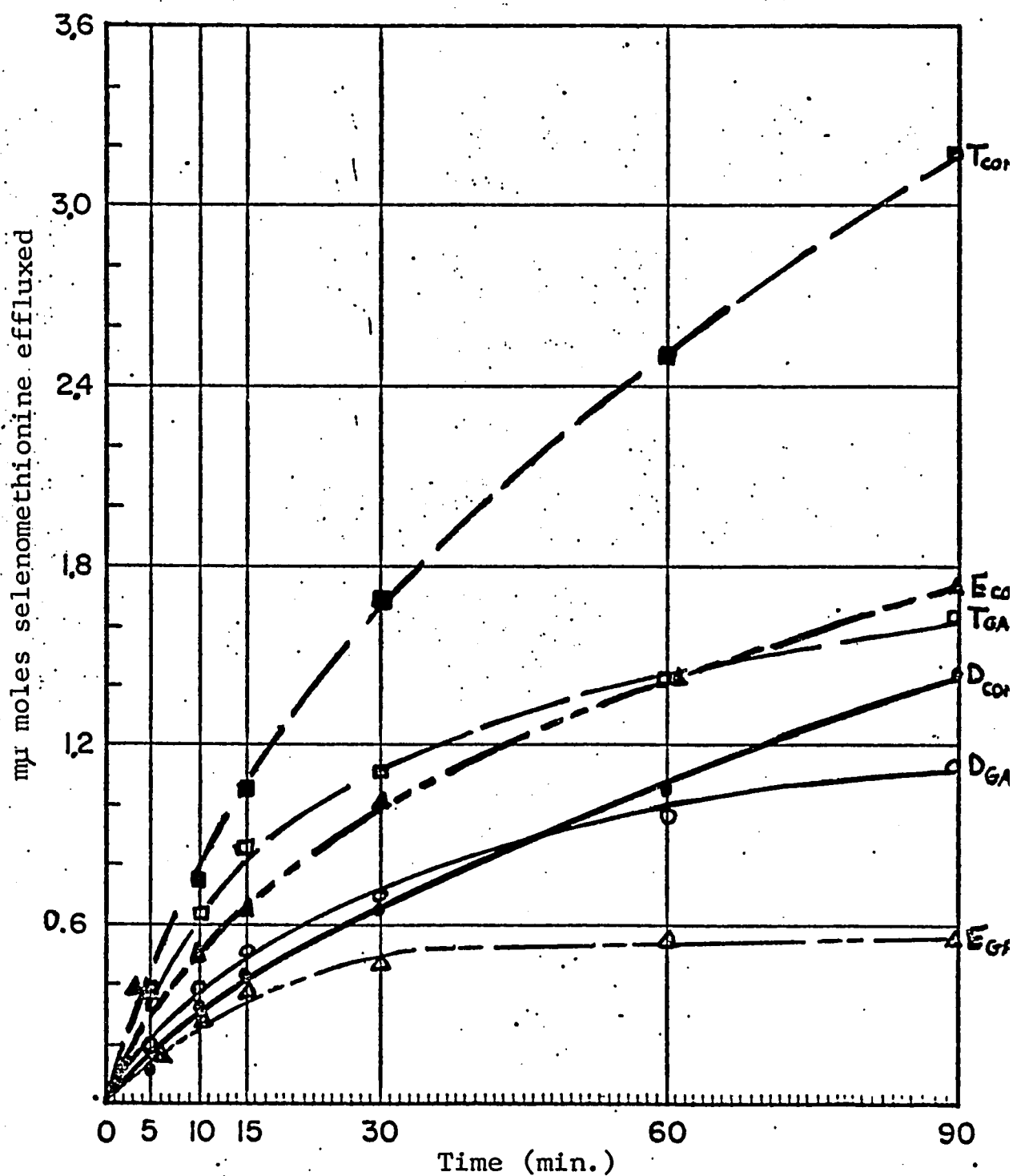


Figure 45. Quantitative efflux of selenomethionine from lenses taken from rats fed a 50% galactose-chow diet for three days.

	<u>Total</u>	<u>Diffusion</u>	<u>Exchange</u>
Control	■ — ■	● — ●	▲ — ▲
Galactose	□ — □	○ — ○	△ — △

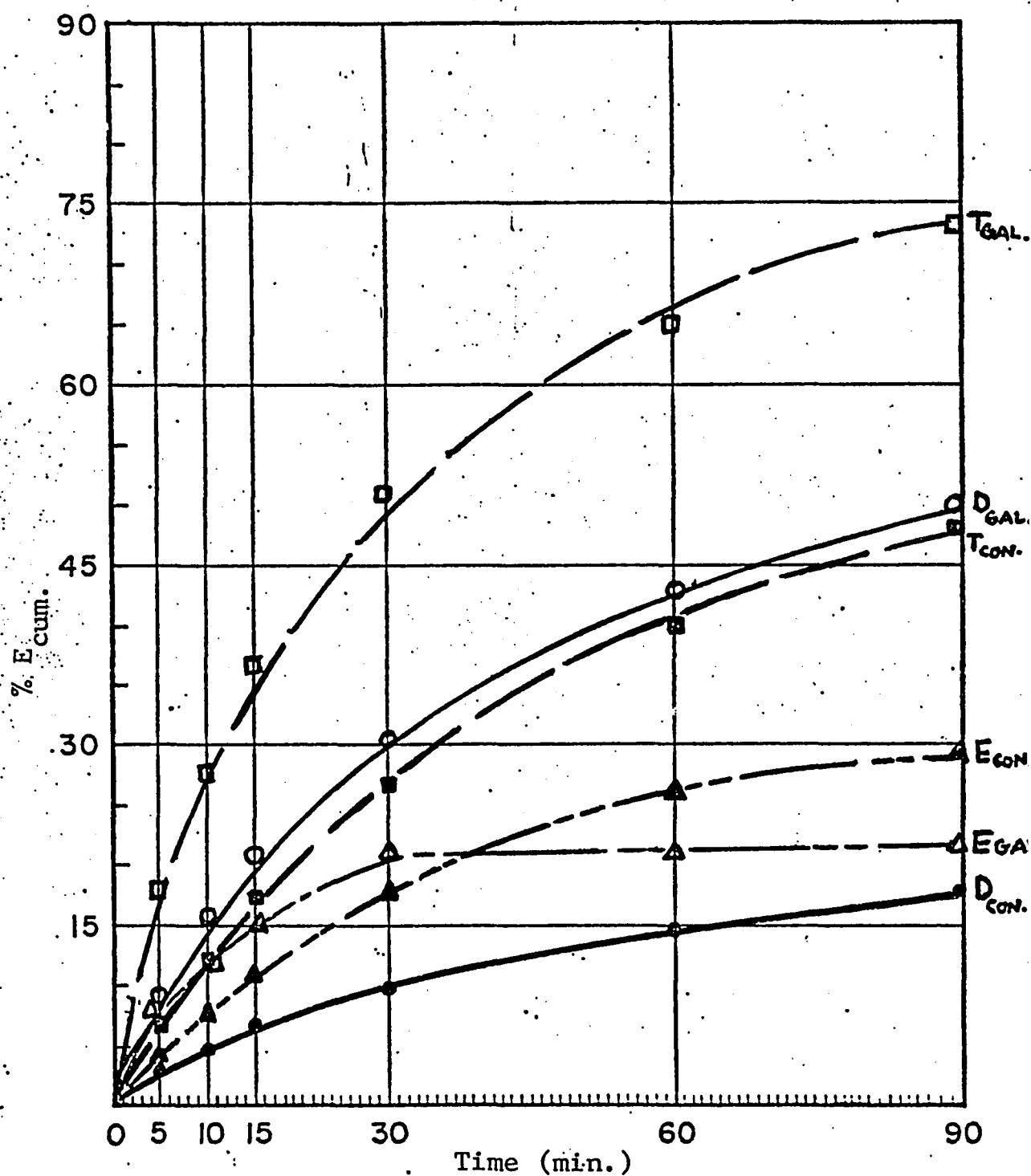


Figure 46. Percent efflux of selenomethionine from lenses taken from rats fed a 50% galactose-chow diet for seven days.

	<u>Total</u>	<u>Diffusion</u>	<u>Exchange</u>
Control	■ — — ■	● — — ●	▲ — — ▲
Galactose	□ — — □	○ — — ○	△ — — △

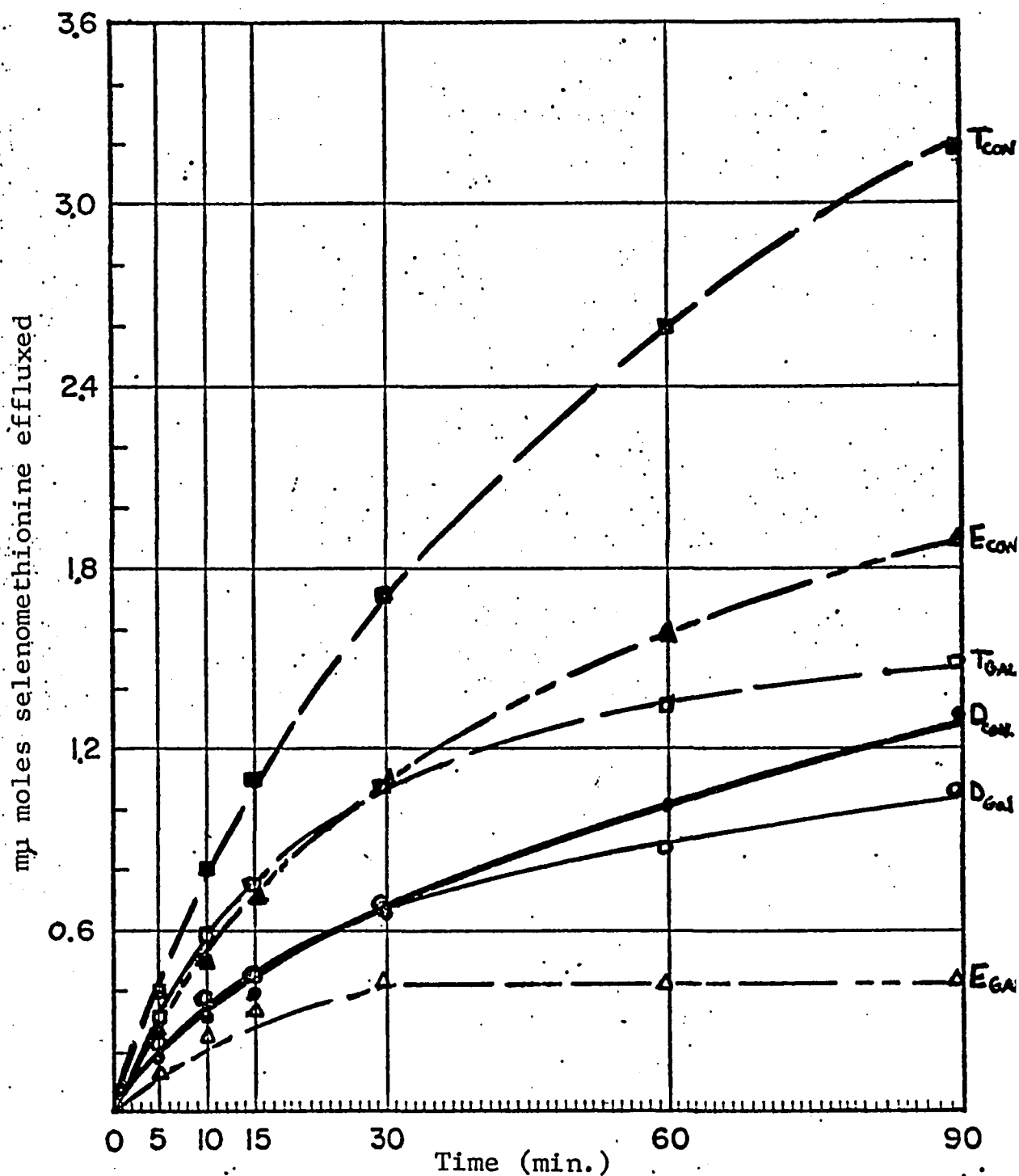


Figure 47. Quantitative efflux of selenomethionine from lenses taken from rats fed a 50% galactose-chow diet for seven days.

	<u>Total</u>	<u>Diffusion</u>	<u>Exchange</u>
Control	■ — — ■	● — — ●	▲ — — ▲
Galactose	□ — — □	○ — — ○	△ — — △

On day three of the diet, the per cent effluxed via exchange diffusion in galactose lenses (E_{gal}) was greater than the controls (E_{con}) for the first 30 minutes of efflux time (Fig. 44). Beyond 30 minutes the E_{gal} value leveled off while the E_{con} continued to increase. Almost identical results were found with lenses from animals fed galactose for seven days (Fig. 46). The corresponding values, expressed in $m\mu$ moles, for exchange diffusion are given in Figure 45 and Figure 47.

I. SELENOMETHIONINE ACCUMULATION AND INCORPORATION INTO PROTEIN AS A FUNCTION OF AGE

Accumulation of selenomethionine by the lens as a function of age was studied. As shown in Figure 48, accumulation by the lens decreased linearly as a function of age between 3 and 44 weeks of age. The curve of uptake, plotted as a function of lens weight, is hyperbolic and concave to the origin (Fig. 49) while the curve representing the number of μ g of selenomethionine accumulated per mg of lens as a function of age is hyperbolic and convex to the origin (Fig. 50).

Per cent incorporation into protein increased for the first few weeks of age and then dropped sharply over the next several weeks (Fig. 51). Beyond the 13th week there was a gradual decrease of per cent incorporation up to 44

weeks of age. Per cent incorporation as a function of lens weight showed an increase in young lenses (17-26mg lens weight) followed by a relatively sharp decrease in per cent incorporation beyond 26mg lens weight (Fig. 52). Per cent incorporation into protein per mg lens weight when plotted against age decreased hyperbolically convex to the origin (Fig. 53).

J. EFFLUX AS A FUNCTION OF AGE

Per cent efflux in old lenses (T_{old}) was greater than in young lenses (T_{young} , Fig. 54). Older lenses had a higher per cent diffusion value (D_{old}) than did young lenses (D_{young}) for all time periods in the experiment. This effect is more pronounced when one looks at the number of $m\mu$ moles effluxed via diffusion (Fig. 55). Older lenses effluxed more selenomethionine (D_{old}) than did younger lenses (D_{young}) even though the older lenses accumulated 25% less selenomethionine (6.0 $m\mu$ moles accumulated by young lenses and 4.5 $m\mu$ moles by old lenses).

The per cent cumulative exchange diffusion values in young and old lenses were similar for the first 60 minutes of efflux (Fig. 54). At 90 minutes the per cent cumulative exchange was slightly higher for the young lenses. There was no discernable difference between young and old lenses in the number of $m\mu$ moles of selenomethionine exchanged

during the first 30 minutes of efflux (Fig. 55). At 60 and 90 minutes the values were higher for the young lenses.

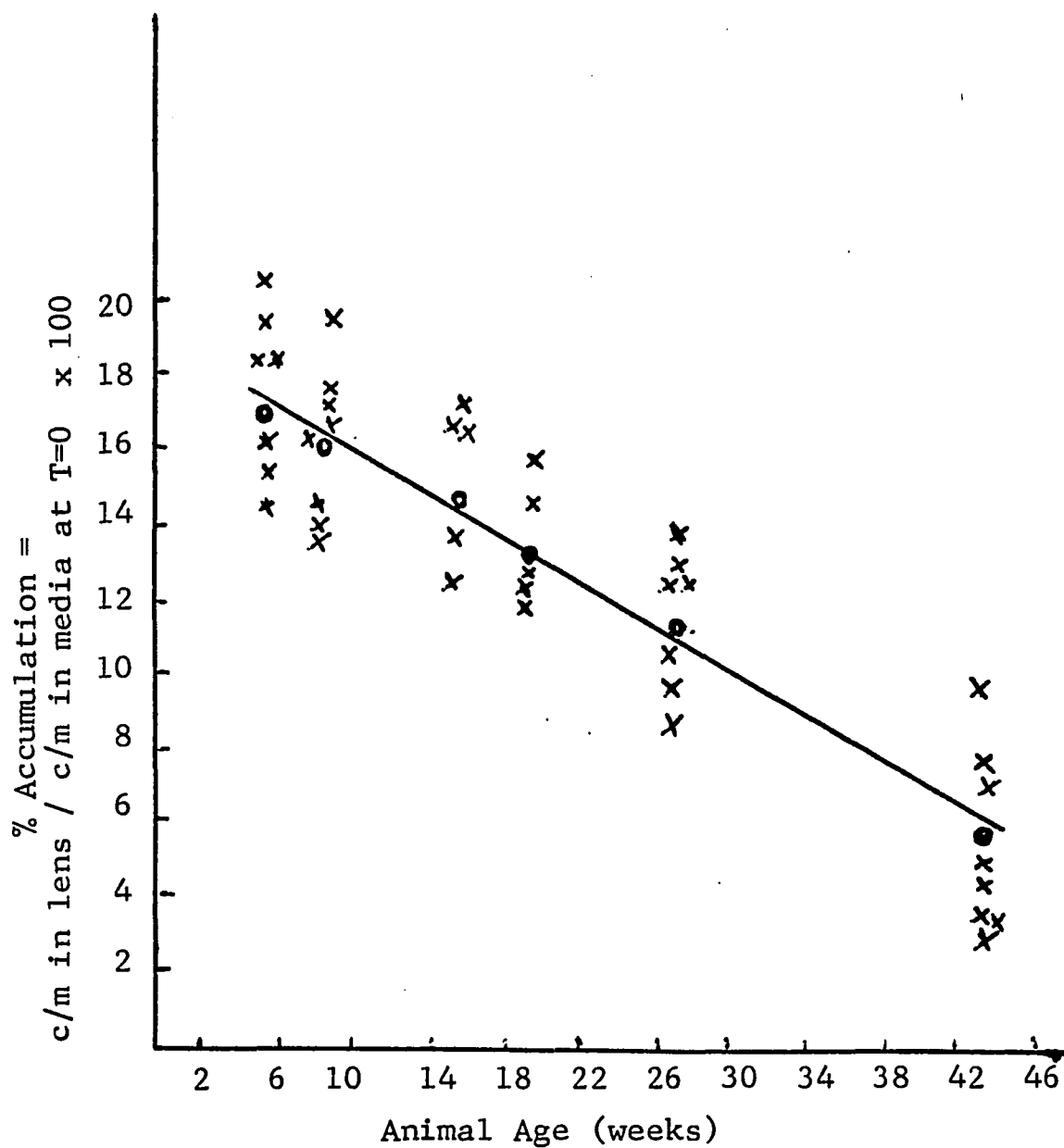


Figure 48. Selenomethionine accumulation as a function of age. Incubation time - 1 hr. Solid circles denote average values.

