The rate and quantity of placental iron transport in swine was observed to be somewhat less than that reported for sheep(5). This could be explained by the differences in physical complexity of the 2 placentae. If placental transfer is governed partly by membrane complexity, then more iron would be expected to traverse the ovine than the porcine placenta, as reported.

Values in Table III show that a standardized litter of 8 pigs contained 119 mg iron at 105 days gestation. Since a total of 280 mg iron was accreted in the total fetal complex during the 105 day period, iron transfer to the total fetal complex per day was calculated as 2.67 mg. Therefore, if available iron was transferred into the placental complex as needed the added daily iron requirement of pregnancy in swine was 2.67 mg. Assuming maternal dietary absorption to be 10%, the sow would need to consume an additional 26.7 mg iron per day to meet these demands for fetal growth and development. The ration utilized in this study contained 463 ppm iron. From these figures it can be calculated, therefore, that an additional 57.6 g feed per day would be required to supply the additional 2.67 mg iron for the developing litter in a pregnant sow.

Summary. Results of radio-chemical studies with 9 pregnant and 3 control gilts dosed intravenously with a single tracer injection of Fe^{59} citrate, and sacrificed after 48 hours, indicated Fe^{59} values to increase 7-fold between 35 and 70, and 5-fold from 70 to 105 days gestation, respectively. Total fetal iron content increased from 0.18 mg at 35 days to 4.3 and 14.9 mg at 70 and 105 days, respectively. Fetal liver and spleen exhibited greatest contribution to hematopoiesis during early gestation, while these organs and red bone marrow became increasingly important as pregnancy advanced. Results of this study indicated that iron is transferred across the placental membranes of swine at each trimester, with quantity and rate increasing progressively with each stage of gestation.

Appreciation is expressed to B. Patterson, B. Cowan and Ann Gaydos for technical assistance.

1. Brunschwig, A. E., *Anat. Rec.*, 1927, v34, 237.
2. Bothwell, T. H., Pribilla, W. F., Mebust, W., Finch, C. A., *Am. J. Physiol.*, 1958, v193, 615.
3. Pommerenke, W. T., Hahn, P. F., Bale, W. F., Balfour, W. M., *ibid.*, 1942, v137, 164.
4. Pond, W. G., Lowery, R. S., Maner, J. H., Loosli, J. K., *J. Animal Sci.*, 1961, v20, 747.
5. Hoskins, F. H., Hansard, S. L., *J. Nutrition*, 1964, v83, 1.
6. Hansard, S. L., Plumlee, M. P., Hobbs, C. S., Comar, C. L., *J. Animal Sci.*, 1951, v10, 88.
7. Barkan, G., Walker, B. S., *J. Biol. Chem.*, 1940, v135, 37.
8. Wong, S. Y., *ibid.*, 1928, v77, 409.
9. Crosby, W. H., Munn, J. L., Furth, F. W., *U. S. Armed Forces Med. J.*, 1954, v5, 693.

Received January 31, 1964. P.S.E.B.M., 1964, v116.

Ethacrynic Acid: A Drug Which Abolishes the Concentrating Ability of the Kidney.* (29144)

K. MACGAFFEY,† L. A. LEZOTTE, JR.,† J. H. SNYDER, E. W. MOORE AND H. JICK
(Introduced by T. C. Chalmers)
Clinical Pharmacology and Renal and Electrolyte Divisions of the Medical Service, Lemuel Shattuck Hospital, Department of Public Health, Commonwealth of Massachusetts, and Department of Medicine, Tufts University School of Medicine

Numerous studies describing the effect of diuretics on the concentrating and diluting operations of the kidney have been reported (1-4). Such studies have led to information regarding localization of action of various diuretics in the tubule. Early, Kahn and

* This investigation was supported by grants from U. S. Public Health Service and by gifts from Merck, Sharp and Dohme Co.

† Work done during tenure of Clinical Pharmacology Traineeships, awarded to Tufts Univ. by Nat. Heart Inst.

