ent in the amount of 175 μg/g. In that F_2 is about ten times more concentrated than F_3T and the concentration of iodine remains approximately the same in both, iodine appears to be really part of the thyrocalcitonin molecule.

We have purified the F_2 fraction still further by precipitating it with 20 percent trichloroacetic acid and then passing it twice through Sephadex column G100 and eluting with 0.1*N* formic acid. In one typical experiment, 35 samples were analyzed by the Kolthoff method and by activation analysis (*9*). The good agreement between the results derived by the two methods (Fig. 1) supports the earlier evidence that iodine is a component of thyrocalcitonin. Plasma calcium was determined by biological assay by injecting into the tail vein of a rat 0.5 ml of the elution liquid from each 10-ml sample. In order to avoid any alterations of its biological properties, we did not evaporate the eluate.

There is a correlation between the iodine content and the potency of the hypocalcemic effect. When the hypocalcemic effect is not present, iodine is not measurable. The maximum amount of iodine is found (samples 17 and 18) simultaneously with a maximum hypocalcemic effect (Fig. 1). The maximum of the iodine curve coincides roughly with the minimum of the plasma calcium curve. This also seems to indicate that the substance containing the hypocalcemic hormone contains iodine in its molecule.

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26 July 1967

Australian Desert Mice: Independence of Exogenous Water

Abstract. *Certain Australian desert mice can survive and may gain weight on a diet of dry seed without drinking water. Urine concentrations for two of the three species studied are the highest recorded for mammals. The kidneys appear to be the major avenue of water conservation.*

Deserts contain as conspicuous faunal elements nocturnal, fossorial rodents. These represent rather divergent familial lines with heteromyids and cricetids predominating in North America, cricetids predominating in Afro-Eurasia, and only murids present in Australia. There is a remarkable degree of morphological and behavioral convergence among these desert rodents. In addition, North American heteromyids and Afro-Eurasian cricetids are physiologically convergent, extreme degrees of water conservation imparting virtual independence of exogenous water (*1*). Since the little-known Australian desert rodents inhabit some of the hottest, driest regions on earth, they too might exhibit unique physiological adaptations in their water economies. We have studied the water economies of Australian hopping mice *Notomys alexis* and *Notomys cervinus* and of the sandy inland mouse *Leggadina hermannsburgensis*.

The *N. alexis* and *L. hermannsburgensis* (mean weight 28.9 ± 5.5 and 12.6 ± 0.9 g, respectively) were collected near Yuendumu Settlement, 296 km west-northwest of Alice Springs, Northern Territory; *N. cervinus* (mean weight 34.7 ± 2.9 g) was collected on Sandringham Station, 160 km north of Birdsville, Queensland. All are from regions of less than 25.4 cm mean annual rainfall; all were collected at the end of a severe drought of 10-year duration.

In the laboratory, animals were housed individually in cylindrical wire-mesh cages; they were provided an excess of mixed bird seed for food (10 percent preformed water by weight). In studies of water deprivation, animals were previously hydrated on fresh apple as a water source and then deprived of water altogether. Initial responses to water deprivation were determined under natural photoperiod at an ambient temperature (T_A) of 19° to 25°C and a relative humidity of 55 to 75 percent, after which animals were transferred to a constant-temperature room on a 12-hour photoperiod at a T_A of 25°C and a relative humidity of 30 to 40 percent; urine, blood, and feces were collected from the animals under the latter conditions. Urine was collected at night in petri dishes placed under the cages and filled with mineral oil to prevent evaporation. Blood was collected in heparinized capillary tubes from a vascular area in the anteriodorsal aspect of the orbit, without permanent injury to the animal. Fecal water content was determined on pellets removed as they were voided by animals held in hand. The pellets were then dried to a constant weight at 100°C. Feces obtained from different mice at the same time were pooled in a single sample.