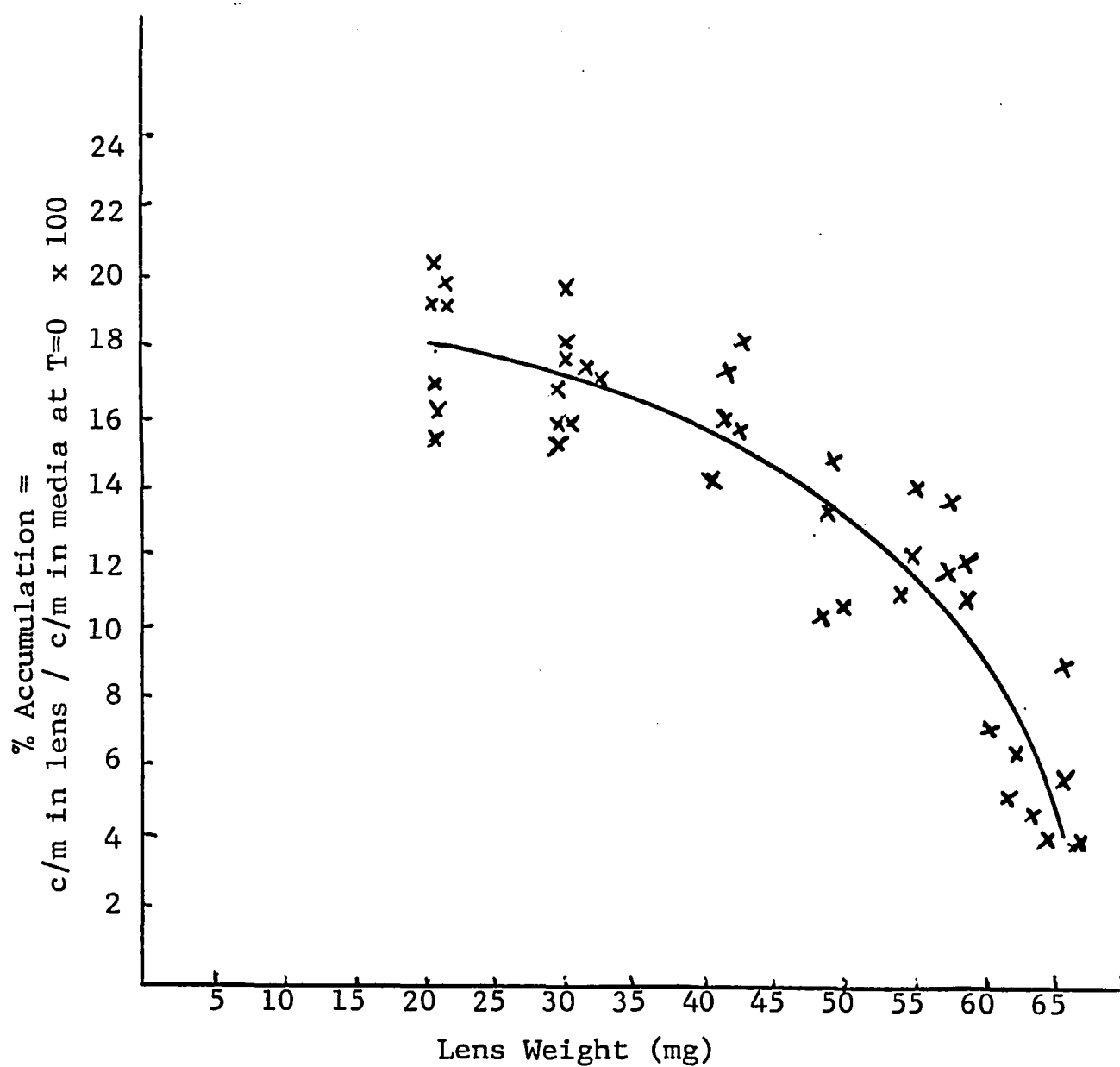


Figure 49. Selenomethionine accumulation as a function of lens weight.

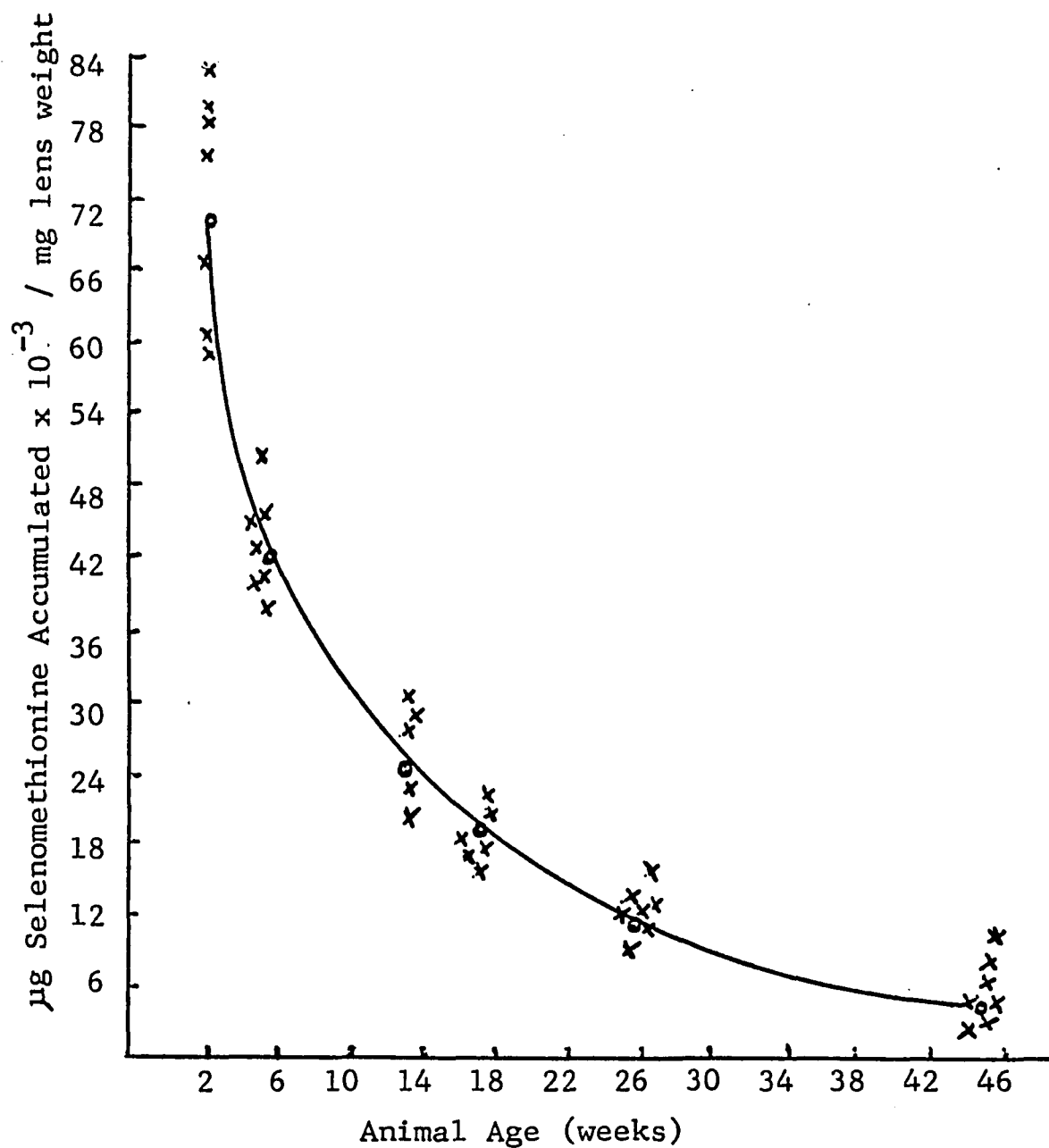


Figure 50. Selenomethionine accumulation per mg of lens as a function of age. Closed circles denote average values.

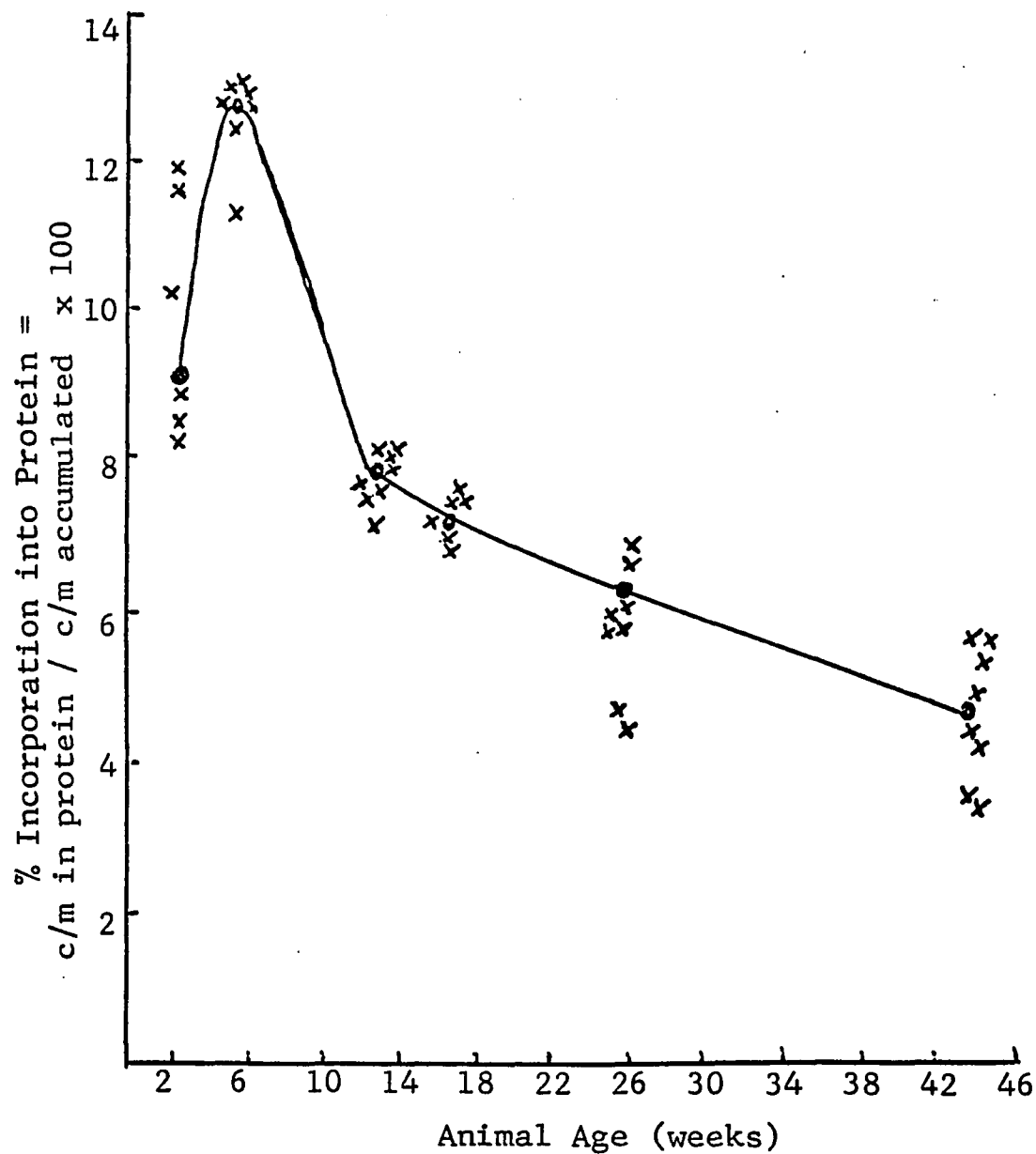


Figure 51. Selenomethionine incorporation into protein as a function of age. Solid circles denote average values.

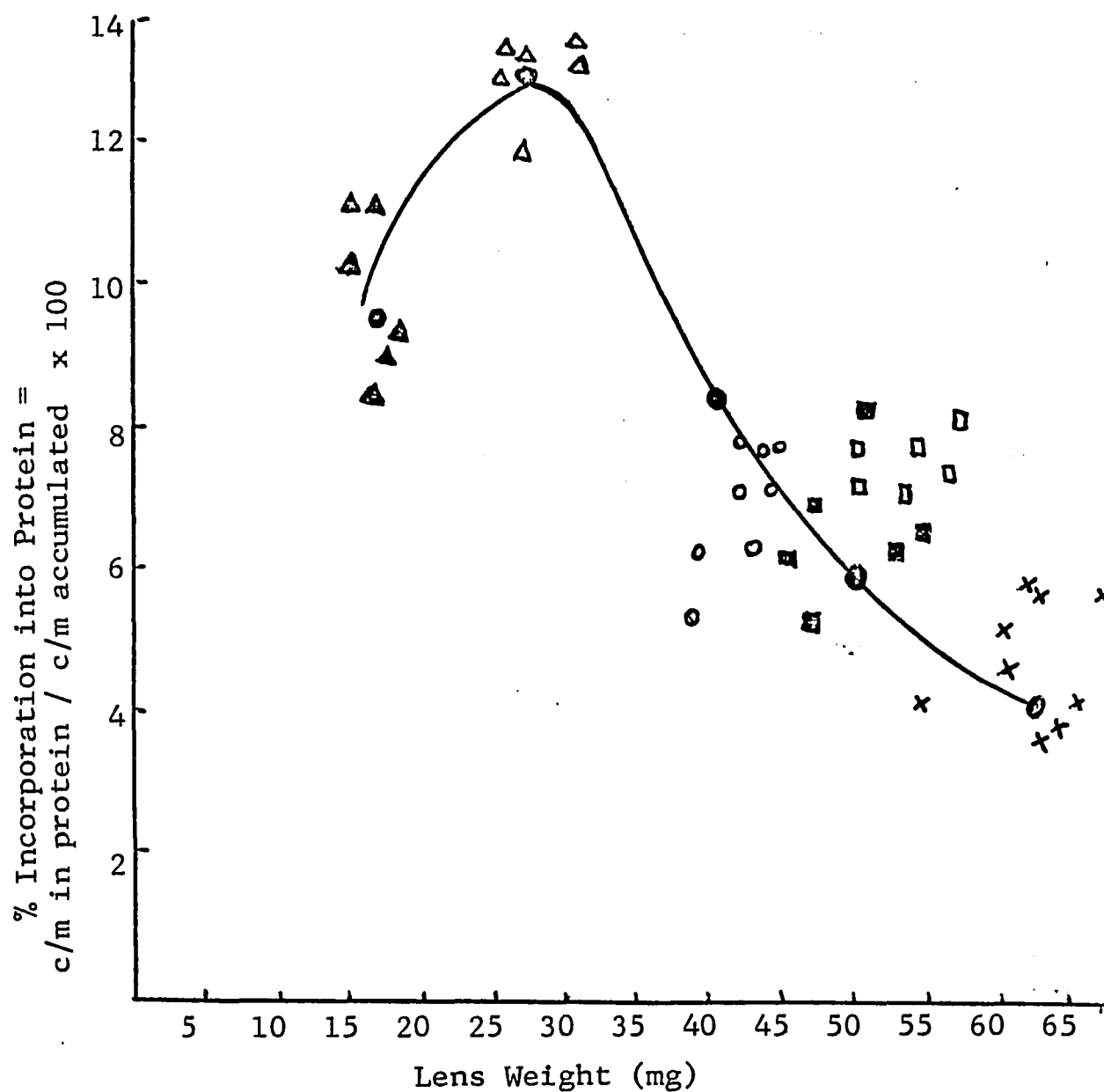


Figure 52. Selenomethionine incorporation into protein as a function of lens weight. Symbols represent lenses taken from rats of the same age.

