The Effect of Antacids on the Absorption of Riboflavin from the Gastrointestinal Tract

A Thesis Presented to the Faculty of the
College of Pharmacy
The University of Houston

In Partial Fulfillment of the Requirements
for the Degree Master of Science
in Hospital Pharmacy

by
Wyatt Hedrick
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ACKNOWLEDGEMENTS

I would like to thank Dr. Stuart Feldman for consenting to be my major professor and for his assistance in the designing and planning of the research study which I undertook. I would also like to express my appreciation toward Pete Cascella, Bill Ravis, Suresh Sachanandani, and Jay Koska, graduate students at the University of Houston, who, along with myself, consented to serve as subjects for the *in vivo* portion of the research study.

ABSTRACT

The effect of various over-the-counter antacids on the rate of gastric emptying was evaluated in both *in vivo* and *in vitro* studies using riboflavin as a test drug. *In vivo* studies, using five male volunteers, showed that only aluminum hydroxide gel had any significant effect ($p<0.05$) on two of three categories observed (time of peak riboflavin excretion and peak rate of riboflavin excretion). No differences in bioavailability of riboflavin were noted with co-administration of the antacids. The percent of riboflavin binding to antacid as a function of both riboflavin concentration and antacid amount was determined in the *in vitro* studies along with the effect of antacid and hydrochloric acid concentration on the pH of riboflavin-antacid suspensions. Magnesium hydroxide exhibited the highest percent binding and pH readings. The smooth muscle relaxant properties of aluminum hydroxide may account for the significant level of delayed gastric emptying noted with this antacid. Although magnesium hydroxide showed no significant difference in riboflavin absorption compared with control values, higher values were observed in 4 out of the 5 subjects for time of peak riboflavin excretion, peak rate of riboflavin excretion, and bioavailability of riboflavin. This suggests that additional studies are needed to evaluate the potential of magnesium hydroxide to alter gastric emptying and drug absorption.

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INTRODUCTION AND LITERATURE SURVEY

Drug interaction is the phenomenon which occurs when the effects of one drug are modified by the prior or concurrent administration of another drug (1). It is an area of considerable importance in the pharmacy profession. There are a number of mechanisms of drug interactions which can occur between two or more drugs that can significantly influence the course of drug therapy for a patient on a multiple drug regimen. Among these mechanisms are: 1) an increase in drug absorption, 2) a decrease in drug absorption, 3) alterations in drug distribution, 4) changes in drug metabolism, 5) changes in the excretion rate of drugs, 6) synergistic interaction, and 7) antagonistic interaction.

An increase in drug absorption can lead to a greater incidence of side effects and toxicity if the drug has a narrow therapeutic index. For example, increased drug absorption has been demonstrated by Peterson and Finland (2) in male subjects from 16 to 60 years of age for sulfadiazine when it is given concurrently with sodium bicarbonate after a meal.

A decrease in drug absorption may present a serious problem for those drugs which are dependent on a specific plasma concentration for therapeutic effect. A classic example of decreased drug absorption occurs when tetracycline is administered with antacids containing divalent or trivalent cations. Of 10,818 patients receiving tetracycline, 572 (5.3%) also received antacids containing calcium, aluminum, or magnesium, with resultant decreased serum tetracycline levels (3). This reduction in gastrointestinal absorption of tetracycline can result in subtherapeutic levels of the antibiotic in the body.

Another example of decreased drug absorption due to a drug interaction is decreased intestinal absorption of thyroxine and triiodothyronine by cholestyramine. Studies on two hypothyroid patients and five normal volunteers

showed that cholestyramine binds to thyroid hormones preventing their absorption across the gastrointestinal tract (4).

The drug interaction between oral anticoagulants and phenylbutazone is a classic example of an alteration in drug distribution leading to an alteration in pharmacologic effect. In vitro experiments have shown that phenylbutazone displaces warfarin from plasma proteins. The increase in plasma levels of free warfarin leads to an increased anticoagulant effect. Phenylbutazone has also been shown to increase warfarin disappearance from the plasma in studies done on three normal adult men aged 18 to 52, a 58-year-old man with coronary artery disease, and a 78-year-old man with coronary artery disease resistant to coumarin anticoagulant drugs (5).

A change in drug metabolism occurs when allopurinol is administered with azathioprine. Nies and Oates (6) attributed the death of a sixty-one year old black male due to hypoplasia of the bone marrow to this interaction. Allopurinol, a xanthine oxidase inhibitor, prevented the conversion of azathioprine to inactive metabolites. Instead, large amounts of 6-mercaptopurine accumulated to cause the toxic effects of thrombocytopenia and leukopenia (6).

An example of a drug interaction involving changes in drug metabolism is the interaction between pyridoxine (vitamin B_6) and levodopa. Pyridoxine accelerates the metabolism of levodopa in the peripheral blood circulation, thus preventing levodopa from reaching the central nervous system. In the central nervous system, levodopa's active metabolite, dopamine, acts to treat the tremors and rigidity caused by Parkinson's disease. This type of drug interaction was demonstrated in a study population consisting of three men and one woman with Parkinson's disease who had achieved stable improvement (7).

The interaction between acetohexamide and phenylbutazone is an example of an interaction resulting in the alteration of the excretion rate of a

drug. The excretion rate of the active metabolite of acetohexamide (hydroxyhexamide) is reduced by phenylbutazone, although the half-life of acetohexamide itself is unaffected. Experiments involving nine patients, seven with mild diabetes and two without diabetes, supported these findings (8).

A synergistic interaction takes place when ethacrynic acid is given with aminoglycoside antibiotics. Increased ototoxicity in the form of moderate to severe permanent hearing loss was observed by Mathog and Klein (9) in three uremic patients after administration of ethacrynic acid and small doses of aminoglycoside antibiotics.

Salicylates administered with probenecid inhibit the uricosuric effects of each medication in an antagonistic interaction. This was shown in tests done on seven gouty and nine nongouty subjects (10).

While not well investigated, drug absorption interactions may present a serious problem in drug therapy, especially in view of the large number of over-the-counter medications taken by the public. An interaction of this nature is more difficult to detect and may be viewed as a therapeutic failure by the physician if the interaction results in decreased absorption of the drug and decreased activity (11).

There are several possible mechanisms of drug absorption interactions. These mechanisms include pH effects on dissolution and ionization; changes in gastric emptying and gastrointestinal motility; formation of complexes, ion pairs and chelates; interference with active transport; disruption of lipid micelles; changes in portal blood flow; toxic effects on gastrointestinal mucosa; changes in volume, composition, and viscosity of secretions; effects on mucosal and bacterial drug metabolism; changes in membrane permeability; and unknown mechanisms (12).

According to the pH-partition theory, drugs that are weakly acidic are

best absorbed in the stomach, and drugs that are weakly basic are best absorbed in the small intestine (13, 14). This is because, in most instances, drugs are more rapidly absorbed as the unionized drug molecule since they are more lipid soluble in this state. Weakly acidic drugs, therefore, would be predominately unionized in the stomach because of the pH 1-3 of gastric fluid. Weakly basic drugs would have greater unionization at the pH (5-8) found in the small intestine. Although the stomach should be the favored area for absorption of weak acids, recent studies have shown these drugs to be well absorbed from the small intestine. For example, aspirin, although weakly acidic, has been reported to be absorbed more rapidly from the small intestine than from the stomach (15).

The gastrointestinal absorption of propantheline, a weak base, is increased when given with sodium bicarbonate (16). Presumably, this is due to an increased rate of gastric emptying which results in propantheline reaching the site of optimum absorption more rapidly in the small intestine.

The formation of complexes, ion pairs or chelates, may result in an increased or decreased absorption for a drug. The absorption of tetracycline is inhibited when it chelates with metals such as calcium or iron (17). On the other hand, when dicumarol chelates with magnesium hydroxide, a more soluble chelate is formed which enhances the oral absorption of dicumarol (18). Ion-pair formation of a quarternary ammonium antiarrhythmic agent with salicylate or trichloroacetate increases absorption of the agent (19). Adsorption of drugs onto kaolin or charcoal (20), or binding to ionic exchange resins (21), can result in a reduction of the gastrointestinal absorption of drugs.

Several drugs are absorbed by an active transport process across the small intestine. Competition at the active site between drugs and nutrients sharing the same transport mechanism may result in a decrease in drug

absorption. For example, their absorption could be blocked by competition at the active site with levodopa or phenylalanine (22). It has been suggested that chlorpromazine may inhibit enzymes involved in the active transport of levodopa (23).

Neomycin inhibits the formation of micelles to limit the solubility of lipids and decrease the absorption of cholesterol, bile acids, and vitamin A (24). A clinically significant interaction could result from a drug which exhibits direct effects on local gastrointestinal blood flow (25) since splanchnic blood flow could be a rate-limiting step in drug absorption (26).

Neomycin, p-aminosalicylic acid, and colchicine may impair the absorption of other drugs due to a toxic effect on intestinal mucosa (27). Drug absorption may also be affected by drugs which change the viscosity, volume, and/or composition of gastrointestinal secretions (28). The reduction of intestinal bacterial flora by some antibiotics can affect drugs that are normally metabolized by the gastrointestinal mucosa or bacterial flora. Danysz and Wisniewski (29) have reported that insulin alters the permeability of the gastrointestinal epithelium and increases the absorption of isoniazid.

The rate of gastric emptying, or the rate at which the stomach empties its contents into the small intestine, may have a significant effect on drug absorption from the gastrointestinal tract. This is especially true of drugs that are weak bases or are absorbed by an active transport process from the small intestine, since absorption from the stomach is insignificant. Some weak acid drugs, such as aspirin, warfarin, barbiturates, and ethanol are also absorbed more rapidly from the small intestine because of the larger surface area in the small intestine (30).

Food delays gastric emptying (31). Because of this, the intestinal absorption of many drugs can be delayed if administered on a full stomach.

Dilute solutions of a drug have been shown to be absorbed more quickly than concentrated solutions of the drug at the same dose due to a more rapid rate of gastric emptying (32).

Propantheline, an anticholinergic agent which delays gastric emptying, decreases the rate of absorption of acetaminophen (33). Acetaminophen is a weakly acidic drug that is largely unionized in gastric and intestinal fluids. Its rate of absorption is directly related to its gastric emptying rate. In contrast, metoclopramide, a drug which accelerates gastric emptying, increases the rate of absorption of acetaminophen (33).

Opposite effects are seen when propantheline and metoclopramide are administered with digoxin (34). This may be due to the slow dissolution of digoxin or its absorption from a limited area of the intestine. The slower rate of gastric emptying induced by propantheline could give digoxin sufficient time to dissolve in the intestine and allow it to remain in contact with its specific area of absorption for a longer period of time. Opposite effects would be expected with metoclopromide.

Antacids are a therapeutic class of drugs which have great potential for drug absorption interactions. Since they may be purchased over-the-counter, they are widely used by the public and may be taken concurrently with a great number of other drugs.

Different types of antacids may have differing effects on the rate of gastric emptying. Aluminum ions have been shown to delay gastric emptying, and a combination antacid containing aluminum and magnesium ions appears to influence gastric emptying less than an antacid containing aluminum hydroxide alone (35). Also, buffered acetylsalicylate test meals (pH 7.0) are emptied more rapidly from the stomach than test meals containing unbuffered (pH 2.8) acetylsalicylic acid (36). An aluminum-magnesium oral sus-

pension antacid (Maalox®) increases the rate of absorption of enteric-coated aspirin (Ecotrin®) when compared to the case where the aspirin product is administered alone (38). A possible explanation for this effect is that the antacid (Maalox® in this case) increases the rate of gastric emptying so that the enteric-coated aspirin can be dissolved sooner in the small intestine for faster absorption.

The proposed research will attempt to determine what effects, if any, various antacids have on gastric emptying rate. The antacids that will be examined are aluminum hydroxide, magnesium hydroxide, and a magnesium-aluminum hydroxide combination.

Table I lists some of the common proprietary over-the-counter antacids containing aluminum or magnesium hydroxide, or both (38).

In order to examine the effect various antacids have on gastric emptying rate, riboflavin will be utilized as the test drug. Riboflavin's suitability as a test drug for this study stems from the fact that riboflavin is absorbed by an apparent active transport process from the proximal portion of the small intestine (39, 40). Because riboflavin absorption occurs only from a certain portion of the small intestine and is dependent on some kind of a carrier enzyme system across the intestinal mucosa, riboflavin absorption is greatly affected by the rate of gastric emptying. The greater the rate of gastric emptying, the less will be the extent of riboflavin absorption and vice versa.

TABLE I. OVER-THE-COUNTER ANTACID PREPARATIONS CONTAINING MAGNESIUM OR ALUMINUM HYDROXIDE[a]

Product (Manufacturer)	Dosage Form	Antacid[b]
Alkets® (Upjohn)	Tablet	MH
Aludrox® (Wyeth)	Tablet, Suspension	AH,MH
Alurex® (Rexall)	Tablet, Suspension	AH,MH
Aluscop® (O'Neal, Jones & Feldman)	Capsule, Suspension	AH,MH
Amphojel® (Wyeth)	Tablet, Suspension	AH
A. M. T.® (Wyeth)	Tablet, Suspension	AH
Antacid Powder® (DeWitt)	Powder	AH
Banacid® (Buffington)	Tablet	AH,MH
Basaljel® (Wyeth)	Suspension, Capsule Tablet	AH
Basaljel Extra Strength® (Wyeth)	Suspension	AH
BiSoDol® (Whitehall)	Tablet, Powder	MH
Camalox® (Rorer)	Suspension, Tablet	AH,MH
Creamalin® (Winthrop)	Tablet	AH,MH
Delcid® (Merrell-National)	Suspension	AH,MH
Dialume® (Armour)	Tablet	AH,
Di-Gel® (Plough)	Tablet, Suspension	AH,MH
Estomul-M® (Riker)	Tablet, Suspension	AH
Flacid® (Amfre-Grant)	Tablet	AH,MH
Gelumina® (Amer. Pharm.)	Tablet	AH
Gelusil® (Warner-Chilcott)	Tablet, Suspension	AH
Gelusil Flavor-Pack® (Warner-Chilcott)	Suspension	AH
Gelusil-Lac® (Warner-Chilcott)	Powder	AH
Gelusil M® (Warner-Chilcott)	Tablet, Suspension	AH,MH
Glycogel® (Central Pharmacal)	Tablet	AH
Kessadrox® (McKesson)	Suspension	AH,MH
Kolantyl® (Merrell-National)	Gel, Tablet, Wafer	AH,MH

[a]Adapted from reference 38.
[b]AH = Aluminum Hydroxide, MH = Magnesium Hydroxide

Table I continued

Product (Manufacturer)	Dosage Form	Antacid[b]
Kudrox® (Kremers-Urban)	Tablet, Suspension	AH,MH
Liquid Antacid® (McKesson)	Suspension	AH,MH
Maalox® (Rorer)	Suspension	AH,MH
Maalox #1® (Rorer)	Tablet	AH,MH
Maalox #2® (Rorer)	Tablet	AH,MH
Maalox Plus® (Rorer)	Tablet, Suspension	AH,MH
Magna Gel® (North American)	Suspension	AH,MH
Magnatril® (Lannett)	Tablet, Suspension	AH,MH
Magnesia and Alumina Oral Suspension® (Philips Roxane)	Suspension	MH
Malcogel® (Upjohn)	Suspension	AH
Marblen® (Fleming)	Tablet, Suspension	AH
Maxamag Suspension® (Vitarine)	Suspension	AH,MH
Mylanta®	Tablet, Suspension	AH,MH
Mylanta - II® (Stuart)	Tablet, Suspension	AH,MH
Noralac® (North American)	Tablet	AH
Nutrajel® (Cenci)	Suspension	AH
Nutrameg® (Cenci)	Suspension	MH
Pama® (North American)	Tablet	AH
Phillips' Milk of Magnesia® (Glenbrook)	Tablet, Suspension	MH
Salcedrox® (Beecham Labs)	Tablet	AH
Silain-Gel® (Robins)	Tablet, Suspension	AH,MH
Syntrogel® (Block)	Tablet	AH,MH
Trimagel® (Columbia Medical)	Tablet	AH
Trisogel® (Lilly)	Capsule, Suspension	AH,MH
Win Gel® (Winthrop)	Tablet, Suspension	AH,MH

The use of urinary excretion data to assess the absorption aspects of drugs from the gastrointestinal tract has been well documented (41). The use of urinary excretion rates to study the pharmacokinetic and biopharmaceutic characteristics of a drug may be examined by observing the following scheme.

$$Xa \xrightarrow{k_a} X \xrightarrow{k_e} Xu$$

Scheme: 1

Where Xa represents the amount of drug at the absorption site in the gastrointestinal tract; k_a is the rate of absorption of drug across the gastrointestional membrane; X is the amount of drug in the body at a certain time, "t"; k_e is the rate of elimination of drug by urinary excretion from the body; and Xu represents the amount of drug in the urine.