Orloff(1) have shown a decrease in free water clearance associated with administration of chlorothiazide in maximally hydrated dogs. The authors suggested that this agent blocked sodium transport in the distal nephron where free water is formed. Porush *et al* (2), and Goldstein *et al*(3) demonstrated that administration of mercurials was unassociated with any effect on the concentrating or diluting operations in man, and suggested a site of action beyond the concentrating and diluting sites. During these experiments(2,3) mannitol, a nonspecific solute diuretic, was demonstrated to produce an augmentation of free water reabsorption (T^cH_2O).

Preliminary studies by Cannon *et al*(5) on the recently synthesized compound 2,3-dichloro-4-(2-methylene butyryl) phenoxyacetic acid, (ethacrynic acid), have indicated that this is a potent natriuretic agent which produces a large increase in osmolar clearance and a slight decrease in free water clearance in hydrated subjects. The authors suggested that both a proximal and a distal tubular action was present. The present studies were designed to study the effect of ethacrynic acid on the concentrating operation.

Materials and methods. Five patients with cirrhosis of the liver and 2 patients with carcinoma were studied. Each patient had substantial fluid retention and was receiving a 500 mg sodium diet containing at least 75 g of protein per day. An indwelling catheter was used in the female patients, but the men were allowed to void spontaneously.

Six female mongrel dogs eating normal *ad lib* diets were studied. The average weight was 15 kg. The animals were anesthetized with i.v. sodium pentobarbital prior to study and an indwelling catheter was placed in the bladder. Blood was obtained at appropriate intervals through an indwelling arterial needle.

All subjects studied were in the hydropenic state having been fasted for 16 hours and given 5 units of vasopressin tannate in oil 12 hours prior to study. Clearance of inulin (C_{In}) was determined in most studies by standard techniques previously described(6). Aqueous pitressin in dosage of at least 200 mU/hr was infused during all studies.

The following studies were carried out:

1. Each subject received a rapid infusion of 10% mannitol to determine the control T^cH_2O curve with a non-specific solute.

2. Ethacrynic acid was given in a single oral dose of 200 mg to 3 human hydropenic subjects, and i.v. in the dose of 1 mg/kg to 4 hydropenic dogs. In humans after the peak volume flow had been obtained a rapid mannitol infusion was superimposed.

3. Ethacrynic acid was given in the same dosage by the same route to hydropenic subjects (4 humans and 5 dogs), after a steady mannitol diuresis had been established.

Osmolality, sodium, potassium, and inulin determinations were made on most blood and urine samples. Laboratory methods have previously been described(6). Solute clearance (C_{osm}) was calculated from the formula $C_{osm} = \frac{U_{osm}}{P_{osm}} \cdot V$ where U_{osm} represents urine osmolality and P_{osm} represents plasma osmolality. T^cH_2O was calculated from the formula $T^cH_2O = C_{osm} - V$.

Results. Since the data appeared comparable in humans and dogs they are discussed together.

A. *Effects on water conservation.* I. Mannitol alone. Hypertonic manntol produced a characteristic rise in T^cH_2O with increasing C_{osm} in all subjects. Values for T^cH_2O at approximately peak osmolar clearances are given in Table I. Representative values of T^cH_2O over a range of C_{osm} from 3 to 16 ml/min in 2 of the 7 human subjects are shown in Fig. 1.

II. Ethacrynic acid alone. When ethacrynic acid was given alone there was a progressive rise in urine volume and C_{osm} which began almost immediately after I.V. administration (4 dogs) and at approximately 20 minutes after oral administration (3 humans). The pre-drug control C_{osm} averaged 1.8 $\pm$ 0.5 ml/min (1 SE) in dogs, and 1.9 $\pm$ 0.23 ml/min in humans. The maximum rise in C_{osm} averaged 7.0 $\pm$ 0.5 ml/min in dogs ($P < 0.01$) and 8.3 $\pm$ 1.6 ml/min in humans ($P < 0.05$).