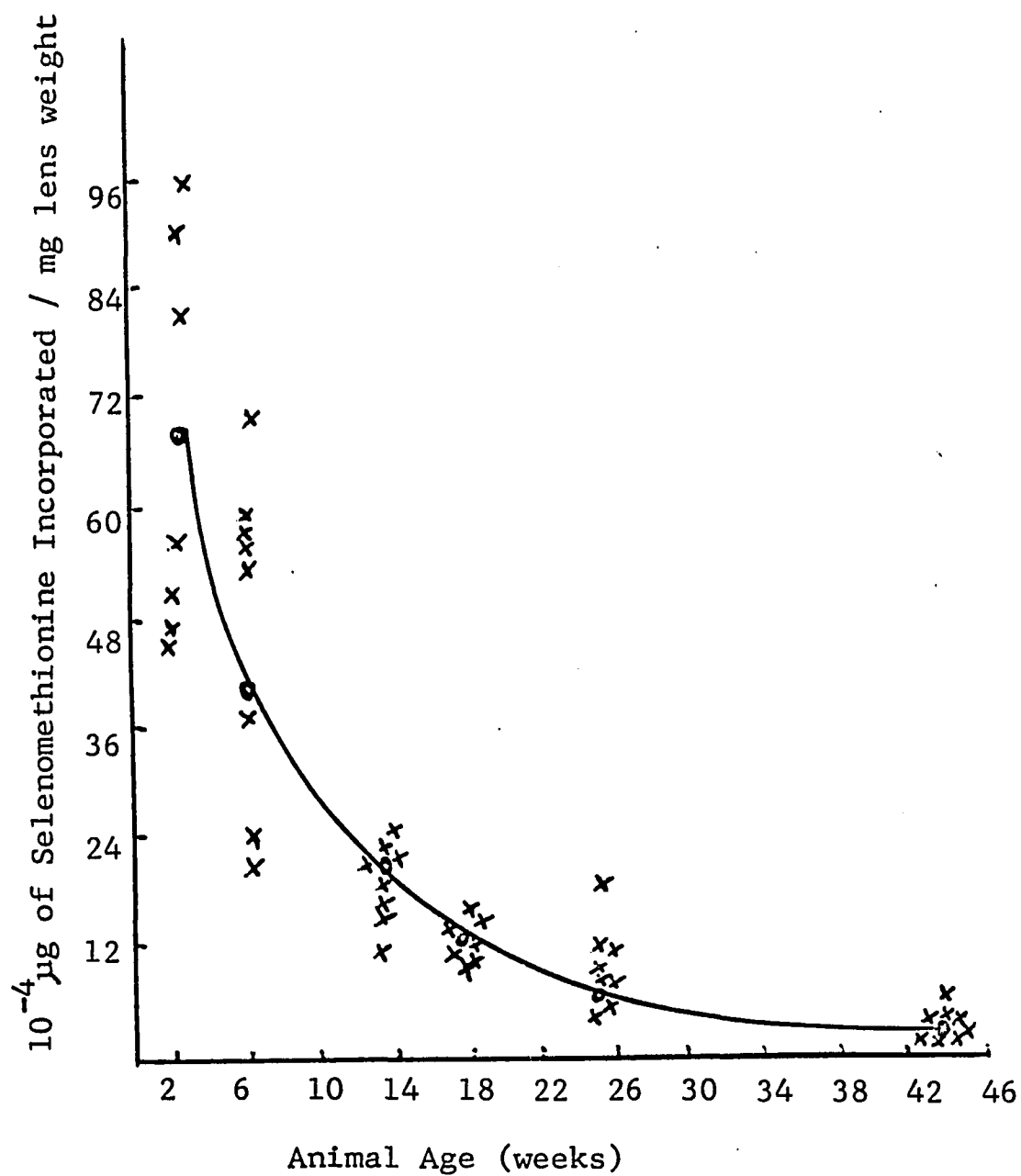


Figure 53. The % incorporation of selenomethionine into protein per mg of lens weight as a function of age.

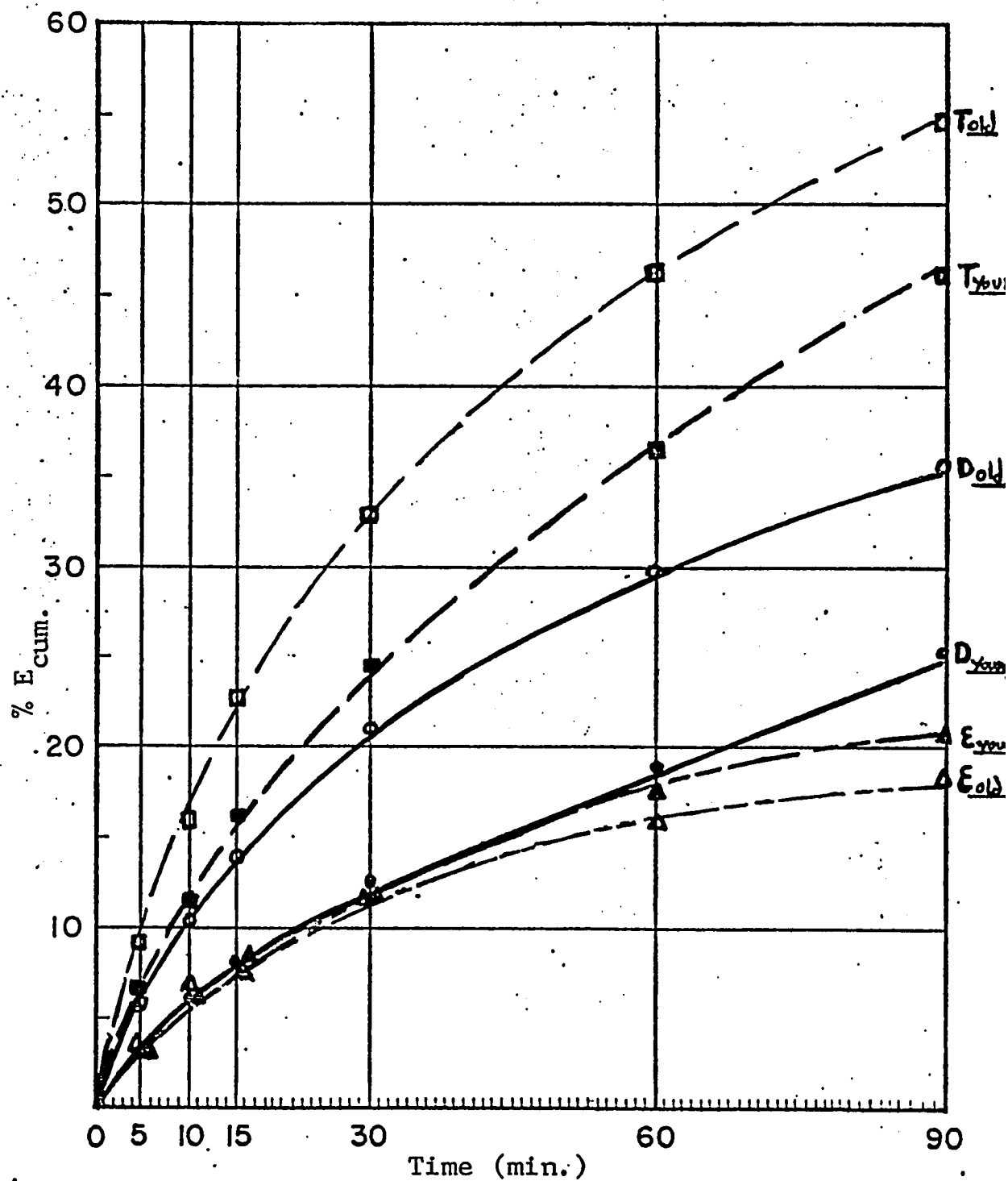
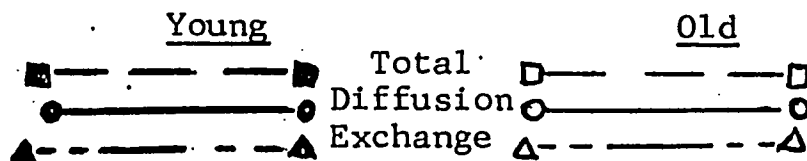


Figure 54. Efflux of selenomethionine from lenses of young (54 days) and old (565 days) rats.



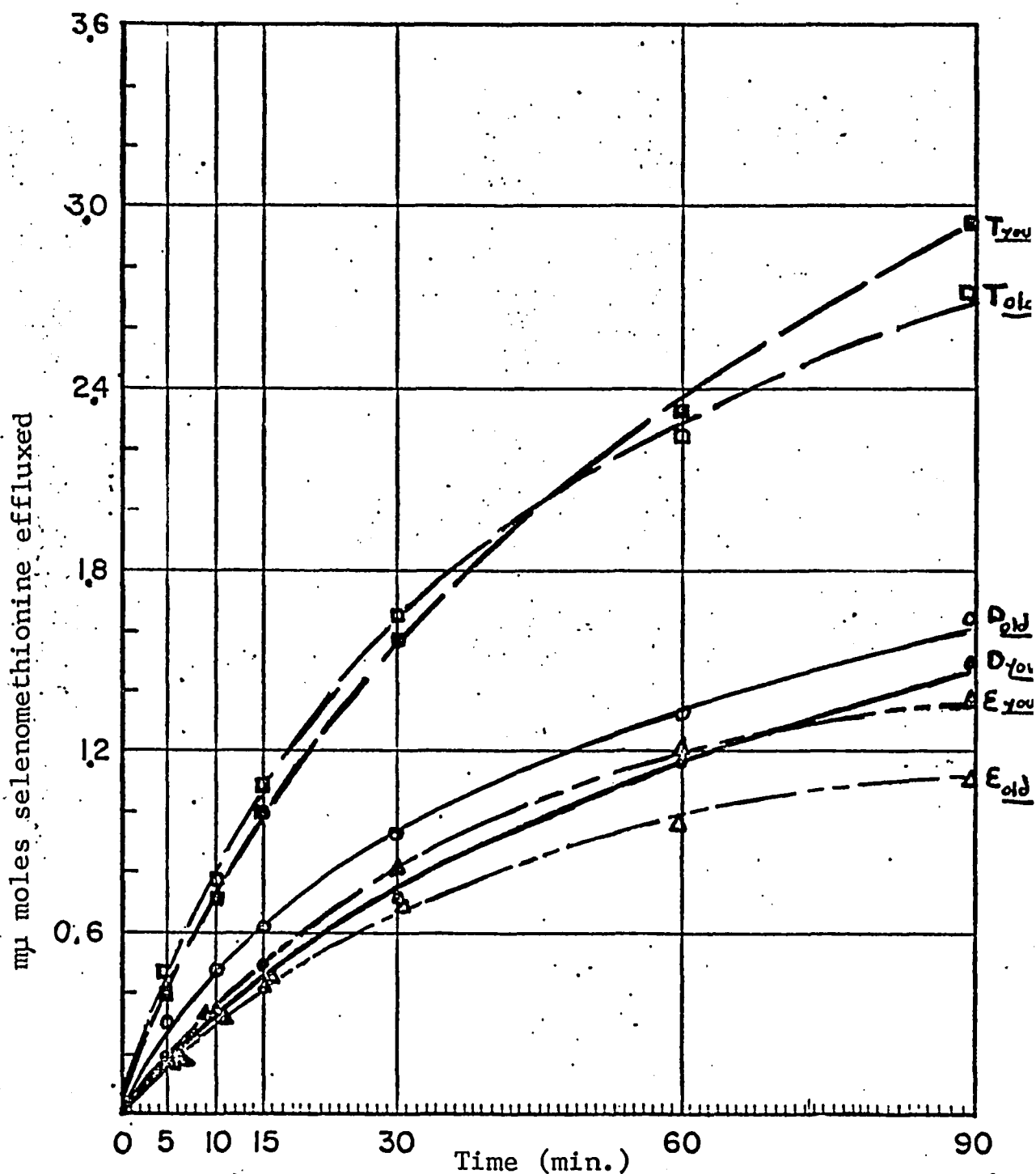
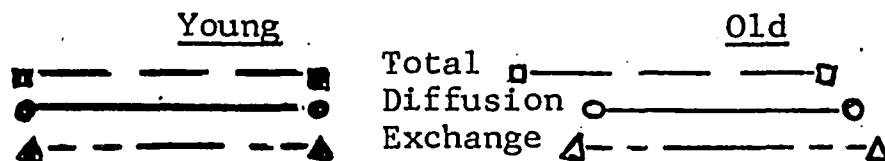


Figure 55. Quantitative efflux of selenomethionine from young and old lenses.



IV. DISCUSSION

Selenomethionine was chosen as the amino acid to be used for these studies since its radioactive analogue Se-75-selenomethionine is a gamma emitter and it mimics methionine with respect to membrane transport and incorporation into protein (Cohen and Cowie, 1957; Spencer and Blau, 1965). Using this gamma emitter one could quickly and easily determine the counts accumulated by the lens and the counts in the TCA-precipitable fraction.

The R_f values for both methionine and selenomethionine in seven different solvent systems were very similar (Table IV). Only the two butanol-acetic acid-water systems were able to distinguish between methionine and selenomethionine with the selenomethionine having the higher R_f value.

Se-75-selenomethionine was determined to be radiochemically pure if chromatographed under carefully controlled conditions. Significant amounts of degradation products were noticed when selenomethionine was dried on the chromatographic paper with hot air (in a technical bulletin issued by Amersham/Searle, 1968, the appearance of two contaminants of selenomethionine after hot air drying was also noted). These degradation products can be eliminated by simply drying under a stream of nitrogen.

An incubation dose containing a sample of Se-75-selenomethionine and carrier selenomethionine was analyzed on the Technicon Amino Acid Analyzer (Fig. 13). Selenomethionine did not elute in the methionine region but migrated in the region of isoleucine where more than 93% of the total radioactivity was found. Some activity was found to be in earlier fractions which corresponded to the areas of methionine oxides (methionine sulfoxide and sulfone). Based on the paper chromatographic data it is likely that the small amounts of breakdown products are a result of thermodegradation in the column (operated at 65°C).

The effluent from the lens which was pre-loaded with Se-75-selenomethionine was also subjected to amino acid analysis (Fig. 14). The distribution profile was the same as that found for the dose. Again, more than 95% of the total activity was in the selenomethionine peak. This clearly indicated that the radioactivity studied in the efflux experiments is the free amino acid and not a breakdown product.

As seen in Figure 20 and Table XVII, unlabeled methionine or selenomethionine when co-incubated with Se-75-selenomethionine yield identical results with respect to counts accumulated or incorporated into protein.

The per cent accumulation of Se-75-selenomethionine by rat lenses increased over the two hour incubation period studied (Fig. 15). In two hours about 25% of the total radioactivity is in the lens even though the lens water represents only 3% of the total volume. Thus the tissue/medium ratio (T/M) is much higher than the diffusion ratio of 1. In this experiment the lens water/medium distribution ratio was about 8, but values of 10 to 15 can be achieved under other experimental conditions. Since the lens is a "pump-leak" system, the per cent accumulated is a function of the radioactivity which enters the lens by active transport, exchange diffusion and non-mediated diffusion, and the radioactivity which is effluxed by diffusion and by exchange diffusion.

The per cent incorporation also increases with time although it seems to level off after 90 minutes (Fig. 16). The conditions of these experiments were designed so that the radioactivity in the protein is small (usually less than 10%) and therefore one can evaluate protein incorporation and transport in the same lens.

Per cent accumulation as a function of temperature is given in Figure 17. The per cent accumulation increased, reached a maximum near 46°C and was greatly reduced at 65°C. The Q_{10} (25-36°C) was 1.7. These results clearly indicate that the overall factor(s) responsible for the

high per cent accumulation is thermolabile. From these experiments one could not determine whether the thermolability is to be attributed to the carrier itself, the metabolic activity or both.

Per cent incorporation into protein had an optimum of 36.5°C and a Q_{10} (26-36°C) of 3.0 (Fig. 17). This system appears to contain thermolabile components as evidence by its decrease at 45°C. Per cent protein incorporation seems to be very temperature dependent.

The increased per cent incorporation from 45°C to 65°C is an artifact due to non-specific binding of selenomethionine degradation products to the protein. The high counts in these samples can be greatly lowered when the precipitate is redissolved in 0.1N NaOH in the presence of selenomethionine (non-labeled) and reprecipitated with TCA. The radioactivity in the TCA precipitates from the 37°C sample changed very little when treated similarly.

Figure 18 illustrates the Lineweaver-Burk plot for accumulation as a function of concentration of selenomethionine in the medium. A straight line was obtained and the K_m and V_{max} were determined to be 2.04mMoles and 0.174 μ moles/hr. respectively for a 27mg lens. The K_m value reported here is based on accumulation data which

include not only the active transport component but also diffusion and exchange diffusion. The apparent K_m and V_{max} values contain an age dependent diffusion component (see discussion on age) and thus should be reported along with the lens age or weight.

As more radioactivity enters the lens, there is a subsequent increase in incorporation of selenomethionine into protein. This incorporation was measured in the above experiment and showed signs of saturability as the level of selenomethionine increased in the lens. A Lineweaver-Burk plot of incorporation into protein ($1/V$) as a function of selenomethionine accumulated ($1/S$) yielded a straight line with K_m and V_{max} values of $0.0714 \mu\text{moles/L}$ for a 27mg lens and $0.00364 \mu\text{moles/40 minutes}$ respectively (Fig. 19). Obviously these values cannot yet be correlated to any specific step of protein incorporation but are a reflection of the rate limiting step under specified conditions (i.e., accumulation values of the lens, lens weight, animal age, amino acid used and incubation time).

Dinitrophenol was found not to inhibit accumulation (Tables V and VI) although, on occasion, uptake was slightly reduced (10-15%). Similar findings using α -AIB have been reported by Kinsey (1965) who attributes the DNP inhibition of transport to some secondary effect of DNP which is not

related to its role as an uncoupler of phosphorylation in oxidative phosphorylation. DNP, however, drastically reduced selenomethionine incorporation into protein.

Lenses pulsed in the presence of 1mM IAA reached a steady state equilibrium at about 2 hours and maintained a T/M ratio of 3.3 (12.4% uptake) as compared to a control T/M ratio of approximately 9.0 (30-33% uptake). The maintenance, by IAA treated lenses, of a 12% accumulation value between 2-4 hours incubation time may indicate (a) only a partial inhibition by IAA (interference of the operation of the glycolytic pathway and/or inactivation of the carrier) and/or (b) a relatively low diffusion out of the lens of selenomethionine (which was accumulated against the concentration gradient prior to total inhibition by IAA).

The inhibition of per cent incorporation into protein appears to be more severe in the presence of DNP than in IAA. Pre-incubation of lenses in 1mM DNP followed by either a 30 or 60 minutes pulse, containing inhibitor, leads to a 69.1% and an 85.3% inhibition respectively when compared to the controls. IAA, under the same conditions, yields a 54.4% and a 75.3% inhibition (Table VI).

Lenses were pulsed in the presence of other inhibitors of protein synthesis (Table V). Both puromycin and chloramphenicol did not affect accumulation but reduced

the per cent incorporation into protein, with the former being the more potent inhibitor. Sodium fluoride at high concentrations (10mM), which inhibits the initiation of translation in protein synthesis, inhibited 83%, while accumulation was not affected. These results indicate that selenomethionine incorporation into protein is related to de novo synthesis of protein.

Other metabolic inhibitors were tested for possible effects on accumulation and protein incorporation (Table V). Iodoacetamide, a sulfhydryl inhibitor, inhibited both accumulation and protein incorporation, but not nearly as effectively as iodoacetic acid. Arsenate did not inhibit either of the two systems under study. Since arsenate acts as a competitive inhibitor of phosphate, the lack of inhibition may be attributed to high concentrations of phosphate in the lens and in the incubation medium (16mM). Sodium azide and sodium cyanide, inhibitors of electron transport, inhibited incorporation into protein. Accumulation was inhibited by cyanide at a 10mM concentration but not at 1mM. The inhibition at high concentrations may be due to some factor other than interference with electron transport since cyanide is generally effective in inhibiting cytochrome c at levels below 1mM (Racker, 1965).