Concentrations of urea in whole blood and urine were determined by the Conway microdiffusion technique. Osmotic pressures of plasma and urine were calculated from the freezing point depressions measured with a Kalber Biological Cryostat. The rates of pulmocutaneous water loss and oxygen consumption were measured simultaneously for 1-hour periods at a T_A of 28°C. Oxygen consumption was measured in an open-air system at a flow rate of 692 cm^3/min with a Beckman Model E2 oxygen analyzer; pulmocutaneous water loss was determined gravimetrically in expired air passed through tubes containing calcium chloride. While the rates of pulmocutaneous water loss reported herein are for hydrated animals, less complete data for animals deprived of water indicate they were no lower. Measure-

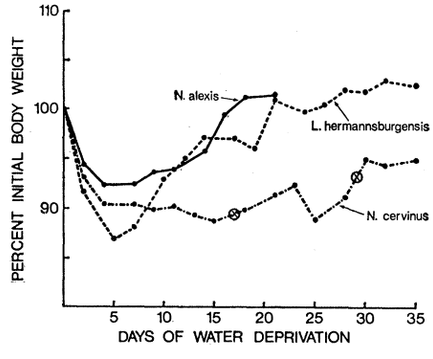

Fig. 1. The mean responses of body weight to water deprivation in ten *Notomys alexis*, eight *N. cervinus*, and nine *Leggadina hermannsburgensis* on a diet of air-dried seed (T_A = 19° to 25°C; relative humidity, 55 to 75 percent). ⊗, day of death of each of two *N. cervinus*.

Table 1. Mean and maximum urine concentrations of rodents on a diet of dry grain (except *C. leucurus* on a diet of sunflower seed and lab chow) without drinking water. Except for the laboratory rat (*R. norvegicus*) all are desert-dwelling species. Measurements of urine urea and osmotic pressure were not necessarily from the same urine sample. Numbers in parentheses are standard errors of the means.

Species	Mean value				Maximum value			
	Urine		Urine : blood ratio		Urine		Urine : blood ratio	
	Urea (mmole/liter)	Osmotic concentration (mosmole/liter)	Urea	Osmotic concentration	Urea (mmole/liter)	Osmotic concentration (mosmole/liter)	Urea	Osmotic concentration
Rodents dependent on water								
Rattus norvegicus (alb.) (*3*)	2070 (±120)	2860 (±120)	87	6.5*	2410	3250*	115	8.1*
Neotoma albigula (*3*) (North America)	1230 (±190)	2100*(±170)	36	5.2	1870	2670*	91	6.7*
Citellus leucurus (*1, 4*) (North America)	2230*	3730 (±160)		9.4*	2860	3900		9.5*
Rodents not dependent on water								
Dipodomys merriami (*3*) (North America)	2420 (±200)	3780 (±280)	202	9.5*	3840	5540*	352	14.0*
Dipodomys merriami (*2*) (North America)		3990		10.2*		4650		11.8*
Dipodomys spectabilis (*3*) (North America)	2340 (±120)	3780*(±130)	190	9.5*	2710	4090*	234	10.4*
Gerbillus gerbillus (*1*) (Egypt)					3410	5500		14.0*
Jaculus jaculus (*1*) (Africa)					4320	6500		16.0*
Notomys alexis (Australia)	3430 (±340)	6550 (±510)	343 (±31)	17.9 (±1.3)	5430	9370	798	24.6
Notomys cervinus (Australia)	2500 (±150)	3720 (±220)	212 (±32)	9.4 (±1.0)	3140	4920	257	14.2
Leggadina hermannsburgensis (Australia)	2760 (±300)	4710 (±820)	242 (±32)	14.7 (±0.8)	3920	8970	381	26.8

* Estimates calculated on the assumption that the urine osmotic pressure equals twice electrolyte concentration plus the urea concentration.

ments of all parameters were made on samples of eight to ten animals of each species.

