controls and 4 experimentals in each exposure, in a manner described by Goldfeder, *et al.* (*6*) for mice and with backscatter made uniform by use of a rice phantom, in accordance with the technique of Ellinger (*3*).

Following irradiation, treatment was continued for the duration of the experiment. Animals dying were autopsied. Clinical symptoms were graded twice weekly with emphasis on visible manifestations of anemia and purpura. Albinism lent itself best for the assessment of such symptoms.

In several experiments involving a total of 230 animals it was observed that, under the conditions described, 220–225 r consistently killed 67% of the animals, with 50% dead within 13 days. With the exception of occasional secondary infections, recovery from radiation injury usually was complete within 30 days; hence the experiments were terminated, although the animals remained under observation for several weeks thereafter.

The data in Table 1 illustrate the effect of "calcium flavonate."

TABLE 1

	Total No. guinea pigs used	Total No. died	Died %	50% death time (days)
Controls	45	30	67	13
Treated	26	9	35	..

The hemorrhagic symptoms (petechial hemorrhages, ecchymoses, generalized purpura) of the treated animals were considerably less marked than those of the controls.

It is concluded that under the experimental conditions described, a flavonoid preparation derived from lemons, administered in the drinking water, reduces the mortality from total-body roentgen irradiation by about half. In our opinion this justifies the employment of smaller laboratory animals than the dog in further studies of this nature and may offer an intact animal assay for vitamin P-like substances. Studies are in progress in attempts to elucidate the molecular configurations necessary for higher activity of the flavonoids and related substances by this and similar techniques.

References

1. Clark, W. G., and Geissman, T. A. *Fed. Proc.*, 1948, **7**, 21.
2. Clark, W. G., and Geissman, T. A. (To be published.)
3. Ellinger, F. *Radiology*, 1945, **44**, 125.
4. Fuhrman, F. A. *Fed. Proc.*, 1948, **7**, 38.
5. Fuhrman, F. A., and Crismon, J. M. *J. clin. Invest.*, 1948, **27**, 364.
6. Goldfeder, A., Cohen, L., Miller, C., and Singer, M. *Proc. Soc. exp. Biol. Med.*, 1948, **67**, 272.
7. Griffith, J. Q., Jr. *Proc. Inst. Med., Chicago*, 1947, **16**, 434.
8. Griffith, J. Q., Jr., Anthony, E., Pendergrass, E. P., and Perryman, R. *Proc. Soc. exp. Biol. Med.*, 1947, **64**, 322.
9. Griffith, J. Q., Jr., Couch, J. F., and Lindauer, M. A. *Proc. Soc. exp. Biol. Med.*, 1944, **55**, 228.
10. Rekers, P. E., and Field, J. B. *Science*, 1948, **107**, 16.
11. Sevin, A. *C. R. Acad. Sci.*, 1943, **216**, 505.

Urinary Flow, Excretion of Solutes, and Osmotic Work During Diuresis of Solute Loading in Hydropenia in Man

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Interest in the study of the osmotic limitations of the kidney has been renewed in the last years after having lapsed since the early studies by Korányi and others (*5*). Gamble, in studies of man during water deprivation (*4*), came to the conclusion that 1,400 milliosmols/liter represented the maximum urinary concentration attainable. Given this fixed maximum value, a minimal urinary volume could then be calculated from any solute load. Studies by McCance and co-workers (*6*) added the information that forced osmotic diuresis in hydropenia produced by urea, sodium chloride or bicarbonate, and potassium chloride increased urinary flow while decreasing the level of urinary osmolarity. The conclusion was reached that for the substances studied the total solute concentration rather than the level of any one solute determined the maximum osmolarity of the urine.

Another aspect of the osmotic relationships of the kidney concerns the work involved to produce the observed concentration differences between urine and plasma. The concept of renal osmotic work was first applied by Dresser (*2*), Galeotti (*3*), and Rhorer (*8*). Later a general thermodynamic treatment of the minimal work necessary for the production of urine was given by Borsook and Winegarden (*1*). Recently Newburgh (*7*) refocused attention on the application of the concept and presented a discussion of the clinical implications of concentration changes which tend to reduce the renal work.