Inhibitors of aerobic respiration, DNP, azide, cyanide, British-Anti-Lewisite, antimycin-A and oligomycin were found to inhibit protein incorporation but not accumulation (Tables V and VII).

From the above data there appear to exist two discrete pools of ATP in the lens, one derived from anaerobic metabolism which drives transport and another derived from aerobic metabolism which furnished energy for protein incorporation. Since a minimum of Krebs' cycle activity has been shown to exist in the lens (Kinoshita and Merola, 1961; Harris and Gruber, 1962), it is not likely that pyruvate is the substrate oxidized by the mitochondria in the lens. Lens mitochondria have been shown to oxidize succinate, glutamate (van Heyningen, 1969b) and α -glycerophosphate (Griffiths, 1966). Glucose has been shown to be converted to glutamate by the lens (Kinoshita and Merola, 1961) and α -glycerophosphate accumulates in the lens in the presence of glucose, the levels being higher under anaerobic conditions (Pirie, 1962). Of all the possible sources of substrate for oxidative metabolism in the lens, the α -glycerophosphate shuttle has received the most attention (Pirie, 1962; Pirie, 1963; Griffiths, 1966; Kinoshita, 1965). All of the inhibitors of oxidative phosphorylation studied

inhibited protein incorporation but not accumulation indicating that protein incorporation is linked to aerobic respiration (i.e., α -glycerophosphate shuttle and/or some portion of the citric acid cycle).

Inhibition of anaerobic respiration, by IAA, also results in an inhibition of protein synthesis. Since glycolysis is the source of $\text{NADH} + \text{H}^+$ and α -glycerophosphate, an inhibition of glycolysis should result in an inhibition of an operative shuttle mechanism (aerobic respiration) which in turn would result in an inhibition of protein synthesis.

Another sulfhydryl inhibitor, N-ethylmaleimide, was found to be a potent inhibitor of accumulation and incorporation at a 10mM concentration (Table VIII). At a 1mM concentration only accumulation was affected. Although IAA and NEM have high affinities for glyceraldehyde-3-phosphate dehydrogenase (the former inhibiting the NAD-bound enzyme and the latter inhibiting the free enzyme, Racker, 1965), it appears that their inhibition of accumulation may involve other sites as well. If the inhibition of protein synthesis was due to the inhibition of the dehydrogenase, then one would expect that the degree of inhibition would be the same for both inhibitors, as was the case with respect to accumulation. It is possible that IAA and NEM differ in their ability to directly

inhibit protein synthesis and/or have multiple modes of inhibition of accumulation (see efflux section). Iodoacetamide, which has a weak affinity for the dehydrogenase, is not as potent an inhibitor as NEM or IAA with respect to accumulation. This would indicate that at least part of the inhibition observed for accumulation is linked to the inactivation of this dehydrogenase.

Ouabain, an inhibitor of the sodium-potassium ATPase, did not affect accumulation of selenomethionine (Table VIII). Although methionine transport has been shown to be 60% sodium dependent in rabbits (Cotlier and Beaty, 1967), the incubation time needed to demonstrate a ouabain effect appears to be greater than any of the incubation times used in our experiments. It is also possible that the A site, which is sodium dependent, is deficient in the rat lens as suggested by Brassil and Kern (1968).

Ten per cent dimethyl sulfoxide (DMSO) proved to be detrimental to the lens by inhibiting incorporation into protein and accumulation (Table VIII). The decrease in protein incorporation may be due in part to an increased permeability which in itself may result in a gross leakage of endogenous amino acids out of the lens.

EDTA was found to inhibit accumulation only after prolonged incubation (Table X). Lenses pulsed in media lacking calcium or magnesium ions for a total of 100

minutes showed no inhibition with respect to transport. Incorporation of selenomethionine into protein was not affected by either the lack of divalent cations in the medium or by EDTA. There appears to be sufficient calcium in the lens to maintain both membrane integrity and calcium-requiring enzyme(s), (e.g., mitochondrial α -glycerophosphate dehydrogenase, Hansford et. al, 1967).

The experimental data presented in Table XI is in agreement with the findings of Brassil and Kern in that those amino acids which ride the A and L site (leucine, alanine, α -AIB) inhibit selenomethionine accumulation, while those amino acids which lack a high affinity for the A and L site (glycine, sarcosine) do not inhibit accumulation. The amino acids histidine and isoleucine probably have a high A and/or L site affinity in the lens since they too inhibit selenomethionine accumulation. Ethionine, the ethyl analogue of methionine, as well as α -methylethionine inhibited the accumulation of selenomethionine. Inhibition also occurred in the presence of the oxidation product of methionine, methionine sulf-oxide (Table XII). It is assumed that these compounds have a relatively high affinity for either the A and/or L site and are inhibiting accumulation competitively.

Those amino acids which inhibited selenomethionine accumulation were found to increase the % selenomethionine incorporation into protein (Table XII). Generally, the greater the inhibition of accumulation the higher the per cent incorporation value. The most plausible explanation of this effect would attribute the increase in per cent incorporation to a change in the specific activity of Se-75-selenomethionine within the lens. Efflux studies (see efflux discussion) reveal that an amino acid which competitively inhibits active transport of selenomethionine is also effective in driving selenomethionine out of the lens via exchange diffusion. Therefore, those amino acids which competitively inhibit active transport of selenomethionine are concomitantly driving endogenous methionine out of the lens which would result in an increase in the specific activity of Se-75-selenomethionine; this in turn would result in an apparent increase of Se-75-selenomethionine incorporation into protein. The absence of this effect with threonine and α -AIB might be attributable to their feeble effectiveness in exchange diffusion, as is the case with respect to accumulation (20% inhibition), which may not significantly alter the specific activity.

The lens is problematic with respect to the elucidation of its efflux systems because of the asymmetrical

nature of its transport systems. Whereas most studies of transport are conducted on symmetrical cell populations (i.e., red blood cells, Ehrlich cells, etc), the lens is composed of a single layer of epithelial cells on the anterior surface which contains the sites for active transport of cations and amino acids, and the sites involved in exchange diffusion. Non-mediated diffusion occurs uniformly from all lens surfaces (Kinsey and Reddy, 1965). In addition, the lens is an organ which increases in density inwardly from peripheral cells to cortex to nucleus; concomitantly there is a progressive decrease in hydration. A pulse of short duration prior to efflux would thus lead to an unequal distribution of the selenomethionine in the lens. The diffusion of the isotope out of the lens would be related to the distribution of the isotope in the lens.

Since the distribution of the amino acid changes with time, the amount of the isotope diffusing out at the surface also changes with time. Figure 32 contains the plot of selenomethionine efflux via diffusion from control lenses (D_{con}). Efflux is plotted as the cumulative percentage of selenomethionine which leaves the lens between 0-90 minutes. The per cent cumulative efflux via diffusion increases with time although the rate decreases with time.

If the data is replotted as a per cent of initial activity remaining in the lens, on semi-log paper, a two-component curve is obtained and not a single linear component one would find for a constant rate of diffusion (Fig. 56, p. 139). The presence of two components may lead one to interpret the shorter life component as being attributed to an adsorption component and/or leakage from the extracellular space, and the longer life component as being the exponential rate of diffusion. However, the components of the curve are not clearly delineated nor do they reflect any marked changes in the rate of efflux, due to the relatively slow rate of exit (i.e., over 80% of the initial activity remains in the lens after 90 minutes of efflux). To delineate more clearly the changes in the rate of efflux with time, a plot of K (rate coefficient) as a function of time was utilized (Fig. 57). When lenses given a pulse of short duration were allowed to efflux into a medium containing no exchangeable amino acids (Fig. 57), the rate coefficient ($K \text{ min}^{-1}$) via diffusion (D) changes with time, decreasing to $1/3$ of the original rate over 90 minutes.

The curve of $K \text{ min}^{-1}$ for diffusion contains at least two components, one of which is prominent during the first 30 minutes of efflux. It is possible that one component

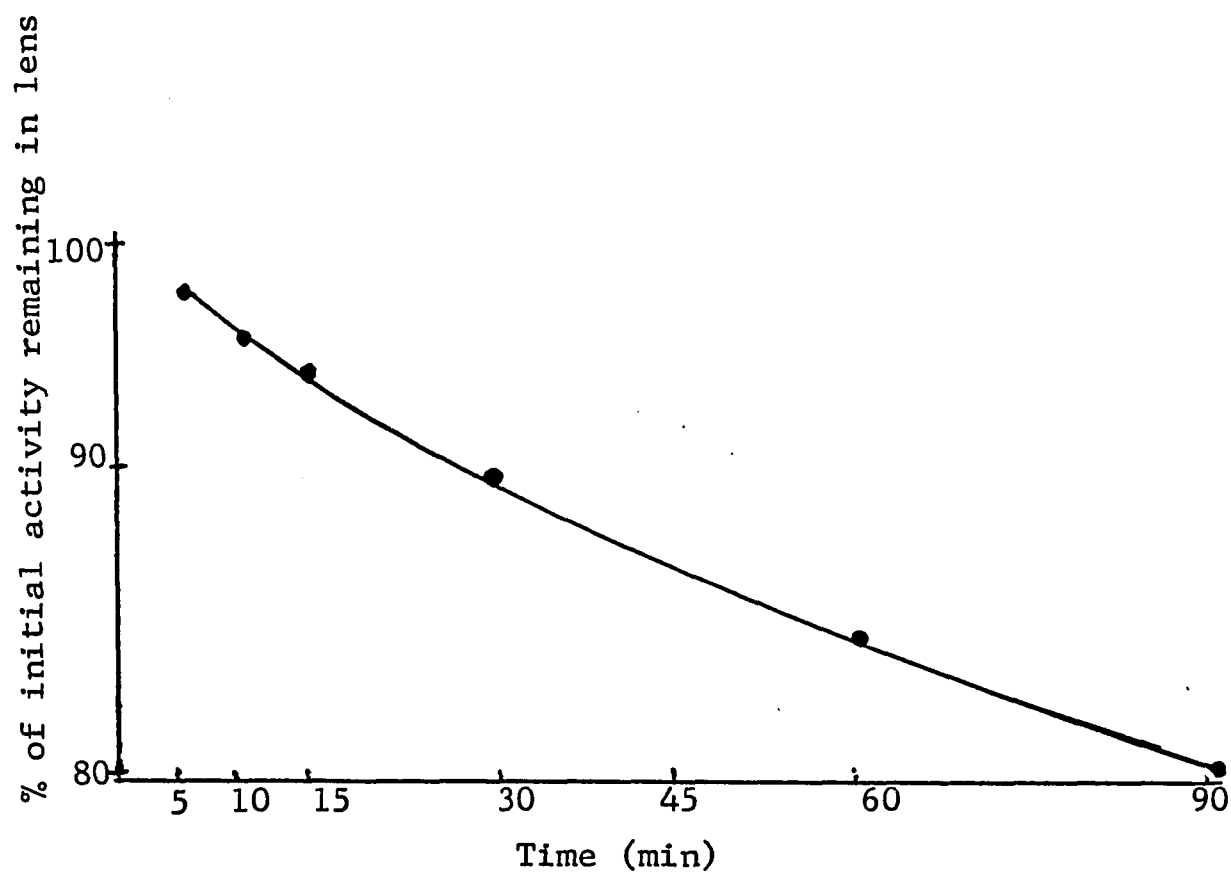


Figure 56. A semi-log plot of the % of initial selenomethionine concentration in the lens during efflux.

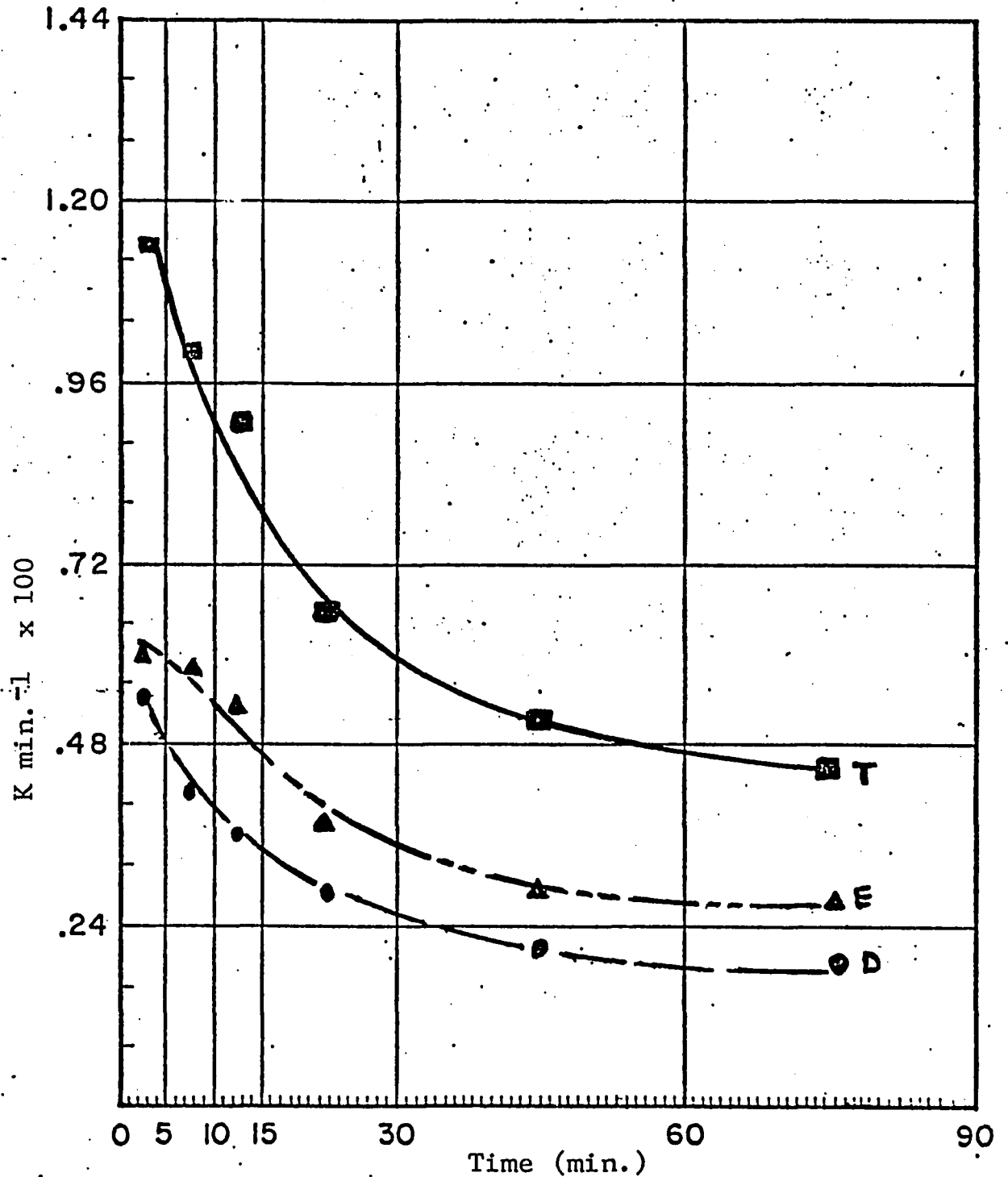


Figure 57. A typical curve of $K \text{ min.}^{-1}$ values as a function of time for non-mediated and exchange diffusion. Values were obtained from ten sets of experiments, each point represents 60 lenses.

may represent, in addition to the two parameters already discussed, the selenomethionine accumulated in the epithelial space (site of active transport); and the other component representing the asymmetrically distributed pool of selenomethionine (more prominent at the anterior portion of the lens) which is distributing itself throughout the lens during the course of efflux. Selenomethionine present in the fiber cells during the earlier periods of efflux would tend to be higher near the anterior surface thereby allowing a greater percentage of selenomethionine to re-enter the epithelium than would diffuse out at the posterior surface which is a greater distance away. This is supported by the exchange component data (E) in Figure 57 where, beyond 15 minutes, the change in the rate of exchange ($K_E \text{min}^{-1}$) begins to parallel the change in the rate of diffusion ($K_D \text{min}^{-1}$) indicating a dependency of exchange diffusion on the re-entry of selenomethionine into the epithelium from the lenticular fiber space.

The elucidation of exchange diffusion was accomplished by the subtraction of the diffusion component (lenses effluxed into media lacking exchangeable amino acids) from the total efflux (lenses effluxed into media containing 0.3mM unlabeled selenomethionine). Variance in a group was minimized by rejecting surgically traumatized lenses after pre-counting for 15 seconds and by minimizing trauma

during the transfer of lenses by use of the lens loop, as described under Materials and Methods. These lens loops were prepared and used in this laboratory since 1967; recently Epstein and Kinoshita (1970) reported the use of a similar lens loop.

Lenses at 0°C exhibited a decrease in both diffusion and exchange diffusion as compared to the 37°C control (Fig. 21). A high temperature of 57°C increased diffusion and caused an initial increase in the rate of exchange followed by total inhibition within 30 minutes (Fig. 22). The changes in diffusion at both 0°C and 57°C may be a reflection of physical changes in the membrane structure. The initial increase in exchange at 57°C might be attributed to a thermal activation of the exchange process. This is followed by thermal inactivation. If one assumes that the exchange carrier and the active transport carrier are the same, then this experiment indicates that the accumulation decrease (as discussed previously, Fig. 17) is related to the thermal destruction of the carrier.

The ability of leucine to drive exchange as effectively as selenomethionine and the inability of lysine and glutamic acid to drive exchange (Fig. 23) correlate well with their effects on accumulation (Table XI). The amino acids which affect accumulation also drive exchange diffusion and those with no effect on accumulation do not affect exchange.

Lenses loaded with different concentrations of selenomethionine leak the same percentage of amino acid at each time interval studied. Accumulation ranged from 2 to 21 μ g which is equivalent to a concentration of 0.5mM to 6.0mM for lenses weighing 30mg and assuming a water content of 60%. The rate coefficient for efflux via diffusion for any specified time period should be independent of concentration. Therefore, as the rate of diffusion changes with time as a result of unequal distribution, the change in the rate coefficient is the same for all concentrations. The extreme curves represent the experimental variation. (Data from Figure 26 is represented in Figure 57.)