First-order absorption and elimination by more than one route of elimination, such as by renal or biliary excretion, and drug metabolism can be illustrated by the following scheme:

$$Xa \xrightarrow{k_a} X \begin{cases} \xrightarrow{k_1} Y \\ \xrightarrow{k_e} Xu \end{cases}$$

Scheme: 2

Where k_1 is the rate of elimination of a drug by extrarenal processes, and Y is the amount of drug eliminated by extrarenal processes.

The rate constants that apply to these pharmacokinetic models are normally considered to be first-order.

The differential equation for the rate of drug absorption is shown in equation 1, and the integrated equation for the amount of drug absorbed from an absorption site is represented by equation 2. In the integrated equation, F represents the fraction of oral dose absorbed, and X_o is the oral dose of the drug.

$$dXa/dt = -kaXa \qquad \text{(Eq. 1)}$$

$$Xa = FX_o e^{-kat} \qquad \text{(Eq. 2)}$$

The differential and integrated equations for the amount of drug in the body according to Scheme 2 are presented in equations 3 and 4 respectively:

$$dX/dt = k_a X_a - KX \qquad \text{(Eq. 3)}$$

$$X = \frac{kaFX_o}{K} \quad (e^{-Kt} - e^{-k_a t}) \qquad \text{(Eq. 4)}$$

where $K = k_e + k_1$

Equation 5 represents the differential equation for the rate of change of unchanged drug in the urine, while equation 6 is the integrated equation for unchanged drug in the urine.

$$dX_u/dt = keX \qquad \text{(Eq. 5)}$$

$$Xu = \frac{kekaFXo}{K} \left[\frac{1}{k_a} + \frac{e^{-Kt}}{K-k_a} - \frac{Ke^{-kat}}{k_a(K-k_a)} \right] \qquad \text{(Eq. 6)}$$

In the theory of a one-compartment model of drug distribution with first-order kinetics of drug absorption and elimination, the rates of absorption and elimination are directly proportional to drug concentration at the site of each process. Urinary excretion rate data can be used to evaluate the rate of drug absorption as well as its bioavailability characteristics. This can be seen by examination of equation 5, where it is shown that urinary excretion rate is directly proportional to the amount of drug in the body. In turn, the amount of drug in the body is directly proportional to the amount of drug being absorbed, as indicated in equation 4. In essence, urinary excretion rate data reflects body levels of a drug and, therefore, absorption and elimination characteristics of the drug.

Evidence exists that riboflavin is absorbed by an active transport process across the gastrointestinal tract (39, 40) and is primarily excreted in the urine (42). Therefore, at doses below concentrations at which saturation of its active transport process occurs, riboflavin will exhibit

the properties of first-order absorption and elimination indicated in the overall discussion given above. At higher doses, riboflavin concentration will be above the saturation point for its active transport process. This means that riboflavin absorption will proceed by a zero-order process independent of riboflavin amount at the absorption site. Because the urinary excretion rate of riboflavin is directly proportional to the amount of riboflavin in the body, which, in turn, is directly proportional to the amount of riboflavin being absorbed at its active transport site, riboflavin is a suitable drug to use for determining antacid effects on gastrointestinal motility.

This case of zero-order absorption and first-order urinary excretion of a drug may be represented by Scheme 3:

$$X_a \xrightarrow{k_o} X \xrightarrow{k_e} X_u$$

Scheme 3

where k_o represents the zero-order rate constant for absorption.

The differential and integrated equations for Scheme 3 and drug absorption are shown in equations 7 and 8. In equation 8, Xo represents the oral dose of the drug.

$$dX_a/dt = -k_o \qquad \text{(Eq. 7)}$$

$$X_a = X_o - k_o t \qquad \text{(Eq. 8)}$$

The differential and integrated equations for drug in the body following zero-order absorption are indicated in equations 9 and 10.

$$dX/dt = k_o - k_e X \qquad \text{(Eq. 9)}$$

$$X = \frac{k_o}{k_e} (1 - e^{-ket}) \qquad \text{(Eq. 10)}$$

The first-order urinary excretion rate equation is the same as equation 5. Equation 11 shows the integrated equation for the amount of drug in the urine following zero-order absorption.

$$X_u = k_o t - \frac{k_o}{k_e} (1 - e^{-k_e t}) \qquad \text{(Eq. 11)}$$

METHODOLOGY

Both *in vivo* and *in vitro* studies were planned to examine the possible interaction between antacids and riboflavin.

Stability. To determine what effects, if any, storage of urine samples in a refrigerator or freezer over a period of time would have on riboflavin concentration in the storage containers, the following *in vitro* experiment was devised. Two one liter solutions were prepared, one solution containing approximately 0.25 mcg./ml. of riboflavin, the other solution being a control with distilled water. Each solution contained 30 ml. of glacial acetic acid. One hundred milliliters of each solution were placed in ten plastic urine bags. One plastic bag sample of each solution was assayed for riboflavin following preparation of the solutions. For the remainder of the plastic bag samples, half were placed in a freezer at -35° C. and the other half in a refrigerator at 7° C. The times for assaying the other samples were 24 hours, 72 hours, one week, and two weeks following preparation of the solutions. Three samples from each plastic bag were assayed for riboflavin content.

In Vivo Studies

Subjects. Five healthy male subjects, ranging in age from 22 to 30 years, and in weight from 145 to 178 lbs., volunteered for the study. None of the subjects was taking vitamin preparations for at least one month prior to the start of the study. The study was conducted over a period of four consecutive weeks in a random cross-over manner with each subject receiving riboflavin control, riboflavin with aluminum-magnesium hydroxide oral

suspension[1], riboflavin with aluminum hydroxide gel[2], or riboflavin with magnesium hydroxide oral suspension[3].

Riboflavin Absorption Study. Each subject collecting riboflavin control urine samples observed the following procedure: A two to four hour blank urine sample was collected the day before taking the riboflavin. The subject fasted overnight. One hour and one-half hour before administration of the drug, 50 ml. of water was ingested. At the time of riboflavin administration, the bladder was voided. Thirty milligrams of riboflavin was suspended in water and ingested with water in one or more rinsings for a total volume of 100 ml. Total urine samples were collected in plastic bags[4] at 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10, 12, and 16 hours after administration, and at convenient intervals up to 36 hours after taking the drug. Food was not ingested for four hours after taking the riboflavin. Fifty milliliters of water was ingested after each collected urine sample for as long as necessary in order to maintain adequate urinary output. Three milliliters of glacial acetic acid were added per 100 ml. of collected urine for purposes of protecting the riboflavin from decomposition. The urine samples were stored in a freezer (-35°C.) or refrigerator (7°C.) until assayed.

Antacid-Riboflavin Absorption Study. For the riboflavin with aluminum-magnesium hydroxide or aluminum hydroxide urine samples, the blank urine sample procedure was repeated by each subject as for the riboflavin control urine samples. One hour before taking the riboflavin with aluminum-

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[1]Maalox,® W. H. Rorer Co.
[2]Amphojel®, Wyeth Laboratories
[3]Milk of Magnesia®, C. H. Philips Co.
[4]Whirl-Pak®, Nasco

magnesium hydroxide or aluminum hydroxide, 50 ml. of water was ingested. One half hour before taking the riboflavin with the antacid, 20 ml. of the antacid along with 30 ml. of water was ingested. At the time of riboflavin ingestion, 20 ml. of antacid followed by 30 mg. of riboflavin in a total of 80 ml. of water was ingested. The collection procedure was identical to that followed for the riboflavin control samples.

During the riboflavin co-administered with magnesium hydroxide portion of the study, the same procedure for collecting urine samples of riboflavin with aluminum-magnesium hydroxide or aluminum hydroxide was followed except that 10 ml. of magnesium hydroxide was taken one half hour before and at the time of riboflavin ingestion. The lower dose of magnesium hydroxide was necessary in order to minimize the possibility of a laxative effect from magnesium hydroxide. At least one week separated each of the riboflavin absorption studies.

Assay. The urine samples were assayed using a modified USP assay for riboflavin (39, 43). One ml. of 2 N acetate buffer (pH 4.8) was placed in each test tube, and five ml. of a diluted urine sample was added. One ml. of 4% w/v potassium permanganate solution was placed in each test tube and mixed. Two minutes later, 1 ml. of 3% v/v hydrogen peroxide solution was added and the solution shaken on a vortex mixer until the solution cleared. Approximately 4 ml. were transferred to cuvettes and fluorometric readings were obtained with a filter fluorometer[5] at a sensitivity of 1, using primary filter 47B and secondary filter 2A-12. A few mg. of sodium hydrosulfite were then added to each cuvette containing the samples to selectively reduce the riboflavin for purposes of obtaining a true riboflavin

[5]Model 111, G.K. Turner Associates, Inc.

fluorometer value. This reading was subtracted from the initial reading.

Using the above assay procedure, a Beer's Law plot was obtained using riboflavin concentrations from 0.1 to 0.5 mcg./ml.

In Vitro Studies

Procedure. Fluorometric and pH readings were recorded for twelve different combinations of riboflavin, antacids, and water or hydrochloric acid. The various studies performed are listed in Table II.

The solutions, with the exception of those from studies four, eight, ten, and twelve, were shaken in a shaker bath for 30 minutes. Afterwards, the solutions were centrifuged for 10 minutes at 1500 RPM.[6] One ml. of the supernatant was diluted to a volume of 25 ml. for fluorometric reading and assayed for riboflavin content. The pH of the supernatant solution was also recorded on a digital pH meter.[7]

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[6] IEC, Model HN-S centrifuge

[7] Beckman pH Meter, Model 4500

TABLE II. COMBINATION OF ANTACID AND RIBOFLAVIN MIXTURES USED IN THE IN VITRO EVALUATIONS.[a]

Study No.	Riboflavin[b]	Antacid[c]	Water
1	15	15	0
2	15	5	10
3	0	15	15
4	15	0	15
			0.01 N HCl
5	15	15	0
6	15	5	10
7	0	15	15
8	15	0	15
			Water
9	5	15	10
10	5	0	25
			0.01 N HCl
11	5	15	10
12	5	0	25

[a] Quantities indicated are in milliliters
[b] Ten micrograms per milliliter
[c] Aluminum-Magnesium Hydroxide (Maalox®), Aluminum Hydroxide (Amphojel®), or Magnesium Hydroxide (Milk of Magnesia®)

RESULTS

In Vivo. The Beer's Law data are listed in Table III, and the Beer's Law plot is shown in Figure 1. The fluorometric readings are directly proportional to the concentrations of the standard solutions, and the Beer's Law plot exhibits the typical linear relationship between fluorescence and concentration.

Calculation of riboflavin amount in each urine sample was made by dividing the corrected fluorometer reading of each sample (initial minus quenched reading) by the slope of the Beer's Law plot and multiplying by the volume of the urine sample (volumes less than 100 ml. were made up to 100 ml.) and the dilution factor.

The rates of urinary excretion of riboflavin for each sample were calculated by dividing the riboflavin amount for a sample by the time interval (hr.) during which the sample was collected. To obtain a true rate of excretion for the riboflavin ingested in each study, the urinary excretion rate per unit time for riboflavin in the blank urine sample for each study was subtracted from each sample rate per time period in each study.

The composite urinary excretion rate versus time plots for the antacid treatment for each subject compared to control studies are shown in Figures 2-16. The plots show peak rates within 0.75-2.75 hours after administration of riboflavin with or without the antacids. The urinary excretion rates declined, with some secondary peaks, from the time of peak excretion rate until about 10-20 hours after administration of riboflavin. The rates of decline for urinary excretion rate varied among the subjects, as reflected in the figures.

The composite cumulative amounts of riboflavin excreted versus time (mid-point) for each subject are shown in Figures 17-21. After initial sharp

TABLE III. FLUORESCENCE READINGS AS A FUNCTION OF RIBOFLAVIN CONCENTRATION

Riboflavin Concentration (mcg./ml.)	Fluorometric Reading[a]
0.1	16
0.2	32
0.3	48.5
0.5	82.5

[a]Initial minus quenched reading

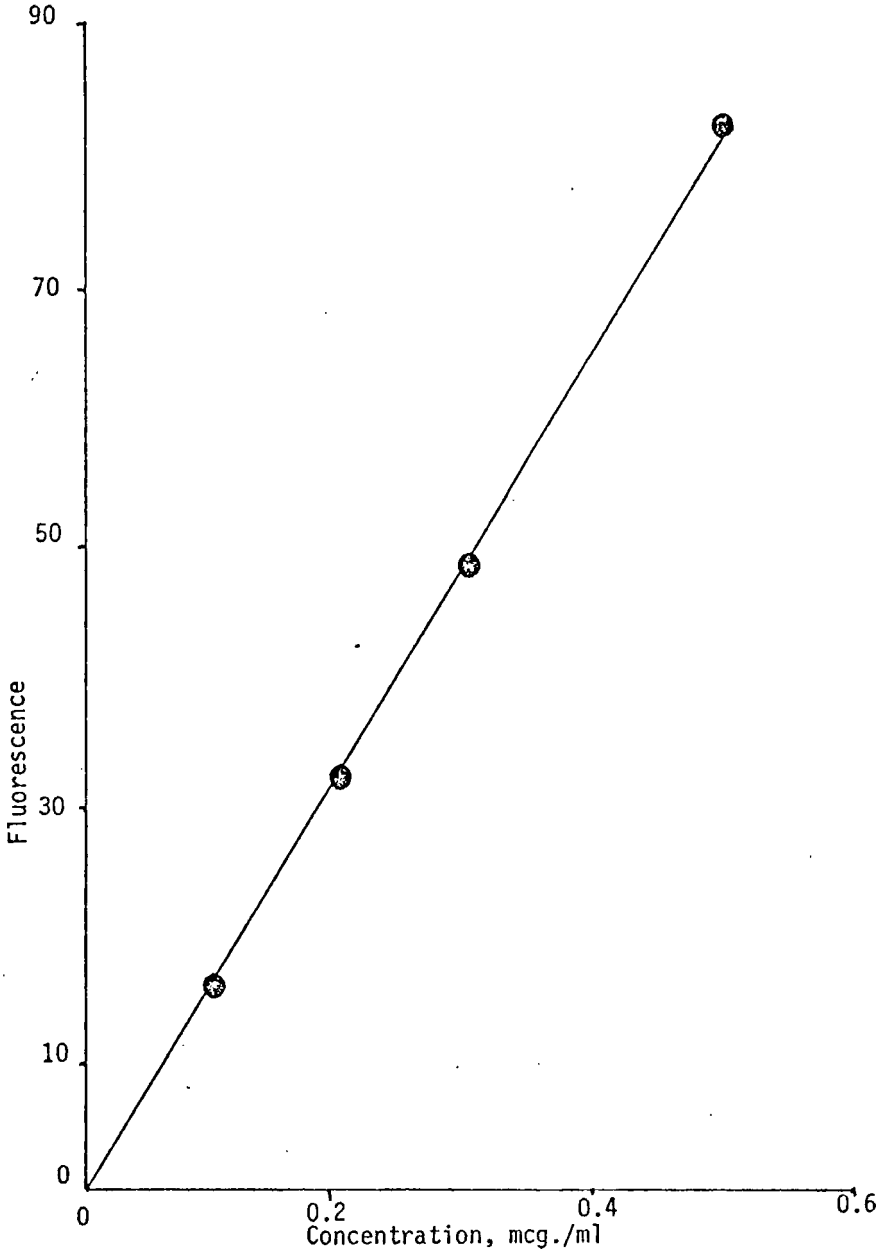

Figure 1. Fluorescence readings as a function of riboflavin concentration.

