The total population will not be sterilized; depending on the individual bird and its susceptibilty to sexual alteration, birds not affected by mestranol will be present and they will produce a normal number of progeny. Adults eating food with grit, will, if they are incubating eggs, add to the chemosterilization program by feeding their young crop milk in which the steroid level is increased, or by passing grit to them in crop food, thereby altering the fertility of the young.

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7. Assessment of treatment on both parents and F_1 raised by treated parents was by statistical comparison with coeval controls of fertility and hatchability of eggs laid, number of days between clutches, gross and histological changes in the reproductive tract due to treatment, and age at first laying of F_1 females. Reproductive tract changes included, in the female, presence of both right and left oviducts (the right is usually absent in untreated birds)—the right oviduct of treated birds was usually incomplete and the left either incomplete or nonsecretory; resorbed ovarian follicles indicating inhibition of ovulation; and immature ovaries. In the male, changes included development of either or both of the usually diminutive Müllerian ducts (future oviducts in the female), flaccid testes with reduced spermatogenesis or azoospermia, and involution of the vasa deferentia.
8. I have tested the effect of mestranol incorporated in grit on hybrid laboratory mice and *Drosophila melanogaster* (representing components of the pigeons' feeding area) in the same form as would be applied for pigeon bait; reproductive potential or productivity or both were not changed when these animals were allowed to feed on prepared bait containing grit with mestranol. Soil microorganisms are very active in degrading mestranol; gas-chromatographic analysis of soil washings after soil treatment with mestranol yielded no trace of the applied mestranol after 6 hours. As an added precaution, the grit particles in all experiments, including those with pigeons, were ultimately coated with a catalyzed resorcinol layer before being incorporated in the bait. Grit ingested by vertebrates lacking gizzard stones was excreted within a few hours.
9. These models are modified from those presented for vertebrates by E. F. Knipling, in *Insect Chemosterilization*, G. C. Labrecque and C. N. Smith, Eds. (Appleton-Century-Crofts, New York, 1968), pp. 7–40.
10. This is a contribution of the Massachusetts Cooperative Wildlife Research Unit (supported by the U.S. Bureau of Sport Fisheries and Wildlife, the Massachusetts Division of Fisheries and Game, the University of Massachusetts, and the Wildlife Management Institute) and the Massachusetts Agricultural Experiment Station.

24 July 1970

Water Transport in the Cloaca of Lizards: Active or Passive?

Abstract. *The withdrawal of water from the lizard cloaca can be a passive process resulting from the colloidal osmotic pressure of the plasma proteins. The forces necessary to withdraw water from lizard urine, the forces prevailing within the cloaca in vivo, and the counterbalancing of these forces by a protein solution placed in the cloaca all are in accord with this hypothesis.*

It is well known that water is withdrawn in the cloaca or lower intestine of birds and reptiles (*1*). Urine, as it leaves the kidney, is liquid (although it may contain crystalline uric acid); in the cloaca water may be withdrawn until the final urinary pellet (dropping) is firm and semisolid.

The mechanism for withdrawal of fluid from tissue spaces, from the intestine, and from the cloaca has been the subject of much discussion (*2*, *3*). The mechanisms commonly considered for water transport in biological systems are either primary active transport of a solute, followed by passive solute-linked water transport, or passive flow by simple physical means, such as flow of water along an osmotic gradient or flow due to hydrostatic pressure. An active transport of water as such (a "water pump") is not presently considered a plausible alternative for transport across animal membranes (with the possible exception of arthropods).

To examine whether water reabsorption in the cloaca can be explained without invoking processes of active transport we selected the desert iguana (*Dipsosaurus dorsalis*). This animal has several advantages; ectothermic animals lend themselves easily to experimental procedures, reptiles are unable to produce hypertonic urine (*4*), and the desert iguana normally lives in arid habitats and produces quite dry pellets of urine (*5*). As will be explained below, the plasma colloidal osmotic pressure could provide a force sufficient to cause withdrawal of water. We therefore wanted to (i) measure the force needed to withdraw fluid from lizard urine and compare this force with the plasma colloidal osmotic pressure; (ii) examine whether the forces prevailing within the cloaca in vivo are in accord with our hypothesis, and whether the intracloacal forces change with induced changes in plasma colloidal osmotic pressure; and (iii) see if the force causing withdrawal of water can be counterbalanced by the introduction into the cloaca of a protein solution of equal colloidal osmotic pressure.