In the initial phase of the diuresis T^cH_2O either was maintained at control levels or

TABLE I. Comparison of Effect of Mannitol and Ethacrynic Acid (E.A.) on T^cH_2O at Comparable Osmolar Clearances.

Subject	Study*	Volume (ml/min.)	C_{osm} (ml/min.)	T^cH_2O (ml/min.)	U_{osm} (mOsm/l)	P_{osm} (mOsm/l)
			I. Human Studies			
1	a.	12.6	16.0	3.4	399	313
	b.	15.8	15.8	0	302	302
2	a.	7.6	10.1	2.5	400	302
	b.	10.8	10.8	0	320	320
	c.	7.9	8.4	.5	324	300
3	a.	17.8	21.2	3.4	363	304
	b.	21.8	21.7	−.1	287	288
4	a.	12.5	15.4	2.9	382	311
	b.	15.5	15.4	−.1	293	295
5	a.	10.0	11.3	1.3	333	294
	b.	11.9	12.0	.1	288	285
6	a.	10.4	12.2	1.9	362	308
	b.	11.7	12.0	.3	312	305
	c.	9.8	10.6	.8	316	286
7	a.	6.0	8.3	2.3	437	315
	b.	8.9	9.3	.4	317	305
	c.	8.7	8.8	.1	305	302
			II. Dog Studies			
A	a.	3.7	6.3	2.6	540	320
	b.	6.1	6.7	.6	364	330
	c.	6.4	6.7	.3	320	305
B	a.	4.5	7.0	2.5	522	337
	b.	7.8	7.9	.1	336	333
	c.	7.5	7.6	.1	318	311
D	a.	10.2	14.8	4.6	500	344
	b.	14.3	14.3	0	354	354
	c.	7.0	7.6	.6	333	308
E	a.	9.0	15.7	6.7	565	324
	b.	15.3	15.5	.2	350	347
F	a.	5.8	9.2	3.4	515	324
	b.	8.9	9.4	.5	358	338
	c.	9.0	9.6	.6	326	305
G	a.	5.6	8.3	2.7	500	336
	b.	8.0	8.2	.2	338	330

* a. = Mannitol alone; b. = Mannitol plus E.A.; c. = E.A. alone.

showed a small transient rise. Following this there was a precipitous fall in U_{osm} to levels very close to plasma in all subjects and T^cH_2O therefore approached zero (Fig. 1) (Table I).

III. Ethacrynic acid and mannitol. When ethacrynic acid was superimposed upon a steady mannitol diuresis (or vice versa) the effects on C_{osm} and T^cH_2O were qualitatively the same as those obtained with ethacrynic acid alone. T^cH_2O at comparable values of C_{osm} for mannitol, ethacrynic acid and the combination are given in Table I. There was a substantial rise in C_{osm} with an abrupt fall in T^cH_2O to zero and in some cases to slightly negative values (Fig. 1, 2). The initial rise or maintenance of T^cH_2O observed when ethacrynic acid was given alone was not observed when it was superimposed on a mannitol diuresis (Fig. 2). The effects on T^cH_2O are further evident in Fig. 3, where mannitol points lie at a greater distance from the isosmotic line, indicating considerable T^cH_2O formation; values for ethacrynic acid cluster around the isosmotic line, indicating T^cH_2O values near zero.

B. *Effect on electrolyte excretion.* In all instances administration of ethacrynic acid

was associated with a massive natriuresis and a moderate kaliuresis, as has been previously reported(5). In the 3 humans receiving the drug alone the increase in sodium excretion ($U_{Na}V$) was 1076 ± 121 μeq/min. When ethacrynic acid was superimposed on a steady mannitol diuresis in 4 humans, $U_{Na}V$ rose 2600 ± 335 μeq/min from control levels with mannitol alone. The increase in potassium excretion (U_KV) averaged 156 ± 71 μeq/min with the drug alone and 302 ± 77 μeq/min when ethacrynic acid was given during a steady infusion of 10% mannitol. Comparable increases in $U_{Na}V$ and U_KV were obtained in dogs (Fig. 2).