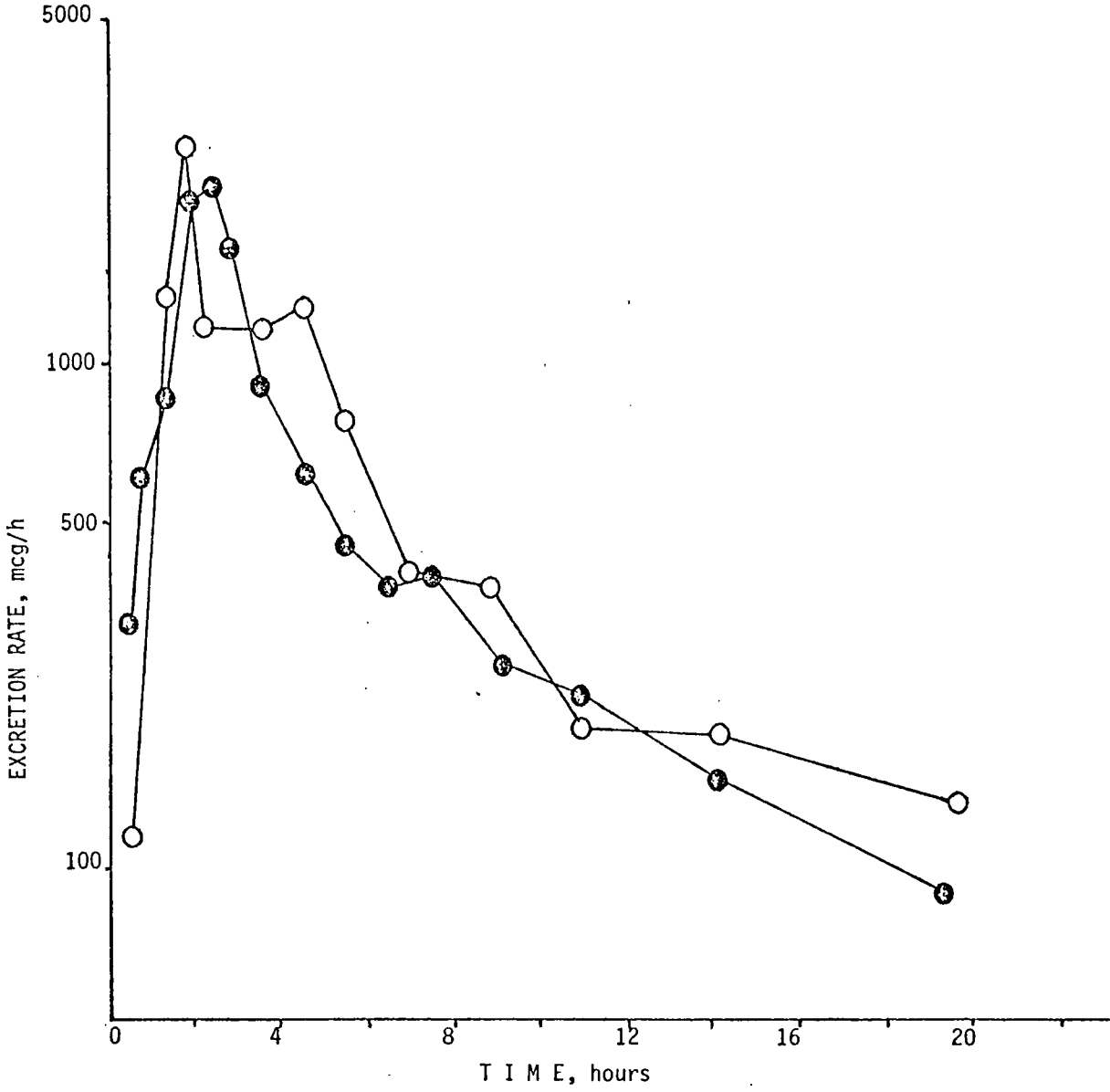

Figure 2. Urinary excretion rate of riboflavin as a function of time for control (●) and aluminum-magnesium hydroxide (○) studies in subject A.

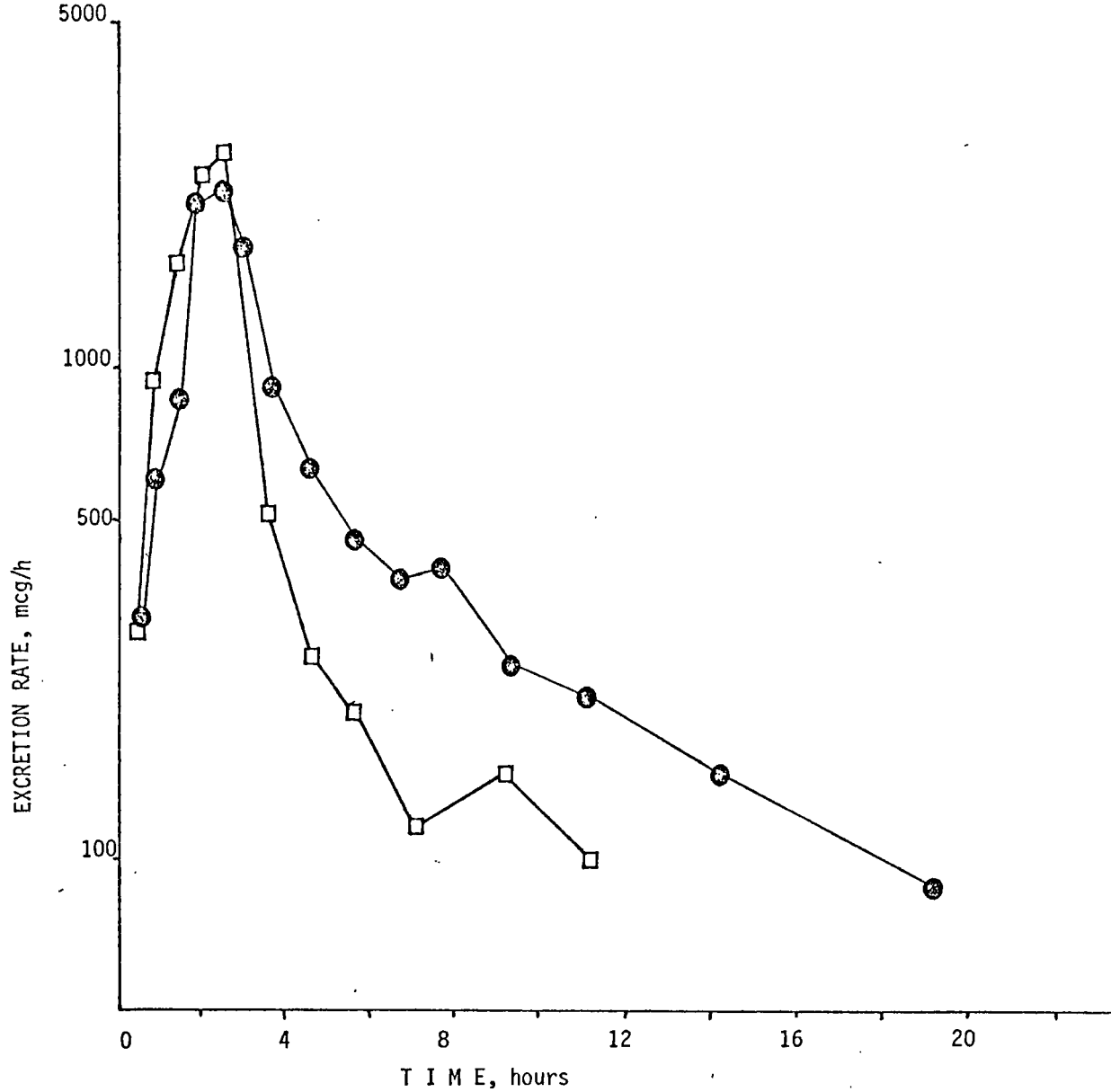

Figure 3. Urinary excretion rate of riboflavin as a function of time for control (⊛) and aluminum hydroxide (□) studies in subject A.

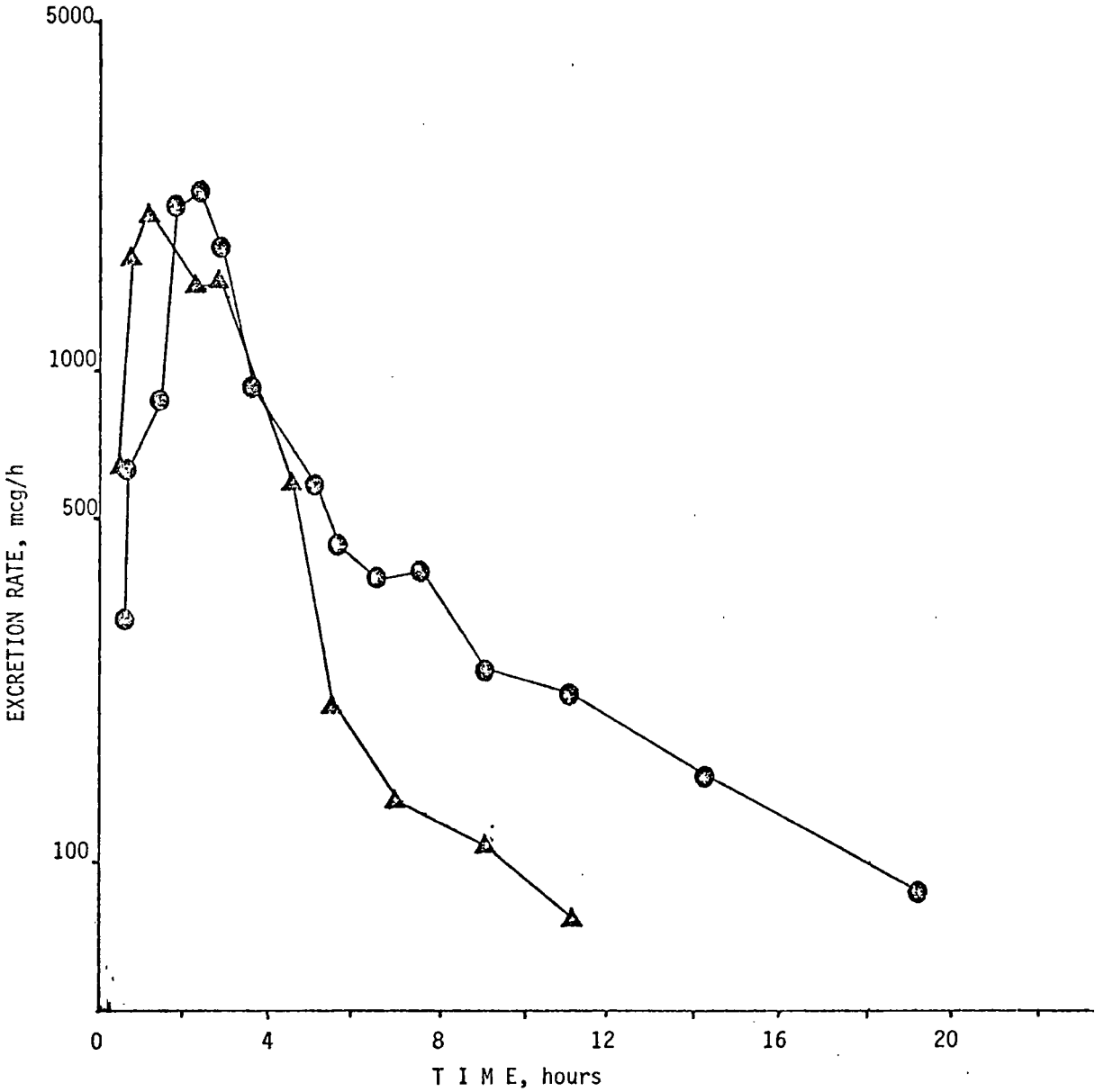

Figure 4. Urinary excretion rate of riboflavin as a function of time for control (●) and magnesium hydroxide (▲) studies in subject A.

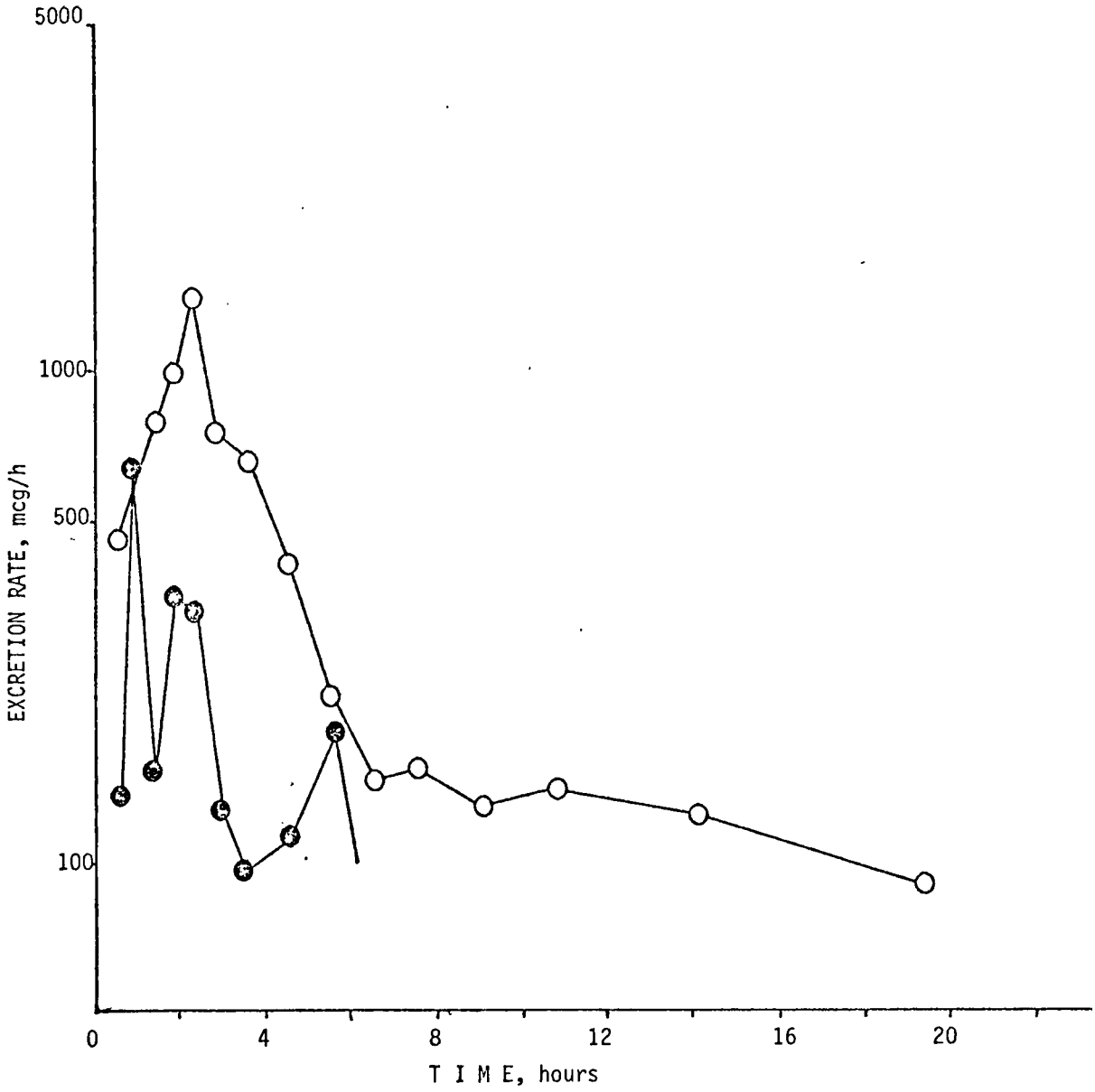

Figure 5. Urinary excretion rate of riboflavin as a function of time for control (●) and aluminum-magnesium hydroxide (○) studies in subject B.