If suction is applied to a liquid, the hydrostatic pressure, relative to atmospheric pressure, becomes negative; we will use the expression "negative pressure" (suction) to mean subatmospheric pressure.

If we attempt to withdraw water from a moistened mass of crystalline uric acid, the necessary negative force increases as the sample becomes drier. The water is held in the capillary spaces of the mass of crystals with increasing force, and progressively greater negative pressures must be applied to remove more fluid. To measure the negative pressures necessary for withdrawal of fluid from a paste of lizard urine, we used the wick method described by Scholander *et al.* (*6*). Clean pellets of lizard urine were dried at 70°C, and distilled water was added to make a suitable paste containing 55 percent water. The wick was suspended in the middle of this mixture, and the container with its contents was placed on a balance. This permitted us to follow the water content of the sample as it began to air-dry while simultaneously recording the negative pressures which developed. The water content of freshly voided urinary pellets, necessary for comparison with the results, was determined by weighing before and after drying at 70°C.

Table 1. Correlation between plasma colloidal osmotic pressure (corrected at 38°C) and cloacal hydrostatic pressure in normally hydrated and dehydrated desert iguanas. Means and S.E.; number of animals in parentheses; *r*, correlation coefficient as calculated with Pearson product-moment correlation.

Lizards	Plasma colloidal osmotic pressure ($mm\text{-}H_2O$)	Cloacal hydrostatic pressure ($mm\text{-}H_2O$)	*r*
Hydrated	215 ± 7.5 (9)	-207 ± 7.6 (9)	+0.96
Dehydrated	267 ± 9.5 (8)	-255 ± 9.6 (8)	+0.98
	$P < .01$	$P < .01$	

The wick method was also used for measuring the negative hydrostatic pressures within the cloaca. The channels in the wick do not impede the movement of ions, proteins, or water, and therefore the wick as such is not sensitive to differences in osmotic pressure. However, if the wick is surrounded by a semipermeable membrane, any hyperosmotic fluid on the outside of the membrane would cause water to leave the wick due to osmotic forces. In this case, then, osmotic forces can be measured with the wick. In the lizard cloaca a similar situation prevails; the cloacal wall takes the place of the semipermeable membrane, and the osmotic force of the plasma proteins would now be the measured force. Diffusible solutes should not influence such a measurement.

Before insertion of the wick into the cloaca, the cloacal region was stimulated with a glass rod to induce evacuation, the animal was anesthetized (4 mg of sodium pentobarbital per 100 g of body weight), and a 50-watt bulb was placed at an appropriate distance to maintain body temperature at 38°C (*7*). The wick was inserted inside a glass tube which was then withdrawn, leaving the wick 2 cm deep in the cloaca. As liquid began to leave the wick, the operator immediately opposed this tendency by applying negative pressure (Fig. 1). When the negative pressure thus applied required no further change for 30 minutes, we considered that maximum negative pressure had been reached. At times two or three attempts were necessary before a satisfactory constant reading was achieved.

Colloidal osmotic pressure was measured with a small osmometer based on the principle of a selectively permeable membrane separating two fluid-filled chambers. We used membranes freely permeable to solute particles of less than 10,000 molecular weight (Diaflow UM-10, Amicon Corp.), which separated one chamber containing lizard plasma from the other containing lizard Ringer solution, the latter being connected to a Sanborn model 268 D differential pressure transducer. This gives a direct measurement of the osmotic pressure of the proteins including ions held by Donnan equilibrium. Calibration was against bovine albumin (1 to 6 g/100 ml in Ringer solution at *p*H 7.4). All values were within 13 mm-H_2O of those predicted for human albumin by Landis and Pappenheimer (*8*), and the precision of the measurement was ± 3 mm-H_2O S.E.

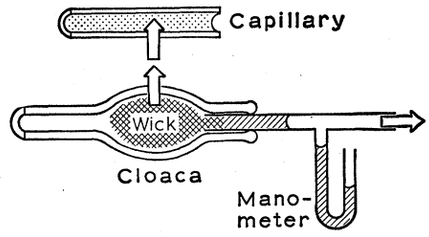

Fig. 1. Diagram showing wick in place in cloaca for measurement of the intracloacal negative pressures. Arrows indicate the direction of forces exerted on the wick fluid, the forces of plasma colloidal osmotic pressure being balanced by the applied negative pressure.

