$r = 0.79$ (Fig. 3). A slightly higher correlation, $r = 0.90$, was obtained when the test period was divided by the control period immediately preceding it. Radioactivity in the urine samples diminished to such low levels after 30 days that reliable slopes could not be constructed.

Discussion. Daily estimation of total exchangeable sodium by a single injection of Na^{22} over prolonged periods would be a welcome addition to our investigative armamentarium. Unfortunately, the progressive rise in Na_E, coupled with inability to correlate levels of Na_E with acute changes in sodium excretion makes this approach impractical. Compared to our finding of an average daily increase of 1.9% in Na_E, Miller(4) *et al.* found a 1.0% increase over a week's period in 4 subjects maintained on a low salt diet. Since external counts over the patella remained constant while serum specific activity diminished, they postulated that this increase in Na_E might be due to increasing exchange of Na^{22} with bone sodium. However, other causes which might account for the rise in Na_E include extrarenal loss of sodium, calculation of serum specific activity when equilibrium has not been attained, and errors in urine volume measurements, all of which may lead to large cumulative errors.

The biological decay curves of serum Na^{22} radioactivity and specific activity paralleled each other. Because of wide daily fluctuations, these curves were not very valuable in depicting alterations of sodium excretion during the short periods used in this study. On the other hand, the cumulative urinary excretion curves of Na^{22} showed good correlation with daily sodium excretion. However, no additional information was provided by the former determination over the latter.

Summary. Biological decay curves of Na^{22} excretion showed good correlation with urinary sodium excretion during alteration of sodium excretion by sodium chloride, DCA and hydrochlorothiazide over periods of 3 to 7 days. During these short intervals, biological decay curves of serum radioactivity and serum specific activity were too variable to allow correlation with urinary sodium excretion. Daily total exchangeable sodium shows a progressive rise and cannot be used to reflect acute changes in sodium balance.

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Effect of Water Deprivation on Nasal Mucous Flow.* (26388)

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The combined action of mucous cells and ciliated cells to form a moving blanket which collects particles and transports them to given places for disposal is present throughout the invertebrate and vertebrate phyla(1). In the respiratory tract of vertebrates this mechanism functions to keep the surfaces free of debris. Most of our knowledge concerning its malfunction relates to the effects of noxious gases and other "external factors" (5). Initially through a chance observation, we have found that degree of systemic hydration of herring gulls and chickens has a marked effect on movement of the mucous

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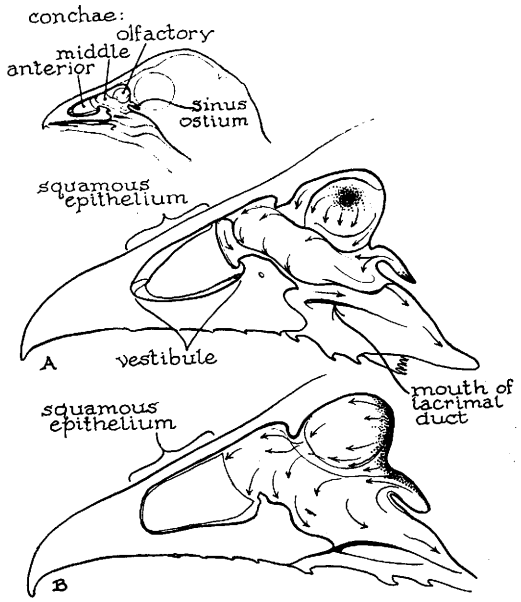

FIG. 1. Structures involved in nasal mucus flow in the chicken as seen in sagittal section. A: Lateral wall. B: Septum. Unless the mucosa is damaged in sectioning, or unless bits of debris such as bone chips or feathers fall onto it, the ink-carrying blanket sweeps antero-radially off the olfactory concha (A) moving as a cohesive blanket yet proceeding fanwise across entire length of the middle concha so that the anteriorly directed upper portion may go along the roof margin to the base of the nasal vestibule, thence ventrally to flow backward along the floor into the choana, joined at the mouth of lacrimal duct by the blanket lining that duct. The main body of the blanket flows over the convexity of the middle concha and inside its scroll, to be funnelled into the pharyngeal current at the base of the concha, joining secretions which have come directly ventrally from the lateral wall and from the maxillary sinus. Septal blanket (B) flows rapidly forward to fan out as shown in diagram. The main portion curves ventrally and then sharply back toward the choanae, while a narrow but strong current continues along roof area to vestibule, where in the living bird it presumably spills onto the non-ciliated squamous lining and out the nares.

blanket in the upper respiratory tract of these birds.

