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Water-Food Interrelationships in Rats Fed Chemically Defined Liquid Diets.* (31699)

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Most studies concerned with thirst, water metabolism and water-food interrelationships have been conducted under conditions of separate feeding of food and water. Under these conditions quantitative relationships between food intake and water intake have been demonstrated and show that restriction of water intake in normal rats depresses food intake and restriction of food intake reduces water intake(1,2).

In contrast, relatively few studies concerning food-water interrelationships have been conducted when both were administered as a single formulation. Adolph and Parmington (3) showed that when food and water were fed together as milk, the water intake was governed by calorie content alone, even though this required ingestion of excess fluid. Feeding a similar milk diet to rats with diabetes insipidus Bruce and Kennedy(4) also showed that food intake depends on caloric content but water intake ultimately depends on the demands imposed by the renal excretion of the products of metabolism. Both studies were of short duration (7-10 days) and both employed extremely dilute diets (less than 10% solids).

Greenstein *et al*(5) developed water soluble completely defined liquid diets containing synthetic amino acids, glucose, vitamins, minerals and ethyl linoleate. Such diets could be useful for studying water-food interrelationships when the two constitute a single physical mixture. The following experiments were

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TABLE I. Composition of Basal Diet.*

Carbohydrates	g/l
Glucose	344.5
Glucono-delta-lactone	13.03
Amino acids	**g/l**
L-arginine • HCl	8.90
L-histidine • HCl • H_2O	2.93
L-isoleucine	6.45
L-leucine	8.70
L-lysine • HCl	8.00
L-methionine	3.03
L-phenylalanine	5.38
L-threonine	4.70
L-tryptophan	1.48
L-valine	6.85
L-aspartate	7.63
L-glutamate, monosodium	13.38
Glycine	6.05
L-proline	4.10
L-serine	7.23
L-tyrosine ethyl ester • HCl	5.08
Total L-amino acids	99.89
Minerals, macro	**g/l**
Ferrous ammonium sulfate	.70
Fructose 1,6-diphosphate, monocalcium	25.00
Magnesium oxide	.38
Potassium hydroxide	3.08
Sodium chloride	3.50
Sodium hydroxide	2.29

Minerals, trace	mg/l
Ammonium molybdate • $4H_2O$	3.0
Cobalt acetate • $4H_2O$	4.5
Cupric acetate • $4H_2O$	7.5
Manganese acetate • $4H_2O$	130.0
Potassium iodide	15.0
Zinc benzoate	11.0
Vitamins, water soluble	**mg/l**
Ascorbic acid	372.5
Biotin	.22
Vit. B_{12} (0.1% trituration)	74.5
Calcium pantothenate	37.25
Choline chloride	1.30 g
Folic acid	.37
Inositol	186.25
Niacin	27.94
Para-aminobenzoic acid	223.5
Pyridoxine • HCl	4.69
Riboflavin	7.45
Thiamine • HCl	3.73
Vitamins, fat soluble	**mg/l**
Vit. A acetate 3000 IU/mg	5.0
Calciferol 40 IU/μg	3.5 μg
Menadione	2.1
α-tocopherol acetate 1 IU/mg	25.0
Polysorbate 80	3.0 g
Ethyl linoleate	2 0 g

Physical Properties of Test Diets

Diet concentration, % solids	Avg osmotic pressure, mOsM/l	Avg specific gravity
50	2860	1.1780
40	2280	1.1459
30	1680	1.1105
20	1020	1.0703
10	570	1.0434

* Total nutrient concentration of basal diet: 50% (w/v). Test diets derived from basal mixture by diluting with distilled water.

conducted to evaluate their suitability for this purpose and to determine whether they can simultaneously satisfy the water and nutrient requirements of the weanling rat.

Materials and methods. Table I shows the composition of the basal diet used in all studies. The concentration of each nutrient is expressed as weight per final volume of diet, the total nutrient concentration being 50%. The protein equivalency (N × 6.25) was 8 g/100 ml and the calculated caloric density was 1.8 calories/ml. The amino acids were provided to simulate a modified whole-egg protein pattern. All diets were prepared with glass distilled water. Each test diet was derived from the basal mixture by diluting with an appropriate amount of distilled water to yield the designated dietary concentration. Diet concentrations were varied in 10% increments from 10% to 50% (w/v) solid nutrients.

In all experiments 10 male, CFE weanling rats with an average initial weight of 62 g were allotted to each dietary regime and were individually housed in wire-bottomed metabolism cages in a temperature controlled room (24-26°C). The diets were administered *ad libitum* in 100 ml Richter tubes. Each tube was placed in a specially designed unit with a sloped bottom to permit quantitative collection of dietary waste. The unit was attached externally to the cage and a small circular opening allowed the animal access to the Richter tube.

TABLE II. Growth Rate, Diet Consumption and Water Consumption of Rats Fed Different Concentrations of a Chemically Defined Liquid Diet With or Without *Ad Libitum* Water.

