RENAL TUBULAR HANDLING OF GLYCEROL AND ETHYLENE GLYCOL IN THE DOG

by

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INTRODUCTION

General Aspects

Glycerol is a ubiquitous substance first discovered in 1779 (29). It is found in food products, beverages and in pharmaceutical preparations as a solvent or vehicle (11, 20, 33). It is occasionally used therapeutically to lower the intraocular tension in humans with glaucoma (32). It is a substance endogenous to mammalians and can be synthesized or obtained from fat metabolism. Glycerol is a relatively non-toxic substance. Toxicity appears only in the case of very large doses, or in the case of subcutaneous injections (20, 33).

Ethylene glycol was first synthesized in 1859 (22). When glycerol was in short supply during World War I, ethylene glycol was studied as a replacement for glycerol, primarily as a solvent or vehicle in pharmaceutical preparations. Toxicity studies however have shown that it is not a safe replacement. Ethylene glycol is a more toxic substance than glycerol and the nephrotoxicity associated with its consumption is often the primary cause of death (14, 20). Ethylene glycol is most familiar for its use in radiator antifreeze.

Glycerol and ethylene glycol are aliphatic straight chained saturated polyalcohols. Table 1 lists some physical properties of these compounds and urea. Urea and ethylene glycol both have the same approximate molecular weight which is about two-thirds the molecular weight of glycerol. Ethylene glycol and glycerol are substantially more soluble in water than urea. Although partition coefficients may vary some between different membranes, in general, ethylene glycol, and urea to some extent, are slightly more lipid

TABLE 1. Physical properties of compounds studied.

Compound	Molecular Weight	Density	Solubility in Water	Olive oil: water Partition Coefficient	Aqueous Diffusion Coefficient D x 10^5
		g/ml	g/100ml		cm^2/sec
Glycerol 	92.1	1.26	∞	0.00007	0.72-0.83
Ethylene Glycol 	62.1	1.12	∞	0.00049	(0.93)*
Urea 	60.1	1.34	119	0.00015	0.94-1.18

Values obtained from three sources (10, 23, 60). *Parenthesis indicates possible misprint in magnitude as expressed in original source (10).

soluble than glycerol. However, all three compounds are relatively lipid insoluble substances. The aqueous diffusion coefficients of all three compounds are similar and approximately proportional to the square root of their respective molecular weights, as predicted from simple diffusion theory.

Studies of the movement of glycerol and ethylene glycol across red blood cell membranes have revealed:

1. In mammalian cells, urea penetrates very rapidly, ethylene glycol penetrates less rapidly, and glycerol slowest of all (10).

2. In most species, including the dog, movement of all three molecules is by simple passive diffusion (10).

3. In other species, such as the human and the rabbit, glycerol movement is too rapid to be accounted for by passive diffusion and carrier mediated facilitated diffusion has been proposed to account for this discrepancy (10, 55).

4. In the facilitated diffusion system, ethylene glycol and some other glycols in high enough concentrations have been shown to be competitive inhibitors of glycerol movement across the membrane (53, 54, 55). It has been proposed (52, 53, 54) that the glycol and glycerol molecules, rich in hydroxyl groups, form a dimer by means of hydrogen bonds. In the case of inhibition, the dimer has an increased molecular size and weight and is thought to have the same, or increased, number of free hydroxyl groups which tend to anchor the dimer in the solvent water and hinder its entry into the lipid membrane according to this concept. The net effect is to inhibit glycerol movement.

5. In a passive diffusion system, such as the ox red cell, glycols in high concentrations may increase the penetration rate of glycerol (52, 53). In the human red blood cell, some glycols, other than ethylene glycol, increase the penetration rate of glycerol (53, 54). It is proposed (53, 54) in this case that the dimer formed between the glycerol and the glycol molecules has less free hydroxyl groups available for hydrogen bonding with water and can thus move across the lipid phase of the membrane more rapidly than the monomers, despite the larger size and weight of the dimer.

For passive movement across a given membrane, probably by movement through pores, it would be expected that urea and ethylene glycol would be more permeable than glycerol.

This might not necessarily be true if glycerol movement occurred by means other than simple passive diffusion.

As discussed above it is possible that the presence of a glycol might affect the movement of glycerol. In a carrier mediated system the presence of a glycol, such as ethylene glycol, might inhibit the movement of glycerol. In a passive diffusion system, a glycol might increase glycerol movement. It is also possible that high concentrations of glycol would be necessary to demonstrate these effects.

In the mammalian kidney (7, 21, 59), urea is quite permeable in the proximal convolution as indicated by the fact that only one-half of the urea filtered is left within the lumen towards the end of the proximal convolution. The amount of urea in the tubular fluid at

the tip of the loop of Henle in the papilla is about equal to the amount that was filtered. In the distal tubule a quantity in excess of the amount of urea filtered may be present in the luminal fluid. Yet in the final urine only about 0.2 - 0.4 of the filtered amount may be present.

These findings are considered to be a consequence of high permeability to urea in the collecting duct with movement of urea into the medullary interstitium and diffusion back into the descending limb of Henle. The distal tubule is believed to be relatively impermeable with respect to urea, even in the presence of antidiuretic hormone (3, 4, 5, 19).

Before a review of the pertinent literature, an outline of symbols and terminology frequently used in renal physiology has been included in the following pages.

SYMBOLS AND TERMINOLOGY FREQUENTLY USED IN RENAL PHYSIOLOGY

EG is an abbreviation used for ethylene glycol.

G is an abbreviation used for glycerol.

In is an abbreviation used for inulin.

PAH is an abbreviation used for para-aminohippurate.

P_x = The concentration of any substance x in the plasma, in mg./ml. of plasma.

U_x = The concentration of substance x in the urine, in mg./ml. of urine.

$\dot{V}$ = The rate of urine flow, in ml./min.

$U_x/P_x = (U/P)_x$ = The U/P ratio of substance x, the concentration of x in the urine divided by the concentration of x in the plasma.

If the U/P ratio is less than 1.0 it is usually interpreted as an indication of active reabsorption of the substance against a concentration gradient by the tubular cells as the urine passed down the tubules. Active reabsorption requires specific energy expenditure by the cell to move the substance against an electrical or chemical gradient.

A U/P ratio greater than 1.0 may be associated with combinations of active transfer, passive diffusion and secretion of the substance into the urine, so that a U/P

ratio greater than 1.0 does not indicate the mechanism involved. However, a substance that moves only by passive diffusion must have a U/P ratio greater than 1.0.

$U_x\dot{V}$ = The amount of x excreted in the urine per unit time, in mg./min.

$U_x\dot{V}/P_x = C_x$ = The clearance of substance x, in ml./min. A measure of the virtual volume of plasma that is cleared of substance x per unit of time. The larger the value is, the more effectively the substance is excreted. If the substance is freely filterable through the glomeruli and is neither reabsorbed nor secreted by the tubules, then the clearance of the substance will be identical with the filtration rate. Inulin meets these specifications.

$U_{In}\dot{V}/P_{In} = C_{In} = GFR$ = The glomerular filtration rate, in ml./min., is equal to the clearance of inulin.

$GFR \cdot P_x = L_x$ = The filtered load of x, the amount of x that is filtered through the glomeruli per unit time, in mg./min.

C_x / C_{In} = The x-to-inulin clearance ratio, the clearance of x relative to the clearance of inulin. Also the fraction of the filtered load of x that is excreted since the amount excreted/filtered load = $U_x\dot{V}/C_{In}P_x = (U_x/P_x)\dot{V}/C_{In} = C_x/C_{In}$ which in its simplest form becomes $(U_x/P_x)\dot{V}/(U_{In}/P_{In})\dot{V} = (U/P)_x/(U/P)_{In}$.

If the x/In clearance ratio is greater than 1.0 it indicates that the quantity of x that appears in the urine exceeds the quantity of x that was filtered. In most cases this would mean that the substance had also been secreted

into the urine. If the clearance ratio is less than 1.0 it indicates that some of the filtered amount has been reabsorbed. It does not indicate the mechanism by which it was reabsorbed. The smaller the clearance ratio is, the larger is the fraction of filtered x that has been reabsorbed. Since the fraction of the filtered load that is excreted and reabsorbed must be equal to 1.0 unless there has been secretion it follows that:

$(1 - C_X/C_{In})$ = the fraction of the filtered load of x that is reabsorbed.

T_X = The transport rate of substance x across the tubular epithelium, in mg./min. It is the difference between the total rate of delivery of x, which is $C_{In}P_X$, and the rate of excretion, which is $U_X\dot{V}$.

Tm_X = The maximum transport rate of x, in mg./min. Demonstration of a Tm is considered evidence that the substance is actively transported. A substance that moves by passive diffusion will not exhibit a Tm (see Appendix B).

REVIEW OF PERTINENT LITERATURE

Glycerol

Several studies have dealt with various aspects of glycerol excretion (15, 18, 30, 35, 45, 63). However, due to the experimental design, these studies do not allow interpretations as to the possible mechanisms or areas of the nephron involved.

Schmengler and Höber (44) in 1933 studied the rate of appearance, in frog urine, of certain organic molecules including glycerol and ethylene glycol. The compounds were perfused to the proximal tubules via the renal portal system. The renal arterial fluid, from which the glomerular filtrate is formed, admixes with the portal fluid at the level of the peritubular capillaries, so that the blood containing the compounds is not filtered but does perfuse the proximal tubule. They observed that glycerol and ethylene glycol moved from the blood into the tubular lumen along their concentration gradients and in accordance with their molecular size. That is, ethylene glycol penetrated more rapidly than glycerol. The permeability of ethylene glycol was about twice that of glycerol. The compounds were not studied simultaneously. There was no evidence that active participation of the tubular epithelium was involved. This was based on the finding that a narcotic, such as phenylurethane, would substantially reduce active transport of phenol red, but not affect glycerol or ethylene glycol movement.

In 1945, on the basis of clearance studies, Sveinsson (57), reported that the percentage of the filtered glycerol that is excreted by

the human kidney is independent of urine flow and plasma concentration, if the plasma concentration is greater than 0.30 mg./ml.[1] At lower plasma levels the percent excreted decreased as the plasma concentration decreased. In one experiment with a very low plasma concentration he obtained U/P ratios for glycerol that were less than 1.0.

He believed that glycerol was a threshold substance which had a low renal Tm. The renal threshold was estimated to be 0.10 mg./ml. of plasma.[2] At the low levels glycerol reabsorption was believed to be primarily by an active mechanism. At levels higher than this the percentage excretion was believed to be primarily influenced by passive reabsorption. From his experiments, which unfortunately did not include determination of the GFR, it was estimated that approximately 45% of the filtered glycerol was excreted when plasma concentrations exceeded 0.30 mg./ml.

Pena and Malvin (37) in 1962 reported studies dealing with the movement of certain compounds, including ^{14}C-glycerol and ^{14}C-urea, from the blood of dogs, across the tubular cells and into the tubular lumen. The procedure was to establish a diuresis and then clamp the ureter for 12 minutes. The labeled substance to be studied was then injected intravenously along with PAH and creatinine. After 2-4 minutes the clamp was released and the stop flow samples were collected. Midway during the collection a blood sample was drawn.

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[1]See Appendix B. Sveinsson (57) also relates that E. Holst (Diss. Kobenhavn, 1943) studied rabbits and found that the percentage of filtered glycerol that was excreted was constant in the same animal irrespective of plasma levels, but that the percentage excreted varied from a small percent up to 60% from animal to animal.

[2]This corresponds to the value reported for rabbits (56).

TABLE 2. Average proximal and distal U/P ratios for C^{14}-labeled compounds.*

Compound	Average Distal U/P	P	Average Proximal U/P	P	No. of Exps.
Urea	0.068± 0.037	0.001	0.300 ± 0.18	0.001	4
Glycerol	0.0176 ± 0.0026	0.001	0.107 ± 0.0011	0.001	3
Inulin	0.0042 ± 0.0037		0.0142 ± 0.008		7

*Data from Table 1 of Pena and Malvin (37). Values are means ± S.D. See text for additional details.

If the labeled compound appeared before (i.e. distal to) the appearance of PAH it was considered to be evidence for diffusion across the distal tubular cells. If it appeared with PAH it was considered evidence for diffusion across only the proximal tubular cells. If it appeared with the creatinine it was considered that the labeled compound entered by filtration only.

In one series of experiments, ^{14}C-glycerol and ^{14}C-urea were studied by the above procedure. In another series, ^{14}C-inulin was injected simultaneously with the creatinine.

The data are presented by the authors as shown in table 2. The P column gives the probability that the average U/P ratio of the labeled compound found in one group of dogs differs only by chance from the average inulin U/P ratios obtained in a different series of dogs. The column labeled distal gives the average activity in counts/min./ml. of all samples obtained before PAH appeared, divided by the activity found in the plasma, also in counts/min./ml. The column labeled proximal gives these ratios for all samples obtained after the appearance of PAH but before the appearance of creatinine.