The studies to be reported were undertaken in order to determine in a broad manner, by the use of loading substances of various kinds, the pattern of urinary flow and excretion of solutes under the condition of water restriction. Furthermore, it was hoped to find out whether under such conditions a biologic maximum of renal osmotic work existed and, if so, whether it was the same or different for varying solutes. Such a maximum of work, if extant, would represent a measure of over-all work capacity of the kidney, with possible physiological and clinical implications.

The subjects were boys, 8–15 years of age, with normal renal function and without major disease. One group of young diabetic patients was included for the study of forced diuresis produced by glucose. The loading substances were administered as a rule in amounts of 500–2,000 milliosmols/1.73 m^2 of body surface, by the oral or, more often, the intravenous route, in concentrated

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solution. The subjects were fasting and had received no water for 16 hrs previous to the experiment, and were excreting urine at rates of 0.5 ml/min or less (except the diabetic subjects). Eleven loading substances were studied: glucose, sucrose, mannitol, sorbitol, sorbose, xylose, urea, creatinine, sodium *p*-aminohippurate, sodium sulfate, and sodium chloride. In some experiments the entire course of diuresis, until the urine flow returned to preloading levels, was followed; in others chief emphasis was laid on observing the periods of maximum flow. Blood and urine were collected at appropriate intervals and were analyzed for the loading solute, as well as sodium, potassium, phosphorus, chloride, urea, and, in some instances, sulfate. On urine the freezing point was also determined, and the total osmolarity at infinite dilution calculated. The solutes determined accounted as a rule for $85 \pm 10\%$ of the total solutes in the preloading urine specimens and for as much as 90–95% of the total during diuresis. The loading solute (or two solutes in the case of salts) accounted usually for $60 \pm 10\%$ of the total. Fifteen subjects were studied in this manner over 111 periods of collection.

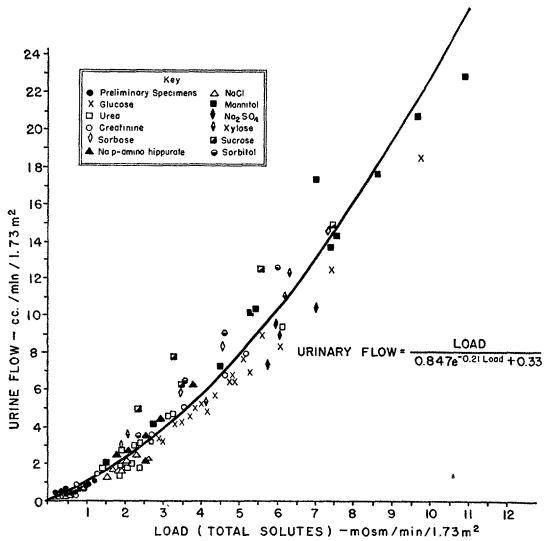

FIG. 1. Urine flow vs. solute excretion in man during hydropenia. Data on 111 periods, including those of minimal flow and those during diuresis forced by various loading substances, are included. The solid line drawn through the points represents the values calculated from the formula,

$$\text{Urinary flow} = \frac{\text{load}}{0.847e^{-0.21\ \text{load}} + 0.33}.$$

