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Diuretic Action of Glycerol in Dogs.* (31880)

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Diuretic drugs that inhibit the renal tubular reabsorption of sodium are often ineffective in edematous patients with hyponatremia. Drugs that could preferentially inhibit the renal tubular reabsorption of water would therefore be useful. Osmotic diuretics such as mannitol and urea cause relatively greater urinary excretion of water than of sodium (1,2) but have certain practical disadvantages clinically.

Glycerol has been given orally to treat glaucoma(3-6) and cerebral edema(6,7). Oral and intravenous glycerol have been noted to cause a diuresis(6-9), the characteristics of which have not previously been reported. Experiments were performed in dogs to characterize the nature and magnitude of this effect of glycerol, prior to its trial as a diuretic in humans.

Methods. Subjects. Twelve female mongrel dogs weighing between 11 and 19 kg were anesthetized with iv pentobarbital 30 mg per kg and then maintained with small supplementary doses. Infusions were given through a polyethylene catheter advanced into the inferior vena cava. Urine was collected from an indwelling catheter; collection periods were initiated and terminated by the instillation and aspiration of air. Heparinized blood was obtained at the midpoint of each clearance period from an indwelling jugular needle.

Glomerular filtration rate (GFR) was usually estimated by the clearance of inulin; the clearance of exogenous creatinine was estimated in those experiments in which fructose was given. Equilibrating infusions were given for between 80 and 150 minutes prior to the control periods in order to assure stability of sodium excretion ($U_{Na}V$). Urine flow (V) was either stable or decreasing slightly during the 2-3 ten minute control periods. Four dogs received constant sustaining infusions of 154 mM NaCl and 4 others were given infusions of 38-51 mM NaCl with 1.5-3.3% glucose or fructose.

Meralluride (0.75 ml) was given intravenously to 3 dogs prior to glycerol, and to 2 others during glycerol diuresis. Another dog received pitressin before glycerol, and one other during glycerol diuresis. The pitressin-treated dogs and 2 of those that received meralluride were given sustaining infusions of 77 mM NaCl with 4-5% mannitol.

Glycerol was added to the sustaining infusions in concentrations of 16-25% (v/v) and was given at a rate of 0.67-1.0 ml/min (0.7-1.3 mOsm/k/min) for 40-60 minutes. Urine collections were restarted 3 to 18 minutes after starting glycerol. Neither hemolysis nor glycosuria were observed.

Analytical. Inulin and creatinine were measured by methods previously reported(10). Sodium and potassium concentrations were measured with an Autoanalyzer, chloride concentrations with a Cotlove chloridometer, and osmolalities with a Fiske osmometer.

Plasma volumes were estimated with T-

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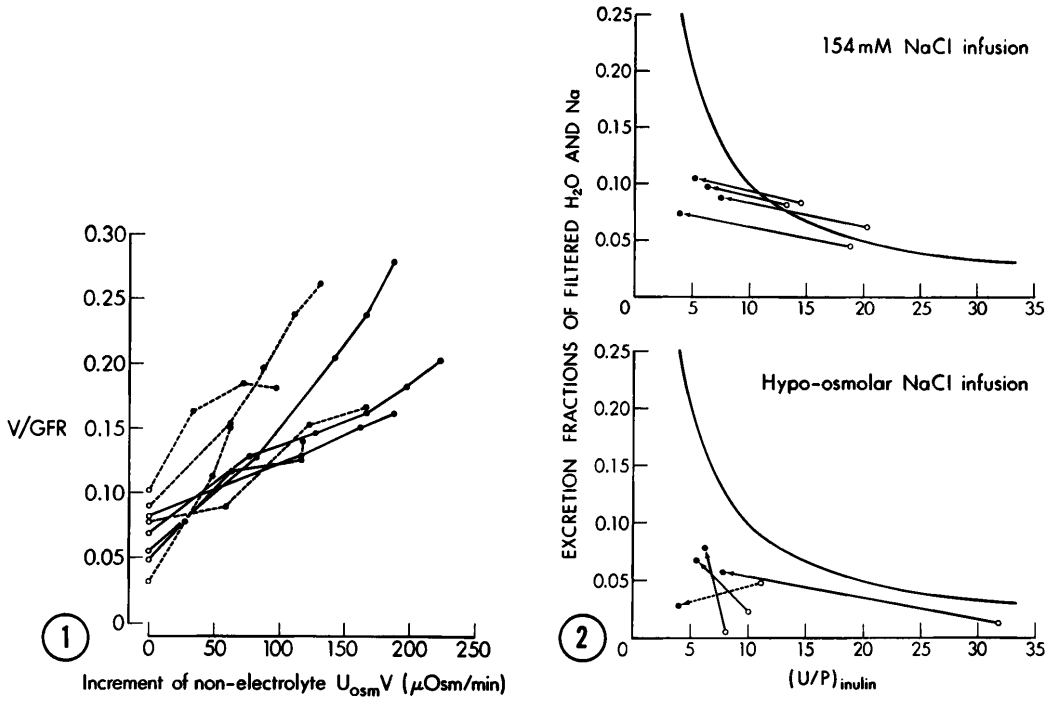