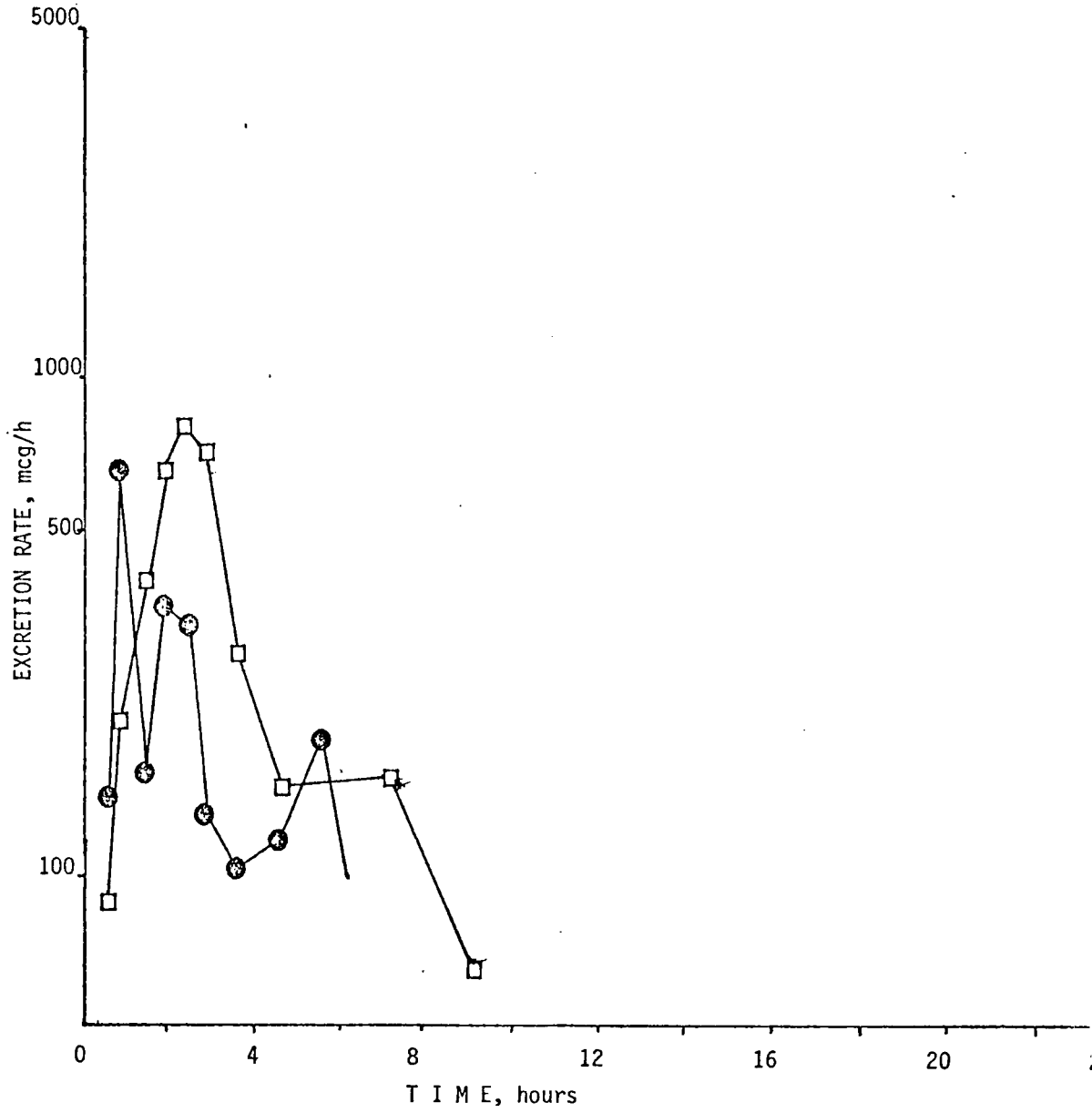

Figure 6. Urinary excretion rate of riboflavin as a function of time for control (●) and aluminum hydroxide (□) studies in subject B.

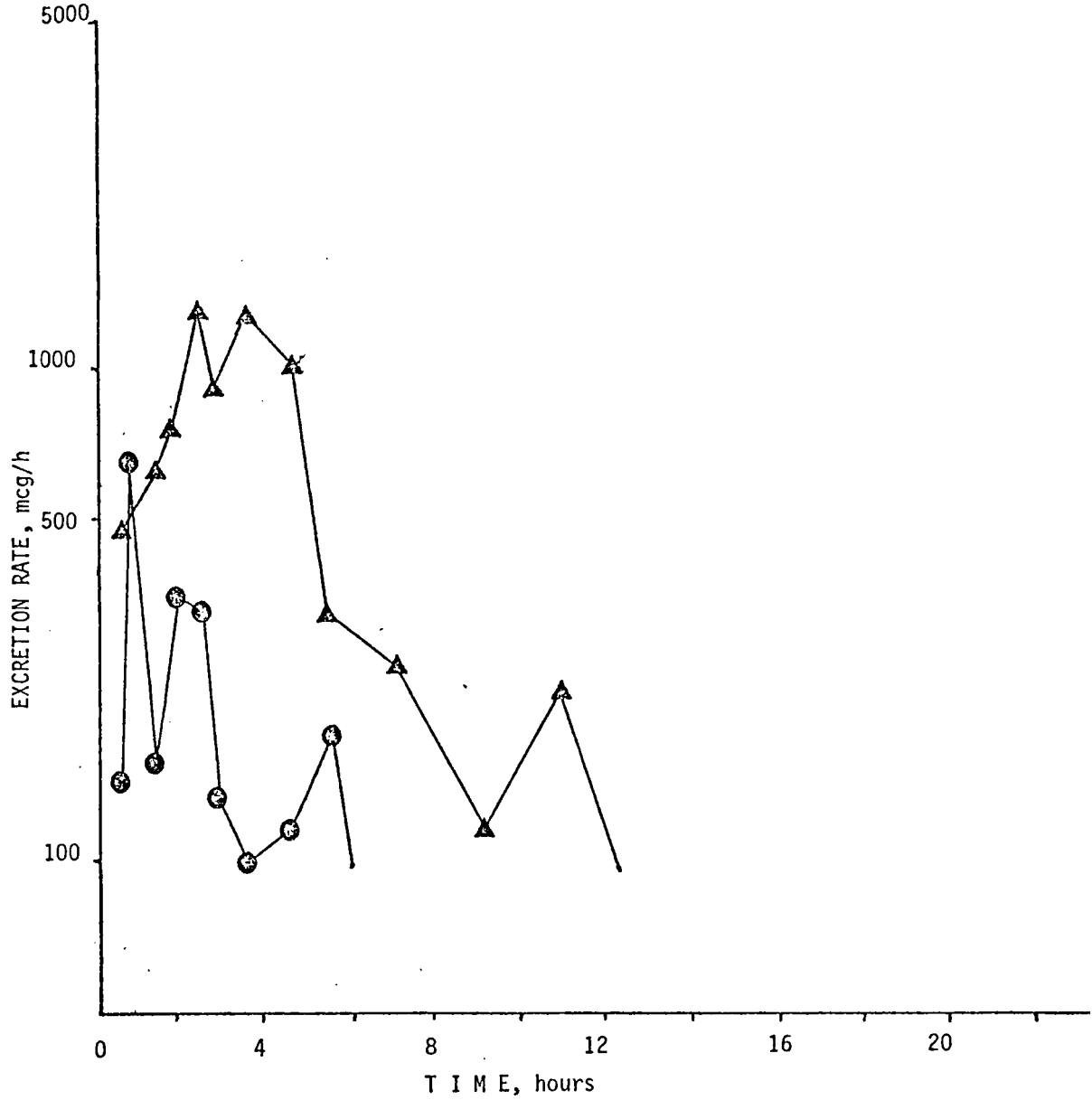

Figure 7. Urinary excretion rate of riboflavin as a function of time for control (○) and magnesium hydroxide (▲) studies in subject B.

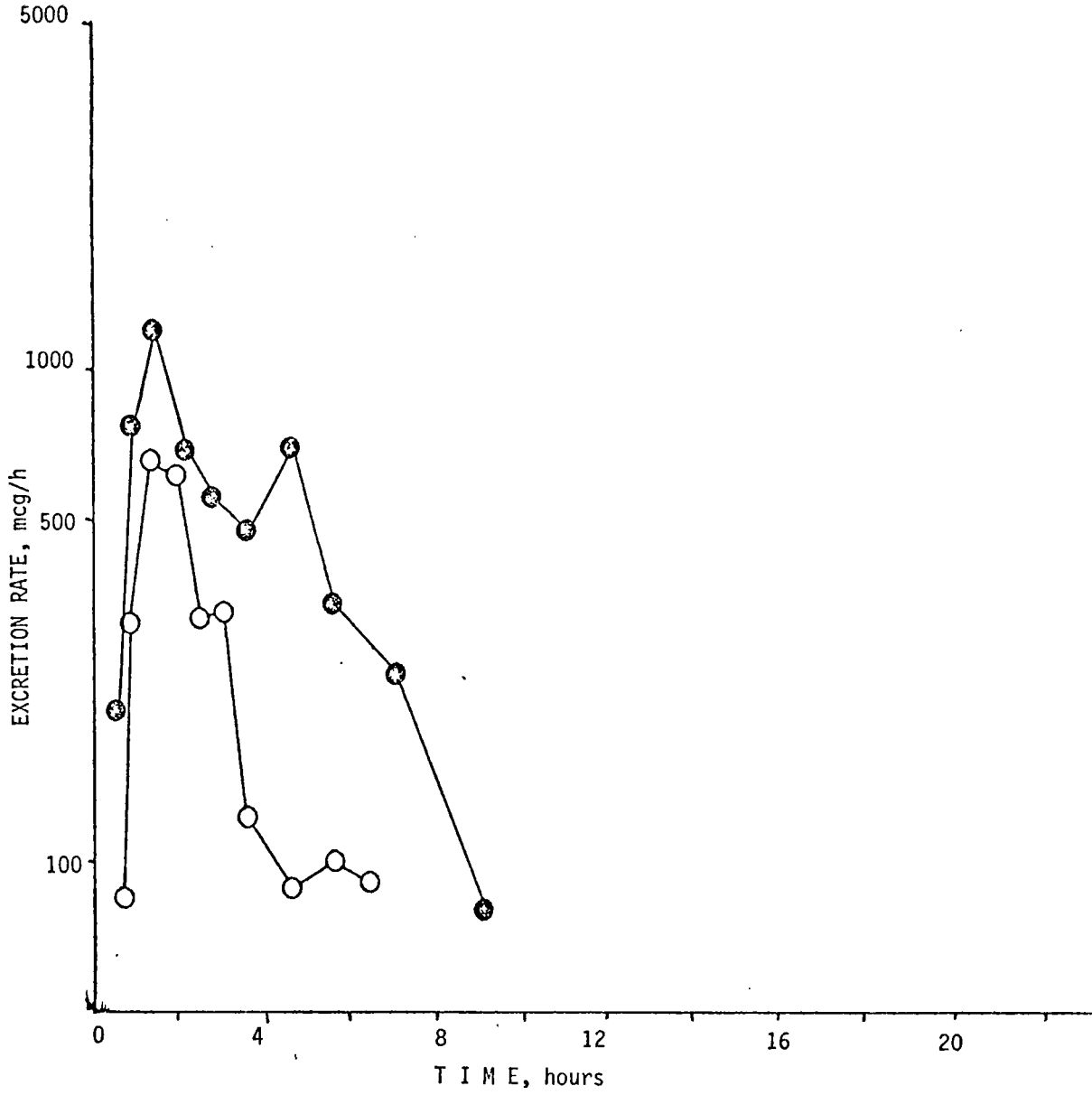

Figure 8. Urinary excretion rate of riboflavin as a function of time for control (●) and aluminum-magnesium hydroxide (○) studies in subject C.

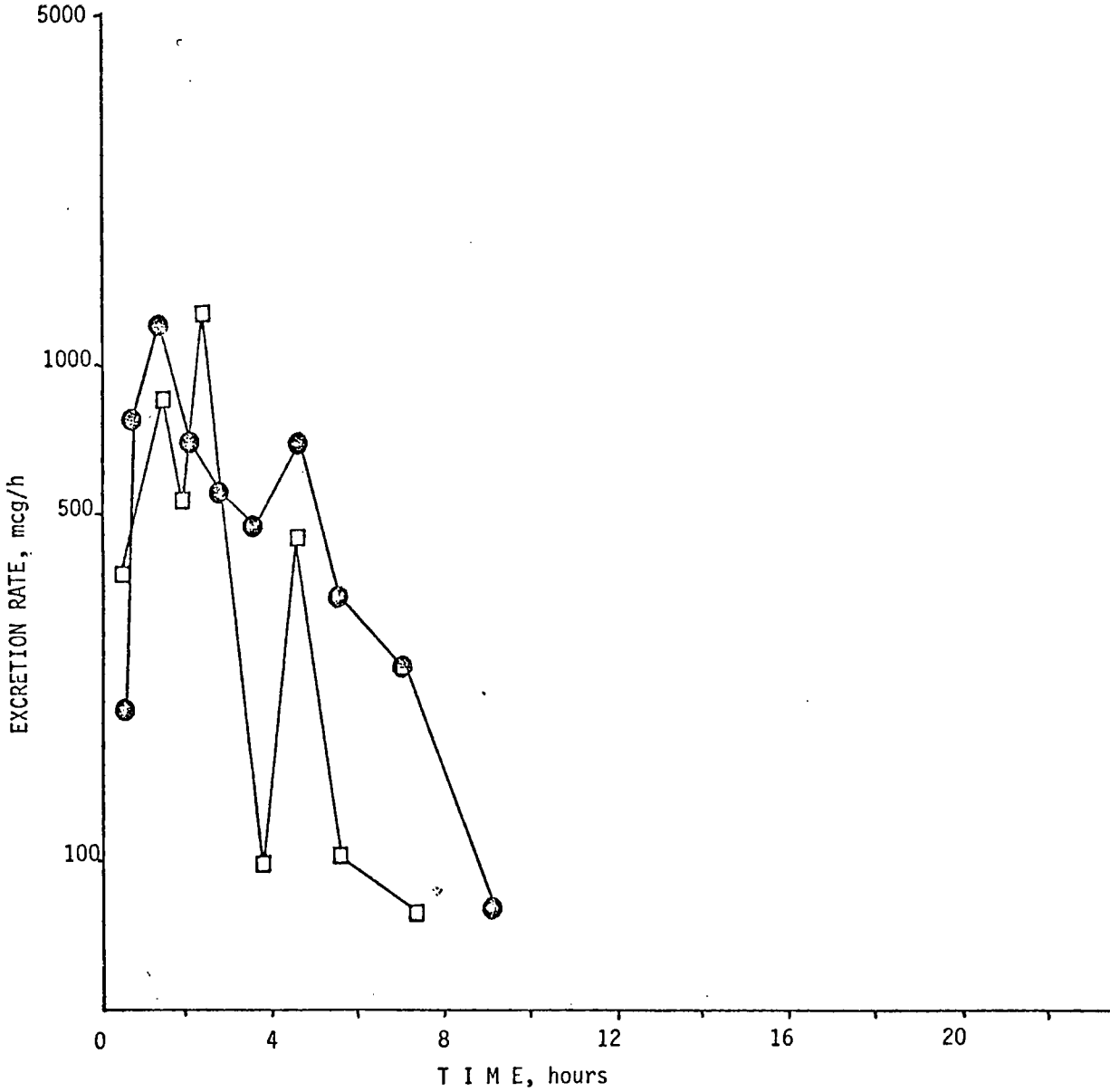

Figure 9. Urinary excretion rate of riboflavin as a function of time for control (●) and aluminum hydroxide (□) studies in subject C.