When lenses loaded with 0.5-0.6mM selenomethionine (as described above) are placed into medium containing 60mg/cc (0.3mM) selenomethionine, the per cent cumulative efflux values are the same for each group within any specified time period. Since the cumulative per cent efflux is the same for diffusion for each group and the total efflux (diffusion + exchange) is the same for each group, the exchange diffusion (T-D) is also identical for each group of lenses. The exchange system appears to be behaving like a simple diffusion system, non-saturable (Fig. 28 and Fig. 29). If one were to assume a one for one exchange then some indication of saturation would be

expected since the substrate on the trans side (0.3mM) is limiting when compared to the concentrations on the cis side (0.5-6.0mM).

There are several possible explanations of this effect. An exchange system may exist which does not involve a one for one exchange but a substantially higher ratio of perhaps 10 to 1. This is most unlikely since such an asymmetrical carrier system would necessitate an energy link required for the alteration of carrier affinities on the cis and trans sides of the membrane. This in essence would be an active pump for the elimination of amino acids from the lens. This seems to be most improbable since no energy requirement appears to be necessary for the operation of exchange (a 33% reduction in available energy in galactosemic lenses does not alter the operation of the exchange system (see section on dietary - efflux)).

The most plausible explanation lies in the nature of the unequal distribution of selenomethionine in the lens. If the bulk of the label is located beyond the epithelium of the lens (behind the area containing the pump) and there is a relatively low rate of diffusion (as compared to the rate of exchange out of the lens) back into the epithelium, then the concentration of selenomethionine in the epithelium may be rate limiting even when the lens as a

whole contains 6mM selenomethionine and the medium is 0.3mM. Furthermore, lenses pulsed in high concentrations of selenomethionine (800 μ g/cc, 4m Molar) would have a greater percentage of the total accumulation due to diffusion (since the K_m of active transport, 2.0mM, has been exceeded). Hence, the relative distribution of selenomethionine at the anterior end, with respect to the total lens, would be decreased as the concentration in the dose is increased.

In a second set of experiments, lenses were loaded with selenomethionine (1.4 μ g/29mg lens; 0.45mM) and allowed to efflux into media containing different concentrations of selenomethionine (0-30 μ g/cc, Fig. 24; 30-120 μ g/cc, Fig. 25). Since the diffusion of selenomethionine out of the lens is independent of the outside concentration, the relative changes observed for the total efflux (diffusion + exchange) can be attributed to exchange. The exchange of Se-75-selenomethionine increased as the concentrations of unlabeled selenomethionine on the outside was increased. Although the increase is generally in the right direction a K_m or V_{max} could not be calculated due to the scattering of data. Two or more populations of exchange carriers with different affinities may be involved.

It was of interest at this point to determine whether any significant contribution was made by exchange diffusion

in the accumulation studies already presented in this paper. Loading the lens with $21\mu\text{g}$ (6mM) selenomethionine (equivalent or slightly greater than the endogenous concentration of all exchangeable amino acids in the lens; Kinsey, 1965), followed by a normal accumulation study pulse ($6.75\mu\text{g}/0.5\text{cc}$ selenomethionine) led to an accumulation value that was no greater than the value obtained from lenses which were not pre-loaded with selenomethionine (Fig. 30). When the outside concentration was raised from $6.75\mu\text{g}/0.5\text{cc}$ (normal dose) to $30\mu\text{g}/0.5\text{cc}$ there was an observable increase in accumulation (25% increase over the unloaded control, Fig. 31). These studies indicate a significant contribution of exchange diffusion to the total accumulation of selenomethionine when there is a high external concentration of amino acid. However, when the amino acid concentration in the dose is low there appears to be little exchange diffusion contributing to the total uptake. Amino acid analysis data (not reported in this paper) indicate no significant increase in endogenous amino acid efflux when the incubation media contains $6.75\mu\text{g}/0.5\text{cc}$ selenomethionine.

The effects of the mercuric ion, iodoacetic acid and N-ethylmaleimide on efflux were studied to elucidate their modes of inhibition. The mercuric ion had no effect on simple diffusion during the time course studied. The

rate of diffusion (K_D) was identical to the values found for controls even though the mercuric ion caused opacification of all lenses within the first 5 minutes of efflux. On the other hand, the mercuric ion was found to be effective in destroying the exchange diffusion system within the first few minutes of exposure. These findings indicate that the inhibition of accumulation by the mercuric ion involves the destruction of the carrier system without altering the permeability of the membrane (Fig. 33).

It was assumed that iodoacetic acid inhibited the accumulation of selenomethionine by inhibiting the enzyme glyceraldehyde-3-phosphate dehydrogenase which in turn leads to the decreased glycolysis. However, exchange diffusion is reduced 40% in the presence of 2mM IAA (Fig. 33). Other experiments have indicated a 30% inhibition of exchange at 1mM concentrations of IAA. Thus IAA may inhibit exchange by reacting with the sulfhydryl group on the carrier. Inhibition of accumulation by IAA does not necessarily imply an inhibition of energetics since the carrier(s) involved in transport are themselves IAA-sensitive (Fig. 33).

Inhibition of exchange diffusion can also be explained by the inhibition of the energetic component of active transport (as proposed by Heinz and Walsh, 1959, Fig. 58). If X went rapidly to an inactive Y, then exchange diffusion as well as active transport would be dependent upon regeneration of the functional carrier, X (Step 5). This model does

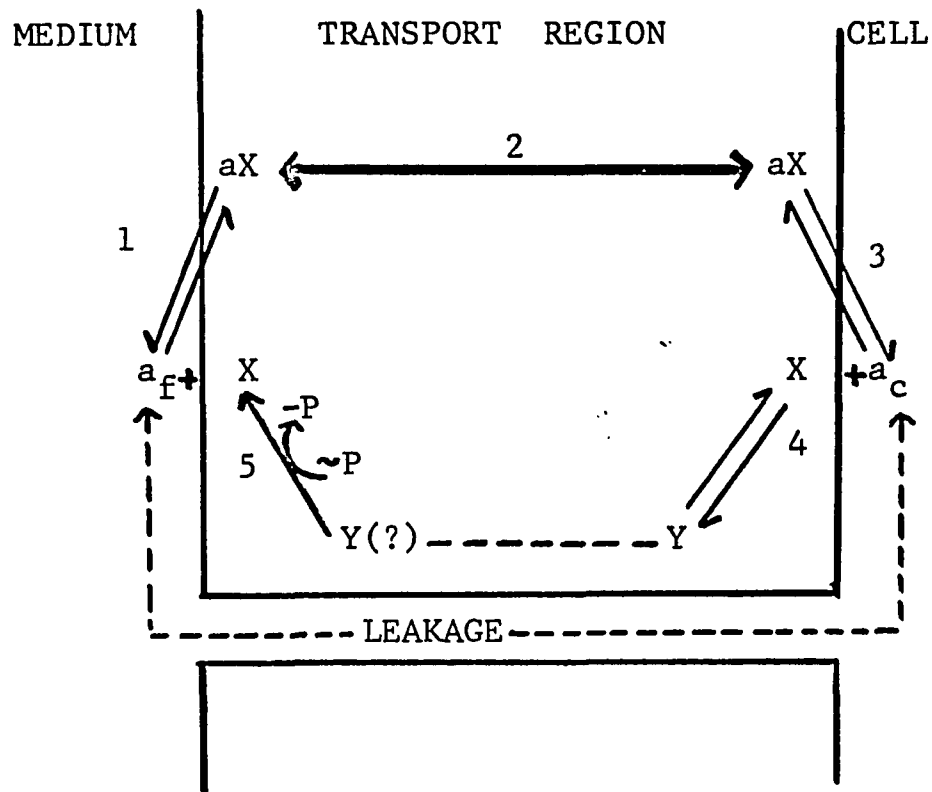


Fig. 58. Schematic model of active transport, associated with exchange diffusion. Five essential steps are involved. The active carrier X combines with the amino acid a (Step 1) to form the complex aX which moves across the transport region to Step 2 and dissociates at the inner side of this region (Step 3). The carrier is catalytically inactivated to become Y (Step 4). New active carrier is formed with the expenditure of metabolic energy (Step 5). Exchange diffusion involves only Steps 1, 2, 3 and 4 and requires that the reactions underlying Steps 1, 3 and 4 are at least partly reversible. (From Heinz and Walsh, 1958).

not explain the results in old and galactosemic lenses where energetics and active transport are decreased while exchange is functioning normally.

Since N-ethylmaleimide (NEM) was found to be a potent inhibitor of accumulation it was of interest to see if it behaved like IAA (Fig. 34 and Fig. 35). NEM proved to be a more potent inhibitor of exchange than IAA, with total inhibition occurring within 30 minutes of incubation. It may be that the relative rates of inhibition of NEM and IAA are related to their respective abilities to bind with the carrier and render it inactive. In addition to the action of NEM on the carrier, there is a delayed increase in diffusion which occurs at about 60 minutes after the start of efflux. The increase in the rate coefficient for the passive diffusion component between 30 and 60 minutes was never observed in any control or experimental set of data other than NEM. The increase in diffusion becomes more apparent when lenses are given a pulse containing NEM and then allowed to efflux into medium which does or does not contain NEM. Both sets show a marked increase in diffusion as compared to the normal rates (Fig. 36). Thus, NEM is more toxic than IAA with respect to exchange carrier inactivation and also alters the permeability of the membrane.

Glucose was found to be necessary for the maintenance of permeability (diffusion) and exchange. Although the relative changes are small between control and glucose "starved" lenses with respect to exchange and diffusion, the observed effects were reproducible (Fig. 37). It appears that glucose is not necessary for relatively short incubation periods and that the endogenous substrate can maintain the lens, with respect to transport, for one hour.

Feeding rats a diet rich in galactose has been shown to induce the formation of lenticular opacities (Patterson, 1954). Prior to the appearance of the opacity, gross changes in the biochemistry and physiology of the lens are observed (see Introduction).

Accumulation of selenomethionine in lenses of galactose fed rats decreased by 30% on the first day of the diet and reached a minimal value (70% decreased) on the third day (Fig. 39). Accumulation remained at this minimum value for the next eleven days. Other investigators have reported similar findings for amino acid accumulation (Cotlier and Beaty, 1968; Reddy, 1965). During this time period, an increase in lens hydration was observed. Galactose lenses increased in wet weight from 19mg on day one of diet to 34.9mg on day fourteen, while controls exhibited an increase in lens weight from 19mg on day one to 26mg on day fourteen.

Per cent incorporation into protein is increased in both the galactose and glucose fed groups. The question arises whether these increases are a result of changes in the specific activity of Se-75-selenomethionine inside the lens due to the lowering of the endogenous pool of methionine. Other investigators in our laboratory (Green and Toran, 1968) had determined the levels of each amino acid in the lenses of animals fed either a 50% glucose-chow diet, a 50% galactose-chow diet, a 50% xylose-chow diet or a 100% chow diet (two-day diets for all groups except the xylose diet which was eight days). The levels of endogenous methionine are given in column B of Table XX (p. 152). The content of methionine in the galactose lens was lowered 88%. However, glucose feeding also lowered the methionine content by approximately 50%. This was unexpected since no change in accumulation was observed as compared to the controls. If one were to assume that the endogenous methionine and the accumulated selenomethionine were distributed uniformly and that the same percentage of each was incorporated into protein, then the total amount of methionine and selenomethionine present in protein for the control and glucose groups would be identical (0.960 μ moles and 0.997 μ moles respectively), while galactose group would incorporate 0.395 μ moles of the amino acids (Table XX). The value obtained for

Table XX. The incorporation of accumulated selenomethionine (SeM) and endogenous methionine (M) into lens protein of control, galactose-fed, glucose-fed and xylose-fed rats. Animals were maintained on a 50%-sugar-50%-chow diet for two days (glucose and galactose diets) or eight days (xylose diet). Controls fed 100% chow. Incubation time 1 hour. Standard incubation dose (6.75 μ g selenomethionine /0.5 cc).

Group	% Uptake of <u>SeM</u> $\frac{\text{SeM in Lens}}{\text{SeM in Dose}} \times 100$	% Incorp. of <u>SeM</u> $\frac{\text{SeM in Protein}}{\text{SeM in Lens}} \times 100$	<u>SeM</u> Uptake mumoles "A"	<u>M</u> in Lens * mumoles "B"	Total <u>SeM</u> & <u>M</u> in Lens, mumoles "A" + "B"	<u>SeM</u> in Protein mumoles	<u>SeM</u> & <u>M</u> in Protein mumoles **
Control	13.41	8.71	4.63	6.50	11.13	0.403	0.960
Galactose	6.75	12.62	2.33	0.80	3.13	0.294	0.395
Glucose	13.42	12.27	4.63	3.50	8.13	0.568	0.997
Xylose	9.40	7.11	3.06	1.10	4.16	0.212	0.295

* - Determined via Technicon amino acid analyzer.

** - Assuming equal distribution of methionine and selenomethionine in the lens.

the galactose group would indicate a smaller net synthesis of protein. However, the activity of the protein incorporation system is greater for the galactose and glucose group than in controls as determined by per cent incorporation.

If one were now to assume that the endogenous methionine pool is relatively uniformly distributed in the lens and that the accumulated selenomethionine is in greater concentration near the anterior surface (as previously indicated) which is the principle site of protein synthesis (Hanna, 1965; Young and Fulhorst, 1966), relative specific activity for control and glucose groups would not be significantly different and the selenomethionine concentration in the lens, (and not the total concentration, methionine + selenomethionine) would be the critical value for determination of protein incorporation. Hence, the glucose group would have a higher net incorporation into protein indicating an increase in the activity of the protein incorporation system.

An increase in the activity of the protein synthetic system for galactose lenses, despite the decrease in net synthesis, is indicated by the comparison of the galactose and xylose values (Table XX). Lenses from xylose fed rats accumulate more selenomethionine than galactose lenses and have the same level of endogenous methionine, but the per

cent incorporation into protein is slightly decreased in the xylose group (7.11%) while the galactose group is increased (12.6%), relative to the controls (8.71%). Grimes and von Sallman (1968) have reported an increase in cell proliferation during the first four days of galactose diet but no change in proliferation for xylose fed animals. Further evidence for an increased protein synthetic activity in galactose fed lenses includes an increase in free and membrane bound ribosomes, increased Golgi apparatus and increased cell proliferation as reported by Kuwabara et. al. (1969). Hanna and O'Brien (1960), utilizing tritiated thymidine, reported an increase in DNA synthesis in the lenticular epithelial layer. Weller and Green (1969) have shown an increase in methionyl -T-RNA synthetase activity during the first six days on diet, with a maximum activity on day two. Also Dische (et. al., 1957) has shown that there is an accumulation of a new soluble protein in the galactose lenses.

The soluble protein in lenses from galactose fed rats has been shown to decrease, while xylose and glucose diets do not result in any change (Packer and Green, 1970, Fig. 59, p. 155). A decrease in soluble protein for both the galactose and xylose group was expected since there is a decrease in net synthesis of protein for both groups. The difference

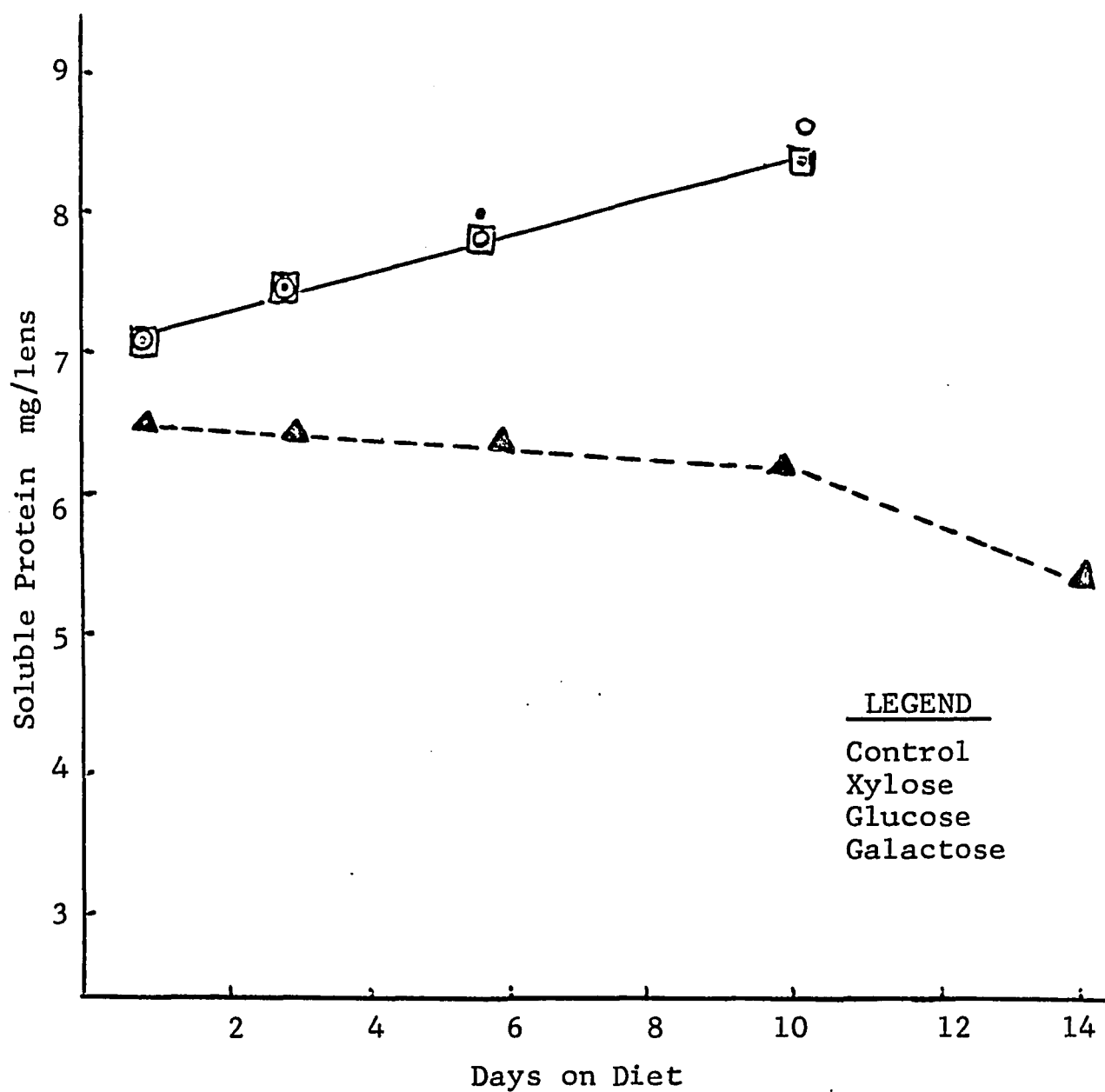


Figure 59. Changes in the soluble protein content of lenses from rats fed a xylose, glucose, and galactose diet, (from Packer and Green, 1970).

in the net content of soluble proteins may be more directly linked to the leakage of protein out of the lens (see dietary efflux discussion) since galactose lenses are considerably more leaky than xylose lenses. In addition, Anderson (1965) has shown that an increase in intracellular sodium and magnesium results in an increased autolytic activity.