As may be seen from Fig. 1, a constant relationship prevailed between solute excretion and urine flow, regardless of the nature of the predominant solute in the urine. This relationship held over the observed range of urine flows from less than 0.5 ml to 24 ml/min/1.73 m². Considering the variety of substances studied, it is evident that the pattern of urinary flow is independent of the nature of urinary excretion, whether it be predominantly by filtration (mannitol, sucrose, xylose, sulfate) or filtration combined with tubular secretion (*p*-aminohippurate), or filtration combined with tubular reabsorption (urea, sodium chloride). A necessary conclusion is also that it is not the quantity of solutes in the glomerular filtrate, but only the amount finding its way into the urine, that determines the urinary flow. Consequently, the urinary flow is independent of the level of the clearance or of the plasma level of any solute. The dependence of urinary flow on solute load ($U \times V$) alone led to the consideration of the form of this relation. The basic assumption that with increasing solute load the urinary concentration decreases asymptotically to the level of the plasma concentration may be expressed as

$$(1)\quad -\frac{\text{d conc.}}{\text{d load}} = k\ (\text{conc.} - B),$$

where k is a proportionality constant and B is the plasma concentration. Integration leads to the expression

$$(2)\quad -\ln(\text{conc.} - B) = k\ \text{load} + A,$$

where A is the integration constant and the other symbols have the same meaning as before. In exponential form and rearranged, the equation is

$$(3)\quad \text{conc.} = A'e^{-k\ \text{load}} + B.$$

Values for the constants A′ and k may be readily derived from equation 2, which is that of a straight line, with A, *i.e.* ln of A′, as the intercept and k as the slope. Choosing 0.33 osmols/liter as the value for B, and using the least squares method for calculating the best straight line, the values of 0.847 for A′ and 0.21 for k were obtained. The final equation is then

$$(4)\quad \text{conc.} = 0.847e^{-0.21\ \text{load}} + 0.33,$$

where urinary concentration is expressed in osmols/liter. From the identity load = concentration × flow, an equation for flow in terms of load may be obtained:

$$(5)\quad \text{urinary flow} = \frac{\text{load}}{A'e^{-k\ \text{load}} + B}.$$

The line drawn through the data of Fig. 1 indicates the calculated curve, which fits the observations for the entire range well.

Calculations of the renal osmotic work by the formula (*3*)

$$(6)\quad W = RT\ V\ (U\ \ln U/P + P - U),$$

where W indicates the work, R is the gas constant, T is the absolute temperature, U and P are the urinary and plasma concentrations, and V is the urinary flow, led to the conclusion that the kidneys were in a relatively resting state in the preloading periods, when minimal urine flow and maximal solute concentration prevailed. The amount of osmotic work could be increased about 10-fold by solute loading up to a maximum value of 4.2 ± 0.5 cal/min/1.73 m² of body surface, a value which could not be exceeded by increasing the load. An increase of

the amount of loading substance administered, while producing increasing urine flow, also resulted in higher plasma level of the solute so that the work remained constant. A maximum value in the same range was observed for the following solutes: glucose, sucrose, mannitol, xylose, sorbitol, creatinine, and sodium sulfate. Sorbose and *p*-aminohippurate were not used in sufficient amounts to produce adequate loads. Maximum work was not obtained with urea or sodium chloride.

From equation 2 it follows that under the conditions of hydropenia here considered, the U/P ratio is the main factor determining work. Given the observed limitations of urinary osmolarity, increasing values of P would necessitate greater rates of urinary flow to produce maximum work. The actual relation between urinary flow and osmolarity, with the osmolarity decreasing with rising flow, is such that beyond a certain level of loading solute in the plasma, which is approximately 100 milliosmols/liter, maximum work can no longer be obtained, no matter how great the amount of loading substance administered. As a matter of fact, the work value may decrease, despite increasing urinary flows, loads, and plasma levels. These relationships serve to explain the failure to produce maximum work with either urea or sodium chloride. On the basis of the work equation and the observed relation between osmolarity and flow of the urine one may also determine by graphic methods the minimal urinary volume at which maximum work will be reached for any plasma level of loading solute under the simplifying assumption that the loading solute accounts for the entire urinary osmolarity.