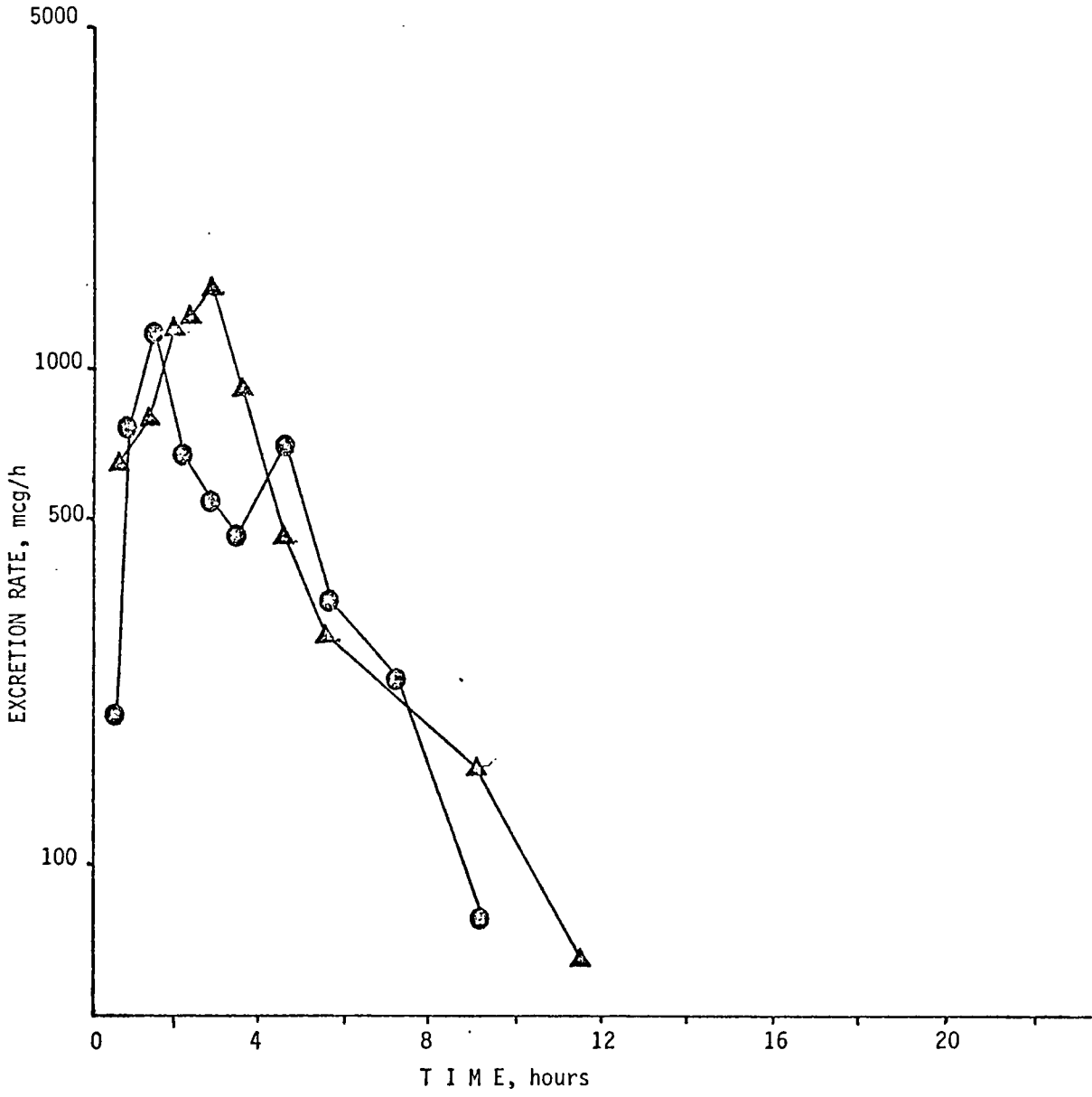

Figure 10. Urinary excretion rate of riboflavin as a function of time for control (●) and magnesium hydroxide (▲) studies in subject C.

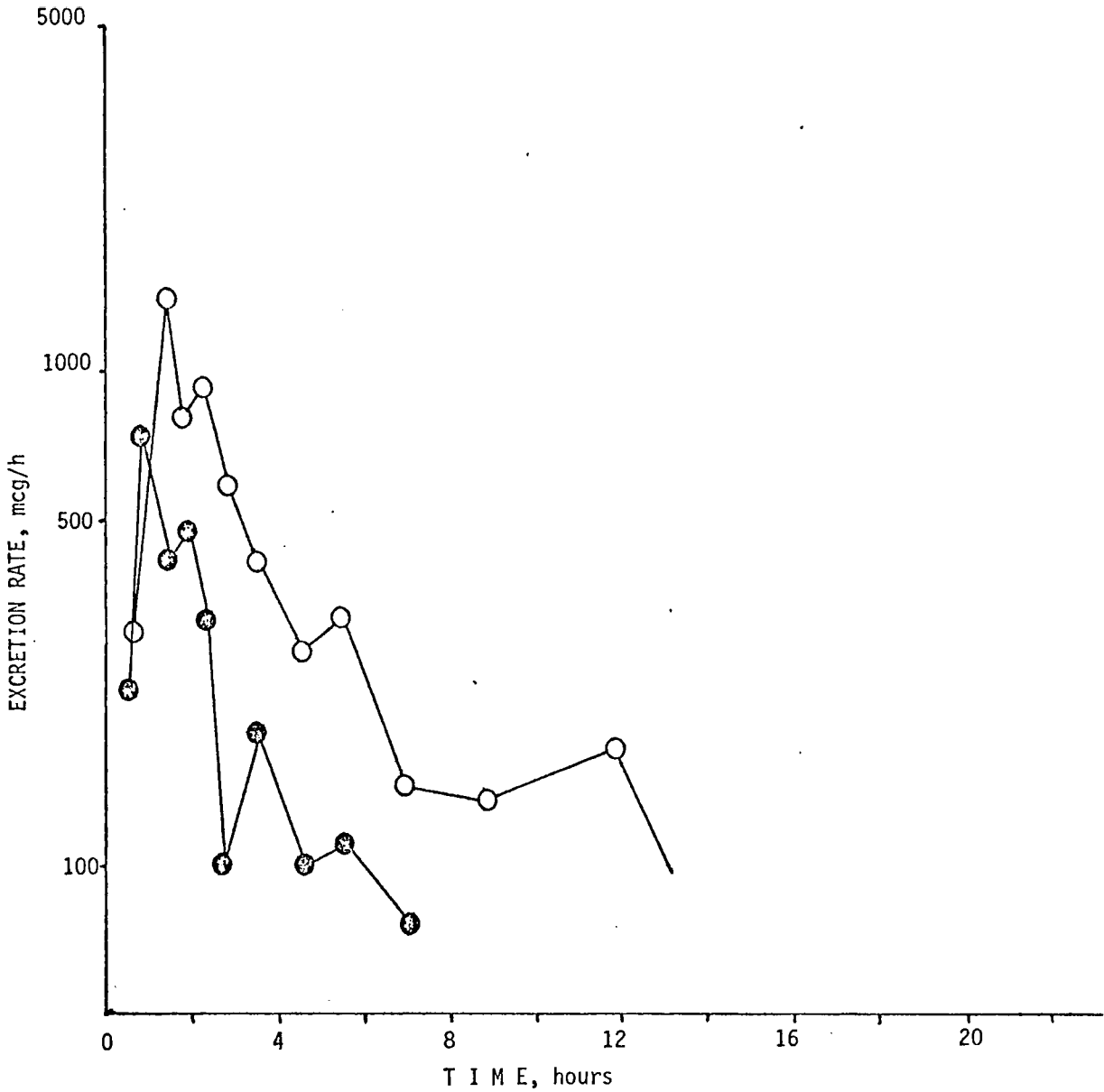

Figure 11. Urinary excretion rate of riboflavin as a function of time for control (●) and aluminum-magnesium hydroxide (○) studies in subject D.

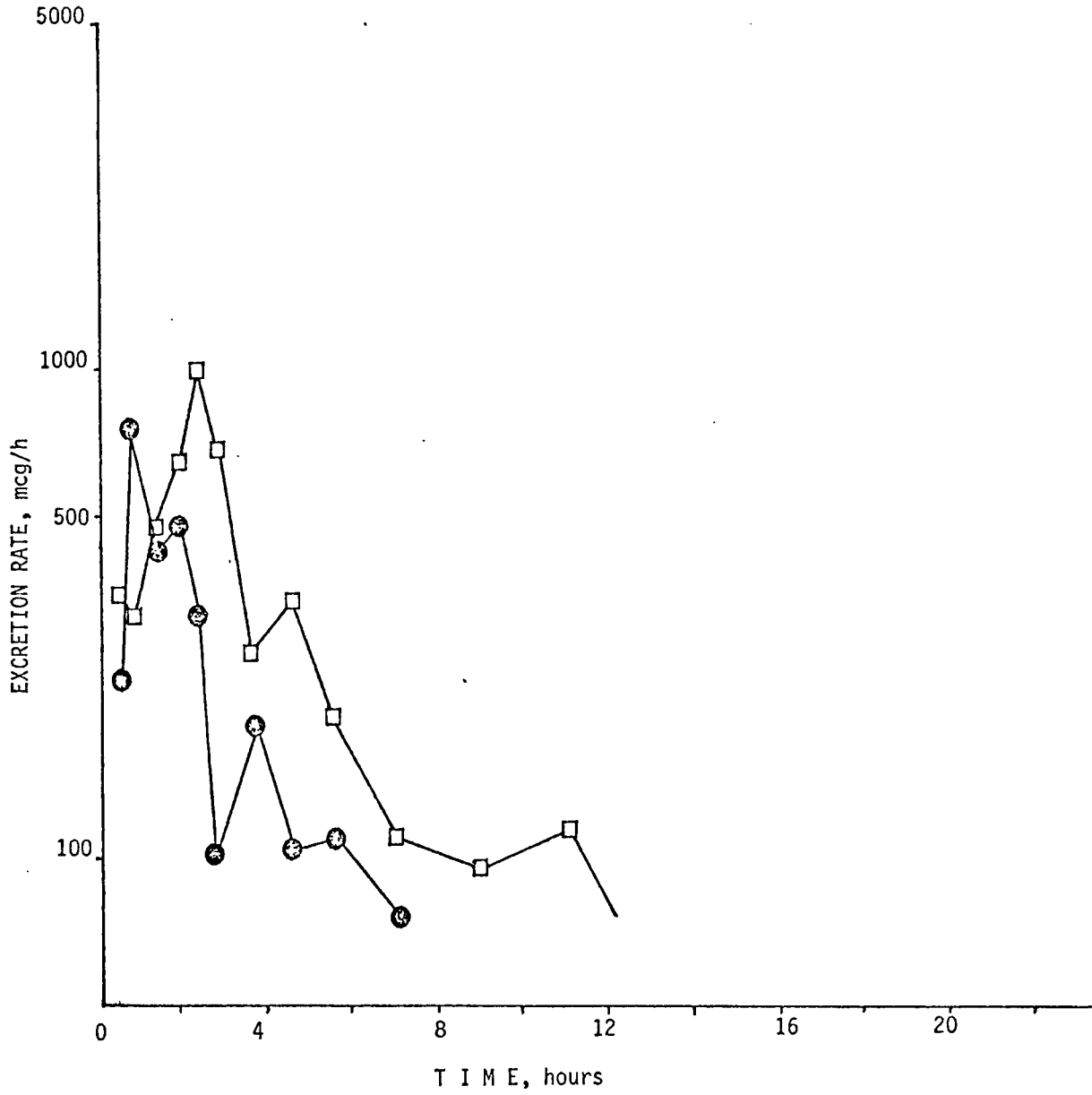

Figure 12. Urinary excretion rate of riboflavin as a function of time for control (✪) and aluminum hydroxide (□) studies in subject D.