They conclude that both glycerol and urea were able to move across the tubular cells into the distal tubular urine, and, to a greater degree, into the proximal tubular urine. The rate of urea movement was greater than that for glycerol. The conclusions with regard to urea are supported by the only stop flow pattern published which indicates that urea did appear in the samples ahead of PAH and creatinine.

When interpreting the glycerol data however, three considerations

are necessary.

First, glycerol has been shown to be metabolized, in the rat, rather rapidly, especially with administration of small doses (12, 17). It may be that some of the radioactivity found in the urine was due to metabolites of glycerol, such as $^{14}CO_2$, rather than ^{14}C-glycerol.

Second, presentation of data in the form of U/P ratios obtained in non-steady state conditions may not allow useful interpretations of the data. This is due to the fact that the plasma concentrations of glycerol and inulin will be decreasing immediately after injection. The plasma sample will contain glycerol and inulin at concentrations lower than the actual values that were present during the time when transtubular movement was presumably occurring. This would artifactually elevate the U/P ratios of both. This would not be an important consideration if the plasma concentrations of glycerol and inulin decreased at the same rate. However, if the plasma concentration of glycerol fell at a greater rate than inulin, then the U/P ratio for glycerol would be falsely elevated and to a greater extent than the U/P ratio for inulin to which it is compared.

Inulin is a large molecule and it would be expected that whenever it left the plasma compartment that the smaller glycerol molecules would also leave and at a greater rate. It would also be expected that glycerol would readily leave the plasma compartment when inulin would be unable to do so. Glycerol, for instance, would enter the red blood cells, an appreciable mass, in addition to being metabolized. The plasma concentration of glycerol would accordingly fall more rapidly than the inulin concentration. This might well lead to a falsely signi-

ficant difference between the U/P ratios for glycerol and inulin.

Third, as the authors themselves point out, the analytical method for detection of ^{14}C is quite sensitive and ^{14}C which was only filtered could be detected before chemically detected creatinine. Since it is not likely that the tubular epithelium would be completely impermeable to all molecules of a substance such as glycerol, it would be expected that a sufficiently sensitive method would indicate that the tubular cells were permeable, to some extent, to the glycerol molecule. It is possible that this may not represent quantitatively appreciable movement of the compound, however, so that for most purposes the tubular epithelium would be considered impermeable to the compound.

The authors also state that glycerol was shown to be actively reabsorbed from the proximal tubule. Unfortunately they do not provide the data that are the basis for this statement and make no additional statements concerning it.

They conclude that glycerol movement from the blood into the tubular lumen was by means of passive movement across the distal tubular cells, and across the proximal tubular cells may have been in part passive, and in part leakage backwards across an active transport system.

Kruhøffer and Nissen (28), working with cats, reported in 1963 that with low filtered loads of glycerol there was essentially complete reabsorption of glycerol with U/P ratios less than 1.0. With increasing filtered loads, there was an increasing rate of reabsorption (i.e. no Tm), and the fraction of the filtered load

reabsorbed approached 0.40. This is in agreement with Sveinsson's (57) interpretation of his data (see Appendix B).

A few stop flow studies were done and interpreted as showing a low distal permeability to glycerol, with glycerol reabsorption occuring in the proximal tubule to the extent that the U/P ratios were less than 1.0. In the single stop flow experiment published, with the exception of the first few distal samples, all U/P ratios of glycerol were less than 1.0 and the minimum glycerol concentration was found in the proximal tubule samples. The plasma glycerol concentration was 0.22 mg./ml. during the experiment.

They postulated that, at low filtered loads of glycerol leading to low urine concentrations, reabsorption took place by diffusion from the lumen into the proximal tubular cell because of metabolic conversion of glycerol within the cell which gave rise to a concentration gradient between lumen and cell. This was designated "conversion reabsorption" and allowed interpretation of U/P ratios less than 1.0 without postulating active reabsorption across the cell membrane. The "conversion reabsorption" hypothesis was based on measurements that indicated that, at low plasma concentrations, there was a greater rate of removal of glycerol from the blood (arterial-venous concentration difference times renal blood flow) than was excreted in the urine. However, discrepancies in the measurement of renal blood flow were occasionally quite large (up to 20%) and the concentration difference between arterial and venous plasma was quite small (about 0.05 mg./ml.) so that there is the possibility of error and low accuracy in the measurements used

in the calculations.

At high filtered loads, leading to high urine concentrations, the glycerol converting enzymes were considered to be saturated and reabsorption then was primarily by the mechanism of transcellular back diffusion of glycerol into the blood, according to the authors.

Ethylene Glycol

With the exception of the work of Schmengler and Höber (44) previously discussed, studies on the possible mechanisms or sites involved in reabsorption of ethylene glycol by the renal tubules have not been published. Previous studies of ethylene glycol have been limited to consideration of its toxic effects or metabolism (16).

Purpose of Present Experiments

The present studies were done in an attempt to define how the kidney deals with glycerol, and with the chemically similar compound ethylene glycol; specifically to determine:

1. the amount of the substance that is excreted in terms of the amount filtered;
2. at what sites the reabsorption occurred, and if possible, the mechanisms involved;
3. if a relatively low concentration of ethylene glycol affects glycerol movement.

The studies were done almost exclusively on the dog, which had not been studied previously with respect to the above considerations. Rabbits were used in one series since, as mentioned above, the rabbit red blood cell handles glycerol, and glycerol with ethylene

glycol present, differently than the dog red blood cell. Thus the possibility that the kidney of different species might handle glycerol, with or without ethylene glycol present, differently, led originally to the plan to study the dog, the rabbit, and possibly other species. However the interesting results that were found early in the dog studies which seemed to require further study, and due to time limitations, it was necessary to limit the study, essentially, to the one species.

METHODS

General

The animals were anesthetized with an intravenous injection of 0.5 ml./kg. of a solution containing 6.0 gm. of powdered sodium pentobarbital diluted to 100 ml. with saline. The commercial preparations of pentobarbital contain 20% propylene glycol which could possibly interfere with glycerol or ethylene glycol movement across membranes (55), and to a minor extent might interfere with determination of glycerol (see Chemical Determinations). Supplementary injections of anesthetic were given as necessary to maintain adequate levels of anesthesia (absence of corneal reflex).

Intratracheal tubes were inserted. All animals were hydrated with 200-500 ml. of saline, given while the surgical procedures were carried out. Arterial blood pressure (carotid artery) was continuously recorded by means of a Statham strain gauge and Grass Polygraph. Sodium sulfate was used to promote a diuresis. Mannitol was not used as a diuretic agent since its presence would interfere with the colorimetric determination of ethylene glycol and glycerol (see Chemical Determinations). All priming solutions were given by intravenous injection. The sustaining solution containing the sulfate was given by intravenous drip into a femoral vein throughout the experiment. The sustaining solution containing the substance to be studied was given by means of a constant infusion pump (Harvard Apparatus Co.), also into a femoral vein.

Urine was collected from polyethylene catheters inserted into both ureters to the renal pelvis. Renal pelvic pressure was recorded during ureteral clamp in the stop flow studies. The

distal end of the ureteral catheter was attached to a three-way stopcock and Statham strain gauge which was connected to the Grass Polygraph. By the appropriate turn of the stopcock urine flow could be stopped, and in effect "the ureter was clamped."

The appropriate blood samples were withdrawn from the carotid polyethylene catheter. Blood samples were obtained midway during urine collections, and before and after ureteral clamp in the stop flow studies. Heparin (Lipo-Hepin, Riker) was used as an anticoagulant.

Commercially available glycerol and ethylene glycol were used without further purification (Merck, or Matheson Coleman and Bell).

Clearance Studies

Preliminary investigations using the clearance technique were carried out on six mongrel male and female dogs weighing 13-25 kg. The composition of the solutions containing the test substances is indicated below:

1. Priming solution (3.0 ml./kg.)

   glycerol: 6.3 - 25.2 gm. of glycerol diluted to 100 ml. with saline.

   ethylene glycol: 5.6 - 22.3 gm. of ethylene glycol diluted to 100 ml. with saline.

2. Sustaining solution (0.78 ml./min.)

   glycerol: 1.9 - 7.6 gm. of glycerol diluted to 100 ml. with saline.

   ethylene glycol plus glycerol: 1.7 - 5.6 gm. of ethylene glycol plus 1.9 - 7.6 gm. of glycerol diluted to 100 ml. with saline.

Priming solutions of sulfate (0.9 osmolar), inulin, and PAH were given, followed by continuous infusion of a sulfate (0.3 - 1.0 osmolar) sustaining solution which also contained inulin and PAH. After a diuresis was established the glycerol priming and sustaining solutions were administered. At least one-half hour was allowed for a steady state to be attained. 2-3 clearance periods were then carried out. The priming solution of ethylene glycol and the sustaining solution of ethylene glycol plus glycerol was then administered. After at least one-half hour, 2-3 clearance periods were again carried out.

It was found that if the priming injection was given as concentrated glycerol that an immediate drop in blood pressure, primarily diastolic, occurred, followed by return of the blood pressure to values above those before injection.

Stop Flow Studies

Dogs. Stop flow studies, as described by Malvin, Wilde and Sullivan (31), were carried out on three male mongrel dogs weighing 16-22 kg. The composition of the test solutions is given below:

1. Priming solution (3.0 ml./kg.)

   glycerol: 25.2 - 34.0 gm. of glycerol diluted to 100 ml. with saline.

   ethylene glycol: 22.3 - 30.2 gm. of ethylene glycol diluted to 100 ml. with saline.

2. Sustaining solution (0.78 ml./min.)

   glycerol: 6.3 - 8.3 gm. of glycerol diluted to 100 ml. with saline.

   ethylene glycol plus glycerol: 5.6 - 7.4 gm. of ethylene glycol plus 6.3 - 8.3 gm. of glycerol diluted to 100 ml. with saline.

The procedure was the same as in the clearance studies with the exception that one-half hour after the test substance was administered, a stop flow procedure, rather than a clearance procedure, was carried out. In one experiment, ethylene glycol was administered first instead of glycerol. The second period was with ethylene glycol and glycerol. In all cases the ureter was clamped for 5-6 minutes.

Rabbits. Stop flow studies were carried out on two rabbits, a 4.0 kg. male and a 4.3 kg. female. The only change in procedure was to administer the sustaining solution with the test substance at a rate of 0.20 ml./min. The ureter was clamped for five minutes.

Postocclusive Injection Studies

In these studies one male and six female mongrel dogs weighing 15-21 kg. were used. The composition of the test solutions is given below:

1. Injection solution (1.5 ml./kg.)

   glycerol: 25.2 gm. of glycerol plus
   5.0 gm. of inulin
   diluted to 100 ml. with saline.

   ethylene glycol: 22.3 gm. of ethylene glycol plus
   5.0 gm. of inulin
   diluted to 100 ml. with saline.

2. Sustaining solution for a 15 kg. dog (0.73 ml./min.)

   glycerol: 6.3 gm. of glycerol plus
   4.1 gm. of inulin
   diluted to 100 ml. with saline.

   ethylene glycol: 5.6 gm. of ethylene glycol plus
   4.1 gm. of inulin
   diluted to 100 ml. with saline.

The procedure was essentially the same as in the stop flow studies

with the exception that creatinine was used in place of inulin for estimation of the GFR. The ureter was occluded as in the stop flow studies. After occlusion for 3-6 minutes, one of the injection solutions was given intravenously over a 50-90 second interval. Four minutes after the injection solution was started, the occlusion was released and the urine samples collected.

In some cases, to obtain additional stop flow data, the same amount of the injection solution was again injected and the appropriate sustaining solution infused. After one-half hour a stop flow study was done as described previously. The ureter was occluded for five minutes in these instances.

Chemical Determinations

1. Chloride (9), creatinine (13), glucose (43), inulin (42), PAH (50), and urea (6) were determined according to standard methods.

2. Glycerol and Ethylene Glycol. Two methods were used:

A. Titration method.

To determine glycerol in the presence of ethylene glycol, a modification of the methods of Shupe (46), and of Newburger and Bruening (36) was developed which allowed the determination of small concentrations in biological fluids. The complete details are given in Appendix A.

B. Colorimetric method.

The method of West and Rapoport (61) for the determination of mannitol was used to determine the amount of glycerol, the amount of ethylene glycol, or the total amount of ethylene

glycol plus glycerol when both were present, in the sample. Additional details are given in Appendix A.