Dietary regime, % solids§	Avg wt gain, g/rat/day	Avg diet intake, ml/rat/day	Avg caloric intake,* cal/rat/day	Avg water intake, ml/rat/day		
				Diet	*Ad libitum*	Total
		Experiment I				
50	3.7 ± .21†	25.3 ± 1.1†	45.5	17.2		17.2
40	4.2 ± .12	38.8 ± .8	55.9	28.7		28.7
30	4.3 ± .14	53.1 ± 1.1	57.3	43.0		43.0
		Experiment II				
50 + H_2O	4.8 ± .10	33.6 ± .7	60.5	22.8	11.3 ± .7	34.1
40 + H_2O	4.7 ± .08	38.8 ± .5	55.9	28.7	10.7 ± 1.3	39.4
30 + H_2O	4.4 ± .08	53.5 ± 1.6	57.8	43.3	9.1 ± 1.5	52.4
		Experiment III‡				
20	3 8 ± .21	100.9 ± 3.7	72.6	88.0		88.0
10	2.4 ± .13	137.3 ± 2.6	49.4	128.5		128.5
20 + H_2O	3.8 ± .17	97.4 ± 2.5	70.1	84.9	6.4 ± 1.0	91.3
10 + H_2O	2.4 ± .11	146.1 ± 1.8	52.6	136.7	3.8 ± .2	140.5

* Calculated using values of 4, 9, and 4 calories per g amino acids, fatty acid and carbohydrate respectively.
† ± S.E.
‡ Values for both 20% diet groups represent 7 rats.
§ Expressed on weight:volume basis.

TABLE III. Volume, Osmotic Pressure and Specific Gravity of Urine from Rats Fed Different Concentrations of a Chemically Defined Liquid Diet With or Without *Ad Libitum* Water.

	Dietary regime, % solids	Avg urine volume, ml/rat/day	Osmotic pressure, mOsM/l	Specific gravity
Experiment I	50	6.4 ± .4*	1881 ± 41.4*	1.0553 ± .0016*
	40	14.0 ± .4	1178 ± 39.2	1.0325 ± .0014
	30	21.7 ± .5	633 ± 19.7	1.0215 ± .0013
Experiment II	50 + H_2O	19.3 ± .6	954 ± 34.0	1.0292 ± .0013
	40 + H_2O	24.0 ± 1.3	740 ± 37.9	1.0214 ± .0008
	30 + H_2O	35.8 ± 2.2	541 ± 15.4	1.0177 ± .0008
Experiment III†	20	59.3 ± 3.6	353 ± 15.8	1.0118 ± .0004
	10	100.0 ± 3.0	136 ± 5.9	1.0069 ± .0006
	20 + H_2O	61.0 ± 2.0	310 ± 4.4	1.0104 ± .0008
	10 + H_2O	103.6 ± 4.2	120 ± 4.6	1.0061 ± .0006

* ± S.E.
† Values for both 20% diet groups represent 5 rats.

In cases where water was provided, it was administered in 200 ml bottles with glass tubes extending into the cage. All *ad libitum* water was glass distilled prior to use.

All experiments were of 28 days duration. In Experiments I and II urine was collected every 48 hours for the first 22 days. In Experiment III, 48 hour collections were made at 7-day intervals for the first 21 days. Measurements were performed on individual samples from each collection. Osmotic pressure was determined by freezing point depression. The data in Table II represent average values for the full 28 days. The data in Table III represent average values over the entire 21- or 22-day collection period.

Results. The results are summarized in Tables II and III. When *ad libitum* water was provided, no differences ($P > 0.01$) in growth were observed between rats consuming 30, 40 or 50% (w/v) diets (Exp. II). The same comparison between rats ingesting 30, 40 or 50% (w/v) diets without drinking water also shows no difference in growth rate (Exp. I). When fed diets of 10% or 20% concentration regardless of access to *ad libitum* water, growth was markedly reduced. Comparison of diets at equivalent concentrations shows that only at the 40% and 50% levels did *ad libitum* water influence growth rate. At each corresponding concentration below 40% growth rates were the same re-

gardless of accessibility to drinking water.

Diet intake increased with decreasing diet concentration whether or not *ad libitum* water was available. At corresponding concentrations below 50%, diet and caloric intakes were equivalent and were not influenced by the availability of drinking water. In all regimes where diet concentrations were 30 and 40% caloric consumption was virtually the same. Rats fed 20% (w/v) diets had the highest caloric consumption of all groups studied.

Diet utilization (g gain/g dry diet consumed) was essentially the same at corresponding concentrations. Only at the 40% level was there an apparent increase (10%) in diet utilization as the result of ingesting drinking water. This group also utilized the diet most efficiently.

In trying to satisfy their caloric needs, rats consumed more of the less concentrated diets. Since each diet contained water and calories, total daily water intake also increased with decreasing diet concentration. *Ad libitum* water intake was similar when rats were fed 40% and 50% diets but then decreased as the diet concentrations decreased. Ingestion of drinking water persisted at the 20% and 10% levels despite the large volume of dietary water consumed.