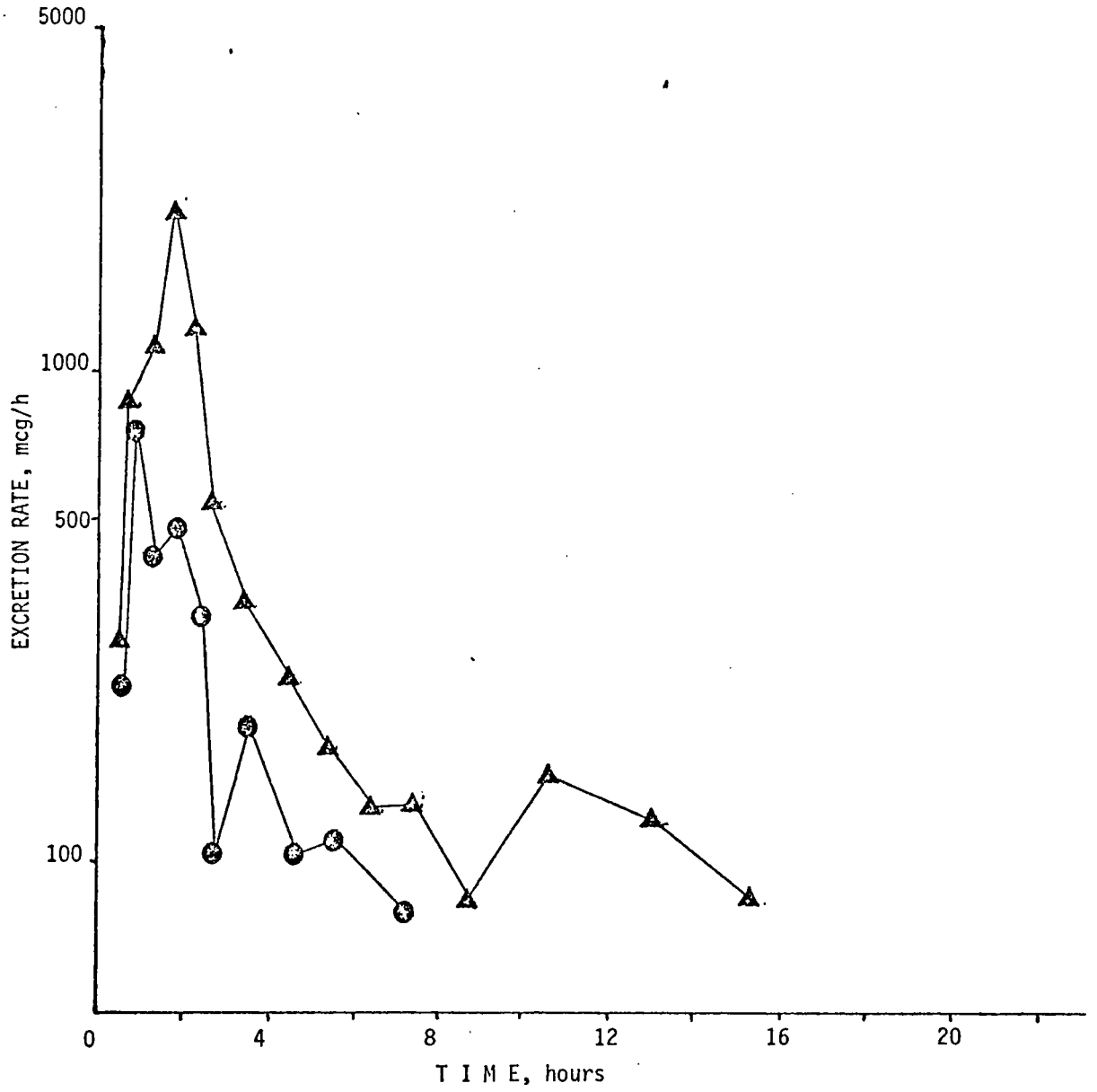

Figure 13. Urinary excretion rate of riboflavin as a function of time for control (●) and magnesium hydroxide (▲) studies in subject D.

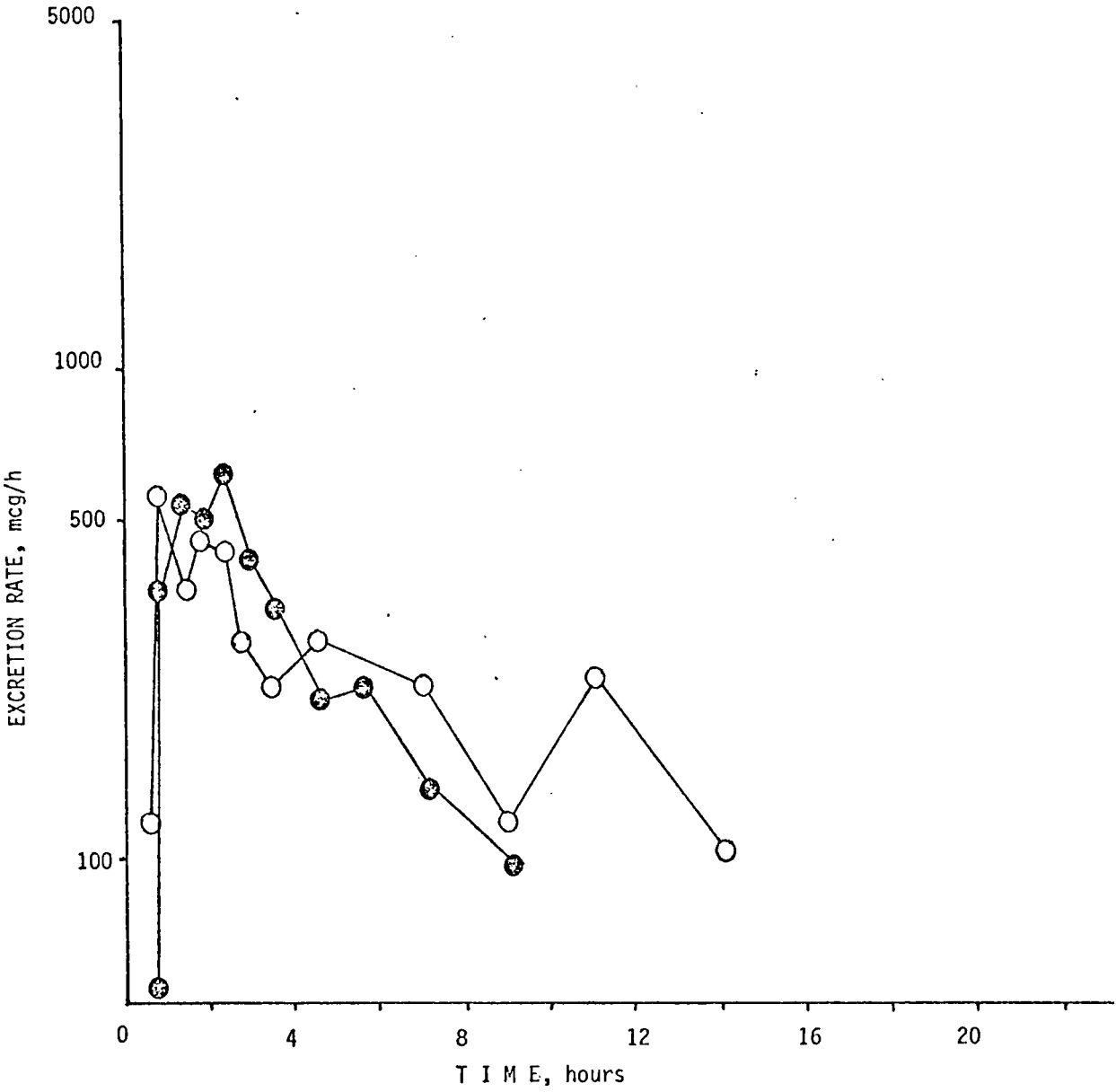

Figure 14. Urinary excretion rate of riboflavin as a function of time for control (●) and aluminum-magnesium hydroxide (○) studies in subject E.

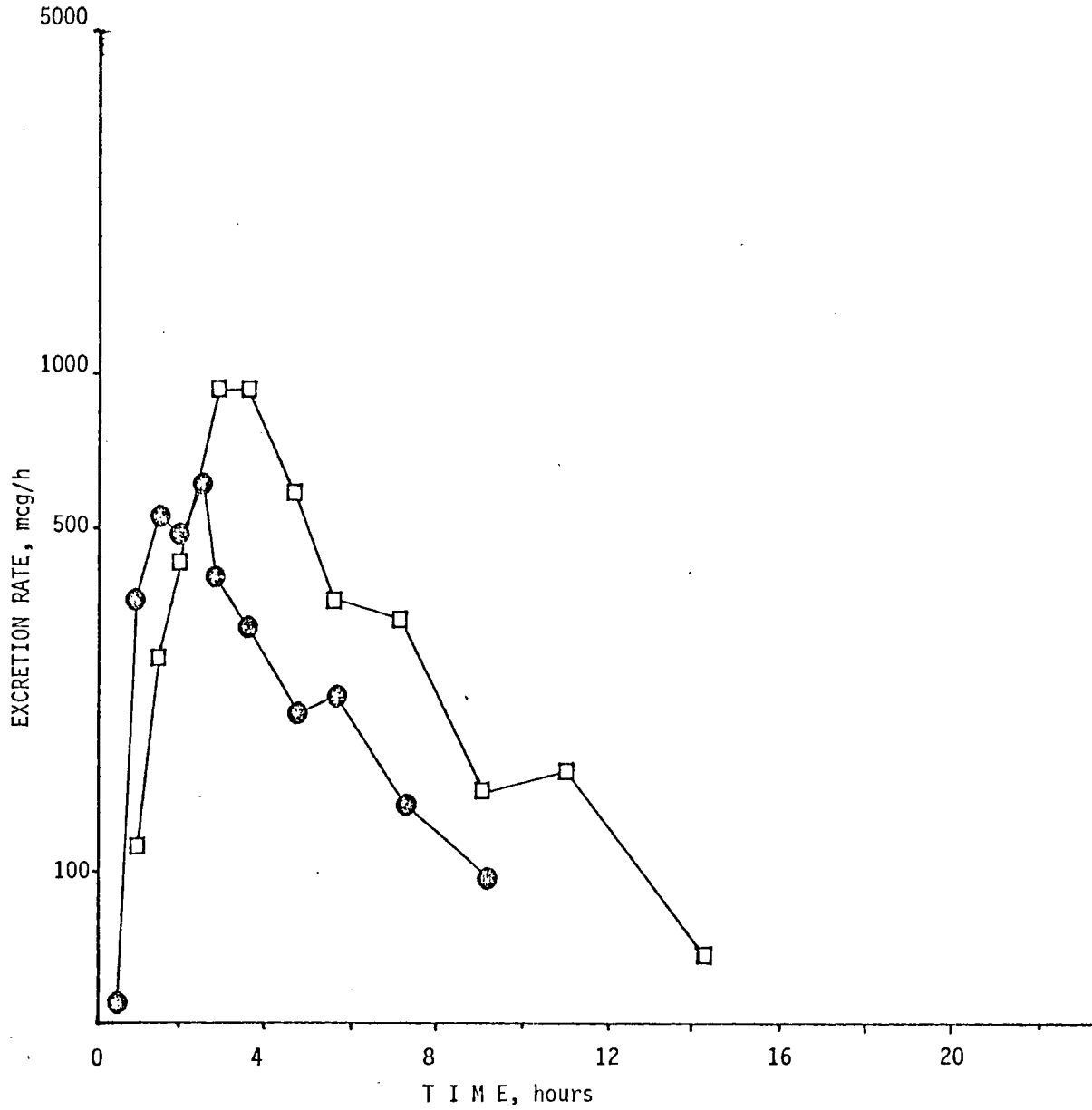

Figure 15. Urinary excretion rate of riboflavin as a function of time for control (✪) and aluminum hydroxide (□) studies in subject E.

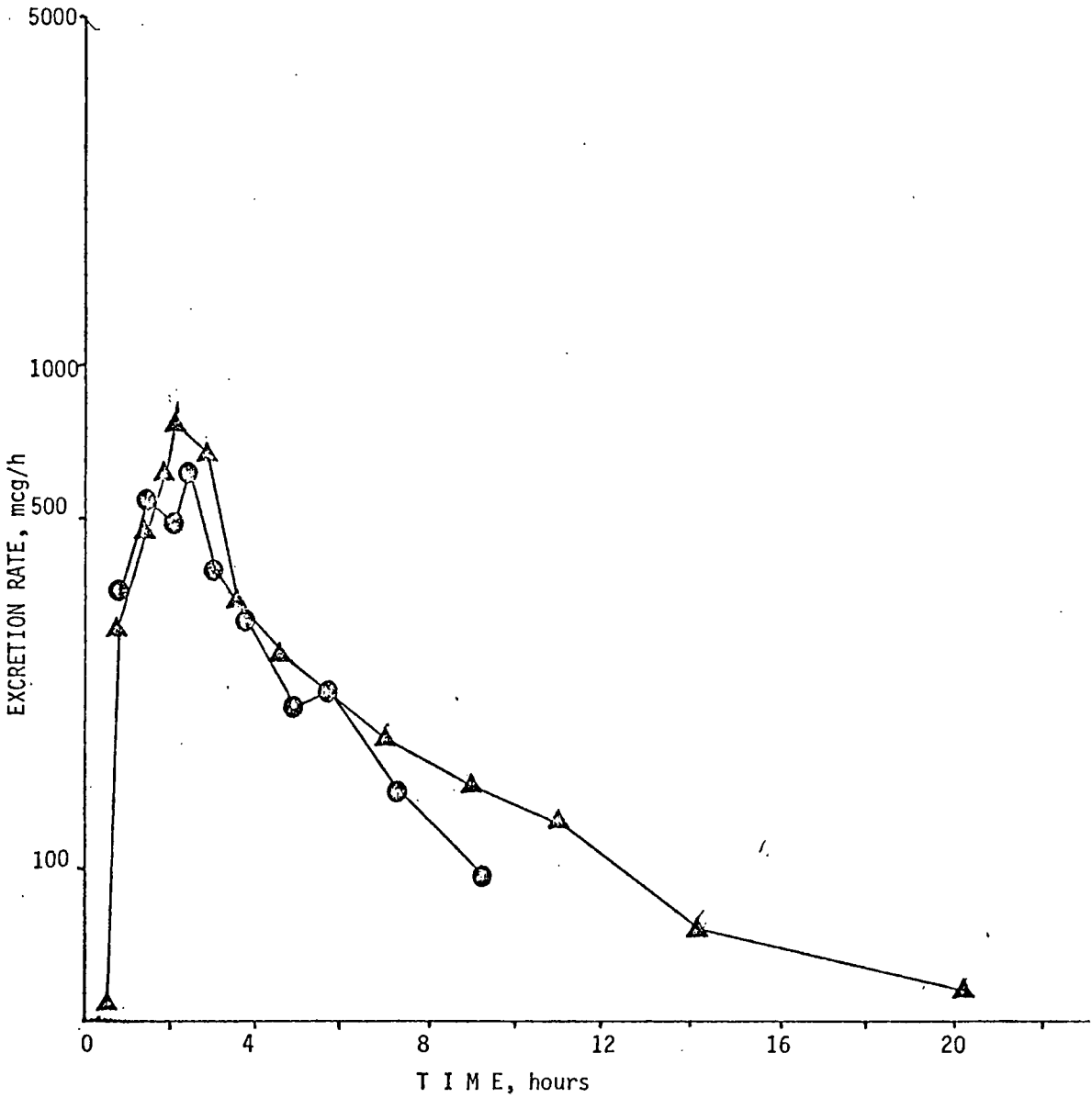

Figure 16. Urinary excretion rate of riboflavin as a function of time for control (⊙) and magnesium hydroxide (▲) studies in subject E.