Inulin clearance (C_{In}) showed a mean decrease after ethacrynic acid of 12.9 ± 2.3 ml/min ($P<0.01$) from mean control values in 5 humans, but the mean decrease in C_{In} in dogs was 6.3 ± 6.8 ml/min which was not statistically significant. The "sodium rejection fraction" (C_{Na}/C_{In}) at the time of maximum diuretic effect with ethacrynic acid alone was 11% and 16% of filtered sodium in 2 humans in whom calculations could be made. When the drug was superimposed on a mannitol diuresis values for $U_{Na}V$ in 3 humans at time of peak drug effect were 32, 34 and 46% of filtered sodium.

Discussion. In the present studies administration of ethacrynic acid was consistently associated with a marked decrease in ability of the kidney to concentrate the urine. According to current concepts of renal physiology concentration of the urine occurs in relation to the following events: sodium is actively transported out of the ascending loop of Henle essentially without water; as a result the medullary interstitium is kept hypertonic to plasma, whereas the tubular fluid entering the distal convoluted tubule is dilute with respect to plasma; in the presence of ADH tubular fluid becomes isotonic to plasma in the distal convolution, and in the collecting duct, where passive diffusion of water into the interstitium occurs, the urine becomes hypertonic.

In hydropenic subjects administration of a nonspecific solute such as mannitol induces a characteristic increase in T^cH_2O as C_{osm} increases. In contrast, the increase in C_{osm} resulting from administration of ethacrynic acid was associated with a fall in T^cH_2O, which approached zero. The abolishment of

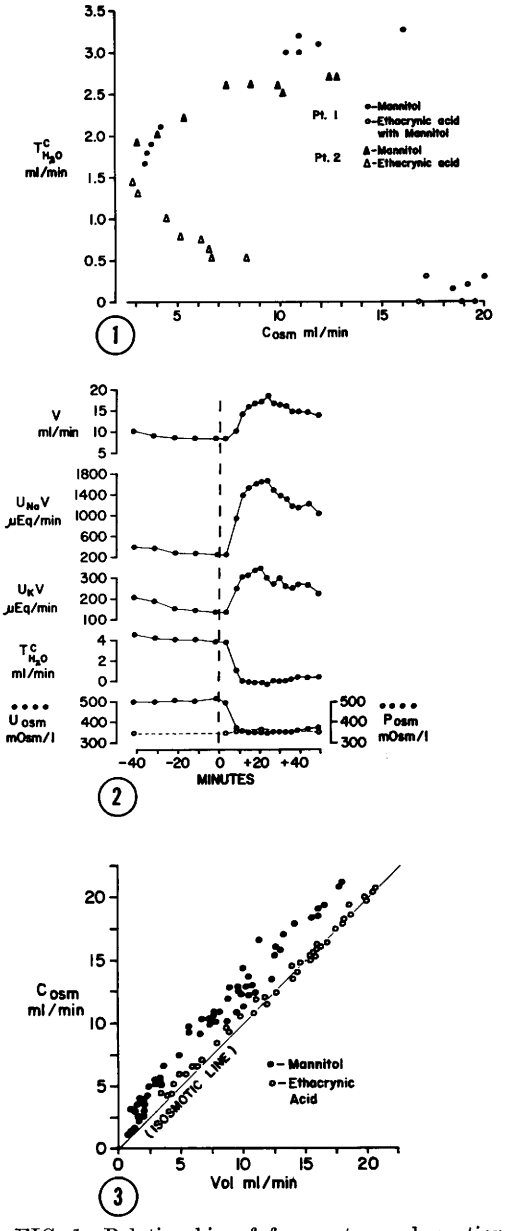

FIG. 1. Relationship of free water reabsorption ($T^c_{H_2O}$) to solute excretion (C_{osm}) during diuresis with ethacrynic acid and mannitol in 2 patients.