It was possible to determine glycerol by either the titration method, or by the colorimetric method if ethylene glycol was not present. Ethylene glycol was determined by the colorimetric method. When ethylene glycol and glycerol were both present in the sample, the colorimetric method was used to measure the glycerol plus ethylene glycol concentration. The glycerol concentration, as determined on an aliquot of the same sample by the titration method, was then subtracted to obtain the ethylene glycol concentration.

RESULTS

Clearance Studies (See protocol Appendix C)

Dogs. Table 3 lists the mean value ± standard error of the mean (S.E.) and the range of the clearance ratios obtained under the various conditions. N is the number of kidneys from which values were obtained and used to calculate the mean value. Also listed is n, the number of dogs in each group, which is not equal to N since both kidneys of some dogs were utilized. Row I gives the mean glycerol/inulin clearance ratio for all dogs studied with only glycerol present. Row II gives the mean glycerol/inulin and endogenous urea/inulin clearance ratios found before ethylene glycol was added and found in the presence of ethylene glycol when the individual ratios were obtained in the same animal. Row III gives the mean ethylene glycol/inulin clearance ratios found with and without glycerol present. The mean ± S.E. and range of the plasma concentrations are listed in a corresponding manner. The P value is the probability that the two mean values preceding it in that row differ only by chance and was determined by use of the t-test (paired or correlated measures used only for data in row II) (51).

When the plasma glycerol concentration was high, usually greater than 0.30 mg./ml., the mean free flow glycerol/inulin clearance ratio was 0.75 for all dogs in which glycerol alone was studied. It was 0.73 during the first (ethylene glycol free) period in the series in which the glycerol/inulin ratios in a given dog before and after addition of ethylene glycol were determined. The mean free flow ethylene glycol/inulin clearance ratio on the other hand was significantly less, 0.53. Excretion of a larger fraction of the filtered

TABLE 3. Mean clearance ratios and plasma concentrations of glycerol, ethylene glycol and urea in the dog under various conditions.

Compound (x)	Clearance Ratio (C_x/C_{In})		P	Plasma Concentration mg./ml.		P
I Glycerol	0.75 ± 0.04* (0.37-0.96) N = 15 n = 10			0.94 ± 0.15 (0.32-2.11) N = 10 n = 10		
II	Ethylene Glycol not present	present		Ethylene Glycol not present	present	
Glycerol	0.73 ± 0.05*† (0.37-0.96) N = 12 n = 7	0.61 ± 0.06# (0.18-0.80) N = 12 n = 7	<0.001	0.96 ± 0.22 (0.32-2.11) N = 7 n = 7	1.13 ± 0.49 (0.36-4.01) N = 7 n = 7	>0.2
Urea	0.71 ± 0.05 (0.54-0.85) N = 9 n = 5	0.70 ± 0.03 (0.47-0.88) N = 9 n = 5	>0.2	0.15 ± 0.02 (0.11-0.22) N = 5 n = 5	0.16 ± 0.02 (0.11-0.23) N = 5 n = 5	>0.2
III	Glycerol not present	present		Glycerol not present	present	
Ethylene Glycol	0.53 ± 0.05† (0.46-0.62) N = 3 n = 2	0.44 ± 0.04# (0.17-0.71) N = 14 n = 8	>0.2	1.17 ± 0.17 (0.86-1.47) N = 2 n = 2	1.21 ± 0.20 (0.72-2.54) N = 8 n = 8	>0.2

Values are mean ± S.E. with range given in parentheses. N is the number of experimental values, n is the number of dogs. *Values statistically not different (P > 0.2),†# Values statistically different (P < 0.01). See text for additional details.

load of glycerol, i.e. glycerol/inulin clearance ratio, was also found on the average (0.61 vs. 0.44, table 3, rows II and III), and with one exception, in the individual experiments, when simultaneous glycerol/inulin and ethylene glycol/inulin clearance ratios were determined. Accordingly, with comparable filtered loads, the tubular reabsorption rates for ethylene glycol were greater than the rates for glycerol (see figures 1 and 2). However, neither the transport rates for glycerol nor for ethylene glycol appeared to reach a transport maxima, Tm, as shown in figures 1 and 2. The transport rates of both ethylene glycol and glycerol appeared to increase as the filtered loads of each increased, over the ranges studied.

With a very low plasma glycerol concentration however, such as 0.09 mg./ml., U/P ratios for glycerol were observed in one study that were less than 1.0, suggesting movement against a concentration gradient.

Also indicated in table 3, row II, is the fact that, to a significant degree, less of the filtered glycerol was excreted when ethylene glycol was also present. On the average, 73% of the filtered glycerol was excreted by these dogs before ethylene glycol was added. With ethylene glycol present, only 61% of the filtered glycerol was excreted by these same animals. In the individual animals, the average glycerol/inulin clearance ratio was never greater after ethylene glycol than beforehand. Comparison of the mean urea/inulin clearance ratios before (0.71) and after (0.70) ethylene glycol does not reveal any change in these values that is associated with the presence of ethylene glycol (table 3, row II).

Figure 1. Reabsorption of glycerol as a function of the filtered load in 8 dogs during 42 clearance periods. Reabsorption, expressed as the tubular transport rate of glycerol, in mg./min./100 gm. of kidney weight, is plotted on the vertical axis. The filtered load of glycerol, in mg./min./100 gm. of kidney weight, is plotted on the horizontal axis. Open triangles represent periods when only glycerol was present. Closed triangles represent periods when ethylene glycol was also present.

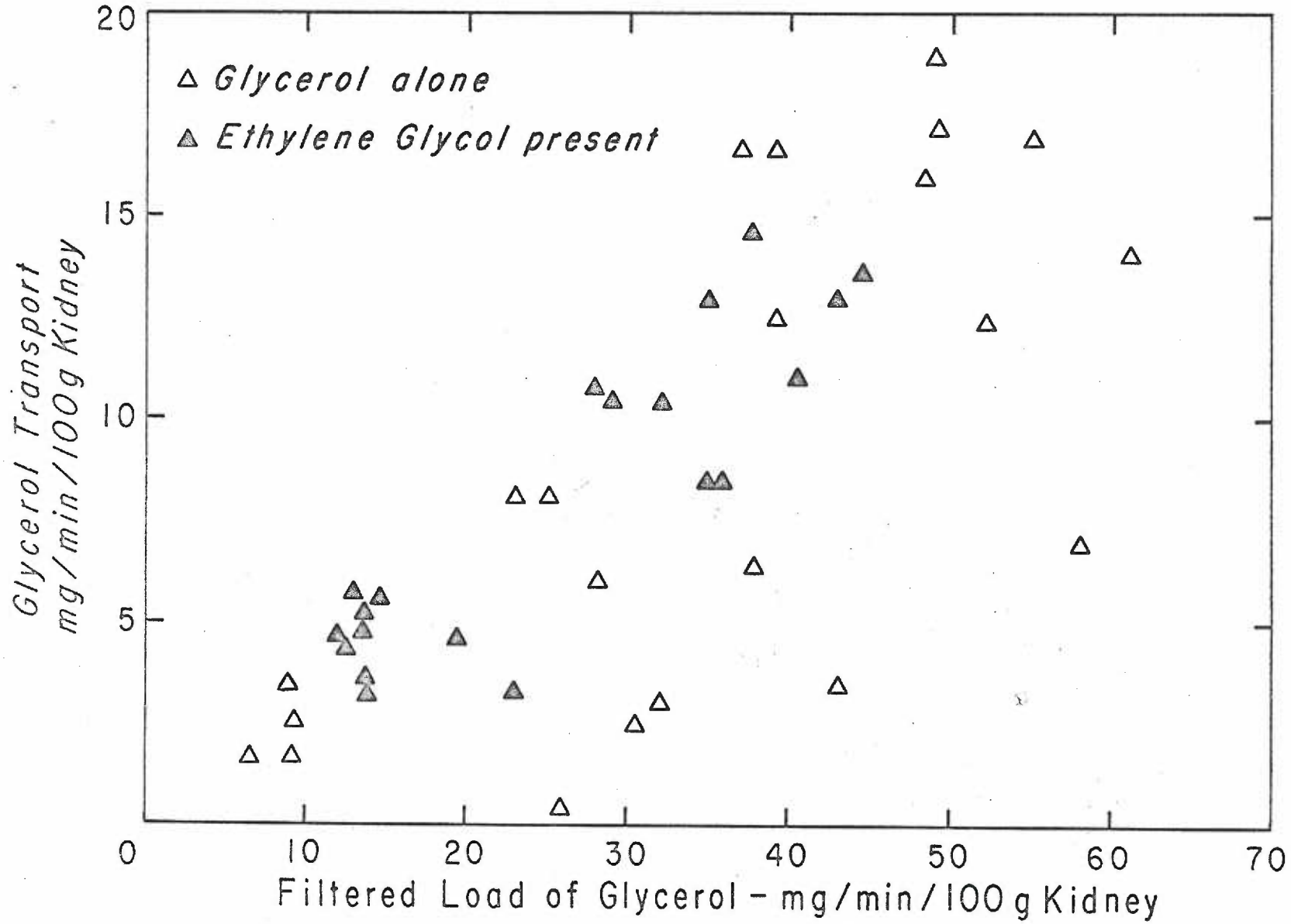

Glycerol alone
Ethylene Glycol present
Glycerol Transport
mg/min/100g Kidney
Filtered Load of Glycerol - mg/min/100g Kidney
0
5
10
15
20
10
20
30
40
50
60
70

Figure 2. Reabsorption of ethylene glycol as a function of filtered load in 6 dogs during 25 clearance periods. Reabsorption, expressed as the tubular transport rate of ethylene glycol, in mg./min./100 gm. of kidney weight, is plotted on the vertical axis. The filtered load of ethylene glycol, in mg./min./100 gm. of kidney weight, is plotted on the horizontal axis.