When deprived of water after a state of optimal hydration, all three species of Australian desert rodents lost weight initially, even at high relative humidities (55 to 75 percent; Fig. 1). The ten *N. alexis* and nine *L. hermannsburgensis* recovered quickly and by 20 days had regained their starting weights. After an initial weight loss eight *N. cervinus* maintained a weight of about 90 percent of the initial value for about 25 days; during this period two of the animals lost weight continually and died, while the remaining six eventually began to regain weight. Then, when transferred, while deprived of water, to a room with constant temperature (T_A = 25°C) and a lower relative humidity (30 to 40 percent), all species continued to gain weight, a clear indication that they are not dependent on exogenous water under these conditions. Even though two *N. cervinus* died after being deprived of water, 75 percent of the sample survived. Thus these animals had a greater tolerance of water deprivation than certain North American *Dipodomys* spp. (*2*), which is generally considered a water-independent genus (*1*).

Analyses of osmotic pressures and urea concentrations of urine and blood of animals deprived of water reveal that all three species of Australian mice concentrate urine to extreme degrees (Table 1). Urine production, though not measured, was conspicuously less in mice deprived of water than it was in those with water, an indication that the kidneys are a significant site of water conservation. The interspecific differences in the capacity to concentrate the urine reflect very closely the differing weight responses to water deprivation (Fig. 1). When compared with rodents from representa-

Table 2. Oxygen consumption, pulmocutaneous water loss, and fecal water content of the laboratory rat (*R. norvegicus*) and several species of desert rodents. Fecal water loss was determined in rodents deprived of drinking water, and pulmocutaneous water loss and oxygen consumption were measured at 28°C. Numbers in parentheses are standard errors of the means.

Species	Oxygen consumption (cm^3/hr)	Pulmocutaneous (mg of H_2O per cm^3 of O_2)	Feces (% H_2O)
Rodents dependent on water			
Rattus norvegicus (alb.) (*1*)	194.4(±6.3)	0.94(±0.03)	68.5
Peromyscus eremicus (*5*)	27.1	1.20	
Citellus leucurus (*4*)		0.53	
Neotoma lepida (*6*)	87.1	2.04	
Rodents not dependent on water			
Dipodomys merriami (*1*)	79.7(±2.1)	0.54(±0.01)	45.2
Dipodomys merriami (*2*)	43.3	0.80	
Dipodomys spectabilis (*1*)	140.9(±7.6)	0.57(±0.03)	
Notomys alexis	62.1(±3.5)	0.91(±0.11)	48.8*
Notomys cervinus	48.3(±4.1)	0.76(±0.08)	51.8*
Leggadina hermannsburgensis	43.3(±6.5)	1.15(±0.11)	50.4*

* Based on single samples of feces collected from eight to ten animals.

tive deserts of the world, the Australian species produce urine whose concentrations are equivalent to or exceed those of other forms that are not dependent on exogenous water (Table 1). In fact, to our knowledge, the values for *N. alexis* and *L. hermannsburgensis* are the highest and second highest, respectively, measured for mammals. The highest single value for urine osmotic pressure (urine, 6340 mosmole/liter; urine : blood ratio, 17 : 1) previously measured was obtained from a Saharan sand rat (*Psammomys obesus*) on a diet of halophytic plants (*1*).

Certain North American desert rodents have unusually low pulmocutaneous and fecal losses of water compared to nondesert forms (*1*). The comparative data, including ours for Australian forms, indicate that water loss may be reduced by the excretion of unusually dry feces in desert rodents deprived of water (Table 2). However, the data available indicate a wide range of pulmocutaneous water loss regardless of habitat and degrees of water independence (Table 2). Expressed as a function of oxygen consumption, which is directly related to the production of metabolic water, the rate of pulmocutaneous water loss was lowest among our animals in the form most dependent on water (*N. cervinus*); the higher rates of *N. alexis* and *L. hermannsburgensis* were comparable to that of the laboratory rat.

While the Australian desert rodents discussed herein will drink water greedily in the laboratory, they are in general not dependent on drinking water under conditions of low relative humidity, moderate temperatures, and a diet of dry, carbohydrate-rich seeds. They appear to owe this independence to extreme renal capacities for concentrating urine and to reduced output of water in the feces. Under natural conditions this efficiency in water conservation, together with a nocturnally active and a diurnally fossorial existence, yield a combination of physiological and behavioral adaptations which ensures survival under the potentially stressful conditions of extreme aridity, diurnal heat, and very low, periodic rainfall characteristic of the Australian desert.