Puromycin inhibited incorporation of selenomethionine into protein in galactose fed and control groups (Table XIV). The inhibition of controls (from 12.9% to 4.55% incorporation) was greater than that found for the galactose group (from 13.11% to 6.56%). This effect (the difference in relative inhibition) would be expected if the number of protein synthetic sites is greater in galactose lenses than in the controls.

Iodoacetic acid inhibited the per cent incorporation into protein in the control lenses to the same degree that it inhibited in glucose fed animals, despite the higher per cent incorporation in the latter group. Dinitrophenol was also an effective inhibitor in both groups but not as effective in the glucose fed group as in the controls. The most surprising effects of DNP and IAA inhibition were with respect to galactose fed animals where the inhibition was not nearly as severe as one finds

in the control. In fact, despite the significantly lower accumulation in galactose lenses, there was a greater number of counts incorporated into protein than in the inhibited controls (see Tables XIV, XV and XVI).

There are two possible explanations for this low inhibition of protein incorporation with DNP and IAA in galactose lenses. It is possible either that energy sources other than glycolysis and aerobic metabolism are involved or that there is an increase in protein synthetic sites (i.e., increased ribosomes, tRNA-synthetase, etc.).

In glucose fed animals, Weller and Green (1969) have shown that tRNA-synthetase activity remains normal. If one assumes that the protein synthetic sites are not increased by a glucose diet but are identical to the controls, then the increase in selenomethionine incorporation may be attributed to a greater availability of ATP. Kinoshita et. al., (1963) have demonstrated that an increase in glucose concentration causes an increase in HMP activity and an increase in the sorbitol pathway but not in the glycolytic activity (Kinoshita, 1965; Lou and Kinoshita, 1967). As a result of the increase in the two pathways, the HMP shunt would serve as the source of NADPH needed for the operation of the sorbitol pathway which in turn furnished NADH which acts as the substrate

for the α -glycerophosphate shuttle. Pirie (1962) has demonstrated that high levels of glucose result in an increase in the level of α -glycerophosphate in the lens which would contribute to an increase in the shuttle activity. In addition, high levels of glucose could result in higher levels of fructose (via the sorbitol pathway) which could result in an increase in the level of glyceraldehyde, which in itself would serve as a substrate for the aldose reductase, thereby increasing the level of NADH. Thus, the decrease in per cent incorporation into protein, in glucose fed rats, in the presence of IAA might be attributed to an inhibition of glycolytic substrate phosphorylation and oxidative phosphorylation, compared with the control lenses. Dinitrophenol, however, inhibits only the oxidative phosphorylation and would not affect the increased glycolytic and hexosemonophosphate shunt activity present in the glucose fed animals. If one now assumes that a small percentage of this substrate phosphorylated ATP, which is increased, contributes to protein synthesis, then the observed differences between the control and glucose fed groups, with respect to DNP inhibition, are explainable.

The increase in activity of the protein synthetic system in the lenses of galactose fed rats, during the

time of initial osmotic damage, appears to be an all out attempt by the lens to repair injured tissue (Fig. 40). This attempt of repair appears to decrease somewhat on days six and ten of the diet as evidenced by a decrease in the per cent incorporation into protein. The sharp rise in per cent incorporation on day fourteen indicates an even greater increase in activity of the protein synthetic system than observed on day two. However, this increase in activity is occurring at a time when the amino acid pool is still minimal and the tRNA-synthetase level is only 25% of the normal activity. The nature of this increase in activity remains to be identified. Sippel (1966b) has reported that oxygen consumption increased at this time, but it is not clear how this relates to protein synthesis.

The changes in per cent incorporation into protein observed with the two different glucose diets (see Materials and Methods) are somewhat perplexing. The glucose-casein diet resulted in a slight decrease in protein incorporation as opposed to an increase which was observed in the glucose-chow diet. The unlabeled analogue used in the dose (methionine or selenomethionine) did not alter the glucose "activation" effect observed with the glucose-chow diet, nor was it altered by the percentage

of glucose present in the diet (35-75% glucose), (Tables XVII and XVIII). Both diets would generally be regarded as complete diets. However, the glucose-casein diet contains approximately 18.8% protein and 8% fat, while the glucose-chow diet, at the best (35% glucose-chow), has 16% protein and 2.6% fat. Lipids have been reported to have two effects on the sorbitol pathway, inhibition of aldose reductase by fatty acids and the ability of glyceraldehyde (derived from fats) to act as a substrate for the aldose reductase (Hayman and Kinoshita, 1965). It is possible that the higher level of fat present in the glucose-casein diet is responsible for a decrease in the sorbitol pathway, thereby decreasing the availability of NADH which is utilized for the increased production of ATP which in turn is responsible for the glucose "activation" effect. It is also possible that the glucose "activation" effect observed with the glucose-chow diet is due to some component which is lacking in the glucose-casein diet or that the glucose-casein diet contains some component (other than lipids) which is responsible for the inhibition of the glucose "activation" effect.

Starvation (48 hours) of animals results in normal accumulation values but decreases incorporation of amino acids into protein. The decrease in protein synthesis may

be related to observed decreases in blood serum amino acid levels as observed by van Heyningen (1959) or to any one of the gross changes in the total physiology which occurs during starvation (Fig. XIX).

Galactose diets caused a sharp rise in the diffusion of accumulated selenomethionine out of the lens (Fig. 42-47). On day one of the diet the diffusion component is increased one and a half fold over the control. By the third day, the diffusion rate is more than two and a half times greater than the control, with 50% of the accumulated selenomethionine diffusing out of the lens within 90 minutes. This appears to be the maximum level of diffusion since no further increase was observed.

The total efflux (diffusion + exchange) of accumulated selenomethionine out of the lens on days 1, 3 and 7 in galactose fed animals is 72% in 90 minutes. There is a greater contribution on day 1, by the exchange component towards the total efflux than there is on days 3 and 7.

The most surprising result was the apparent increase in exchange diffusion in the galactose fed group (on an absolute basis and on the basis of per cent exchanged, Fig. 42 and Fig. 43). This increase in the exchange diffusion is maintained on days 3 and 7, when expressed as per cent of accumulated selenomethionine effluxed via

exchange diffusion. For all days studied, the exchange in galactose lenses begins to level at a point when 45% of the accumulated selenomethionine has been effluxed. This decrease in exchange appears to be related to the unavailability of the remaining selenomethionine which, in galactose lenses, may have diffused even more rapidly to other parts of the lens as compared with the controls (the rate coefficients of diffusion for galactose lenses are two to three times greater than control values, see Table XXI, p. 163).

The question arises whether there is an increase in the number of exchange sites, in the galactose lenses, which are responsible for the net increase in exchange on day 1, or for the relative increase in exchange on days 3 and 7. It is possible that there is an increase in the number of exchange sites if one considers the active transport carriers as potential exchange carriers. Hence, any loss of the energy component of active transport might result in the loss of the anisotropic nature of the active carrier thus rendering it capable of becoming an exchange carrier, or even a facilitative carrier. However, if the active carriers were converted into facilitative carriers, the facilitation would occur during diffusion conditions (no amino acids on the trans side of the membrane).

Table XXI. The rate constants ($K \text{ min.}^{-1}$) of diffusion and exchange diffusion for control and galactose-fed rats. Animals were maintained on the galactose-chow diet for one day. Data taken from the experiment shown in figure 43.

Diet and Efflux Component	Kmin. ⁻¹ x 100					
	Efflux Time Intervals (min.)					
	0-5	5-10	10-15	15-30	30-60	60-90
Control-Total (D + E)	1.048	0.859	0.823	0.609	0.466	0.401
Control-Diffusion (D)	0.493	0.421	0.371	0.261	0.229	0.212
Control-Exchange (E)	0.555	0.438	0.452	0.348	0.237	0.189
Galactose- Total (D + E)	2.863	2.346	2.081	1.361	1.000	0.899
Galactose-Diffusion (D)	1.389	1.233	1.015	0.789	0.564	0.537
Galactose-Exchange (E)	1.474	1.113	1.066	0.572	0.436	0.362
D _{Control} / D _{Galactose}	0.36	0.34	0.37	0.33	0.40	0.40

The presence of amino acids on the trans side could increase the efflux if the carriers were to move faster when loaded than when unloaded as proposed for the transport of glucose in red blood cells by Mawe and Hempling (1965).

Another explanation of the high exchange diffusion activity would relate the rate of exchange to the rate of diffusion from the fibers into the epithelium (site of exchange). As deduced from the data presented in this paper, the accumulation in the lens is asymmetrical, with the bulk of the label, prior to efflux, being located near the anterior surface. Hence, the backflux from this pool into the epithelium (localized site of exchange diffusion) would determine the quantity of selenomethionine present in the epithelium at any given time.

The change in concentration of selenomethionine in the epithelium as a function of time can be expressed by the following equation:

$$\frac{dC_e}{dt} = (C_f - C_e)K_{Df} - (C_e - C_{med})K_{De}$$

where: C_e = concentration in the epithelium
 C_f = concentration in the fibers
 C_{med} = concentration in the medium
 K_{Df} = diffusion coefficient of the fibers
 K_{De} = diffusion coefficient of the epithelium

As the fibers swell there is an increase in their permeability (K_{Df}) which results in an increase in the selenomethionine concentration in the epithelium. The kinetics of the system are complex since several parameters are unknown both during the course of the experiment and during cataractogenesis (i.e., location and quantity of damaged fibers; distribution of selenomethionine in the damaged fibers, normal fibers and epithelium; and the degree of damage to the fiber in any region).

The results shown in Figure 43 can best be explained by the following series of events. As efflux progresses, the relative ratio of μ moles of selenomethionine in control/galactose lenses increases (the initial per cent accumulations for control/galactose were $\frac{6.34\%}{4.54\%}$ or a ratio of 1.4). The relative concentrations in the lenses at the end of 30 minutes efflux for control/galactose was $\frac{6.34 - 6.34(0.27)}{4.54 - 4.54(0.45)} = 1.85$. The increase in the relative ratio is due to a greater percentage of efflux from galactose lenses than from control lenses (i.e., in 30 minutes galactose lenses lost 45% of their initial accumulation while the controls lost only 27% as seen in Figure 42). The ratio of K_{min}^{-1} , via diffusion, for control/galactose at any time can be calculated from Table XXI. On day one, the control/galactose ratio at all times is approximately

0.35 (Table XXI). At earlier time periods (i.e., first five minutes), when the ratio of the concentrations of selenomethionine in the lenses of control/galactose is 1.4 and the ratio of the corresponding K_{min}^{-1} is 0.35, the relative concentrations available for exchange at the surface of control lenses as compared to galactose lenses is 50% ($1.4 \times .35$). At this time, one would expect the control lenses to be effluxing approximately 50% of the selenomethionine that the galactose lenses efflux. The actual value obtained was approximately 70% (Fig. 43) which may be a reflection of the differences in the distribution of selenomethionine in the two groups.

Beyond 30 minutes the ratio of selenomethionine concentrations in control/galactose lenses increases to a value greater than the maximum flux ratio of galactose/control necessary to maintain equal concentrations for the two groups at the site of exchange. As a result of this decrease in effective concentration, in galactose lenses, beyond 30 minutes efflux, one would expect and find a decrease in the exchange component as compared to controls.

In summary, the increase in the intralenticular diffusion in galactose lenses leads to an increase in the re-entry of amino acid into the epithelium which leads

to an apparent increase in exchange diffusion. Furthermore, the data have clearly indicated that on day one, at least, there is no impairment of the exchange carrier system(s) which are responsible for the transport of selenomethionine.

Figure 60 contains the data of Green and Toran (1968) for the efflux of selenomethionine from lenses of rats fed a 50% xylose-chow diet for seven days. There is an increase in the diffusion rate for xylose animals (xylose- $\%E_{cum}$, 90 min. = 33.4%; control- $\%E_{cum}$, 90 min. = 24.9%). The severity of the leak is not as great in xylose lenses as compared to galactose lenses (50% out via diffusion in 90 minutes). It is possible that the nature of the permeability change in galactose lenses is severe enough to allow the leakage of protein, while the change in "xylose" permeability may only result in the leakage of smaller molecules (i.e., cation, amino acids) out of the lens. This may help explain the data of Packer and Green (1968) who reported a decrease in soluble protein in galactose lenses but not in xylose lenses. The exchange diffusion system in xylose lenses appears to be functioning normally, as was the case for galactose lenses.

The incubation of lenses in a standard dose followed by a 90 minute efflux in the presence of 100mg% galactose resulted in no change in the diffusion or exchange diffusion

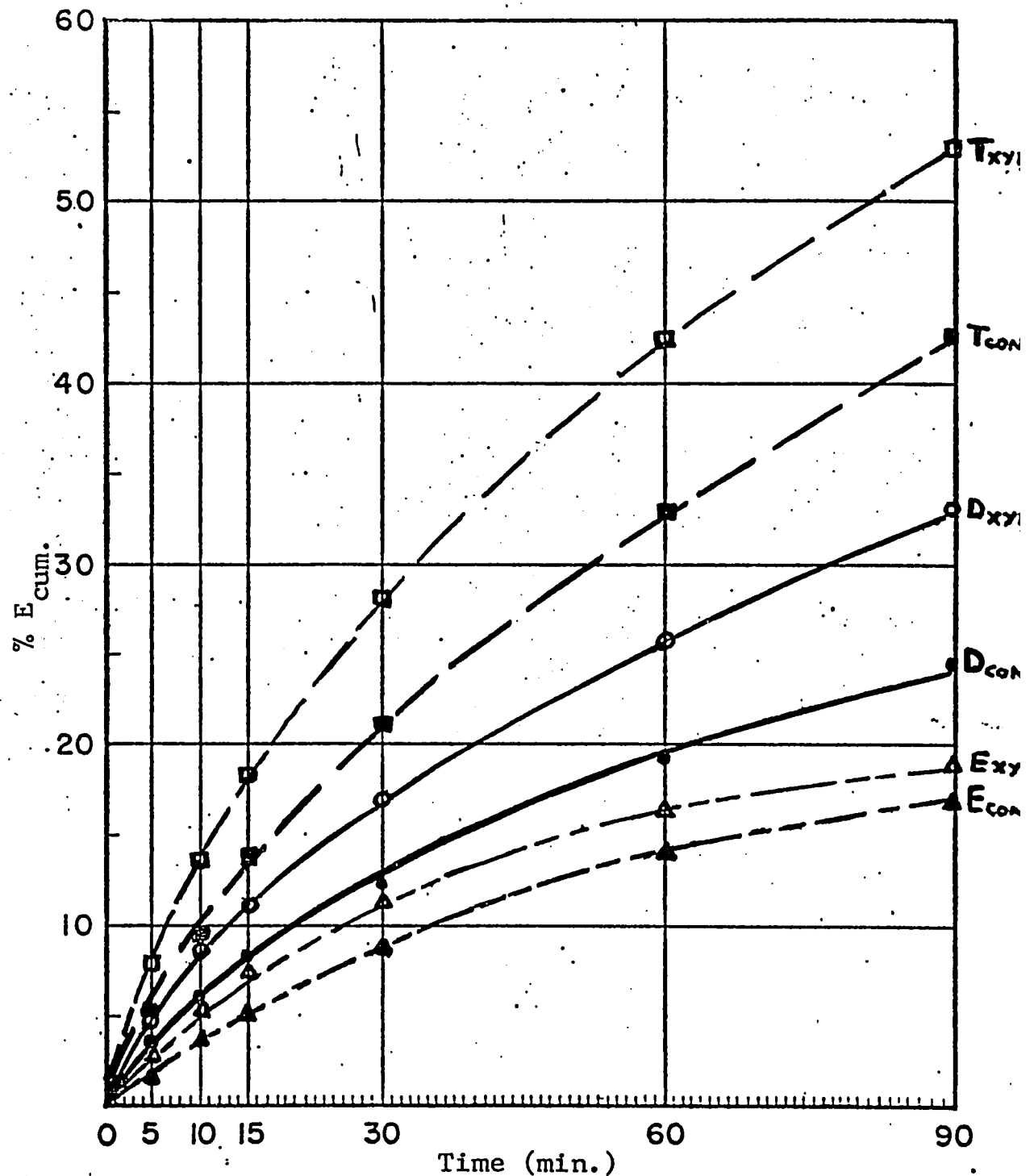


Figure 60. Percent efflux of selenomethionine from lenses taken from rats fed a 50% xylose-chow diet for two days, (from Toran and Green, 1968).

	<u>Total</u>	<u>Diffusion</u>	<u>Exchange</u>
Control	■ — — ■	● — — ●	▲ — — ▲
Galactose	□ — — □	○ — — ○	△ — — △

components as compared to the controls. This is in agreement with the findings of Kinoshita (1965) who demonstrated that α -AIB transport was not impaired during the first several hours of incubation in galactose.

Accumulation of selenomethionine decreases linearly as a function of age, while its incorporation into protein rises sharply in the first few weeks of life and then begins to decrease linearly at about 13 weeks of age. There are several parameters which can contribute to these effects. The surface area and volume of the lens increase linearly during growth, while the ratio of surface area/volume decreases slightly (Toran and Scherzer, 1968, Fig. 61). An increase in surface area leads to an increase in leak (Fig. 54), thereby requiring an increased activity of the transport system to maintain intralenticular amino acid concentrations. However, the amino acid concentration decreases slightly with age indicating a lack of a compensatory increase in the active transport system. Changes in accumulation and incorporation into protein may also be related to changes in available energy.