The time to reach 95 percent of the final reading was 3 to 4 minutes, and all readings were taken after 15 to 30 minutes. Blood samples were taken by heart puncture, and 0.2 ml of plasma was used in the sample chamber.

The negative hydrostatic pressure necessary to withdraw liquid from lizard urine is given in Fig. 2. As water evaporated from a sample, the balancing pressure changed. Until the mixture dried to 50 percent water, the hydrostatic pressure within the sample was not measurably different from atmospheric (0 mm-H_2O), but upon further drying the hydrostatic pressure of the liquid within the mass dropped sharply, and when the mixture contained about 45.5 percent water, it was −250 mm-H_2O (approximately equal to the colloidal osmotic pressure of the plasma). This water content is nearly identical to the water content of freshly dropped urinary pellets of animals kept on a diet in which water is restricted. Such pellets contained 45.5 percent H_2O ± 1.7 S.E. ($n = 11$; range, 43.9 to 50.0 percent H_2O) (*9*). Thus, we conclude that the amount of water withdrawn from the cloacal contents of hydropenic lizards is consistent with the hypothesis that the plasma proteins provide the moving force.

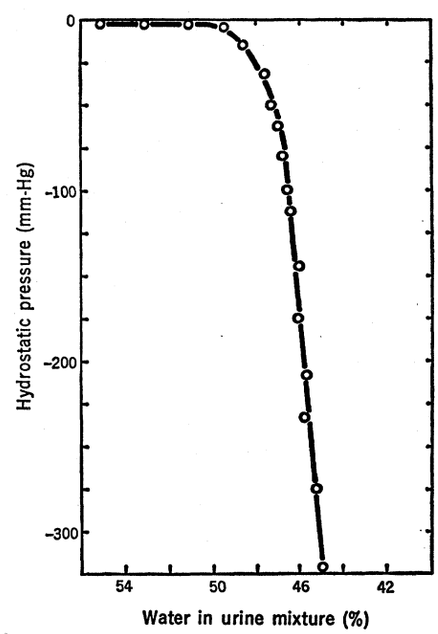

Fig. 2. The negative hydrostatic pressure necessary to balance the matrix forces in a moist mass of lizard urine as it gradually loses water by evaporation. Note the precipitous drop in pressure when the water content of the urine decreases below 50 percent.

The measured intracloacal negative pressures are nearly identical with the plasma colloidal osmotic pressure, and changes in the plasma colloidal osmotic pressure are reflected in the pressures measured within the cloaca (Table 1). To achieve these changes nine lizards were deprived of lettuce and mealworms (their main water supply). They lost 1.5 percent of their body weight per day and were considered dehydrated when they had lost 20 percent of their initial body weight. The mean plasma colloidal osmotic pressure of the dehydrated lizards increased to 267 mm-H_2O (Table 1), as compared with 215 mm-H_2O in a control group (*10*). The negative pressures measured in the cloaca of the two groups of lizards closely followed the plasma colloidal osmotic pressure with correlation coefficients near unity.

If the plasma colloidal osmotic pressure actually provides the driving force for withdrawal of water, the negative pressures should be abolished if an equivalent amount of protein is present within the cloaca. To test this we filled the wicks with 5 percent bovine albumin in Ringer solution (*11*) to equalize the colloidal osmotic potential on both sides of the cloacal membrane. The wick pressure in the cloaca of ten lizards with protein-filled wicks was now −10 mm-H_2O ± 8 S.E. (range, −56 to +16). In other words, when the cloaca contained a protein solution with a colloidal osmotic pressure similar to that of the plasma, the negative pressure (and presumably the driving force for water withdrawal) was abolished.

In view of these results, the colloidal osmotic pressure of the plasma is strongly implicated as the driving force for withdrawal of water from the cloaca (*12*). Forces similar to those acting on urine presumably also act on fecal material. The mechanism is analogous to one of the often suggested hypotheses for withdrawal of liquid from the in-

testine, as proposed by Starling (*2*). Such reabsorption includes the movement of low-molecular-weight solutes, primarily ions. If large amounts of ions are reabsorbed in this way, the need arises for an extrarenal avenue for electrolyte excretion, and such a mechanism exists in the form of the nasal salt gland of birds and reptiles. The possible relation of this gland to cloacal function was previously discussed by Schmidt-Nielsen *et al.* (*13*), who suggested that it may be an evolutionary prerequisite necessary for fully exploiting the potential of uric acid excretion for water conservation.