Fig. 1. Relation between excretion fraction of filtered water and urinary osmolal excretion. —— 154 mM NaCl infusion; - - - - hypo-osmolar NaCl infusion; O average control values, ● values during glycerol infusion.

Fig. 2. Relation between excretion fractions of filtered water and sodium and $(U/P)_{inulin}$. The hyperbolic curves show that V/GFR was inversely proportional to the decrease of $(U/P)_{inulin}$ during diuresis. The open (control) and black (glycerol diuresis) points refer to values for the sodium excretion fraction. – – – – Na values in hypokalemic dog (see text).

1824 in 5 experiments(11). Blood packed cell volumes (PCV) were measured in all experiments after 30 minutes centrifugation at 3000 rpm. Blood hemoglobin concentrations (Hb) were determined using a cyanmethemoglobin method(12).

The plasma water content and the Donnan factor were not used to convert the measured plasma Na and K concentrations to their respective concentrations in glomerular filtrate. Omission of these corrections had no important effect upon the calculation or interpretation of the observed changes. The contribution of electrolytes to urinary osmolality was calculated as $2 \times (U_{Na} + U_K)$, neglecting the small error introduced by not correcting for the activity coefficient of NaCl.

Results. Diuresis always started within 5 minutes of beginning the glycerol infusion and its magnitude was related to the increased urinary solute excretion. This relationship was evident when the excreted fraction of filtered water (V/GFR) was compared with either total $U_{osm}V$ or with the increment in urinary non-electrolyte osmolal excretion above control values (Fig. 1). The increment of non-electrolyte osmolal excretion = $(U_{osm}V)$ — [(2 × $U_{(Na + K)}$ V) + (average non-electrolyte $U_{osm}V$ during control periods)]. Plasma osmolality increased an average of 13% over control values (range 7-23%) during glycerol infusion. Plasma sodium increased slightly during glycerol infusion in 2 of 4 dogs receiving 154 mM NaCl, and decreased slightly in 1 of 4 receiving hypo-osmolar NaCl. Osmolal clearance (C_{osm}) increased and free water clearance decreased during glycerol diuresis.

When 154 mM NaCl was infused throughout, urine sodium concentrations during the control periods were 125 mM per L or higher and *decreased* during glycerol diuresis. When 38-51 mM NaCl was infused throughout,

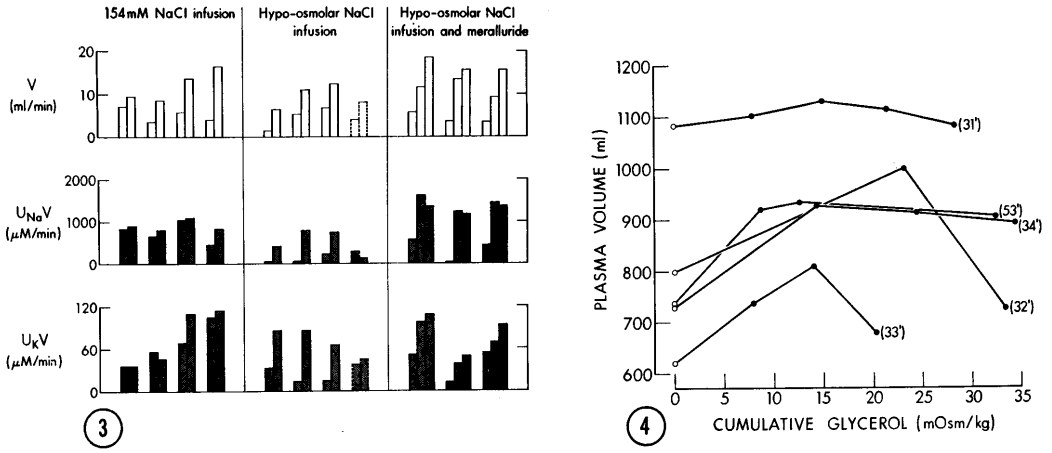

Fig. 3. Urine volume and sodium and potassium excretion during glycerol diuresis. Each bar set gives the average of the control values (left), and the peak values during glycerol diuresis (right) in one dog. The center bars in the mercurial experiments give the average of the peak mercurial diuresis results. The dash-encompassed values in the hypo-osmolar NaCl group refer to the hypokalemic dog.