Protein synthesis has been shown to occur primarily at the anterior surface of the lens, and to a lesser extent in the cortex and nucleus (Nordmann, 1954). As the animal ages there is an increase in lens weight (Fig. 61) and a reduction in the percentage of lens volume

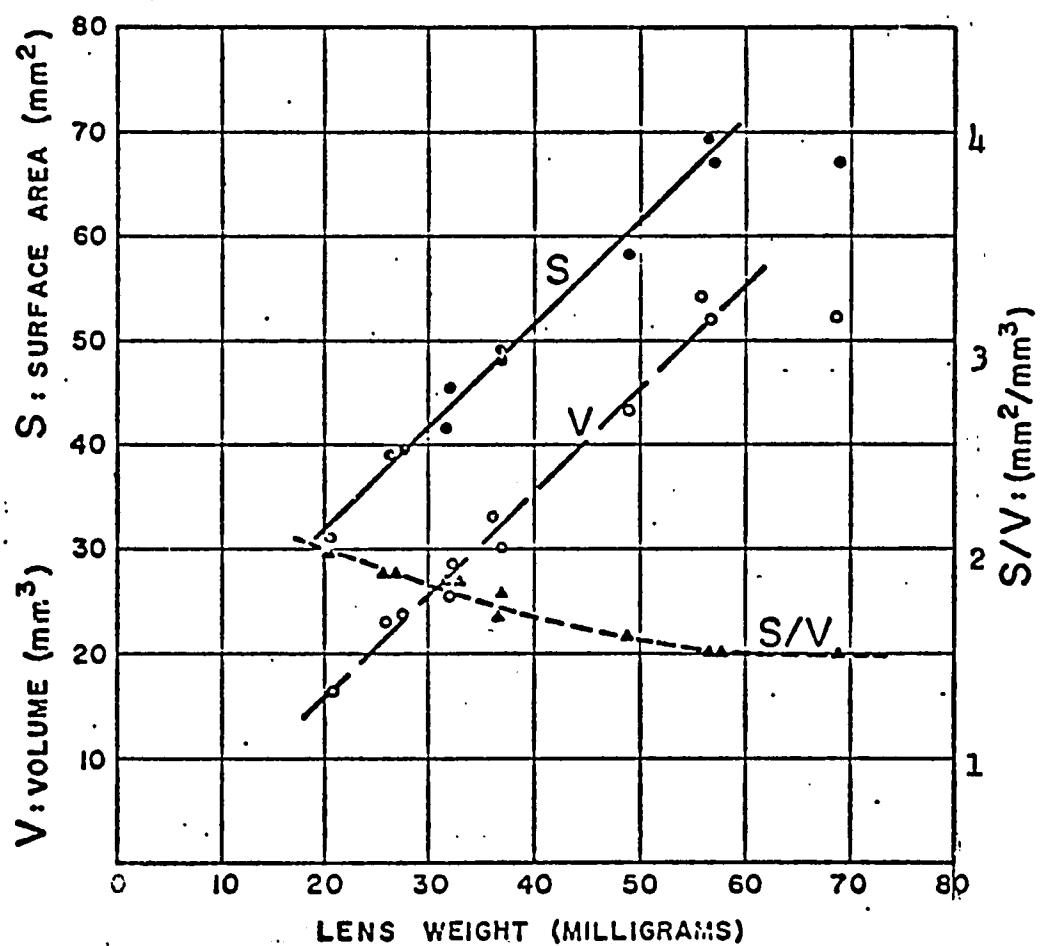


Figure 61. Surface area, volume and surface area to volume ratio of the rat lens as a function of lens weight. (from Toran and Scherzer, 1968).

which is effective in amino acid incorporation into protein. Accumulation and incorporation into protein, when plotted as a function of lens weight, could be misleading since the major sources of metabolic energy and the protein synthetic sites are generally confined to a small area of the lens, with the remainder of the lens being less active.

The observed decrease in selenomethionine incorporation may represent a shift from the growth to the maintenance state of the lens. A decreased rate of growth would not necessarily require the high levels of amino acids required during the rapid growth phase early in life.

Figure 54 and Figure 55 show the diffusion and exchange diffusion rates for young (54 days, 36 mg) and old (565 days, 68 mg) lenses. The rate of diffusion is increased 48% in older lenses (90 minute value). The surface areas of young and old rat lenses are 47mm^2 and 70mm^2 respectively as calculated from Figure 61. The % $E_{\text{cum}}/30\text{min.}/\text{mm}^2$ surface for young and old lenses are identical (young, $\frac{13\%}{47\text{mm}^2} = 0.30\%/\text{mm}^2$; old, $\frac{21\%}{70\text{mm}^2} = 0.30\%/\text{mm}^2$). Thus, the integrity of the membrane in both young and old lenses appears to be identical in that the diffusion per mm^2 of lens does not change.

The exchange diffusion system appears to be functioning at the same efficiency for both the young and old lenses. This would indicate that the carrier system(s) involved in active transport (assuming that they are identical to those of exchange) are not impaired with increasing age and that any decrease observed in activity would be attributable to an insufficient source of energy and/or an increase in diffusion. Diffusion increases in both the galactosemic and older lenses, for different reasons. Galactosemic lenses exhibit an increase in the diffusion coefficient (K_D) while old lenses exhibit an increase in surface area with no change in the diffusion coefficient.

V. SUMMARY

Se-75-selenomethionine was used to study amino acid accumulation, diffusion, exchange diffusion and protein incorporation in the rat lens.

The Q_{10} (26-36°C) for accumulation and protein incorporation were 1.7 and 3.0, respectively. The apparent K_m of accumulation was calculated to be 2.04 $\mu\text{moles/L}$ and the V_{max} value was 116 $\mu\text{moles/40 minutes}$ for a 27 mg lens. Protein incorporation was found to be saturable as the intralenticular level of selenomethionine increased. An apparent K_m of 71 $\mu\text{moles/27 mg lens}$ and a V_{max} of 3.6 $\mu\text{moles/40 minutes/27 mg lens}$ was determined for protein incorporation.

Competitive inhibitor studies indicate that selenomethionine, like methionine, has a high affinity for the L carrier site and a low affinity for the A site. The amino acids: leucine, isoleucine, alanine, histidine tertiary leucine α -methylethionine and ethionine, the ethyl analogue of methionine, inhibited accumulation and increased the per cent incorporation of selenomethionine into protein. The increase in per cent incorporation in presence of effective competitors is probably related to changes in the specific activity of the isotope in the lens. Glycine, glutamic acid, lysine, α -AIB, sarcosine, diaminopimelic

acid, taurine, and methionine sulfone had no effects on either accumulation or per cent incorporation into protein.

Inhibitors and uncouplers of oxidative respiration (i.e., cyanide, azide, dinitrophenol, oligomycin, antimycin A and BAL) do not affect accumulation but cause a marked inhibition in protein incorporation. The energy necessary for active transport is probably derived from anaerobic metabolism (glycolysis) while the energy required for protein synthesis is derived from aerobic metabolism (probably the α -glycerophosphate shuttle).

Several sulfhydryl inhibitors were found to inhibit accumulation and protein incorporation. Iodoacetic acid appears to inhibit accumulation by direct inactivation of the amino acid carrier (as determined by exchange diffusion studies) and by affecting glycolysis. N-ethylmaleimide was found to be a more effective inhibitor than IAA. N-ethylmaleimide was also found to increase non-mediated diffusion after prolonged incubation.

Calcium and magnesium appear to be present, in the lens, in sufficient concentrations to maintain membrane transport and protein incorporation for short incubation periods.

Diffusion and exchange diffusion were determined by loading lenses with Se-75-selenomethionine and allowing

them to efflux into media which either lack or contain an exchangeable amino acid. Kinetic studies demonstrated changes in the rate coefficients of diffusion and exchange diffusion in lenses pulsed for one hour prior to efflux. Changes in the slope of the rate coefficient, with time, for exchange diffusion paralleled the changes in the rate coefficient of diffusion indicating a dependency of exchange on intralenticular diffusion. The inability to demonstrate saturability of exchange diffusion may be related to a high K_m and/or the asymmetry of selenomethionine distribution in the lens.

Those amino acids which inhibited accumulation were also capable of driving exchange diffusion. Mercuric chloride produced an opacification of the lens as well as a complete inhibition of exchange diffusion. However, the integrity of the membrane was not altered since non-mediated diffusion was unchanged. Sulfhydryl inhibitors, IAA and NEM, were found to inhibit exchange diffusion. Preincubation of lenses in the presence of NEM resulted in a marked increase in non-mediated diffusion.

Galactose feeding results in a marked decrease in accumulation of amino acids and net incorporation into protein. Changes in accumulation could be detected within the first 24 hours of the diet. Maximum inhibition of

accumulation was reached by the third day and was maintained over the next eleven days. Per cent incorporation into protein in the galactosemic lens was found to be increased, to varying degrees, during the two weeks of diet.

Comparison of galactosemic and xylosemic lenses indicates that the increase in per cent incorporation of Se-75-selenomethionine into protein in the former group is related to an increase in the number of protein synthetic sites. An increase in protein synthetic sites is further indicated by the differences observed in puromycin, IAA and DNP inhibition in lenses from normal, galactose fed and glucose fed rats.

Glucose diets do not cause any changes in selenomethionine accumulation but do initiate an increase in protein incorporation. This increase does not appear to be related to changes in specific activity nor to an increase in the number of protein synthetic sites, but to an increase in the energy supply.

Non-mediated diffusion is increased two to three fold by the third day of galactose feeding. This increase in diffusion is maintained over the next four days. Exchange diffusion appears to increase in the galactosemic lens. However, the increase in exchange diffusion seems to be related to increased fiber permeability which results in an

increased intralenticular diffusion which in turn results in an apparent increase in the epithelial selenomethionine concentration. This implies that the carrier itself is not altered during cataractogenesis and that the decreased accumulation of selenomethionine is related to an increase in diffusion with a possible diminution in energetics.

The feeding of rats a diet consisting of "50% glucose-50% chow" was found to increase protein incorporation; while a "50% glucose-casein" diet resulted in a decreased protein incorporation. Differences in the lipid content and/or the presence of an inhibitor or essential nutrient may be responsible for these observations.

Selenomethionine accumulation decreased linearly with age. This decrease in accumulation is related, in part, to the observed increase in non-mediated diffusion. The increased diffusion is directly related to the linear increase in surface area with age. Relative rate coefficients of diffusion/mm² are identical for young and old lenses, unlike the galactosemic lenses where an increase in the rate coefficient is observed. Thus the integrity of the membrane is not altered during aging. Exchange diffusion in old lenses is the same as that found in young lenses.

Per cent protein incorporation was found to increase during the first few weeks of life and then began to decrease linearly beyond the thirteenth week. The change in protein incorporation in older animals appears to be related to a diminished protein synthetic activity and not to decreased amino acid accumulation.

VI. BIBLIOGRAPHY

1. Amersham / Searle Corp., 1968, Technical Bulletin RP-1, Nov., Des Plaines, Illinois
2. Anderson, E.I., 1965, Dual Cation Activation of Bovine Lens Autolysis, Invest. Ophth., 4:181.
3. Barber, G.W., 1968, Free Amino Acids in Senile Cataractous Lenses; Possible Osmotic Etiology, Invest. Ophth., 7:564.
4. Becker, B., 1962, Accumulation of Rubidium-86 by the Rat Lens, Invest. Ophth., 1:502.
5. Bernstein, L., Kerrigan, M. and Maisel, H., 1966, Lactic Dehydrogenase Isozymes in Lens and Cornea, Exptl. Eye Res., 5:309.
6. Blau, M., 1961, Biosynthesis of Se-75-Selenomethionine and Se-75-Selenocystine, Biochim. Biophys. Acta., 49:389.
7. Blau, M. and Bender, M.A., 1962, Se-75-Selenomethionine for Visualization of the Pancreas by Isotope Scanning, Radiology, 78:974.
8. Bonting, S.L., Simon, K.A. and Hawkins, N.M., 1961, Studies on Sodium-Potassium-Activated Adenosine Triphosphatase. 1. Quantitative Distribution in Several Tissues of the Cat, Arch. Biochem., 95:416.
9. Brassil, D. and Kern, H.L., 1968, Characterization of the Transport of Neutral Amino Acids by the Calf Lens, Invest. Ophth., 7:441
10. Bremer, J. and Natori, Y., 1960, Behavior of some Selenium Compounds in Transmethylation, Biochim. Biophys. Acta., 44:367
11. Charlton, J. and van Heyningen, R., 1968, An Investigation into the Loss of Proteins of Low Molecular Size from the Lens in Senile Cataracts, Exptl. Eye Res., 7:47

12. Christensen, H.N., 1962, "Biological Transport!" W.A. Benjamin, New York.
13. Chylack, L.T. Jr., Kinoshita, J. and Kasabian, R., 1970, Nature and Distribution of Two Distinct Forms of Hexokinase within Mammalian Lens, Exptl. Eye Res., 10:250.
14. Chylack, L.T. and Kinoshita, J., 1972, The Interaction of the Lens and Vitreous. I. The High Glucose Cataract in Lens-Vitreous Preparations, Exptl. Eye Res., 14:58.
15. Cohen, G.N. and Cowie, D.B., 1957, Total Replacement of Methionine by Selenomethionine in the Proteins of Escherichia coli, C.r. hebd. Séanc. Acad. Sci. Paris, 244:680.
16. Conconi, F., Bank, A. and Marks, P., 1966, Polyribosomes and Control of Protein Synthesis: Effects of Sodium Fluoride and Temperature in Reticulocytes, J. Mol. Biol., 19(2):525.
17. Cotlier, E., 1964, Synthesis of Creatine Phosphate by the Crystallin Lens, Am. J. Ophth., 57:63.
18. Cotlier, E. and Beaty, C., 1967, The Role of Na⁺ Ions in the Transport of α -Aminoisobutyric Acid and other Amino Acids into the Lens, Invest. Ophth., 6:64.
19. Cotlier, E. and Beaty, C., 1968, The Transport of C-14- α -Aminoisobutyric Acid in Galactose Cataracts in Rat and Rabbit Lenses Incubated in High Galactose Media, Invest. Ophth., 7:77.
20. Cotlier, E. and Becker, B., 1965, Rubidium-86 Accumulation and Dulcitol Distributions in Lenses of Galactose-Fed Rats, Exptl. Eye Res., 4:340
21. Coulombre, A.J., 1965, Experimental Embryology of the Vertebrate Eye, Invest. Ophth., 4:411

22. Cowie, D.B. and Cohen, G.N.,1957, Biosynthesis by *Escherichia coli* of Active Altered Proteins Containing Selenium instead of Sulphur, *Biochim. Biophys. Acta.*, 26:252.
23. Dardenne, U. and Kirsten, G.,1962, Presence and Metabolism of Amino Acids in Young and Old Lenses, *Exptl. Eye Res.*, 1:415.
24. Davson, H. and Duke-Elder, W.S.,1948, The Distribution of Reducing Substances between the Intra-ocular Fluids and Blood Plasma, and the Kinetics of Penetration of Various Sugars into these Fluids, *J. Physiol.*, 107:141.
25. Dische, Z.,1965, The Glycoproteins of the Bovine Lens and their Relation to Albuminoid, *Invest. Ophth.*, 4:759.
26. Dische, Z., Ginevra, Z. and Youlus, J.,1957, Studies on Protein and Protein Synthesis during the Development of Galactose Cataracts, *Am. J. Ophth.*, 44(Part II):332.
27. Eguchi, G.,1964, Electron Microscopic Studies on Lens Regeneration. II Formation and Growth of Lens Vesicle and Differentiation of Lens Fibers, *Embryologia*, 8:247.
28. Epstein, D.L. and Kinoshita, J.H.,1970, The Effect of Diamide on Lens Glutathione and Lens Membrane Function, *Invest. Ophth.*, 9:629.
29. Estabrook, R.W. and Pullman, M.E., eds.,1967, "Methods of Enzymology". Vol. X Oxidation and Phosphorylation, Academic Press, New York.
30. Farkas, T. and Roberson, S.,1964, The Effect of Temperature on the Glucose Uptake and Glucose Utilization of Isolated Rat Lenses, *Exptl. Eye Res.*, 3:198.

31. Feldman, G.E. and Feldman, L.S.,1965, New Concepts of Human Lenticular Lipids and Their Possible Role in Cataracts, Invest. Ophth.,4:162.
32. Francois, J. and Rabaey, M.,1958, Permeability of the Capsule for the Lens Proteins, Acta Ophth. (Kbh), 36:837.
33. Gorthy, W., 1968, An Electron Microscopic Study of the Rat Lens Epithelium, Exptl. Eye Res., 7:394.
34. Green, H. and Solomon, S.A.,1957, Effect of Age upon Metabolism, Arch. Ophth., 58:23.
35. Green, H. and Solomon, S.A.,1959, Hexokinase of Rabbit Lenses, Arch. Ophth., 61:616.
36. Green, M. and Toran, N.,1968, unpublished data.
37. Griffiths, M.K.,1966, The Components of an α -Glycerophosphate cycle and their Relation to Oxidative Metabolism in the Lens, Biochem. J., 99:12.
38. Grimes, P. and von Sallman, L.,1968, Lens Epithelium Proliferation in Sugar Cataracts, Invest. Ophth., 7:355.
39. Hanna, C.,1965, Changes in DNA, RNA and Protein Synthesis in the Developing Lens, Invest. Ophth., 4:480.
40. Hanna, C. and Keatts, H.,1968, Cell Migration in the Adult Rat and Rabbit Lens, Exptl. Eye Res., 7:244.
41. Hanna, C. and O'Brien, J.E.,1960, Studies on Galactose Cataract Formation Utilizing Thymidine-tritium, A.M.A. Arch. Ophth., 64:708.
42. Hansford, R.G. and Chappell, J.B.,1967, The Effect of Ca^{2+} on the Oxidation of Glycerol Phosphate by Blowfly Flight-Muscle Mitochondria, Biochem. Biophys. Res. Commun., 27:686.
43. Harris, J.E. and Becker, B.,1965, Cation Transport in the Lens, Invest. Ophth. 4:691.