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21 August 1967

Sulfhydryl Groups and Estradiol-Receptor Interaction

Abstract. *The characteristic ability of rat uteri to take up tritiated estradiol in vitro or to retain estradiol previously incorporated either in vivo or in vitro is destroyed by treating the tissue with various sulfhydryl-blocking reagents. The two radioactive estradiol-receptor complexes, observed in uterine homogenates in the supernatant fraction and in an extract of the nuclear fraction, respectively, are disrupted by brief exposure to organic mercurials in the cold. Sulfhydryl groups of uterine receptor substances apparently play a vital role in estradiol binding, perhaps indirectly through contribution to receptor conformation.*

It is now well established that estrogen-responsive tissues, such as uterus and vagina, contain minute amounts of apparently unique components called "estrogen receptors" which show a striking affinity for estradiol and certain other estrogens, both in vivo (*1*) and in vitro (*2, 3*). Strong but reversible interaction of hormone with receptor, without chemical transformation of the steroid molecule, appears to be an early step—if not the initial one—in the uterotrophic process (*3, 4*). Our study demonstrates that sulfhydryl groups of the receptor substance are essential to its ability to associate with estradiol (*5*).

On exposure to dilute ($10^{-10}M$) solutions of tritiated estradiol in Krebs-Ringer-Henseleit (KRH) glucose buffer (*3*), the uterine horns from immature rats rapidly accumulate radioactive steroid until the concentration in the tissue is several hundred times that of the medium (Fig. 1). Slit uterine horns of 24-day-old rats were stirred for 1 hour at 38°C in 500 ml KRH glucose buffer (*p*H 7.3) alone or containing $0.001M$ iodoacetamide (*IA*), *N*-ethylmaleimide (*NEM*), or *p*-hydroxymercuribenzoate (*PHMB*), after which they were stirred for various periods at 38°C in 500 ml of the buffer containing $10^{-10}M$ estradiol-6,7-H^3 (specific activity, 57 mc per micromole; solution contains 12,500 disintegrations per minute per milliliter). Radioactivity is determined by combustion of freeze-dried tissues by modified Schöniger technique to produce tritiated water counted in liquid-scintillation spectrometer.

As shown in Fig. 1, incubation of the uteri with iodoacetamide markedly decreases their ability to take up estradiol, whereas similar treatment with *N*-ethylmaleimide or *p*-hydroxymercuribenzoate, even for as little as 15 minutes, completely eliminates the characteristic affinity of the tissue for estrogen. The latter agents reduce the amount of radioactivity entering the tissue to that observed with rat diaphragmatic muscle, a nontarget tissue in which the small uptake of radioactivity is not affected by treatment with sulfhydryl reagents. Similar abolition of estradiol uptake is effected when $0.001M$ *p*-hydroxymercuribenzoate or methylmercurihydroxide are present during exposure of the uteri to the hormone solution.

Tritiated estradiol, previously incorporated into rat uteri, either after subcutaneous injection of the hormone in the animal or by exposure of the iso-

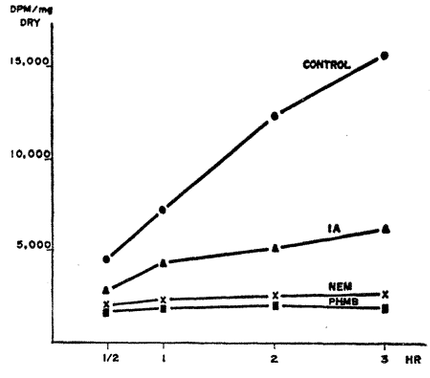

Fig. 1. Effect of sulfhydryl reagents on uptake of estradiol by uterine tissue in vitro. Each point represents the median value of five horns.