Fig. 4. Plasma volume changes during glycerol infusion. Cumulative infused glycerol is plotted along the abscissa. Open circles — control values. The numbers in parentheses refer to the total time of glycerol infusion to that point.

urine sodium concentration usually *increased* during glycerol diuresis, except in one dog with hypokalemia. This animal appeared malnourished, had a plasma K concentration of 2.7 mM per L, and its urine was hypo-osmolar to its plasma even during the height of glycerol diuresis. Except in this hypokalemic dog, urine sodium concentrations were between 50 and 100 mM per L during glycerol diuresis regardless of the sodium concentration in the infused fluid.

Fig. 2 shows the relation between the excretion fractions of filtered water (the hyperbolic curves) and of filtered sodium (the connected points, $U_{Na}V/F_{Na}$), and the urine to plasma concentration ratios (U/P) for inulin in the dogs receiving 154 mM NaCl and in those receiving hypo-osmolar NaCl. The hyperbolic curve that relates V/GFR and $(U/P)_{in}$ derives from the mathematical relationship between these two parameters. $V/GFR = V/(UV/P)_{in} = (P/U)_{in}$. Thus, $(V/GFR) \times (U/P)_{in} = (P/U)_{in} \times (U/P)_{in} = 1$. When the excretion fraction of Na is equal to that of water, the points indicating $U_{Na}V/F_{Na}$ lie *on* the hyperbolic curve, in which case $U_{Na} = P_{Na}$. When U_{Na} was less than P_{Na}, less of the filtered Na than of the filtered water was excreted and the values for $U_{Na}V/F_{Na}$ fell in the area between the origin and the curve. During infusions of 154 mM NaCl (Fig. 2, top) proportionately more filtered sodium than filtered water was usually excreted during the control periods, whereas these findings were reversed during glycerol diuresis. When hypo-osmolar NaCl was infused (Fig. 2, bottom) relatively more filtered water than filtered sodium was excreted during the control periods and also during glycerol diuresis.

Fig. 3 shows the values of V, $U_{Na}V$ and U_KV during control periods and at the height of glycerol diuresis. Urine volume increased more than $U_{Na}V$ in the group receiving 154 mM NaCl, whereas in the dogs that received hypo-osmolar NaCl the increases in both V and $U_{Na}V$ were considerable except in the hypokalemic animal. Urinary potassium excretion usually increased during glycerol diuresis (Fig. 3), U_KV/F_K reaching values as high as 0.5. There were, nevertheless, no significant changes in the concentration of plasma potassium during glycerol infusion. Urinary Cl concentration was proportional to U_{Na} in all experiments.

Plasma volume first increased (Fig. 4), and then tended to plateau or decrease during continued glycerol administration. Hemoglo-

bin concentration and PCV decreased proportionately during glycerol infusion indicating that the decreases of PCV were not secondary to erythrocyte shrinkage.

When meralluride was given *before* glycerol to 3 dogs (2 receiving 77 mM NaCl and 1 receiving 46 mM NaCl) urine volume increased by 100 to 260% and $U_{Na}V$ by 174 to 5800%, while GFR increased by only 8 to 40%. The infusion of glycerol during peak sustained mercurial diuresis resulted in a decrease of GFR toward control pre-mercurial levels. Fig. 3 shows that despite this decrease of GFR, V increased by 15 to 59% above the levels during peak mercurial diuresis; $U_{Na}V$ decreased slightly (average 9%). Administration of meralluride to 2 other dogs *during* glycerol diuresis resulted in modest additional increases of V (8% and 22%), and of $U_{Na}V$ (16% and 9% respectively).

One dog was given a single dose of 1 unit of pitressin intravenously during glycerol diuresis, and another was given a sustaining infusion of 100 μunits per kg per minute before and during glycerol diuresis. The diuresis continued unchanged despite a modest fall of GFR in both animals.