As expected, urine volume increased with increased diet intake (Table III). Urinary osmotic pressure and specific gravity reflected diet concentration and accessibility to supplemental water. Osmotic pressure and specific gravity correspondingly decreased with decreasing diet concentration. Diluting the diet between concentrations of 50% and 30% had a more pronounced effect on lowering the urinary osmolarity when *ad libitum* water was denied than when fed to the animals. On the other hand, the magnitude of this change was the same regardless of accessibility to *ad libitum* water when the diet was diluted to similar concentrations between 30% and 10%. In all cases where *ad libitum* water was permitted, the urinary osmotic pressure was below 1000 mOsM/l and the specific gravity was below 1.03. When *ad libitum* water was unavailable only rats ingesting diets at concentrations of 30% or lower had urinary values falling within this range.

Discussion. The results suggest that several mechanisms are operative in regulating diet intake and diet utilization when rats are fed a chemically defined liquid diet as the sole source of water and solid nutrients. These mechanisms are related to caloric density of the diet, diet osmolarity, and the physical capacity of the rat.

Depending upon diet concentration and availability of drinking water rats apparently consumed diets to fulfill their energy requirements. Only at a diet concentration of 50% in the absence of *ad libitum* water did the hypertonicity of the ingested fluid seem to cause a reduction in caloric intake and a corresponding decrease in growth rate. Rats fed a 40% diet without access to drinking water also grew slowly when compared to their counterparts allowed drinking water. However, in this case caloric intake was not reduced. Apparently when rats consume a 40% or 50% diet without access to drinking water, the osmolarity of the gastrointestinal fluids becomes excessive, resulting in reduced nutrient utilization and stress upon renal excretion. This is reflected in the high osmolarity of urine specimens from rats ingesting 40% or 50% diets without drinking water. In this connection, it may be noteworthy that only at diet concentrations of 30% or lower did rats which were denied *ad libitum* water produce urines with an average osmolarity and specific gravity below 1000 mOsM/l and 1.03 respectively.

Based on caloric intake one might expect better growth from rats fed the 10% or 20% diets, especially in view of the very high caloric intake of the 20% groups. The relatively poor growth observed may be partially due to an elevated energy expenditure caused by the increased urine output and increased physical activity associated with ingesting large volumes of fluid. In addition, the ingestion of large volumes of nutrient solution probably caused rapid passage through the body and decreased cellular utilization of the transported nutrients.

Finally, one can estimate the maximum dietary concentration which will permit good growth when a chemically defined diet of this type is the sole source of water and solid nutrients. Table II shows that rats fed a

40% diet without drinking water do not grow as well as those fed a 50% diet with drinking water. While this may be attributed to the somewhat lower caloric intake of the former group it is more likely the result of inadequate water intake since rats ingesting an equal volume of the same 40% diet with *ad libitum* water grew as well as the 50% group and even utilized the diet more efficiently (g gain/g dry diet consumed). Since the solid nutrient intake was equal for the groups fed the 40% diet (approximately 16 g) it may be deduced that under these conditions the minimal water requirement compatible with good diet utilization and growth is represented by the 40% + *ad libitum* water regime. This value corresponds to about 39 ml/rat/day. The total volume of a water soluble diet combining 39 ml of water and 16 g of solids into a single source would be 48 ml. This is equivalent to a diet containing 33% (w/v) solids and would represent the maximum dietary concentration capable of simultaneously satisfying the water and solid nutrient requirement of the weanling rat.

Summary. A chemically defined liquid diet was found to be well suited for studying water:nutrient interrelationships. When supplied as the sole source of water and solid nutrients such a diet can satisfy the nutritive requirements of the growing rat. In this study rats fed diets containing 30% (w/v) or 40% (w/v) solids as the sole source of water and nutrients appeared healthy and grew well. Under the same conditions a 50% (w/v) diet proved hypertonic, promoted slower growth and produced highly concentrated, hypertonic urine. More diluted (10% and 20%) diets were also inadequate, but, in this case, the cause was probably poor diet utilization. When drinking water was provided with a diet containing 50% (w/v) solids, good growth was obtained and urinary specific gravity and osmolarity were reduced.

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Reversal of Post-Thymectomy Wasting Syndrome with Multiple Thymus Grafts in Diffusion Chambers.* (31700)

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Previous experiments in this laboratory demonstrated that wasting syndrome in thymectomized mice can be reversed by intact multiple syngeneic or allogeneic thymus grafts (1,2). Osoba and Miller(3) and Levey *et al* (4) have shown that a non-cellular thymic factor can stimulate lymphoid maturation and development of normal immune capacity in thymectomized mice prior to the onset of wasting. The following studies were performed to determine whether thymic humoral mechanisms, apart from direct cellular mechanisms, are effective in reversing wasting syndrome and restoring immunologic capacity in thymectomized mice.

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Materials and methods. Thymectomy or sham-thymectomy was performed on inbred C3H/HeJ mice during the first 12 hours of life, employing hypothermic anesthesia. The onset of wasting was determined by the appearance of diarrhea, ruffled fur, hunched posture, a sustained weight loss of at least 7 days duration or a failure to gain weight for 10 days or more, and a progressive lym-