44. Harris, J.E. and Gruber, L.,1962, The Electrolyte and Water Balance of the Lens, Exptl. Eye Res., 1:372.
45. Hatcher, H. and Andrews, S.,1970, Changes in Lens Fatty Acid Composition during Galactose Cataract Formation, Invest. Ophth., 9:801.
46. Hayman, S. and Kinoshita, J.,1965, Isolation and Properties of Lens Aldose Reductase, J. Biol. Chem., 240:877.
47. Heinz, E.,1954, Kinetic Studies on the "Influx" of Glycine-1-C¹⁴ into the Ehrlich Mouse Ascites Carcinoma Cell, J. Biol. Chem., 211:781.
48. Heinz, E. and Walsh, P.M.,1958, Exchange Diffusion, Transport, and Intracellular Level of Amino Acids in Ehrlich Carcinoma Cells, J. Biol. Chem., 233:1488.
49. van Heyningen, R.,1959, Formation of Polyols by the Lens of the Rat with Sugar Cataracts, Nature, 184:194.
50. van Heyningen, R.,1962a, The Sorbitol Pathway in the Lens, Exptl. Eye Res., 1:396.
51. van Heyningen, R.,1962b, The Lens in "The Eye" Vol. I, Davson, H. ed., Academic Press, New York.
52. van Heyningen, R.,1965, Some Glycolytic Enzymes and Intermediates in the Rabbit Lens, Exptl. Eye Res., 4:298.
53. van Heyningen, R.,1969a, Xylose Cataracts: A Comparison between the Weanling and the Old Rat, Exptl. Eye Res. 8:379.
54. van Heyningen, R.,1969b, The Lens in "The Eye" Vol. I, Davson, H. ed., Academic Press, New York.
55. Hockwin, O.,1965, Enzyme Activities in Relation - ship to Age and Phosphorylated Intermediates in Energy Metabolism, Invest. Ophth. 4:496.

56. Hockwin, O. and Berghoff, P., 1966, Untersuchungen Verschiedener Fermentaktivitäten Menschlicher Linsen in Abhängigkeit von der Lokalisation der Linsentrübung, Klin. Mbl. Augenheilk, 149:87.
57. Hockwin, O., Schmutter, J. and Müller, H.K., 1963, Untersuchungen über Gewicht und Volumen Verschieden Alter Rinderlinsen, Graefes, Arch. Ophth., 166:136.
58. Inui, Y. and Christensen, H.N., 1966, Discrimination of Single Transport Systems. The Sodium Sensitive Transport of Neutral Amino Acids in Ehrlich Cells, J. Gen. Physiol., 50:203.
59. Keiding, S. and Mellempgaard, 1972, Dose Dependence of Galactose Cataracts in the Rat, Acta Ophth. 50, FASC. 2, Munksgaard, Copenhagen:174.
60. Kern, H.L., 1962, Accumulation of Amino Acids by Calf Lens, Invest. Ophth., 1:368.
61. Kern, H.L., 1965, The Transport of Sugars into the Lens, Invest. Ophth., 4:680.
62. Kern, H.L., 1970, Efflux of Amino Acids from the Lens, Invest. Ophth., 9:692.
63. Kern, H.L. and Brassil, D., 1968, Effect of Sodium Ions on the Transport of Neutral Amino Acids by Calf Lens, Invest. Ophth., 7:452.
64. Kern, H.L., Roosa, P. and Murray, S., 1962, Evidence for Active Transport of Alkali Metal Cations by Calf Lens, Exptl. Eye Res., 1:385.
65. Kinoshita, J.H., 1955, Carbohydrate Metabolism of the Lens, A.M.A. Arch. Ophth., 54:360.
66. Kinoshita, J.H., 1965, Cataracts in Galactosemia, Invest. Ophth., 4:786.
67. Kinoshita, J.H., Barber, G.W., Merola, L.O. and Tung, B., 1969, Changes in the Levels of Free Amino Acids and Myo-Inositol in the Galactose-Exposed Rat, Invest. Ophth. 8:625.

68. Kinoshita, J.H. and Dillen, I., 1959, The Effects of Age on the Carbohydrate Metabolism of Bovine Lens, Acta: XVIII, Conc. Ophth. Belge, 744.
69. Kinoshita, J.H., Futterman, S., Satoh, K. and Merola, L.O., 1963, Factors Affecting the Formation of Sugar Alcohols in Ocular Lens, Biochim. Biophys. Acta, 74:340.
70. Kinoshita, J.H., Kern, H.L. and Merola, L.O., 1961, Factors Affecting the Cation Transport of Calf Lenses, Biochim. Biophys. Acta, 47:458.
71. Kinoshita, J.H. and Merola, L.O., 1961, The Utilization of Pyruvate and its Conversion to Glutamate in Calf Lens, Exptl. Eye Res., 1:53.
72. Kinoshita, J.H. and Merola, L.O., 1964, Hydration of the Lens during the Development of Galactose Cataract, Invest. Ophth., 3:577.
73. Kinoshita, J.H., Merola, L.O. and Dikmak, E., 1962a, Osmotic Changes Caused by the Accumulation of Dulcitol in Lenses of Rats Fed with Galactose, Nature, 194:1085.
74. Kinoshita, J.H., Merola, L.O. and Dikmak, E., 1962b, Osmotic Changes in Experimental Galactose Cataracts, Exptl. Eye Res., 1:405.
75. Kinoshita, J.H., Merola, L.O. and Dikmak, E., 1962c, The Accumulation of Dulcitol and Water in Rabbit Lenses Incubated with Galactose, Biochim. Biophys. Acta, 62:176.
76. Kinoshita, J.H., Merola, L.O. and Hayman, S., 1965, Osmotic Effects on the Amino Acid-Concentrating Mechanisms in the Rabbit Lens, J. Biol. Chem., 240:310.
77. Kinoshita, J.H., Lorenzo, O., Merola, O. and Tung, B., 1968, Changes in Cation Permeability in the Galactose-Exposed Rabbit Lens, Exptl. Eye Res., 7:80.

78. Kinoshita, J.H. and Wachtl, C.A., 1958, A Study of C-14-Glucose Metabolism of the Rabbit Lens, J. Biol. Chem., 233:5.
79. Kinsey, V.E., 1965, Amino Acid Transport in the Lens, Invest. Ophth., 4:691.
80. Kinsey, V.E. and Reddy, D.V.N., 1962, Transport of Amino Acids into the Posterior Chamber of the Rabbit Eye, Invest. Ophth., 1:355.
81. Kinsey, V.E. and Reddy, D.V.N., 1963, Studies on the Crystalline Lens X. Transport of Amino Acids, Invest. Ophth., 2:229.
82. Kinsey, V.E. and Reddy, D.V.N., 1965, Studies on the Crystalline Lens XI. The relative role of the Epithelium and Capsule in Transport, Invest. Ophth., 4:104.
83. Kinsey, V.E., Wachtl, C., Kuck, J.F.R. Jr. and Reddy, D.V.N., 1958, in "Concil. Ophthalmol. Acta", 18:865.
84. Kuck, J.F.R. Jr., 1961, The Formation of Fructose in the Ocular Lens, Arch. Ophth., 65:840.
85. Kuck, J.F.R. Jr., 1963, Sugar and Sugar Alcohol Levels in the Aging Rat Lens, Invest. Ophth., 2:607.
86. Kuck, J.F.R. Jr., 1965, Carbohydrates of the Lens in Normal and Precataractous States, Invest. Ophth., 4:638.
87. Kuck, J.F.R. Jr., 1970, in "Biochemistry of the Eye", Graymore, C.N., ed., Academic Press New York.
88. Kuwabara, T., Kinoshita, J.H. and Cogan, D.G., 1969, Electron Microscopic Study of Galactose Induced Cataract, Invest. Ophth., 8:133.
89. Lehninger, A.L., 1965, "The Mitochondrion", W.A. Benjamin, New York.

90. Lerman, S.,1960, Pathogenetic Factors in Experimental Galactose Cataract, Arch. Ophth., 63:132.
91. Lerman, S.,1965, Carbohydrate Metabolism in the Rat Lens as Related to the Age of the Animal, Arch. Ophth., 65:181.
92. Lerman, S. and Devi, A.,1961, The Incorporation of Labeled Amino Acids into Lens Protein of Normal, Galactose and Xylose Fed Rats, Am. J. Ophth., 51(Part II):1012.
93. Lerman, S., Donk, J. and Pitel, M.,1962, Further Studies on the Metabolism of the Rat Lens, Growth, 26:103.
94. Lerman. S. and Heggeness, F.M.,1961, The Metabolism of Xylose by the Ocular Lens of the Rat, Biochem. J., 79:224.
95. Lerman, S. and Zigman, S.,1965, The Metabolism of the Lens as Related to Aging and Experimental Cataractogenesis, Invest. Ophth., 4:643.
96. Lerman, S., Zigman, S. and Forbes, W.,1968, Insoluble Protein Fraction of Lens, Exptl. Eye Res., 7:444.
97. Lessinger, A. and Mandel, P.,1960, Incorporation of Methionine-S-35 in the Crystalline of Young and Aged Animals, J. Physiol.(Paris), 52:150.
98. Levari, R., Wertheimer, E. and Kornblueth, W., 1964, Interrelation between the Various Pathways of Glucose Metabolism in the Rat Lens, Exptl. Eye Res., 3:99.
99. Lou, M.G. and Kinoshita, J.H.,1967, Control of Lens Glycolysis, Biochim. Biophys. Acta, 141:547.
100. Li, L. K. and Spector, A.,1972, Studies on the Reaggregation of Isolated Subunits of Calf Lens Alpha-Crystallin, Exptl. Eye Res., 13:110.

101. Mawe, R.C. and Hempling, H.G.,1965, The Exchange of C-14-Glucose across the Membrane of the Human Erythrocyte, J. Cell. and Comp. Physiol., 66:95.
102. Mok, C. and Waley, S.G.,1968, N-Terminal Groups of Lens Protein, Exptl. Eye Res., 7:148.
103. Morner, C.T.,1894, Untersuchungen der Protein-Substanzen in den Leichtbrechenden Medied des Auges, I, Ztschr. Physiol. Chem., 18:61.
104. Müller, H. and Kleifeld, O.,1956, The Phosphorous Metabolism in the Lens, Am. J. Ophth., 42:431.
105. Mudd, S.H. and Cantoni, G.L.,1957, Selenomethionine in Enzymatic Transmethylations, Nature,London, 180:1052.
106. Nordman, J.,1954, "Biologie du Cristallin", Masson et Cie, Paris.
107. Oxender, D.L. and Christensen, H.N.,1963, Distinct Mediating Systems for the Transport of Neutral Amino Acids by the Ehrlich Cell, J. Biol. Chem., 238:3686.
108. Packer, D. and Green, M.,1970, unpublished data.
109. Painter, E.P.,1947, A Synthesis of Selenium Analogues of d,l-Methionine and d,l-Homocysteine, J. Am. Chem. Soc., 69:2009.
110. Papaconstantinou, J.,1967, Molecular Aspects on Lens Cell Differentiation, Science, 156:338.
111. Paterson, C.A.,1970a, Sodium Exchange in the Crystalline Lens, I. Relative Exchange in Capsule, Cortex and Nucleus, Exptl. Eye Res., 10:151.
112. Paterson, C.A.,1970b, Efflux of ^{22}Na and ^{86}Rb from the Crystalline Lens, Exptl. Eye Res., 10:331.
113. Patterson, J.W.,1953, Cataracts Caused by Carbohydrates, Am. J. Ophth., 36: No.6, II, 143.

114. Patterson, J.W.,1954, Hyperglycemia and Galactose Cataracts, Am. J. Physiol., 177:541.
115. Patterson, J.W.,1955, Effect of Blood Supply on the Development of Cataracts, Am. J. Physiol., 180:495.
116. Patterson, J.W.,1965, A Review of Glucose Transport in the Lens, Invest. Ophth., 4:667.
117. Patterson, J.W. and Bunting, K.W.,1963, Factors Limiting Glycolysis in the Rat Lens, Invest. Ophth., 2:612.
118. Patterson, J.W. and Bunting, K.W.,1965, Changes Associated with the Appearance of Mature Sugar Cataracts, Invest. Ophth., 4:167.
119. Patterson, J.W. , Patterson, M.E. and Kinsey,E.V., 1965, Lens Assay on Diabetic and Galactosemic Rats Receiving Diets that Modify Cataract Development, Invest. Ophth., 4:98.
120. Patterson, J.W., Patterson, M.E. and Bunting, K.W., 1962, Experimental Carbohydrate Cataracts, Exptl. Eye Res., 1:411.
121. Pirie, A.,1962, Metabolism of Glycerophosphate in the Lens, Exptl. Eye Res., 1:427.
122. Pirie, A.,1963, Metabolism of α -Glycerophosphate in Lens, Biochem. J., 88:48.
123. Pirie, A.,1965, Epidemiological and Biochemical Studies of Cataract and Diabetes, Invest. Ophth., 4:629.
124. Pirie, A. and van Heyningen, R.,1956, "Biochemistry of the Eye", Blackwell Scientific, Oxford.
125. Racker, E.,1965, "Mechanisms in Bioenergetics", Academic Press, New York.
126. Reddy, D.V.N.,1965, Amino Acid Transport in the Lens in Relation to Sugar Cataracts, Invest. Ophth., 4:700.

127. Reddy, D.V.N.,1966, in "Biochemistry of the Eye", XX International Congress of Ophthalmology, Tutzing Castle.
128. Reddy, D.V.N.,1967, Distribution of Free Amino Acids and Related Compounds in Ocular Fluids, Lens, and Plasma of Various Mammalian Species, Invest. Ophth., 6:478.
129. Reddy, D.V.N.,1970, Studies on Intraocular Transport of Taurine. II. Accumulation in the Rabbit Lens, Invest. Ophth., 9:206.
130. Reddy, D.V.N. and Kinsey, V.E.,1962a, Studies on the Crystalline Lens IX. Quantitative Analysis of Free Amino Acids and Related Compounds, Invest. Ophth., 1:635.
131. Reddy, D.V.N. and Kinsey, V.E.,1962b, Transport of α -AIB into Ocular Fluids and Lens, Invest. Ophth., 1:41.
132. Reddy, D.V.N. and Kinsey, V.E.,1963, Transport of Amino Acids into Intraocular Fluids and Lens in Diabetic Rabbits, Invest. Ophth., 2:237.
133. Reddy, D.V.N., Rosenberg, C. and Kinsey, E.V., 1961, Steady State Distribution of Free Amino Acids in the Aqueous Humors, Vitreous Body and Plasma of the Rabbit, Exptl. Eye Res., 1:175.
134. von Sallman, L.,1966, Effect of Age on Cell Division, H-3-Thymidine Incorporation, and Diurnal Rhythm in the Lens Epithelium of Rats, Invest. Ophth., 5:560.
135. Satoh, K.,1972, Age-Related Changes in the Structural Proteins of Human Lens, Exptl. Eye Res., 14:53.
136. Shrift, A., Nevyas, J. and Turndorf, S.,1961, Mass Adaptation of Selenomethionine in Populations of *Chlorella vulgaris*, Pl. Physiol.,Lancaster, 36:502.

137. Sippel, T.O.,1962, Pyridine Nucleotides in Normal and Diabetic Rat Lenses, Exptl. Eye Res., 1:368.
138. Sippel, T.O.,1965, Energy Metabolism in the Lens during Aging, Invest. Ophth., 4:502.
139. Sippel, T.O.,1966a, Changes in Water, Protein, and Glutathione Contents of the Lens in the Course of Galactose Cataract Development in Rats, Invest. Ophth., 5:568.
140. Sippel, T.O.,1966b, Energy Metabolism in the Lens during Development of Galactose Cataracts in Rats, Invest. Ophth., 5:576.
141. Sippel, T.O.,1966c, in "Biochemistry of the Eye", Symp., Basel. S. Karger, A.G.
142. Sippel, T.O.,1967, Enzymes of Carbohydrate Metabolism in Developing Galactose Cataracts of Rats, Invest. Ophth., 6:59.
143. Smith, M.G.,1962, Polyol-dehydrogenases. 4. Crystallization of the L-Iditol Dehydrogenase of Sheep Liver, Biochem. J., 83:135.
144. Spector, A.,1965, The Soluble Proteins of the Lens, Invest. Ophth., 4:579.
145. Spector, A. and Kinoshita, J.H.,1964, The Incorporation of Labeled Amino Acids into Lens Proteins, Invest. Ophth., 3:517.
146. Spencer, R.P. and Blau, M.,1962, Intestinal Transport of Selenium-75-Selenomethionine, Science, New York, 136:155.
147. Srinivasan, B.D. and Harding, C.V.,1965, Cellular Proliferation in the Lens, Invest. Ophth., 4:452.
148. Thoft, R.A. and Kinoshita, J.H.,1965, The Effect of Calcium on Rat Lens Permeability, Invest. Ophth., 4:122.

- 149. Toran, N. and Green, M.,1968, unpublished data.
- 150. Toran, N. and Scherzer, N.,1968, unpublished.
- 151. Turchetto, E., Sola, M. and Lenaz, G.,1963, Gas-Liquid Chromatography of the Fatty Acids of the Lipids of the Crystalline Lens, Exptl. Eye Res., 2:160.
- 152. Waley, S.G. and van Heyningen,1962, Purification and Properties of a Neutral Proteinase in the Lens, Exptl. Eye Res., 1:343.
- 153. Weller, C. and Green, M.,1969, Methionyl-tRNA Synthetase Detected by Se-75-Selenomethionine in Lenses from Normal and Galactose-Fed Rats, Exptl. Eye Res., 8:84.
- 154. Wortman, B. and Becker, B.,1956, Enzymatic Activities of the Lens, Am. J. Ophth., 42:342.
- 155. Young, R.W. and Fulhorst, H.W.,1966, Regional Differences in Protein Synthesis within the Lens of the Rat, Invest. Ophth., 5:288.