Discussion. The onset of diuresis following glycerol administration was rapid. Its magnitude was related to the increment in non-electrolyte solute excretion (Fig. 1), attributable largely to unreabsorbed glycerol in the renal tubules. Exogenous glycerol has been shown to be partially reabsorbed by the proximal renal tubules in cats(13) and in humans(14). The mechanism of this reabsorptive process is uncertain, but it may be one of passive diffusion related to metabolism of glycerol within the renal tubular cells(13). Other non-electrolytes (for example, urea) may also have been excreted in increased amounts during the diuresis but these probably represented only a small portion of the increment in $U_{osm}V$. Urine sodium and potassium excretion usually increased (Fig. 3), and $U_{Cl}V$ paralleled $U_{Na}V$. The following findings were characteristic of an osmotic diuresis: 1) urine Na concentrations were between 50 and 100 mM per L, and 2) there was a relatively larger increase of V than of $U_{Na}V$ during 154 mM NaCl infusion (1, 2, 15).

Changes of plasma sodium concentration *during* the infusion of an osmotic diuretic depend upon 1) degree of permeation of the agent into cells, and 2) the external balance of sodium and water, which depends in turn upon rates of infusion and of urine excretion of sodium and water. Plasma sodium concentrations changed very little during glycerol infusion even though plasma osmolality increased. The infusion of solutes that penetrate cells poorly (*e.g.,* mannitol) tends to cause a decrease of plasma sodium as water shifts from cells to extracellular fluids. Complete information concerning the permeability of cells to glycerol is unavailable but human erythrocytes appear to be very permeable(8) whereas certain "tissue compartments", such as the brain and anterior chamber of the eye, are presumably less readily penetrated by glycerol(3-7). The net loss of water was greater than the net loss of sodium during glycerol infusion in the dogs that received infusions of 154 mM NaCl; slight increases of plasma sodium (of 5 and 8 mEq per L) were observed in 2 of these 4 dogs. In those that received hypo-osmolar NaCl, the negative balance of sodium was somewhat greater than that of water in 3 dogs; in only 1 of them was a slight decrease of plasma sodium (of 8 mEq per L) observed, however.

Following an osmotic diuresis, plasma sodium concentration should increase because of the relatively greater excretion of filtered water than of sodium. Osmotic diuretics might therefore be useful clinically in hyponatremic edematous patients who are refractory to drugs that inhibit renal tubular reabsorption of sodium. An increase of plasma sodium concentration following an osmotic diuresis might result in restoration of responsiveness to conventional saluretic drugs.

The use of the available osmotic diuretics presents certain practical problems in the management of patients with edema. Urea MW 60) can be given orally as a 50% solution but it is unpalatable. Mannitol (MW 180) can be given only intravenously in a maximum concentration of 25%. Both substances therefore entail the concomitant administration of relatively large volumes of

water. Glycerol (MW 92, specific density 1.26) can be given orally in high concentrations (70-100%) so that it is possible to administer very large quantities. It is partially metabolized to glucose(16) and chronic administration in rats has been reported to increase plasma triglycerides(17). However, its anticipated clinical use as a diuretic would be for brief periods so that these considerations should not pose major problems.

Plasma volumes first increased during the infusion of glycerol and then reached a plateau or decreased (Fig. 4). The levelling off was presumably a consequence of the following factors: 1) approaching equilibrium between the rate of glycerol infusion on the one hand and its rates of urinary excretion and intracellular distribution on the other, and 2) increasing negative external balance of sodium and water with continuing diuresis.

Summary. Glycerol produced an osmotic diuresis when given intravenously to dogs. The magnitude of the diuresis was proportional to the increased urinary solute excretion, and urine sodium concentration ranged between 50 and 100 mM per L. When glycerol was given together with a mercurial diuretic there were additive effects on water excretion, but sodium excretion was not enhanced beyond that produced by the mercurial.

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Nutritional Requirements for Reproduction of a Temperature Sensitive Nematode, Reared in Axenic Culture.*† (31881)

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Two strains of the free-living nematode *Caenorhabditis elegans,* a self-fertilizing hermaphrodite, show a differential nutritional requirement for reproduction(1,2) which is a function of temperature(3). Both strains reproduce normally when grown between 10°-18°C under axenic conditions in a liver medium. When the temperature is raised to 20°C, reproduction of the heat-sensitive or Bergerac(2,3) strain is variable; at 23°-25°C the worms grow to adult size, but no reproduction occurs. The worm's heat-sensitive period commences at the onset of the last molt. The heat-resistant or Bristol(1,3) strain reproduces consistently at the above

* This paper is dedicated to the memory of Dr. Ellsworth C. Dougherty who has guided me in this research. The material reported herein was used in a thesis submitted to the Graduate Division of the University of California by the author, in partial fulfillment of requirements for the Ph.D. degree.

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