Substituting the value of flow from equation 5 and using the identity $U = \frac{\text{load}}{\text{flow}}$, an equation is obtained in which both U and V are expressed in terms of load:

$$(7)\quad W = RT\ \text{load}\left(\frac{\ln A' e^{-k\ \text{load}} + B}{P} + \frac{P}{A' e^{-k\ \text{load}} + B} - 1\right),$$

in which the symbols have the same meaning as before. This equation permits one to relate directly work and solute load, and, by definition, urinary volume, for any given plasma level of loading solute on the same assumption as before, namely, that the loading solute accounts for the entire urinary osmolarity. One may also calculate the minimal urine volume at which maximum work will be reached.

The studies of glucose diuresis in diabetic subjects, beyond offering an explanation of the polyuria of diabetes, have a bearing on the problem of the cause of electrolyte loss during uncontrolled glycosuria. It was found that the urinary losses of sodium and chloride increased 4-fold above control levels during glucose diuresis, whereas the potassium losses were unchanged. Urea diuresis, on the other hand, did not affect the rate of electrolyte excretion.

At present studies are planned on the osmotic limitations and the work of the kidney at the other extreme of the osmotic relationships, *i.e.* during diuresis of water loading when the work of electrolyte conservation is at maximum.

Details of the foregoing studies will be published shortly.

References

1. Borsook, H., and Winegarden, H. M. *Proc. nat. Acad. Sci., Wash.*, 1931, **17**, 3.
2. Dresser, H. *Arch. exp. Path. Pharmakol.*, 1892, **29**, 303.
3. Galeotti, G. *Arch. Physiol.*, 1902, 200.
4. Gamble, J. L. *Proc. Amer. phil. Soc.*, 1944, **88**, 151.
5. Korányi, A. *Z. klin. Med.*, 1897, **33**, 1; 1898, **34**, 1.
6. McCance, R. A. *J. Physiol.*, 1945, **104**, 196; Hervey, G. R., McCance, R. A., and Tayler, R. G. O. *Proc. physiol. Soc.*, 1946, **104**.
7. Newburgh, J. D. *J. clin. Invest.*, 1943, **22**, 439.
8. Rhorer, L. *Arch. ges. Physiol.*, 1905, **109**, 375.

Effects of the Antithyrotoxic Factor of Liver on Growth and Survival of Immature Rats Fed Massive Doses of Thyroactive Materials[1]

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Available data indicate that liver contains one or more factors other than the known B vitamins that prolonged survival and counteracted the growth retardation of immature rats fed massive doses of desiccated thyroid

TABLE 1

Dietary component	Diet A	Diet B
Extracted liver residue*	0.0	10.0
Casein†	22.0	22.0
Salt mixture‡	4.5	4.5
Sucrose	73.5	63.5

To each kilogram of the above diets were added the following synthetic vitamins: thiamine hydrochloride, 72 mg; riboflavin, 9 mg; pyridoxine hydrochloride, 15 mg; calcium pantothenate, 67.2 mg; nicotinic acid, 60 mg; 2-methylnaphthoquinone, 5 mg; and choline chloride, 1.2 gm. (In view of the increased requirements for thiamine, pyridoxine, and pantothenic acid in the hyperthyroid rat (*2*), the B vitamins in the present experiment were administered in excessive amounts in order to assure an adequacy of these factors in the diet.) Each rat also received 3 times weekly the following supplement: cottonseed oil (Wesson), 500 mg; α-tocopherol acetate, 1.5 mg; and a vitamin A-D concentrate (Nopco Fish Oil Concentrate, assaying 800,000 U.S.P. units of vitamin A and 80,000 U.S.P. units of vitamin D/gm) containing 50 U.S.P. units of vitamin A and 5 U.S.P. units of vitamin D.

* Extracted Liver Residue, Wilson Laboratories, Chicago, Illinois.

† Vitamin Test Casein, General Biochemicals, Inc., Chagrin Falls, Ohio.

‡ Salt Mixture No. 1 (*6*).

(*1, 3, 4*). Since liver feeding did not prevent the rise in oxygen consumption following thyroid administration (*3*), the question arises whether liver actually exerts an antithyrotoxic effect or whether it might not be counteracting other noxious substances present in the desiccated

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