FIG. 2. Results of a representative study (Dog D) showing effects of 14 mg of IV ethacrynic acid superimposed on a steady mannitol diuresis. (E.A. given at time zero.)

FIG. 3. Effects of ethacrynic acid and mannitol on $T^c_{H_2O}$ formation in humans.

T^cH_2O by this agent is not prevented by prior or concomitant administration of mannitol. This latter finding indicates that decreased delivery of sodium to the loop of Henle cannot be responsible for the observed effect on T^cH_2O.

One explanation for the abolishment of concentrating ability by ethacrynic acid is that the drug blocks sodium transport in the ascending loop of Henle, with the result that the medullary interstitium rapidly becomes isotonic to plasma and T^cH_2O is abolished. Another possibility is that the drug decreases the permeability of the collecting duct membrane to water, even in the presence of maximal ADH, so that tubular fluid entering from the distal convolution does not come into osmotic equilibrium with the medullary interstitium. Since the decrease in T^cH_2O is accompanied by a profound sodium diuresis, it seems more reasonable to explain the two phenomena through a single mechanism, *i.e.*, a blocking of sodium transport in the ascending loop of Henle. Such an action would not only eliminate the formation of T^cH_2O, but also deliver more sodium into the urine. The hypothesis that ethacrynic acid decreases collecting duct permeability to water seems unlikely since one would have to postulate a separate action to explain the natriuresis. Furthermore, the urine obtained in the present studies following ethacrynic acid was essentially isosmotic to plasma, indicating that the distal tubular membrane was permeable to water, so that the tubular fluid reached equilibrium with the surrounding interstitium in the cortex. The second hypothesis would then require a selective effect on the collecting duct membrane as differentiated from the distal tubular membrane, which is unlikely. Further evidence against this hypothesis was reported by Cannon *et al*(5) who showed that this drug in hydrated subjects was associated with decreased free water clearance, which might suggest an increase in membrane permeability rather than a decrease. While neither of these hypotheses is established by the present data, the first seems more likely for the reasons given.

The magnitude of the sodium diuresis seen with ethacrynic acid strongly suggests that the drug inhibits sodium reabsorption elsewhere in the tubule besides the loop of Henle. Cannon *et al*(5) observed an average peak "sodium rejection fraction" of 22% of filtered sodium in a group of subjects consisting of 2 normals, 2 patients with cardiac failure and 2 patients with cirrhosis. A somewhat smaller fraction (11 and 17%) was noted in 2 humans in the present studies. The present data showing that the initial sodium diuresis is associated with no change or a transient but significant increase in T^cH_2O when ethacrynic acid is given alone also suggest that this agent blocks sodium reabsorption at site(s) other than the loop of Henle, and that this action may precede the effect in the loop by a brief period of time. This action is apparently not demonstrable when the drug is given with mannitol (Fig. 2).

Summary. Studies to demonstrate the effect of ethacrynic acid on the urine concentrating ability of the kidney in maximally dehydrated humans and dogs have been described. Results indicate that the drug essentially abolishes T^cH_2O in association with a massive sodium diuresis. The most likely explanation of the effect on urine concentration is an inhibition of sodium transport in the ascending loop of Henle. Additional sodium blocking action at a site(s) other than the loop of Henle is postulated to account for the magnitude of the sodium diuresis.

Addendum. Since submission of this article for publication, Goldberg *et al* (*J. Clin. Invest.*, 1964, v43, 201) have reported that ethacrynic acid markedly impaired the urine concentrating mechanism in normal human subjects.

We wish to thank Miss Ann Long and Mrs. Margaret Wilcox for supervising the care of the patients on the metabolic unit and Mrs. Antigone Letsou for dietary supervision.