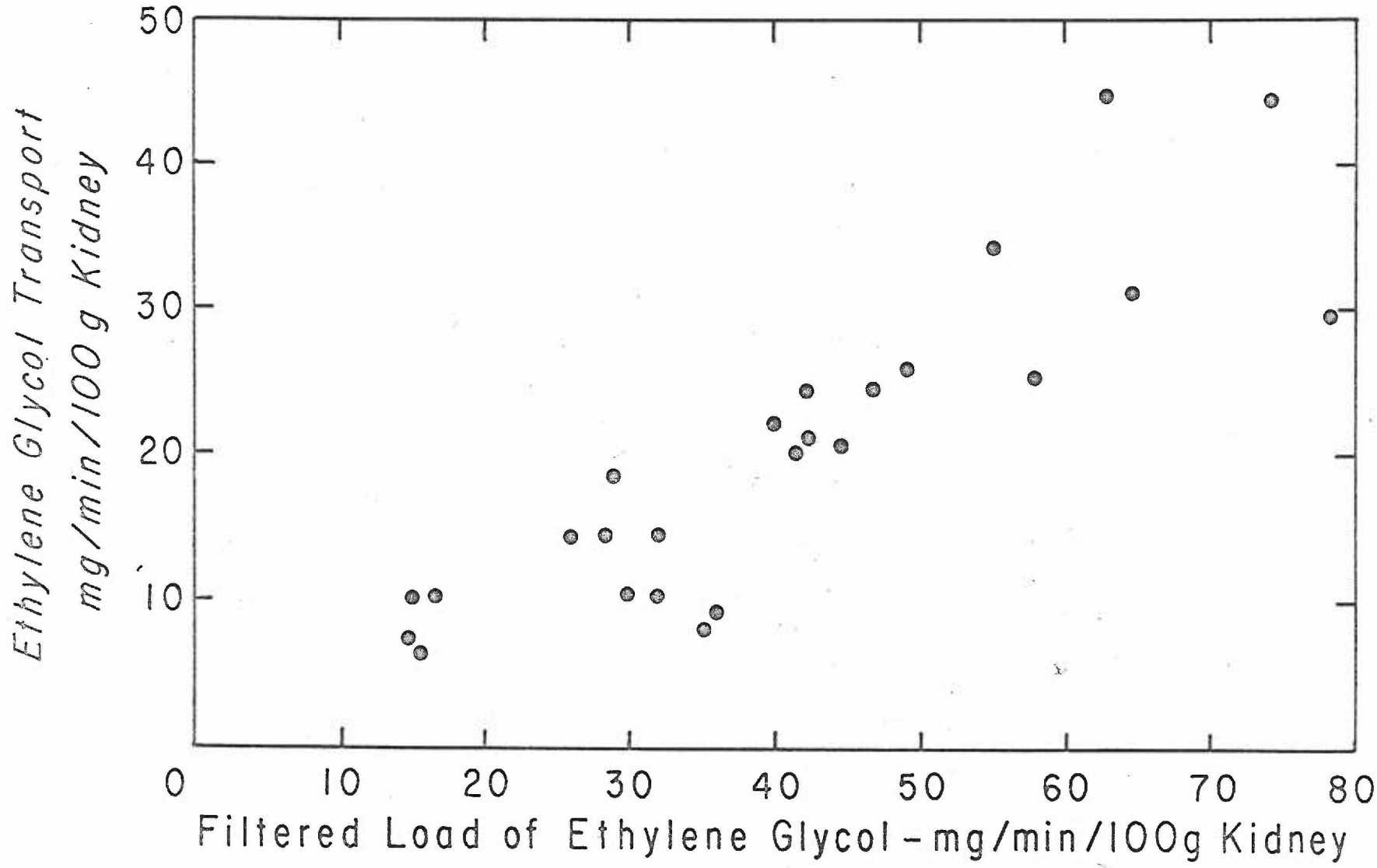
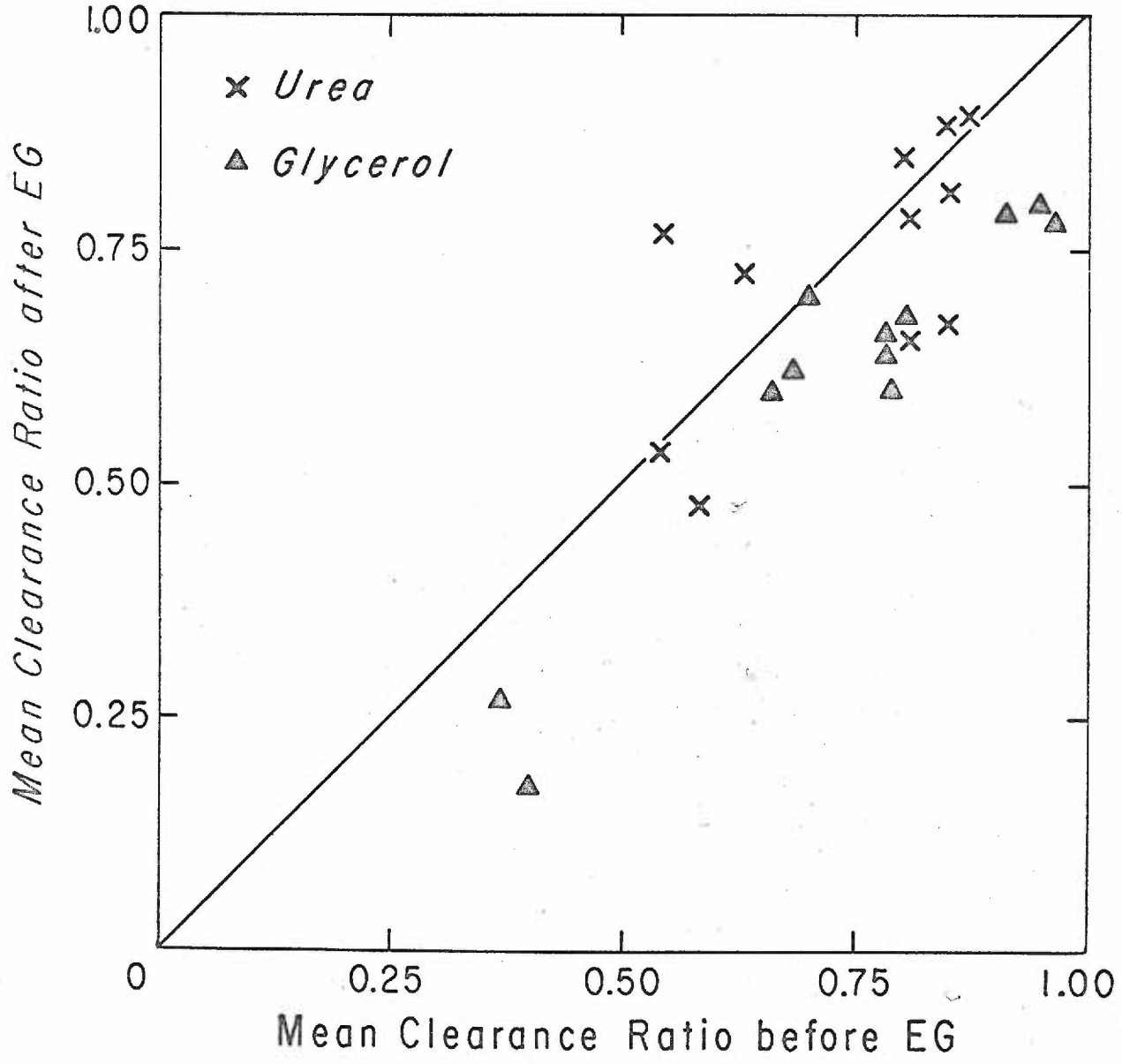

Ethylene Glycol Transport
mg/min/100 g Kidney
50
40
30
20
10
0
10
20
30
40
50
60
70
80
Filtered Load of Ethylene Glycol - mg/min/100g Kidney

Figure 3. The mean endogenous urea/inulin and glycerol/inulin clearance ratios after addition of ethylene glycol (vertical axis) as a function of the mean clearance ratio before addition of ethylene glycol (horizontal axis). Urea (11 kidneys in 7 dogs) is represented by an x, glycerol (12 kidneys in 7 dogs) by the closed triangle. Points on the diagonal line indicate no change in the clearance ratio occurred. Points above the line indicate a larger clearance ratio (more excretion), points below the line a lower clearance ratio (less excretion), after addition of ethylene glycol.


1.00
0.75
0.50
0.25
0
0.25
0.50
0.75
1.00
× Urea
▲ Glycerol
Mean Clearance Ratio after EG
Mean Clearance Ratio before EG

This is also indicated in figure 3 in which the mean clearance ratios obtained after the addition of ethylene glycol are plotted against the mean ratios obtained before ethylene glycol was added. If there were no differences in the fraction of filtered glycerol excreted before and after addition of ethylene glycol, then the points would fall along the 45 degree line. This is found to be the case when the urea/inulin clearance ratios before and after ethylene glycol are plotted in this manner. It is not the case when the glycerol/inulin ratios are plotted. All points except one lie below the line indicating that the average glycerol/inulin clearance ratios were less in the presence of ethylene glycol in all experimental kidneys except one in which there was no change.

The mean ethylene glycol/inulin clearance ratio found when only ethylene glycol was studied was 0.53. When ethylene glycol was studied in the presence of glycerol, the ethylene glycol/inulin clearance ratio was 0.44. Statistical analysis indicates that this difference was not significant (table 3, row III). In the one study done on the same animal, the average ethylene glycol/inulin ratios obtained after addition of glycerol (0.54 and 0.47) were actually slightly greater than those obtained before glycerol was added (0.50 and 0.46).

The plasma values for glycerol, ethylene glycol and urea are also tabulated in table 3. The mean plasma concentrations of glycerol, and of ethylene glycol, and of urea, were not significantly different during comparable clearance periods.

Rabbits. Table 4 lists, in a manner similar to table 3, the

TABLE 4. Mean clearance ratios and plasma concentrations of glycerol and ethylene glycol in two rabbits under various conditions.

Compound (x)	Clearance ratio (C_x/C_{In})		Plasma Concentration mg./ml.	
	Ethylene Glycol		Ethylene Glycol	
	not present	present	not present	present
Glycerol	0.79 ± 0.02#* (0.71-0.93) N = 4	0.85 ± 0.03* (0.77-0.94) N = 4	1.32 ± 0.18 (1.13-1.50) N = 2	1.24 ± 0.09 (1.16-1.33) N = 2
Ethylene Glycol	Glycerol present 0.66 ± 0.03# (0.51-0.91) N = 4		Glycerol present 1.26 ± 0.28 (0.98-1.54) N = 2	

Values are mean ± S.E. with range in parentheses. N is the number of experimental values. * Statistically not different ($P > 0.2$). # Statistically different at $P < 0.05$.

values obtained in the rabbits. At high plasma concentrations, the glycerol/inulin clearance ratio was 0.79, a value just slightly greater than that found in the dog. The mean ethylene glycol/inulin clearance ratio, in the presence of glycerol, was 0.66, a higher value than occurred in the dogs (0.44) when glycerol was present. As occurred in the dog, the fraction of the filtered glycerol excreted, i.e. the glycerol/inulin clearance ratio, 0.79, was statistically greater ($P<0.05$) than the fraction of ethylene glycol excreted (0.66).

Glycerol U/P ratios less than 1.0 were not observed at these high plasma levels and no experiments were carried out at low plasma concentrations.

In contrast to the dog, the mean free flow glycerol/inulin clearance ratio after the addition of ethylene glycol, 0.85, was actually slightly greater than before, 0.79, but was not statistically different ($P>0.2$), as indicated in table 4. The presence of ethylene glycol was associated with an increase in the average glycerol/inulin clearance ratio in one animal, and with a decrease in the other.

Stop Flow Studies

Dogs. Figures 4 and 5 are representative stop flow patterns obtained from two different animals and are typical of the data obtained during the stop flow studies (8 dogs, 14 glycerol and ethylene glycol stop flow patterns.) The clearance ratios are plotted on the vertical axis and the accumulated urine volumes on the horizontal axis. Fluid that was trapped in the distal segment during the period of the ureteral clamp may be identified by

Figure 4. Stop flow patterns of glycerol, ethylene glycol, endogenous urea and inulin in the dog. The clearance ratios of glycerol (G), ethylene glycol (EG), and urea (U), and the U/P ratios of inulin (In), are plotted on the vertical axis. The accumulated urine volumes are plotted on the horizontal axis. U1 and U2 represent, respectively, the pre- and postocclusive free flow samples. The arrows indicate the accumulated urine volumes at which the minimum chloride/inulin and maximum PAH/inulin ratios occurred. Glycerol was present during period I, glycerol and ethylene glycol during period II. The plasma glycerol concentration during the experiment was about 0.7 and 0.4 mg./ml., the plasma ethylene glycol concentration about 1.0 mg./ml.