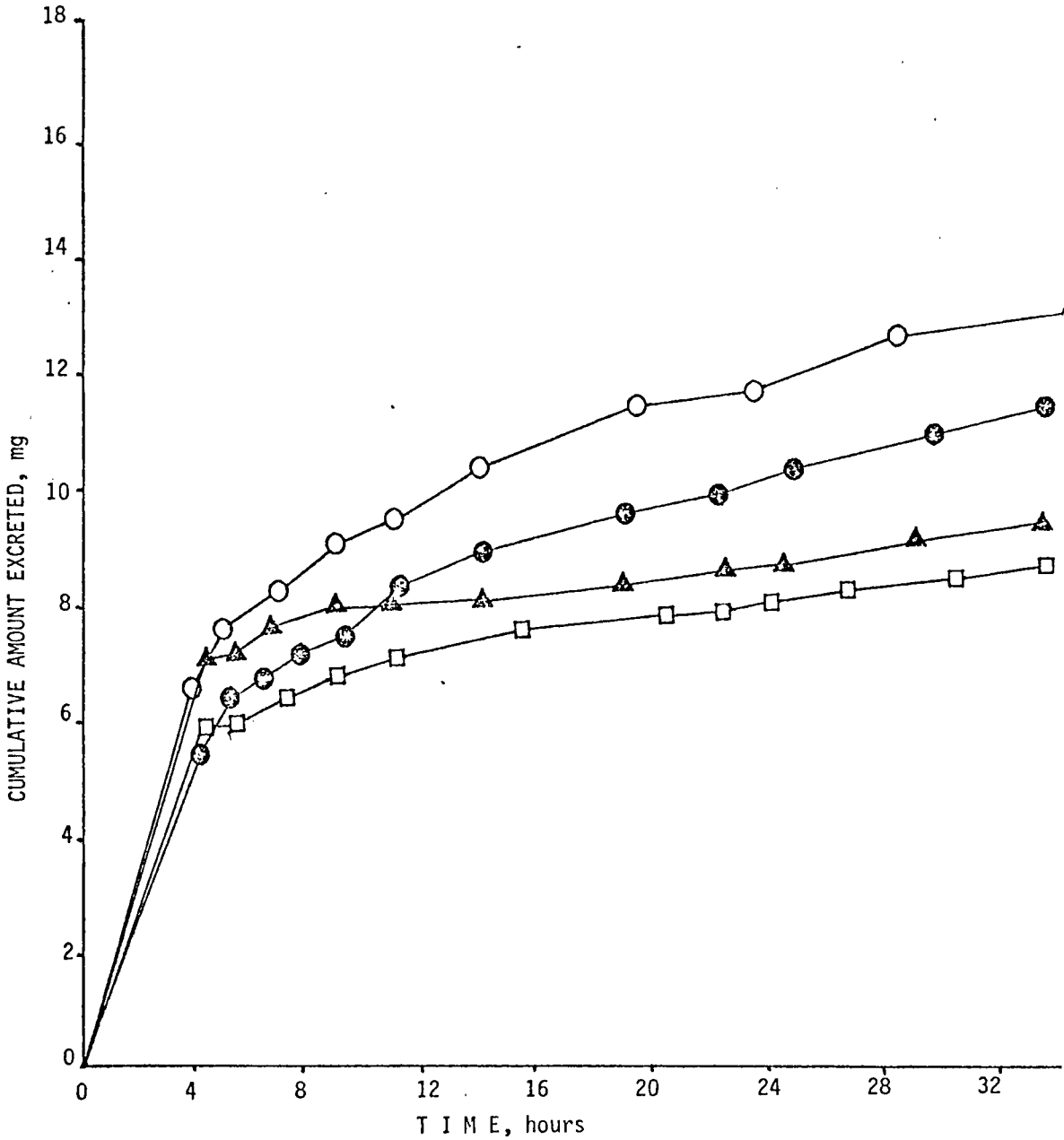

Figure 17. Cumulative amount of riboflavin excreted as a function of time for control (⊙), aluminum-magnesium hydroxide (○), aluminum hydroxide (□), and magnesium hydroxide (△) studies in subject A.

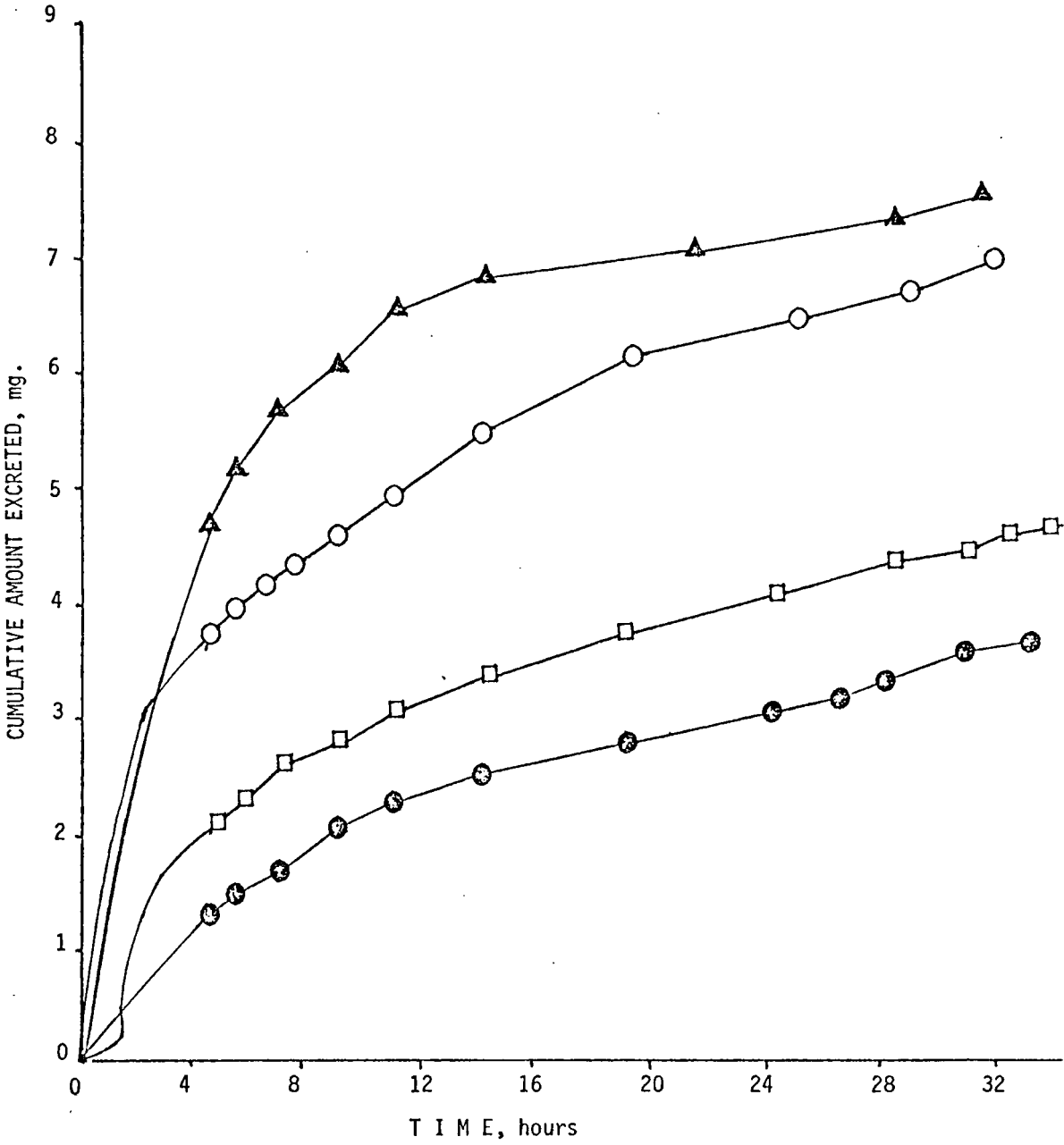

Figure 18. Cumulative amount of riboflavin excreted as a function of time for control (○), aluminum-magnesium hydroxide (○), aluminum hydroxide (□), and magnesium hydroxide (△) studies in subject B.

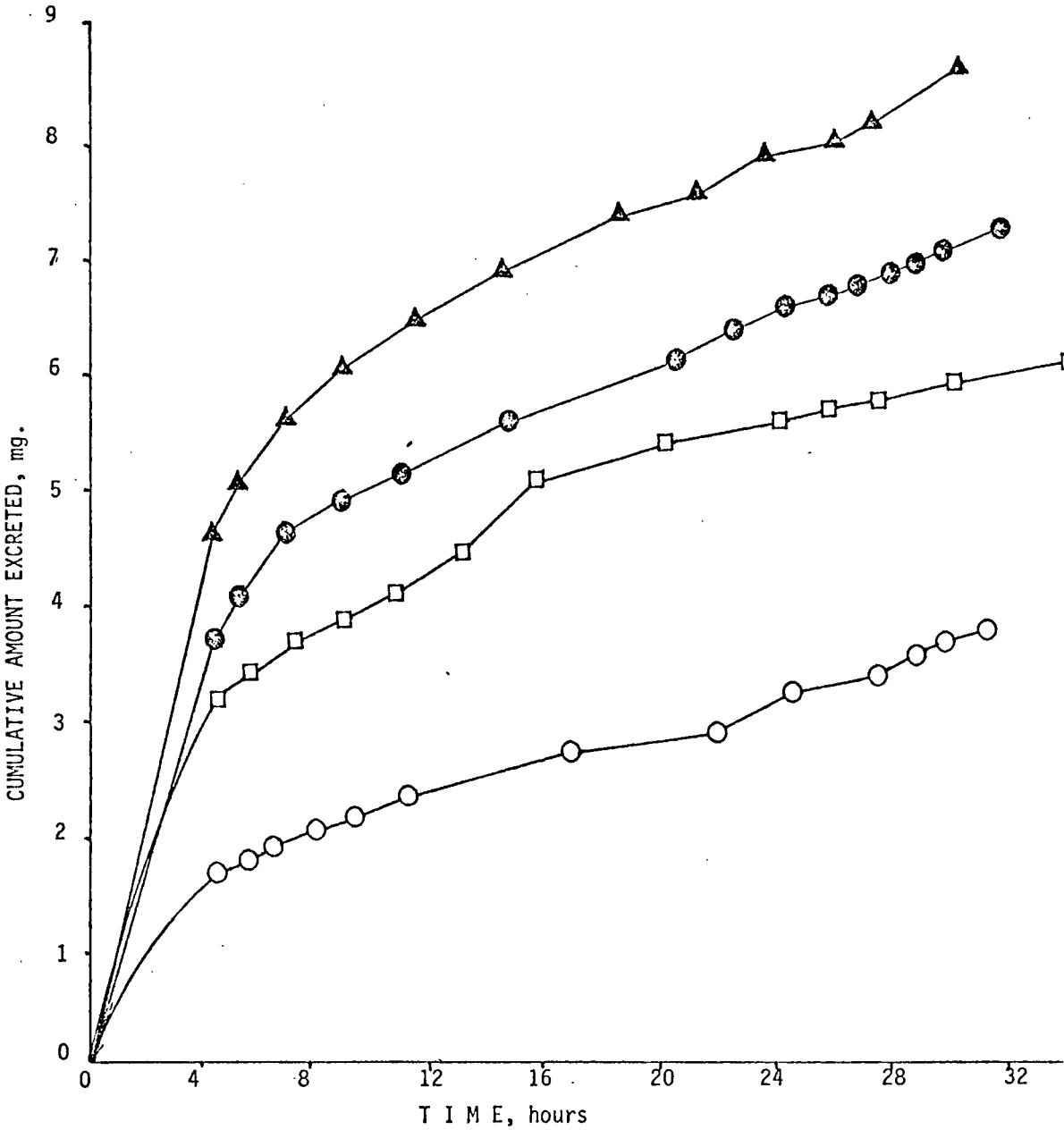

Figure 19. Cumulative amount of riboflavin excreted as a function of time for control (❁), aluminum-magnesium hydroxide (○), aluminum hydroxide (□), and magnesium hydroxide (▲) studies in subject C.

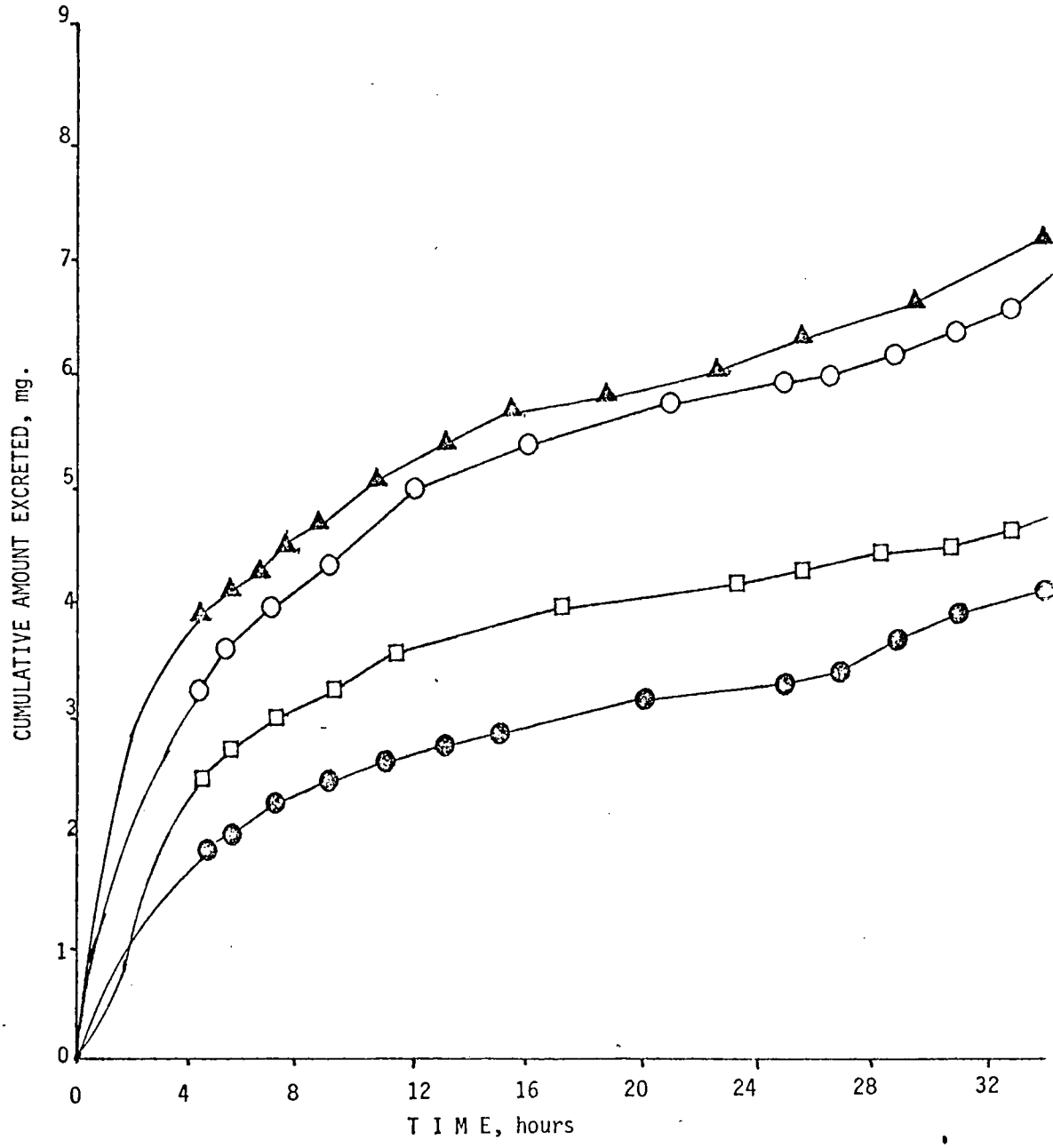

Figure 20. Cumulative amount of riboflavin excreted as a function of time for control (⊙), aluminum-magnesium hydroxide (○), aluminum hydroxide (□), and magnesium hydroxide (△) studies in subject D.