1. Earley, L. E., Kahn, M., Orloff, J., *J. Clin. Invest.*, 1961, v40, 857.

2. Porush, J. G., Goldstein, M. H., Eisner, G. M., Levitt, M. F., *ibid.*, 1961, v40, 1475.

3. Goldstein, M. H., Levitt, M. F., Hauser, A. D., Polimeros, D., *ibid.*, 1961, v40, 731.

4. Au, W. Y. W., Raisz, L. G., *ibid.*, 1960, v39, 1302.

5. Cannon, P. J., Ames, R. P., Laragh, J. H., *J.A.M.A.*, 1963, v185, 854.

6. Jick, H., Snyder, J. G., Finkelstein, E. M., Cohen, J. L., Moore, E. W., Morrison, R. S., *J. Clin. Invest.*, 1963, v42, 1561.

Received February 5, 1964. P.S.E.B.M., 1964, v116.

Relationship of the Adenovirus Erythrocyte-Receptor-Modifying Factor to the Type-Specific Complement-Fixing Antigen. (29145)

JULIUS A. KASEL AND MARGRET HUBER

Department of HEW, Public Health Service, Nat. Inst. Health, National Institute of Allergy and Infectious Diseases, Laboratory of Clinical Investigations, Bethesda, Md.

Infection of tissue cultures with adenovirus types 1, 2, 4, 15 and possibly type 5 results in formation of a factor demonstrable by its capacity to reduce the agglutinability of human O erythrocytes for 3 of the adenovirus serotypes which agglutinate these cells (1,2). This substance, termed the "erythrocyte-receptor-modifying factor," was distinguishable physically, serologically, or biologically from the infectious particles, the soluble group complement-fixing antigen(3, 4,5) and the cell-detaching factor(6). Since the erythrocyte-receptor-modifying factor was neutralized type-specifically, experiments were done to determine its possible relationship to the soluble type-specific complement-fixing antigen(4,5). This report describes results of these experiments.

Materials and methods. Virus preparations. Stock virus pools were prepared from prototype strains of adenovirus types 1 and 2. These strains, which had been previously propagated in cultures with medium containing 5% chicken serum, were passaged an additional 5 times (0.1 ml of 1:100 dilution) in HeLa tube cultures* with Eagle's basal medium(7). Following the final passage, cultures were frozen and thawed 5 times, pooled, centrifuged for 10 minutes at 2000 rpm and the supernatant fluid stored at —70°C until used.

Virus suspensions were reduced 100-fold in volume with polyvinylpyrrolidone (PVP)† by the method described by Kohn(8). This procedure was carried out at room temperature. Prior to use, suspensions were dialyzed overnight at 4°C against 0.01 M phosphate buffer solution pH 7.2.

Antisera preparation. Serum-free adenovirus suspensions prepared with either primary rabbit kidney or hamster kidney cultures were used to prepare antisera in rabbits as previously described(3).

Chromatography procedure. Fractionation of adenovirus suspensions was done by column chromatography with DEAE-cellulose‡ at room temperature, essentially using the procedure described by Wilcox and Ginsberg (5). Following addition of a suitable volume of concentrated and dialyzed adenovirus suspension to a DEAE column which had been previously equilibrated with 0.01 M phosphate buffer pH 7.2, fractions were collected by stepwise elution. Twenty-five ml of 0.01 M phosphate buffer solution pH 7.2 (0.0 M NaCl) and buffer solutions containing increasing concentrations of NaCl were added to the column and the effluents collected separately under conditions of free-flow. Each fraction was reduced in volume 10-fold by dialysis against PVP.

Assay of eluates. Each eluate was assayed for biologic activity, and presence of complement-fixing antigens against rabbit antiserum prepared with a crude type 1 suspension and human antiserum from volunteers experimentally infected with adenovirus type 26. The tests for demonstrating erythrocyte-receptor-modifying (ERM) activity and inhibition

* Tissue cultures were obtained from Microbiological Associates, Inc., Bethesda, Md., and Flow Laboratories, Rockville, Md.

† General Aniline and Film Corp., Linden, N. J.

‡ Eastman Organic Chemicals, Rochester, N. Y.