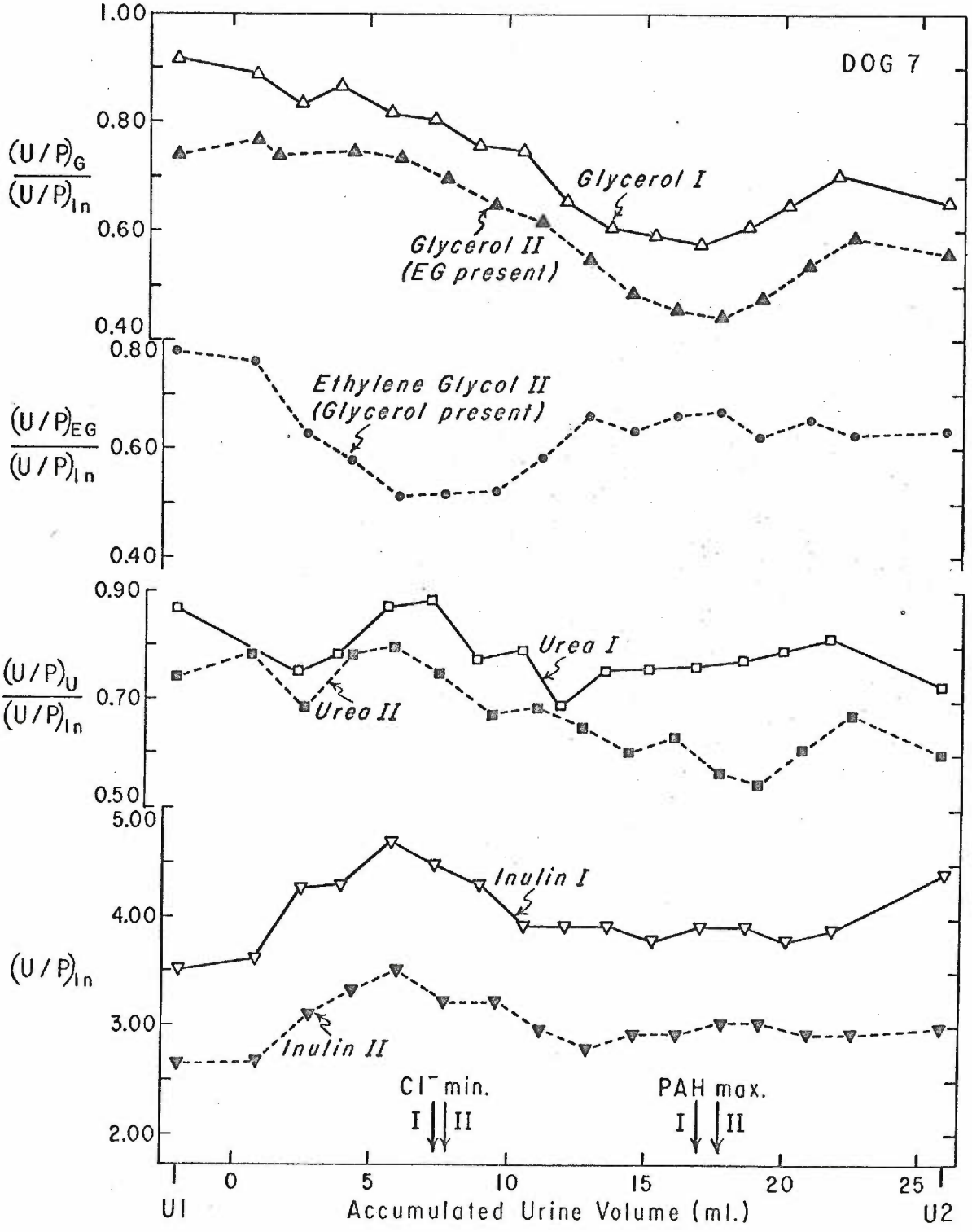
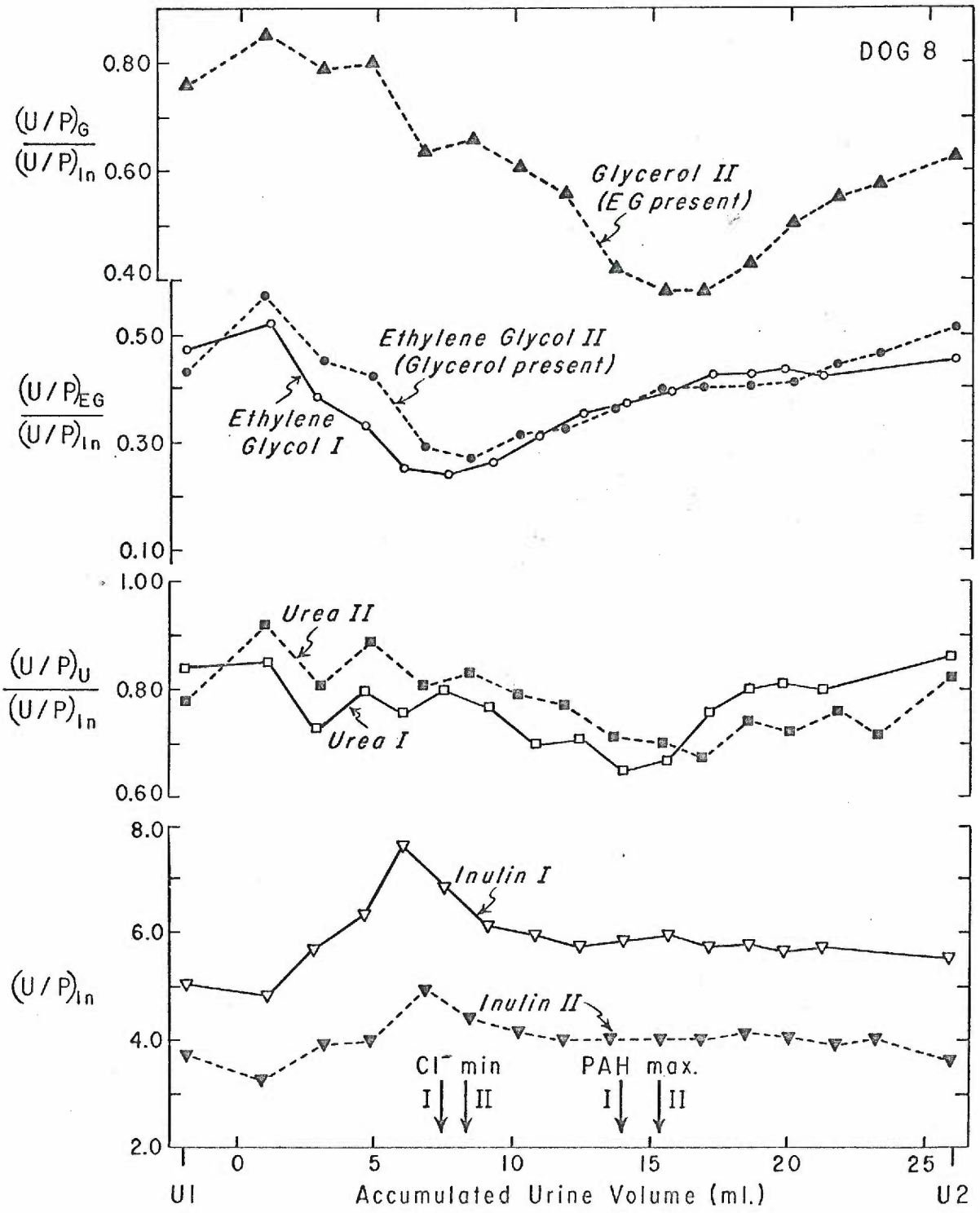

DOG 7
1.00
0.80
0.60
0.40
(U/P)G / (U/P)In
Glycerol I
Glycerol II
(EG present)
0.80
0.60
0.40
(U/P)EG / (U/P)In
Ethylene Glycol II
(Glycerol present)
0.90
0.70
0.50
(U/P)U / (U/P)In
Urea I
Urea II
5.00
4.00
3.00
2.00
(U/P)In
Inulin I
Inulin II
Cl⁻ min.
I II
PAH max.
I II
0
5
10
15
20
25
UI
Accumulated Urine Volume (ml.)
U2

Figure 5. Stop flow patterns of glycerol (G), ethylene glycol (EG), endogenous urea (U) and inulin (In) in the dog, plotted as in figure 4. Ethylene glycol (plasma concentration about 0.9 mg./ml.) present during period I, ethylene glycol (plasma concentration about 1.0 mg./ml) and glycerol (plasma concentration about 0.9 mg./ml.) during period II.


DOG 8
$\frac{(U/P)_G}{(U/P)_{In}}$
0.80
0.60
0.40
Glycerol II
(EG present)
$\frac{(U/P)_{EG}}{(U/P)_{In}}$
0.50
0.30
0.10
Ethylene Glycol II
(Glycerol present)
Ethylene
Glycol I
$\frac{(U/P)_U}{(U/P)_{In}}$
1.00
0.80
0.60
Urea II
Urea I
$(U/P)_{In}$
8.0
6.0
4.0
2.0
Inulin I
Inulin II
Cl⁻ min
I
II
PAH max.
I
II
0
5
10
15
20
25
UI
Accumulated Urine Volume (ml.)
U2

the low chloride concentration of the samples collected after release of the clamp. Similarily, fluid trapped in the proximal segment during this period may be identified by the high PAH concentration in the samples collected after release of the clamp. The accumulated urine volumes at which the minimum chloride/inulin and maximum PAH/inulin clearance ratios occurred, are indicated by arrows.

The minimum glycerol/inulin clearance ratios, indicating reabsorption, were observed in the tubular fluid samples containing the maximum PAH/inulin values. Reabsorption of glycerol in the proximal tubule during the period of ureteral clamp is shown by the decline of the stop flow pattern above the arrows indicating maximum PAH/inulin ratios. Reabsorption of glycerol in the proximal tubule was found in all dogs studied. The stop flow patterns did not indicate that there was any appreciable glycerol reabsorption in the distal nephron during the period of clamp (figures 4 and 5).

In one stop flow study, with a plasma glycerol concentration of 0.05 mg./ml., the proximal fluid samples contained glycerol concentrations that were less than the plasma concentration. This suggests that glycerol was able to move against a concentration gradient in the proximal tubule.

Figures 4 and 5 also show that, in contrast to glycerol, the minimum ethylene glycol/inulin clearance ratios occurred in the samples with the minimum chloride/inulin ratios, indicating that ethylene glycol was reabsorbed in the distal nephron during the period of ureteral clamp. Of particular interest is that fact that in the proximal tubule, where the larger glycerol molecule was reabsorbed,

there did not appear to be any appreciable reabsorption of the smaller, chemically similar, ethylene glycol molecule.

Figure 4 shows the typical stop flow patterns for glycerol obtained before and after ethylene glycol was added. In both cases reabsorption of glycerol occurred in the proximal tubule. However, when ethylene glycol was present less of the filtered glycerol was excreted as indicated by the generalized depression of the glycerol stop flow pattern during period II. This was a consistent finding in the stop flow studies and at no time did the two curves overlap. This decrease in the stop flow pattern (glycerol/inulin clearance ratios) occurred despite the fact that the U/P inulin ratios usually decreased (figures 4 and 5), and urine flow rates increased, during period II. This would indicate that less water was reabsorbed during period II and imply a decrease in the average urinary glycerol concentration. This would appear to indicate that the average concentration gradient for any possible passive diffusion of glycerol was actually lower during period II, than during period I.

Again, as indicated by the clearance studies, no consistent effect on endogenous urea excretion associated with the presence of ethylene glycol was seen in the stop flow patterns (figure 4 and 5). Nor were there changes in the stop flow patterns of PAH and chloride.

Figure 5 shows the stop flow patterns for ethylene glycol obtained before and after glycerol was added. In this instance, as in the clearance studies, an effect on ethylene glycol excretion associated with the presence of glycerol was not evident in the stop flow patterns.

An interesting finding in a few cases was that the endogenous

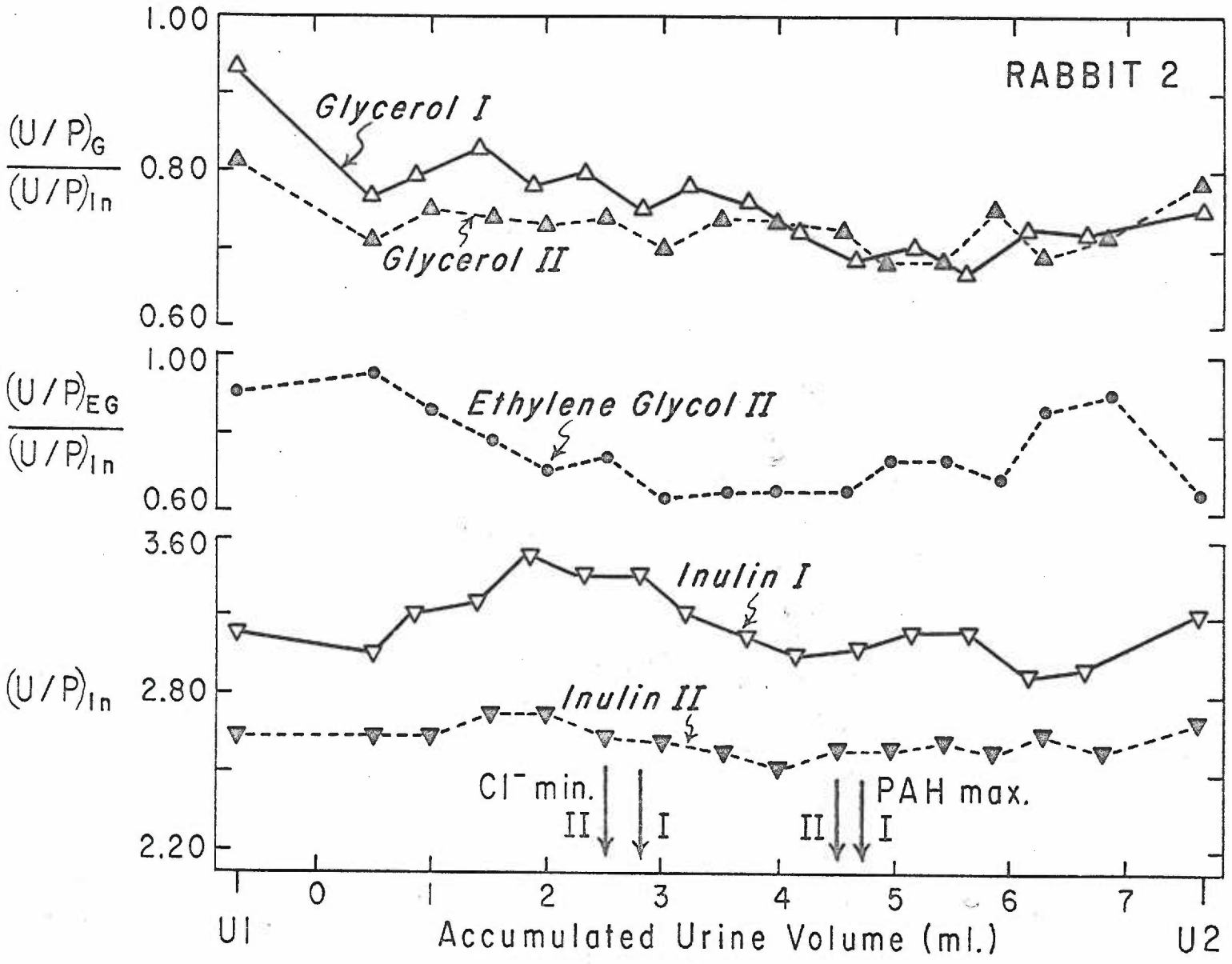
urea/inulin clearance ratio in some of the distal nephron samples was greater than 1.0.