Water movement due to the plasma colloidal osmotic pressure does not exclude other possibilities, such as active transport of ions followed by passive movement of water. Skadhauge (*14*), for example, has demonstrated active sodium reabsorption in the chicken cloaca and lower intestine, and Junqueira *et al.* (*15*) have similarly implicated sodium transport in snakes. It should be noted, however, that all our results are compatible with the conclusion that the plasma proteins alone can provide the necessary force for water transport in the cloaca, and that none of our evidence suggests the necessity to invoke active transport or other alternate mechanisms.

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10. The control group was deprived of food and water for 2 days before testing because their cloacal contents would otherwise have had too much liquid for the wick method. After 2 days the readings were consistent, although the lizards were not sufficiently dehydrated to increase significantly the plasma colloidal osmotic pressure.
11. The measured colloidal osmotic pressure of 5.0 percent bovine albumin at *p*H 7.4 was 268 ± 3 mm-H_2O (S.D.).
12. The force acting on the cloacal contents can also be regarded as being that which constitutes the sum of the force provided by the colloidal osmotic pressure of the interstitial fluid and the negative hydrostatic pressure of the interstitial spaces, as described by A. C. Guyton [*Circ. Res.* **12**, 399 (1963)]; Scholander (*6*); and S. B. Strømme, J. E. Maggert, P. F. Scholander [*J. Appl. Physiol.* **27**, 123 (1969)].
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26 June 1970

Demonstration of Biological Activity of a Murine Leukemia Virus of New Zealand Black Mice

Abstract. *Although New Zealand Black mouse embryo and adult tissues show evidence of murine leukemia viral particles and antigens, efforts to demonstrate biological activity of a murine leukemia virus by standard methods have proved negative. Cocultivation of tissues of these mice with non-virus-yielding hamster cells transformed by Moloney sarcoma virus, however, has resulted in the rescue of a pseudotype sarcoma virus, presumably carrying the New Zealand Black mouse leukemia virus coat. This virus has an unusual host restriction, producing foci of cell alteration only in rat cells.*

New Zealand Black (NZB) mice and their F_1 hybrids with New Zealand White (NZB/NZW) mice develop a spectrum of autoimmune phenomena, including Coombs-positive hemolytic anemia and immune-complex type nephritis, resembling human systemic lupus erythematosus (*1, 2*). Abundant "C-type" particles characteristic of murine leukemia virus have been described in these mice (*3*), and the times of appearance of circulating G+ murine-leukemia-virus antigen and its antibody have been correlated with the times of onset of anemia and nephritis, respectively (*4*).

The great majority of murine leukemia virus strains propagate on either BALB/c or NIH Swiss mouse embryo cells (*5*), with production of the murine leukemia virus group-specific complement-fixing antigen (*6*), and formation of syncytia on cocultivation with XC cells (a line of rat cells infected with Rous sarcoma virus) (*7*). High-leukemic mouse strains, such as AKR and C58, contain the group-specific murine leukemia virus antigen in secondary mouse embryo cultures and adult tissues, which consistently release infectious murine leukemia virus and form syncytia when cocultivated with XC cells (*8*). New Zealand mouse embryo tissue cultures and adult organs contain naturally occurring murine leukemia virus antigen in amounts comparable to the high-leukemic strains. However, neither recovery of infectious virus nor formation of XC cell syncytia has been observed, indicating the absence of murine leukemia virus biological activity using conventional methods despite the presence of antigens and "C-type" particles in NZB tissue.

Another approach to the demonstration of murine leukemia virus biological activity involves the use of a hamster tumor cell line (HT-1) transformed by the Moloney strain of murine sarcoma virus (M-MSV). These cells lack the murine leukemia virus group complement-fixing antigen and show no evidence of virus production by bioassay or electron microscopy (*9*). Upon cocultivation with mouse leukemia virus–infected mouse or rat cells, the sarcoma virus genome is rescued from HT-1 cells, and enveloped in the type-specific coat of the murine leukemia virus used for the rescue (*9*). The rescued pseudotype sarcoma virus is recognized in tissue culture by the production of foci of transformation. Since NZB cells contain murine leukemia virus antigen, a rescue experiment was attempted by cocultivating HT-1 cells with NZB cells in tissue culture. Murine leukemia virus growth has been shown to be subject to host range restrictions (*5*); accordingly, the cocultivation fluids were assayed on BALB/c, NIH, and NZB mouse embryo cells. In addition, cells derived from rats were used, since these cells, in contrast to mouse cells, can detect