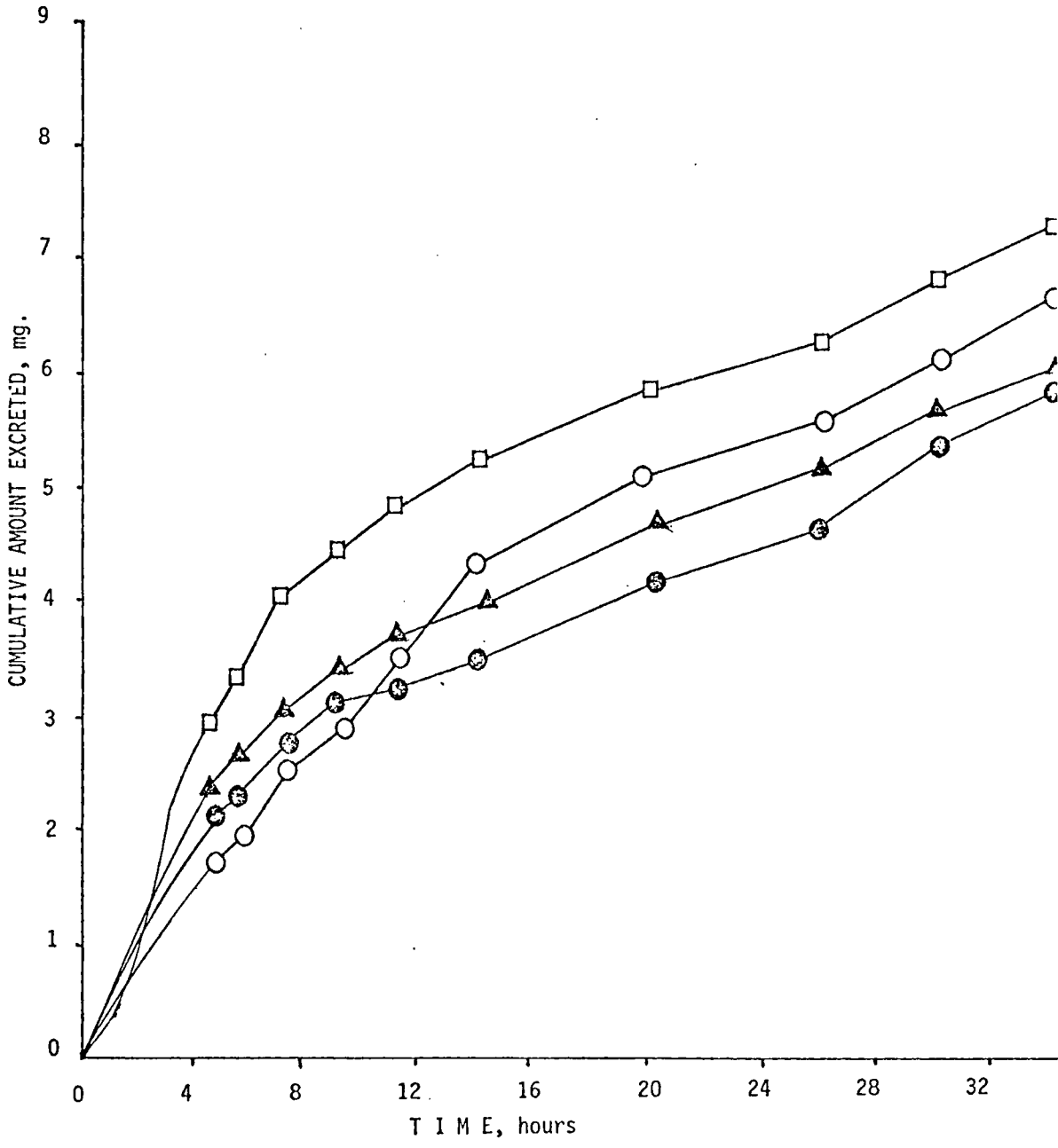

Figure 21. Cumulative amount of riboflavin excreted as a function of time for control ( ), aluminum-magnesium hydroxide ( ), aluminum hydroxide ( ), and magnesium hydroxide ( ) studies in subject E.

rises in amount of riboflavin excreted, the plots tended to level off at later times. The times of peak urinary excretion rate of riboflavin for each subject under each experimental condition, along with the means and standard deviations for each study, are shown in Table IV. Based upon the averaged data, aluminum hydroxide gel administration resulted in the longest time to peak riboflavin excretion. This was followed by time to peak for magnesium hydroxide. The control studies with riboflavin alone and the antacid treatment with aluminum-magnesium hydroxide resulted in the shortest time to peak urinary excretion rates of riboflavin.

Table V shows the peak excretion rates for each subject under the different experimental studies plus the means and standard deviations for each study. With reference to the mean values, magnesium hydroxide administration resulted in the greatest peak excretion rate for riboflavin, with aluminum hydroxide and aluminum-magnesium hydroxide co-administration producing approximately the same peak excretion rates for riboflavin. The riboflavin controls showed the lowest peak excretion rates in these studies.

Magnesium hydroxide administration resulted in the highest bioavailability for riboflavin excretion as measured by the amount excreted in the urine. This was followed, in order, by aluminum-magnesium hydroxide co-administration, riboflavin control, and aluminum hydroxide gel. These results are summarized in Table VI.

Paired-t tests were performed between the control and antacid with riboflavin studies for each subject in each category of data: availability, time of peak, and peak rate. Significant differences ($p < 0.05$) were observed between the control and aluminum hydroxide treatment for the time of peak and peak rate data.

From the <u>in vitro</u> experiments, the mean amount of riboflavin in each

TABLE IV. TIME TO PEAK RIBOFLAVIN[a] EXCRETION RATE IN CONTROL AND ANTACID TREATED SUBJECTS (hr).

Subject	Control	Aluminum-Magnesium Hydroxide	Aluminum Hydroxide	Magnesium Hydroxide
A	2.25	1.75	2.25	1.75
B	0.75	2.25	2.25	2.25
C	1.25	1.25	2.25	2.75
D	0.75	1.25	2.25	1.75
E	2.25	0.75	2.75	2.25
Mean	1.45	1.45	2.35[b]	2.15
S.D.	0.75	0.57	0.22	0.41

[a]Riboflavin Dose = 30 mg.
[b]$p < 0.05$, Paired t - test compared to control.

TABLE V. PEAK EXCRETION RATE OF RIBOFLAVIN[a] IN CONTROL AND ANTACID TREATED SUBJECTS (mcg./hr.).

Subject	Control	Aluminum-Magnesium Hydroxide	Aluminum Hydroxide	Magnesium Hydroxide
A	2394	2811	2826	2337
B	673	1476	822	1391
C	1247	653	1301	1512
D	787	1437	1010	2176
E	631	552	968	800
Mean	1146	1386	1386[b]	1643
S.D.	739	904	824	624

[a]Riboflavin Dose = 30 mg.
[b]$p < 0.05$, Paired t - test compared to control.

TABLE VI. BIOAVAILABILITY OF RIBOFLAVIN[a] IN CONTROL AND ANTACID TREATED SUBJECTS[b].

Subject	Control	Aluminum-Magnesium Hydroxide	Aluminum Hydroxide	Magnesium Hydroxide
A	38.0	43.7	29.1	31.1
B	12.1	23.2	15.6	24.9
C	24.0	12.5	20.1	28.7
D	13.6	23.4	16.1	23.8
E	19.4	21.8	24.1	19.9
Mean	21.4	24.9	21.0	25.7
S.D.	9.3	10.2	5.1	3.9

[a]Riboflavin Dose = 30 MG.
[b]Cumulative amount riboflavin excreted/dose X 100

study, the percent of riboflavin bound to antacid, and the mean pH of each study were calculated from the five samples in each study. The amount of riboflavin in each sample was calculated as described in the in vivo studies except that the dilution factor was excluded. The percent of riboflavin bound to antacid was calculated by subtracting the amount of riboflavin free in the presence of antacid from the amount of riboflavin in a control study and dividing that value by the amount of riboflavin in the appropriate control set.

The percent riboflavin binding in the presence of each antacid as a function of riboflavin concentration and antacid amount is shown in Table VII. The percent binding of antacid to riboflavin was comparable at both riboflavin concentrations in each study. Riboflavin binding to magnesium hydroxide was the highest followed by aluminum hydroxide and aluminum-magnesium hydroxide.

The data for percent antacid binding to riboflavin as a function of antacid amount, indicates that the greater the antacid amount, the greater the binding of riboflavin to antacid. Once again, as in the preceding data, magnesium hydroxide exhibited the highest percent of binding to riboflavin, followed, in order, by aluminum hydroxide and aluminum-magnesium hydroxide.

The studies to examine the effect of adding 0.01 N HCl on the pH of the riboflavin-antacid mixtures are included in Table VIII. The inclusion of magnesium hydroxide in the mixture resulted in the highest pH readings, followed, in order, by aluminum-magnesium hydroxide and aluminum hydroxide.

In all of the above in vitro experiments, hydrochloric acid was added to approximate the effect of the neutralizing capabilities of each antacid and the effect on riboflavin binding. The effect of the addition of 0.01 N HCl on the suspension pH is also included in Table VIII.

TABLE VII
PERCENT RIBOFLAVIN BOUND AS A FUNCTION OF RIBOFLAVIN AND ANTACID CONCENTRATION AND VEHICLE.

Antacid Combination[a]	Initial concentration of Riboflavin	
	1.7 mcg/ml	5.0 mcg/ml
AMH (15)	-8.80 (3.83)[b]	2.20 (2.68)[b]
AMH (5)		0.20 (5.07)
AMH(15) + HCl(10)	4.80 (9.50)	7.00 (3.67)
AMH (5) + HCl(10)		0.60 (2.88)
AH (15)	27.4 (5.22)	29.0 (2.74)
AH (5)		5.60 (7.27)
AH (15) + HCl(10)	21.2 (7.12)	20.2 (6.91)
AH (5) + HCl(10)		9.72 (7.79)
MH (15)	75.8 (4.09)	77.4 (9.45)
MH (5)		69.6 (13.2)
MH (15) + HCl(10)	72.2 (5.07)	71.6 (9.26)
MH (5) + HCl(10)		64.6 (4.93)

[a]Numbers in parenthesis equal volume of antacid or 0.01N HCl used.
AMH = aluminum magnesium hydroxide, AH = Aluminum hydroxide, MH = Magnesium hydroxide

[b]%Riboflavin Bound, Mean ± S.D.

TABLE VIII
EFFECT OF ANTACID AND HCl CONCENTRATION ON
pH OF RIBOFLAVIN - ANTACID SUSPENSIONS

Antacid Combination[a]	5 ml[b]	15 ml[b]
AMH	8.29 (0.05)[c]	8.26 (0.04)[c]
AMH + HCl (10)	7.61 (0.05)	8.21 (0.03)
AH	6.74 (0.12)	7.02 (0.10)
AH + HCl (10)	5.98 (0.18)	7.07 (0.08)
MH	9.62 (0.13)	9.59 (0.08)
MH + HCl (10)	9.58 (0.08)	9.65 (0.09)

[a]Numbers in parentheses equal volume of 0.01 N HCl used. AMH = aluminum - magnesium hydroxide, AH = aluminum hydroxide, MH = magnesium hydroxide.
[b]Volume of antacid
[c]Mean pH reading ± S.D.

TABLE IX. STABILITY OF RIBOFLAVIN UNDER VARYING STORAGE CONDITIONS

Time (days)	No.	Refrigerator (7 °C) (mcg/ml)	Freezer (-35 °C) (mcg/ml)
0	3	0.248 ± .011	0.248 ± .011
1	3	0.230 ± .007	0.245 ± .009
3	3	0.220 ± .020	0.260 ± .003
7	3	0.213 ± .012[a]	0.232 ± .010
14	3	0.228 ± .009	0.265 ± .016

[a] $p < 0.05$, "t"-test compared to day "0"

The stability data, presented in Table IX indicates the means and standard deviations of the stock solution and the various riboflavin solutions under the different time intervals and storage conditions. The controls (no riboflavin present) had no fluorometric readings.

DISCUSSION

In order to investigate whether the storage of riboflavin in the plastic bags would lead to a problem in stability, the studies outlined in the experimental section were run. From the data presented in Table IX it would appear that storage conditions (freezer or refrigerator) or the time interval before the riboflavin assay had no significant effect on riboflavin concentration in the urine samples, nor was there any significant binding of riboflavin to the plastic bag under the storage conditions. No fluorescence due to leaching of plasticizers from the bags was observed. These studies confirm that riboflavin stability would not be a problem during the time period from sample collection to assay.

The usual recommended daily allowance for riboflavin is 1-2 mg (44). Because the dose of riboflavin given to the subjects in the _in vivo_ experiments was 30 mg., and because the subjects had not taken vitamins for at least 30 days and had an overnight fast, the urinary excretion data for riboflavin can be said to reflect the elimination of riboflavin ingested during the experiments.

The results presented in Figures 2-16 are similar to the data reported by Levy and Jusko (39, 40) for riboflavin administered as a suspension to man. Inspection of the data presented in these findings for all five subjects reveals a number of smaller peaks in the excretion rate _versus_ time profiles after the intial peak. These peaks are most likely due to the bile - mediated enhanced absorption of riboflavin (42). It is of interest to note that these "peaks" occur at the approximate time of meal ingestion (4 and 8 to 10 hours after the dose).

The cumulative amount of riboflavin excreted _versus_ time plots shown in Figures 17 to 21 clearly shows the differences in bioavailability

of riboflavin from each treatment. Riboflavin absorption from the control studies ranged from 12.1 to 38.0 percent with a mean value of 21.4 percent. This is in good agreement with the 15 percent recovery reported by Levy and Jusko (40).

Aluminum hydroxide gel administration resulted in a significant ($p < 0.05$) delay in the time of peak excretion rate compared to control values (average increase of 0.9 hour) based upon a paired "t"-test. This is consistent with the reported activity of aluminum ion as a smooth muscle relaxant (36). The smooth muscle relaxing effect of aluminum hydroxide would reduce the gastric emptying rate and result in a delay in the time of riboflavin reaching the optimum absorption site in the small intestine. Corresponding to the delay in time of peak excretion rate of riboflavin with co-administration of aluminum hydroxide gel, an increase in the magnitude of the peak rate was noted. This is consistent with a reduction in the amount of riboflavin reaching the absorption site in the small intestine per unit time period and would allow for greater absorption over a given time period. No increase in bioavailability of riboflavin was noted in aluminum hydroxide gel treated subjects.

The administration of magnesium hydroxide suspension produced the highest overall peak excretion rate and bioavailability of riboflavin based upon mean values. However, these differences were not significant when evaluated by a paired "t"-test, although an increase in both peak excretion rate and bioavailability was noted in four out of the five subjects studied. Additional subjects would be necessary to determine if magnesium hydroxide suspension does alter riboflavin absorption. It is important to note that in studies in rats (45) which were administered magnesium hydroxide by gastric intubation, gastric volume was increased by 99% over control values

following administration of the antacid. This was reportedly due to an increase in water flux into the gastric pouch following antacid administration (45). The increase in volume could result in a decrease in the percent of gastric contents entering the small intestine. A larger increase in gastric volume (197%) was noted following aluminum hydroxide administration.

Also of importance are the in vitro binding studies (Table VII). These studies have revealed a number of important facts. For example, the in vitro data shows that riboflavin produced the highest percent binding to magnesium hydroxide (approximately 70%). Therefore, less riboflavin should be available for absorption in the small intestine and a lower peak excretion rate and lower bioavailability of riboflavin should result. However, it has been reported (46) that many complexation or adsorption interactions of drugs are completely reversible and therefore, do not alter drug absorption or bioavailability. A similar case may be made for the in vitro binding of riboflavin to aluminum hydroxide gel where between 6 and 27 percent of the vitamin was bound depending upon the initial concentration of antacid. No riboflavin concentration dependence was noted with magnesium or aluminum hydroxide.

What is puzzling is the apparent absence of an interaction between riboflavin and the magnesium-aluminum hydroxide combination. This may indicate that the interaction of riboflavin with the suspension dose forms may involve an interaction with some other ingredient in the suspension containing the antacids since if both magnesium and aluminum hydroxide interact with riboflavin one would expect the combination to also bind riboflavin. Further studies are necessary to explore this phenomenon.

Another possibility for an alteration of riboflavin absorption in the presence of antacids is the increased volume of the gastric contents due

to the antacids. If this occurs in man, the increased volume may result in an increase in dissolution rate of riboflavin in the gastrointestinal tract and result in a change in bioavailability.

The results of the present study would indicate that administration of antacids may result in a change in gastric emptying and therefore may produce a change in the absorption of a second drug administered in the presence of the antacids. The potential importance of this interaction needs further study.

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