Rabbits. The stop flow samples obtained from the two rabbits studies showed only small and inconsistent decreases in the glycerol/inulin clearance ratios in the tubular fluid samples to indicate the area where glycerol reabsorption took place. In one, the ratios did decrease slightly in the proximal fluid samples to show a slight fall in the stop flow pattern (figure 6). In the other, the PAH/inulin clearance ratio was reaching a maximum value while the chloride/inulin ratio was reaching a minimum value, indicating that filtration had continued during the period of ureteral clamp which may have obscured any reabsorption that occurred in the proximal tubule.

In both animals the ethylene glycol/inulin clearance ratios decreased in the distal fluid samples indicating that reabsorption had occurred in the distal nephron. This is shown in the ethylene glycol stop flow pattern in figure 6. The ethylene glycol clearance ratios in the proximal fluid samples were obscured, in one case, by the continuing filtration mentioned above. In the other case, the clearance ratios in the proximal fluid samples remained at about the value found in the distal fluid samples (figure 6) and was possibly due to the low urine flow and subsequent removal of ethylene glycol from the proximal fluid samples as they slowly moved through the distal nephrons. On the other hand, it may indicate that ethylene glycol is also reabsorbed in the proximal tubule of the rabbit.

As indicated by the clearance studies, the presence of ethylene glycol was not associated with a decrease in the glycerol/inulin

Figure 6. Stop flow patterns of glycerol (G), ethylene glycol (EG) and inulin (In) in the rabbit, plotted as in figure 4. Glycerol present during periods I and II, ethylene glycol during period II. Plasma concentrations about 1.0 mg./ml.


RABBIT 2
(U/P)G / (U/P)In
1.00
0.80
0.60
Glycerol I
Glycerol II
(U/P)EG / (U/P)In
1.00
0.60
Ethylene Glycol II
(U/P)In
3.60
2.80
2.20
Inulin I
Inulin II
Cl⁻ min.
II
I
PAH max.
II
I
0
1
2
3
4
5
6
7
UI
Accumulated Urine Volume (ml.)
U2

clearance ratios in the rabbit. This was also indicated in the stop flow patterns. In one animal, the stop flow pattern for glycerol, obtained in the presence of ethylene glycol, was consistently higher than the pattern obtained before addition of ethylene glycol. In the other animal (figure 6), there was no consistent difference between the two glycerol stop flow patterns.

The rabbit studies may suggest similarities, and differences, between the rabbit and canine kidney with regard to the excretion of these substances. However, these studies, based on only two rabbits, and low urine flows in one case, and replacement filtration in the other, do not provide an adequate basis for justifiable conclusions in this respect. Consequently discussion of results will be limited almost exclusively to the findings in the dog.

Postocclusive Injection Studies

Dogs. Figure 7 represents the data obtained from a study in which glycerol and inulin were injected after the ureter was clamped. Similar results were obtained in 3 other dogs.

Accumulated urine volumes are plotted on the horizontal axis. The urinary glycerol and inulin concentrations, expressed as a percentage of the final free flow urinary glycerol or inulin concentrations, are plotted on the vertical axis. It was predicted that if the tubular cells have an appreciable permeability to glycerol that it should appear in the tubular fluid samples ahead of inulin under these conditions.

Figure 7 shows that glycerol was not detected in the tubular fluid samples before inulin the glomerular marker and indicates that

Figure 7. Appearance in stop flow samples of glycerol and inulin injected intravenously during ureteral occlusion. Data plotted as in figure 4 with the exception that the concentrations of glycerol (closed circles) and inulin (open circles), expressed as a percentage of the postocclusive free flow urinary glycerol or inulin concentrations, are plotted on the vertical axis. After the ureter had been clamped 4 minutes, glycerol and inulin were injected over a 90 second interval. Total period of clamp was 8 minutes.

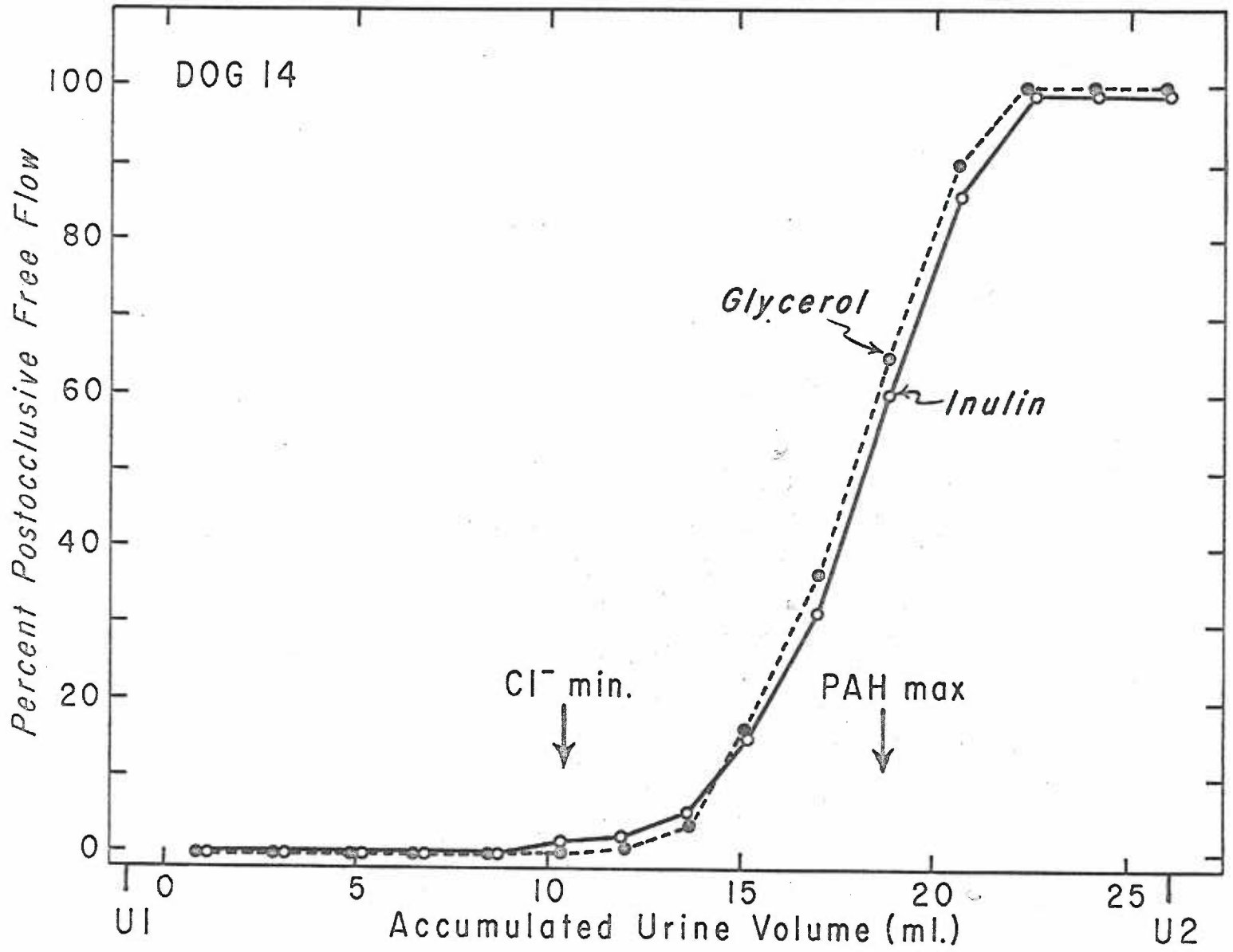
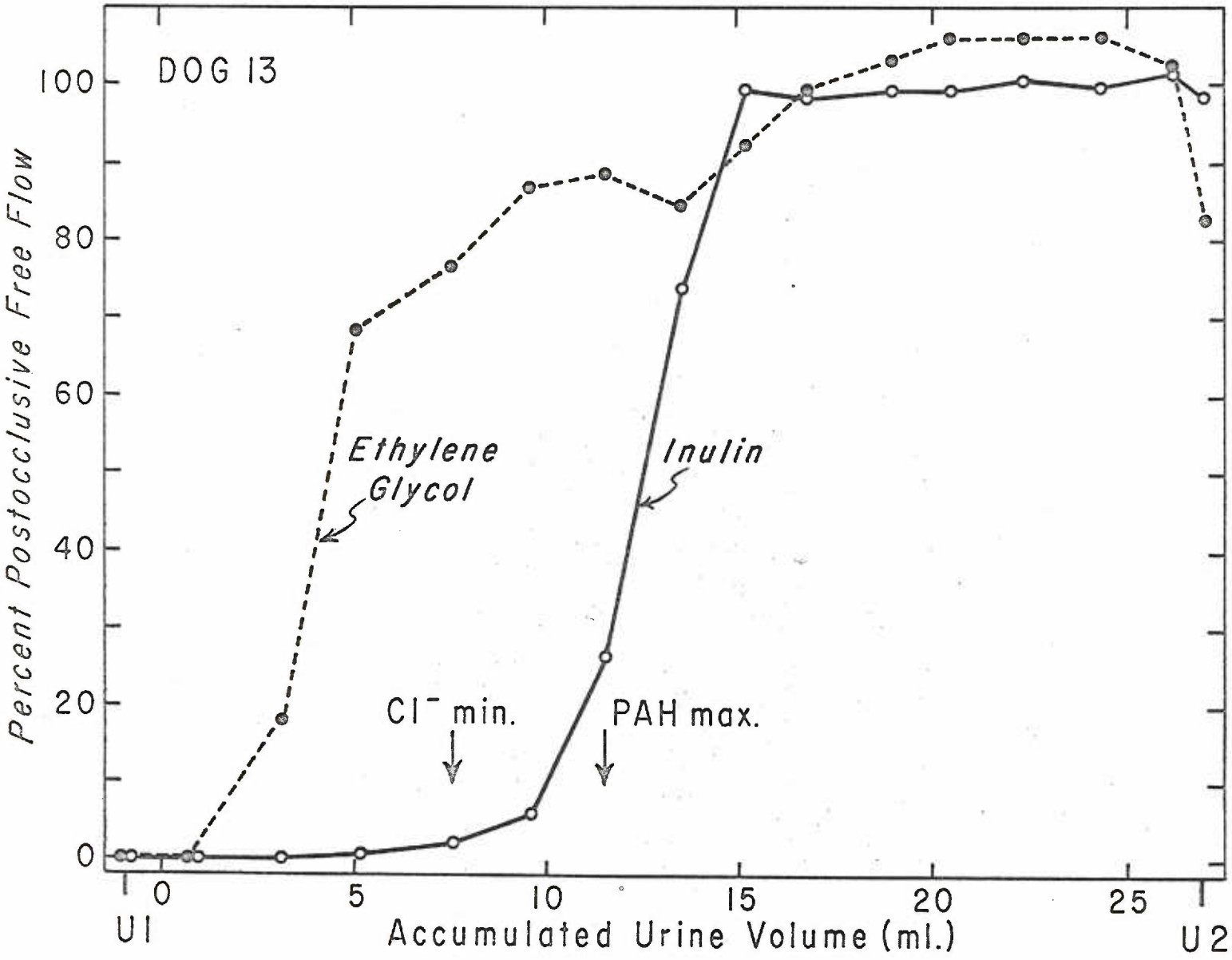

DOG 14
Percent Postocclusive Free Flow
100
80
60
40
20
0
Glycerol
Inulin
Cl⁻ min.
PAH max
0
5
10
15
20
25
U1
Accumulated Urine Volume (ml.)
U2

Figure 8. Appearance in stop flow samples of ethylene glycol (closed circles) and inulin (open circles) injected intravenously during ureteral occlusion. Data plotted as in figure 7. 3 minutes after the ureter was clamped, ethylene glycol and inulin were injected over a 55 second interval. Total period of clamp was 7 minutes.


DOG 13
Percent Postocclusive Free Flow
100
80
60
40
20
0
Ethylene Glycol
Inulin
Cl⁻ min.
PAH max.
0
5
10
15
20
25
U1
Accumulated Urine Volume (ml.)
U2

the glycerol found in the samples was probably filtered at the glomerulus together with inulin and was not transported across proximal or distal tubular cells, from the peritubular capillaries, in sufficient quantity during the period of ureteral clamp to be detected.

Figure 8 shows the typical results obtained when ethylene glycol and inulin were injected postocclusively. Similar results were obtained in all 3 dogs studied. In these cases ethylene glycol was detected in substantial quantities before inulin in the tubular fluid samples. Figure 8 shows that the sample which contained 86% of the final free flow ethylene glycol concentration contained only 6% of the final inulin concentration. These studies indicated that ethylene glycol moved from the blood into the lumen of the distal nephron along its concentration gradient during the period of ureteral clamp. It was not possible to determine from this type of study if ethylene glycol moved across the proximal tubular cells from blood to lumen.

DISCUSSION

Glycerol

The results of the clearance studies indicate that glycerol is reabsorbed by the renal tubules of both the dog and rabbit (clearance ratios less than 1.0). This has been shown or implied by the findings of others working with the rabbit* (56), cat (28), dog (37) and man (57). However studies on the fractions of the filtered load of glycerol actually excreted or reabsorbed by the dog or rabbit have not been previously reported or available.* In the cat (28) and in the human (57) it has been found that the fraction of filtered glycerol excreted, the glycerol/inulin clearance ratio, at no time exceeded 0.60. This was not found to be true in the species studied in the present experiments. The average fraction of the filtered glycerol that was excreted by the rabbit (0.79) and dog (0.75) was similar and exceeded the upper values reported for the cat and human.

With the wide overall (0.37 to 0.96, Table 3) and individual (0.65 to 0.92, figure 4) ranges obtained in the dogs at the higher plasma concentrations, it is not possible to state that the fraction of filtered glycerol excreted reaches a constant value with increases in the plasma glycerol concentration, or filtered load, as has been stated to occur in other species (28, 57). Such a relation would hold for a substance subject to only passive reabsorption (see Appendix B).

In the dog, the tubular transport rate of glycerol increased as the filtered load increased, over the ranges studied (figure 1). This

* As pointed out under Review of the Literature, Sveinsson (57) indicated that Holst had found the glycerol clearance ratio to be less than 0.60 in the rabbit.

has also been reported to occur in the cat (28) and is evident in the data available in human subjects (57).

The stop flow patterns seem to indicate in the dog that glycerol is reabsorbed in the proximal tubule and is relatively impermeable distally. This is in agreement with the single stop flow experiment data from the cat published by Kruhøffer and Nissen (28). Stop flow patterns have not been published previously for the dog.

In the frog, the proximal tubular cells are passively permeable to glycerol presented to the tubules via the peritubular capillaries (44). The interpretation given the data of previous postocclusive injection studies done on dogs (37) might seem, at first, to be at variance with the present injection studies. These authors believed the data showed that glycerol moved from the blood into the proximal tubular lumen and also, to a lesser extent, into the lumen of the distal nephron. Mention has already been made in the Review of the Literature concerning the possible difficulties in interpretation of their data in the form presented. Mention was also made that use of very sensitive analytical methods might indicate that movement occurred but this might not represent appreciable movement in a quantitative sense. The present studies indicated that neither the cells of the proximal or distal nephron were sufficiently permeable to glycerol to allow its detection before inulin in the tubular fluid samples (figure 6), whereas appreciable permeability to ethylene glycol in the distal nephron could be detected (figure 8). This is interpreted as indicating that quantitatively appreciable permeability to glycerol present in the blood is not present in either the distal or

proximal cells and is in agreement with the stop flow studies which indicated that glycerol was not reabsorbed in the distal nephron.

It has been stated that glycerol is actively reabsorbed by the kidney of the human, (57) and the dog (37), although the basis for the statement in the latter case is not clear. The data available on the cat (28) are also compatible with active reabsorption of glycerol.

In the present studies active reabsorption of glycerol in the proximal tubule of the dog is suggested by the following:

1. Glycerol U/P ratios less than 1.0 were observed in a clearance study and in the proximal fluid samples in a stop flow study. This indicates that glycerol was transported against a concentration gradient. Glycerol U/P ratios less than 1.0 have been reported in the cat (28) and in the human (57) and probably occur in the rabbit (56). The concept of metabolic conversion of glycerol (28) has been used to account for reabsorption at low, but not high, filtered loads of glycerol. This possibility has not been ruled out by the present experiments. However, it is unlikely to be the mechanism involved at high filtered loads (28).

2. Failure to demonstrate movement of glycerol from the blood into the proximal tubular lumen (postocclusive injection studies). This movement would be expected to occur if the proximal tubular cells were passively permeable to glycerol. The weak secretory movement of creatinine in male dogs (58) and movement of urea from blood to proximal tubular lumen in dogs (1, 37) can be detected by this type of study, and the procedure does not prevent blood flow from reaching all parts of the nephron.

3. Failure to demonstrate reabsorption of ethylene glycol in the proximal tubule (stop flow studies). If the proximal tubular cells were passively permeable to glycerol then the smaller, chemically similar molecule, ethylene glycol, would be expected to be passively permeable in this area. In a membrane that was passively permeable to both ethylene glycol and glycerol, it would also be predicted that the smaller ethylene glycol molecule would penetrate at a faster rate than the larger glycerol molecule. Such were the findings in the proximal tubule of the perfused frog kidney (44). Since the proximal tubular cells of the dog did not appear to be even passively permeable to ethylene glycol, let alone allow faster penetration of ethylene glycol, it is not likely that glycerol was reabsorbed by passive mechanisms in the proximal tubule.

It has also been stated in the literature (28, 37, 57) that the cells of the canine distal nephron are passively permeable to glycerol and that a passive component to glycerol reabsorption exists in the proximal tubule of other animals at high concentrations. The present studies do not indicate that cells in either the proximal or distal nephron of the dog are passively permeable to glycerol over the wide range of concentrations employed in these studies. The stop flow patterns (figures 4 and 5) and postocclusive injection studies (figure 7) did not indicate that there was any appreciable movement of glycerol across cells of the distal nephron. In the proximal tubule, the reabsorption of glycerol which occurred is not believed to be a simple passive process for the reasons mentioned above. This would be true irrespective of the glycerol concentration since if under any

conditions glycerol was passively permeable, ethylene glycol should also be passively permeable.

It is also interesting to note that urea, which is passively permeable in the distal nephron (collecting duct) and is able to accumulate in the renal medulla, is able to enhance the renal concentrating ability, but that glycerol, which the present studies indicate is not permeable in any part of the distal nephron, is not able to enhance the renal concentrating ability of dogs (41).

Ethylene Glycol

Clearance and stop flow studies of ethylene glycol excretion have not previously been reported. Schmengler and Höber (44) have presented evidence that the proximal tubular cells of the frog are passively permeable to ethylene glycol present in the peritubular capillaries.

The clearance studies show that ethylene glycol is reabsorbed by the renal tubules of both the dog and rabbit. The dogs, on the average, excreted about 50% of the ethylene glycol that was filtered. At high plasma levels in the dog, ethylene glycol was less efficiently excreted (0.53) than glycerol (0.75) and accordingly, at comparable filtered loads the tubular transport rates for ethylene glycol were greater than the rates for glycerol (figures 1 and 2). As was the case with glycerol, the tubular reabsorption rate of ethylene glycol increased as the filtered load increased, over the ranges studied.

The stop flow studies indicated that ethylene glycol was reabsorbed in the distal nephron. However, reabsorption was not found to take place in the proximal tubule during ureteral clamp in the dog.

This is in contrast to glycerol which was reabsorbed in the proximal tubule but not the distal.

The postocclusive injection studies in the dog showed, in contrast to the results obtained with glycerol, that the cells of the distal nephron are highly permeable to ethylene glycol so that it moves rapidly from the blood into the lumen of the distal nephron in quantitatively appreciable amounts. Urea, a molecule of similar size, has also been shown to move from the blood into the tubular lumen after postocclusive injection (1, 37). Movement of tritium-labeled water from blood into the distal nephron occurs in a similar manner (62).

The results of the ethylene glycol studies are in accord with reabsorption by passive movement of ethylene glycol along its concentration gradient in the distal, but not the proximal, nephron. Glycerol, on the other hand, is reabsorbed in the proximal, but not the distal, nephron, probably by an active transport process.

Glycerol and Ethylene Glycol

The clearance and stop flow studies in the dog did not indicate that ethylene glycol inhibited reabsorption of glycerol. They did indicate that the presence of ethylene glycol was associated with a decrease in the fraction of the filtered glycerol that was excreted (table 3, figure 4). That is, more of the filtered glycerol was reabsorbed when ethylene glycol was present. Statistical analysis indicates that this was not likely a chance observation.

To explain the effect of ethylene glycol on glycerol penetration

across the red blood cell membrane Stein (52, 53, 54) has proposed that glycerol and ethylene glycol form a dimer. The concentrations used in Stein's experiments are in substantial excess of the concentrations used in the present experiments. Also, it was only in the red blood cell without a glycerol carrier that an increase in glycerol penetration was noted when a glycol was present. The indications from the present study are that glycerol penetrates the renal tubular epithelium by an active process. Movement of glycerol by facilitated diffusion in the red blood cell is inhibited by ethylene glycol. Even if in the present experiments, ethylene glycol formed a dimer with glycerol which led to an increased reabsorption of glycerol, then it would be expected that ethylene glycol would also be reabsorbed at the same time. However, there was no indication from the stop flow studies that this took place.

The doses of ethylene glycol used in the present investigations were substantially below amounts associated with known toxicity (16, 24, 25, 34). The presence of ethylene glycol was not associated with changes in the clearance ratios, or stop flow patterns, of PAH, chloride or urea so that it seems unlikely that ethylene glycol had a nonspecific toxic effect on the renal tubules. Even if ethylene glycol were toxic to the tubular epithelium at these concentrations it might be expected that the fraction of filtered glycerol excreted would increase rather than decrease, if glycerol reabsorption is an active process. If ethylene glycol led to an increase in glycerol reabsorption by passive means, then evidence of ethylene glycol reabsorption, and increased urea reabsorption, would be expected.

However, in these experiments neither of these phenomena were observed.

It is conceivable that a decrease in the filtered load of glycerol, coupled with a continuation of a relatively constant glycerol transport rate, during the periods when ethylene glycol was present, could lead to a decrease in the glycerol/inulin clearance ratio. This was not felt to be the mechanism by which the glycerol/inulin clearance ratio decreased in the presence of ethylene glycol since the filtered load of glycerol actually was greater during the period when ethylene glycol was present in almost half the cases. Supporting this was the finding that the mean filtered load (in mg./min.) of glycerol before, 13.8 ± 2.4 (S.E.) was not significantly different ($P > 0.2$) than the mean filtered load after, 12.3 ± 3.4, the addition of ethylene glycol.

In any event there is insufficient evidence from the present studies to elucidate the mechanism which causes the decrease in the fraction of filtered glycerol excreted when ethylene glycol is present. It is suggested that none of the possibilities previously mentioned (i.e. ethylene glycol - glycerol dimer formation, ethylene glycol toxicity, or a fall in the filtered load of glycerol with a constant transport of glycerol) play a major role.

Urea

Urea/inulin or urea/creatinine clearance ratios greater than 1.0 have been observed by others (1, 2, 8, 26, 27, 40). With the exception of errors in the chemical determination, or secretion, a possible explanation is that during the stop flow period, urea, to which the collecting duct is highly permeable, accumulates in the medulla, with

release of the stop flow, tubular fluid samples with lower urea concentrations arrive in the collecting ducts and the direction of the gradient is reversed so that urea moves from the interstitium into the collecting duct containing these samples which may then contain urea in amounts greater than was filtered (1, 26).

SUMMARY AND CONCLUSION

The renal tubular reabsorption of glycerol, and the chemically similar compound, ethylene glycol, was studied in dogs, and in two rabbits, by means of clearance and stop flow methods. Localization of movement from blood to tubular lumen was also studied in the dog by intravenous injection of glycerol or ethylene glycol midway during a period of ureteral clamp.

1. Glycerol was reabsorbed in the proximal tubule of the dog and possibly the rabbit. Reabsorption of glycerol was not apparent in the distal nephron.

2. Ethylene glycol, in contrast, was reabsorbed in the distal nephron of the dog and rabbit. It was not reabsorbed in the canine proximal tubule.

3. At low plasma glycerol concentrations evidence was obtained that glycerol was reabsorbed against a concentration gradient.

4. At high plasma levels, more of the filtered glycerol, than ethylene glycol, was excreted.

5. Movement of glycerol from peritubular capillaries into the proximal or distal tubular lumen in quantitatively appreciable amounts was not detected.

6. A relatively large movement of ethylene glycol from peritubular capillaries into the lumen of the distal nephron was detected.

7. Tubular transport maxima were not observed for either glycerol or ethylene glycol over the range of filtered loads present.

8. The presence of ethylene glycol was associated with a decrease in the fraction of filtered glycerol excreted.

The results indicate that two similar nonelectrolytes, glycerol and ethylene glycol, are handled differently by the dog kidney. Glycerol, but not ethylene glycol, is reabsorbed in the proximal tubule, probably by an active transport mechanism. In contrast, ethylene glycol, but not glycerol, is passively reabsorbed in the distal nephron. A mechanism to account for the decrease in glycerol excretion when ethylene glycol was present is not suggested by the present studies.

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APPENDIX A

Chemical Determination of Glycerol and Ethylene Glycol.

1. Titration Method

This method is a modification of semimicro volumetric methods outlined by Shupe (46) and by Newburger and Bruening (36) and allows determination of glycerol when ethylene glycol is present in the sample. Their methods were designed for analysis of cosmetic ingredients and required a period greater than one hour to perform. Modifications were developed which permitted the rapid determination of small concentrations in biological fluids. The chemical reactions involved correspond to the following equations:

Glycerol		Potassium Periodate		Formaldehyde		Potassium Iodate				Formic Acid
H_2COH $HCOH$ H_2COH	+	$2KIO_4$	$\longrightarrow$	$2HCHO$	+	$2KIO_3$	+	H_2O	+	$HCOOH$

Ethylene Glycol								
H_2COH H_2COH	+	KIO_4	$\longrightarrow$	$2HCHO$	+	KIO_3	+	H_2O

Propylene Glycol								Acetaldehyde
H_2COH $HCOH$ H_2CH	+	KIO_4	$\longrightarrow$	$HCHO$	+	KIO_3	+	CH_3CHO

The formic acid, which is formed only when glycerol is oxidized by the periodate, is determined with sodium hydroxide after the excess periodate is utilized by addition of propylene glycol. The pH is monitored with a Radiometer pH Meter.

Known amounts of glycerol are added to the sample blanks to obtain a standard curve from which the concentration in the experimental samples can be read.

The urine samples can be analyzed for glycerol directly. Glycerol can be determined directly in the plasma, or as was most often the case, in a perchloric acid ($HClO_4$) filtrate of plasma. The advantages of a perchloric acid filtrate are discussed below.

Reagents:

0.02 M KlO_4	4.0 N H_2SO
0.02 N NaOH	6.7 N KOH
0.02 M H_2SO_4	0.8 M $HClO_4$

concentrated propylene glycol

Procedure:

1. Transfer appropriate sample, in duplicate, to a 10 ml. beaker.
2. Dilute with 0.9% saline to an adequate volume.
3. Place magnetic stirring rod in beaker.
4. Place beaker on stirring platform, insert pH electrode into solution and stir continuously.
5. Adjust plasma samples to pH 4 or less and allow 2 minutes for CO_2 to be driven off. This step is not necessary with the plasma perchloric acid filtrate samples.
6. Adjust all samples to pH 6.000 with the 0.02 M H_2SO_4 or 0.02 N NaOH reagents.
7. Add 2.0 ml. of 0.02 M KlO_4. Wait 90 seconds.
8. Add two drops of concentrated propylene glycol to stop the

reaction.

9. Titrate back to pH 6.000 with 0.02 N NaOH by means of a syringe microburet.

Preparation of Perchloric Acid Filtrate:

1. To 4.0 ml. of 0.8 M $HClO_4$ and 1.0 ml. of water, add 1.0 ml. of plasma.

2. Centrifuge and remove supernatant.

3. Add 0.5 ml. of 6.7 N KOH to 5.0 ml. of supernatant.

4. Chill in refrigerator for 1 hour and centrifuge to precipitate $KClO_4$.

5. Add one drop of 4.0 N H_2SO_4 to supernatant of step 4 to obtain acid pH.

Use of the perchloric acid filtrate of plasma provided three advantages over direct use of plasma. The titration end point was more sensitive with the buffering proteins removed. CO_2 from the samples was driven off due to the acidity of the filtrate. The plasma blank value determined on the perchloric acid filtrate was usually 30-40% lower than the value obtained by direct titration of plasma.

Preliminary investigation revealed that 96-100% of the glycerol in an aqueous perchloric filtrate could be recovered. The aqueous filtrate blank value was zero.

92-109% (mean 100%) of the glycerol in plasma perchloric acid filtrates was recovered. The mean plasma blank value determined on perchloric acid filtrates of plasma from 12 dogs was 0.20 mg./ml. It was found that glucose contributed significantly to this plasma blank value. Glucose however is oxidized more slowly than glycerol.

Stopping the reaction with propylene glycol 1.5 minutes after adding KIO_4, when only 50% of the glucose present has been oxidized, decreases the plasma blank value by 20-40%. 98% of the glycerol is oxidized in 1.5 minutes.

The experimental plasma filtrate blank value, uncorrected for glucose (mean 0.09 mg./ml. in 8 dogs), was subtracted from the value found in the experimental samples containing glycerol. This makes it unnecessary to correct for the glucose present in the experimental samples unless the plasma glucose concentration changes during the experiment. Plasma glucose determinations were done so that corrections could be made if necessary.

Glucose is not usually detected in the urine samples of the dog so that a glucose correction was not necessary. It was found however that glucose was present in rabbit urine and it was necessary to make corrections for the varying glucose concentrations in the stop flow urine samples from the rabbits.

95-98% of the glycerol added to urine samples was recovered. The blank urine value varies with the urine flow rate. With flow rates less than 1.0 mg./min., the blank value was usually greater than 0.40 mg./ml. in the dog. Flow rates above 2.0 ml./min. usually were associated with blank values less than 0.40 mg./ml. (mean 0.23 mg./ml. in 9 samples) and flows greater than 3.0 ml./min. were associated with values less than 0.20 mg./ml. (mean 0.10 mg./ml. in 9 samples). The blank value continues to decrease as flow increases, so that with flows greater than 6.0 ml./min. the value often was zero.

The presence of urea, PAH, heparin, ethylene glycol and formaldehyde did not affect the determination of glycerol. The presence of

creatinine may give a falsely low value, and inulin a falsely high value, with this procedure. However if the aliquot used for the determination has less than 0.20 mg. of creatinine, and less than 1.0 mg. of inulin, their presence does not affect the glycerol determination.

2. Colorimetric Method (61).

This method makes use of the fact that compounds with adjacent partially oxidized carbon atoms at the end of a chain are further oxidized with periodate to yield formaldehyde. The formaldehyde is then measured colorimetrically. Both glycerol and ethylene glycol yield formaldehyde under these conditions (see chemical equations under Titration Method above). The mean recovery of known amounts in the plasma filtrate was 99% (98-103%).

Glycerol was determined by either the titration or colorimetric method. Ethylene glycol was determined with the colorimetric method. When both glycerol and ethylene glycol were present in the sample, the colorimetric method was used to determine the total ethylene glycol plus glycerol concentration. The glycerol concentration, as determined on an aliquot of the same sample by the titration method, was then subtracted to obtain the ethylene glycol concentration.

Agreement between the colorimetric and titration methods was good. The mean difference between the plasma values obtained by the two methods was 3.4% $\pm$ 0.8 (S.E.), the mean difference between the urine concentrations was 3.4% $\pm$ 0.4 (S.E.).

APPENDIX B

Certain authors (28, 57) have observed that:

1) the transport rate of glycerol increases as the plasma concentration, or filtered load, of glycerol increases;

2) the fraction of the filtered glycerol excreted (or reabsorbed) tends to become constant and independent of the filtered load or plasma concentration of glycerol.

These observations are considered to be an indication that glycerol is reabsorbed by simple passive diffusion. This section considers the above observations in terms of active and passive transport.

1. Transport

   a. Passive

   The tubular transport rate, T_d, of a substance that is passively permeable to the tubular cells should be proportional to the concentration difference between the tubular fluid and plasma.

$$T_d = K\,(TF_x - P_x)$$

$$T_d = KP_x\,[(TF/P)_x - 1]$$

T_d is the transport rate of a passively diffusing substance x. K is a permeability constant. TF_x is the tubular fluid concentration. P_x is the plasma concentration. $(TF/P)_x$ is greater than 1.0 for a passively reabsorbed substance.

In general, an increase in the filtered load of x is accomplished by increasing the plasma concentration of x. As the above equation indicates, T_d should increase as the plasma concentration of x increases if the substance is passively reabsorbed and water reabsorption remains constant

(which implies a constant $(TF/P)_x$ ratio).

b. Active

The transport rate of an actively reabsorbed substance (e.g. carrier mediated transport) will also increase with increases in the plasma concentration, or filtered load, until the point is reached at which the carrier is "saturated". Once this point of saturation is reached, as reflected by the Tm, further increases in the plasma levels will not result in further increases in the transport rate.

However, transport maxima have not been demonstrated for all substances known to be actively transported (39). In such a case, the transport rate continues to increase as the plasma levels increase. Also, if the transport rate approaches the maximum rate gradually, as is the case with a titration curve (T_x vs. P_x) which shows a high degree of "splay", carrier mediated transport increases over a wide range of plasma concentrations (38, 48, 49).

Thus evidence that the transport rate increases with increases in the plasma concentration or filtered load can be consistent with either active or passive transport.

2. Fraction of Filtered Load Excreted

a. Passive

The fraction of the filtered load that is excreted is given by the clearance ratio:

$$C_x/C_{In} = U_x\dot{V}/GFR \cdot P_x = (U/P)_x/(U/P)_{In}.$$

One minus this quantity is the fraction of the filtered load

that is reabsorbed.

For a passively reabsorbed substance, the $(U/P)_x$ ratio is a function of water reabsorption and T_x. The degree to which water is reabsorbed is indicated by the $(U/P)_{In}$ ratio. For a given $(U/P)_{In}$ ratio, fractional water reabsorption is constant and the $(U/P)_x$ ratio is also approximately constant. The clearance ratio, $(U/P)_x/(U/P)_{In}$, would accordingly be constant and independent of the plasma concentration of a substance that is reabsorbed by simple passive mechanisms.

b. Active

In the case of a substance reabsorbed by active mechanisms, once the transport maximum is reached, the fraction of the filtered load that is excreted will rise with increases in the plasma concentrations. This can be seen from the following equations which state that the rate at which the substance is filtered ($C_{In} \cdot P_x$) is equal to the rate at which it is removed from the tubular fluid (T_x) plus the rate at which it appears in the urine ($U_x\dot{V}$):

$$C_{In} \cdot P_x = T_x + U_x\dot{V}$$

$$C_{In} = T_x/P_x + U_x\dot{V}/P_x = T_x/P_x + C_x$$

It is clear that when transport is at a maximum value (Tm_x), T_x/P_x will approach zero as P_x increases and C_x will approach C_{In} at high P_x values. The C_x/C_{In} ratio would not remain constant as P_x is increased, until a value of 1.0 is reached. If however the transport rate did not reach a maximum value at any P_x achieved in the experiment (see 1 b

above), then T_x would increase with increasing plasma levels and the C_x/C_{In} ratio might be relatively constant for a substance that is actively transported (47, 48, 49).

Thus evidence that the clearance ratio is constant with increasing plasma concentrations or filtered loads can be consistent with either active or passive transport.

Appendix C. Protocol and results of a clearance experiment (dog 5, a 13.6 kg. female).

Time, min.	$\dot{V}$	P_G	$U_G\dot{V}$	C_G	P_{EG}	$U_{EG}\dot{V}$	C_{EG}	P_{In}	C_{In}	C_G/C_{In}	C_{EG}/C_{In}	C_u/C_{In}
-67	Priming dose of 1.7 gm. Na_2SO_4, 1.2 gm. inulin and 0.1 gm. PAH, followed by constant infusion of 0.17 gm. Na_2SO_4, 0.03 gm. inulin and 1.2 mg. PAH, per min.											
-28	Priming dose of 10.3 gm. glycerol, followed by constant infusion of 0.05 gm. glycerol/min.											
0-6	3.8	100	15.9	16.0				44	22.4	0.72		0.83
6-13	3.8	(92)	14.0	15.2				(45)	22.1	0.69		0.80
13-18	3.8	84	12.8	15.2				46	21.5	0.71		0.77
18-23	Priming dose of 9.2 gm. ethylene glycol, followed by constant infusion of 0.04 gm. ethylene glycol/min.											
55-60	5.1	78	11.1	13.8	113	12.4	10.9	52	19.6	0.70	0.58	0.87
60-65	5.2	(80)	10.9	13.5	(125)	13.0	10.4	(54)	19.6	0.69	0.53	0.82
65-70	5.3	82	10.9	13.2	137	11.4	8.3	55	19.9	0.66	0.42	0.82

Only values obtained from left kidney are presented (kidney weight 36.8 gm.) Values in parentheses are averages of the preceding and succeeding values. $\dot{V}$ = urine flow in ml./min., P = plasma concentration in mg./100 ml., $U\dot{V}$ = excretion rate in mg./min., C = clearance value, G = glycerol, EG = ethylene glycol, In = inulin, C_u = urea clearance.