Rate and pattern of flow of the blanket of mucus within the nasal chambers of wild-caught subadult Herring Gulls, *Larus argentatus,* were observed on freshly killed birds during the summer at Marine Biological Laboratory, Woods Hole, Mass. They were anesthetized with Nembutal, the upper skulls were sagittally sectioned so that the septum was intact on one half and the conchal structures on the other, and an even sheet of colloidal India ink diluted with normal saline (2 parts ink, 1 part saline), was spread on the posterior limit of the septal mucosa, on the rim of the maxillary sinus ostium, and on the medial surface of the olfactory concha. Motion of the blanket could then be observed in the dissecting microscope as the foci of ink were incorporated into it and were swept along the established ciliary pathways (see Fig. 1 and accompanying text).

Two gulls in succession were found to have such flaccid veins that intravenous anesthesia could be accomplished only after much frustration. When ink was placed on the mucosae of these birds in the usual way, it lay immobile. The mucosal surfaces were coated with thick glassy mucus, there was no visible beating of cilia, and a few thin wisps of ink slowly sank downward through the viscous coat to puddle on the mucosa. Enquiry revealed that these 2 gulls were from a group that came to our attention some time after they had been brought in, due to an unintended delay in notification. Gulls used for investigation at the Laboratory are caught by hand at their island breeding site (a procedure which involves a rigorous chase and considerable emotional excitement on the part of the birds), secured in burlap bags, and brought to the mainland where they are maintained in outdoor cages until required. Most of them are large immature birds not quite able to fly but nearly as large as their volant mates. We habitually used them as soon as they were brought in. This group, then, were not only disturbed as a result of the trapping experience but were dehydrated as well. The remaining gulls in the group were given water, and later the same evening 2 of them were tested to check the result of rehydration. The movement of the blanket appeared normal in both.

The next "catch" of gulls was the last of the season. Of 4 birds of nearly equal sizes, 2 were deprived of water for 12 hours after being brought in, and 2 were fed and watered in the usual way. Of the water-deprived birds, the nasal mucous blanket moved very sluggishly in one and essentially not at all in the other. No ciliary motion was visible

through the thick coats of mucus, but when bits of mucosa were stripped off and observed in the phase microscope, ciliary beating was plainly seen. There was swift movement in the 2 control gulls over a healthy mucosa shimmering with rapidly beating cilia. The mean rate of motion of the blanket in all of the normally nourished birds was about 10 mm/minute.

On returning to Baltimore, a flock of 8-week-old White Leghorn chickens was divided into experimental and control groups, the latter being fed and watered in the routine way. Experimental birds were deprived of water, but not their dry mash feed, for 4, 16, 24, 36, 48, and 72 hours; 4 birds were in each deprived group, each of which had 2 concomitant controls, and 2 additional birds were included in the 72 hour group in order to observe the effect of rehydration. Using the same procedure described for the gulls, at 4 hours there was no apparent change in rate of mucus transportation; at 16 hours there was noticeable sluggishness in various areas in each of the deprived birds but not in the same area in each bird. At 24 hours there was essentially no motion of the blanket in 3 birds and fair but erratic motion in the fourth. At 36 and at 48 hours motion of the blanket was uneven throughout, and mean rate in any area did not exceed one millimeter a minute. At 72 hours the pattern of flow was again uneven throughout, but along one particular pathway that was consistent for all 4 birds it moved at a mean rate of nearly a millimeter a minute. This was along the upper lateral wall of the olfactory chamber. The pull of the ink-laden areas in all of the birds deprived for 16 hours or more was characterized by a sluggish hesitancy quite unlike the smooth swift flow of a healthy mucus-ciliary current. Control birds in each group showed this normal flow, rate of which in the chickens as in the gulls was an average 10 mm/minute. The 2 chickens which had been deprived of water for 72 hours were rehydrated, then killed after 36 and 53 minutes respectively. Motion of the mucous blanket was apparently normal in both rate and pattern of flow on both birds; no electrolytes were added to the water of rehydration.

Brisk ciliary motion could be seen at all times on all of the deprived as well as on all of the control chickens. None of these at any phase showed the degree of viscous glazing observed in the dehydrated gulls, though some thickening, puddling, stickiness, and glassiness was visible grossly on most of the deprived birds. It may be that the degree of dehydration was greater in the gulls. Both gulls and chickens were treated with ink within less than 90 seconds after decapitation. All observations were at room temperature and no attempt was made to control humidity.

The mechanism by which mucus remains in position as though anchored to the mucosa, while cilia continue to beat, is not understood. A clue may have been supplied by some current studies of whole mounts and sections of nasal organs of dehydrated baby chicks in which the mucous sheet was fixed *in situ*. Groups of chicks were deprived of water for 24, 48, and 72 hours. One or 2 from each group showed very thick, glassy, bubbly mucus. From each group, some were used for study of ink transportation, some were fixed for histology, and some were prepared according to Moe's method(2) which allows direct examination of surface mucous glands in whole mounts. Mucus was fixed *in situ;* whole septa and whole conchae were dissected out, stained with periodic acid-Schiff reagent, cleared, and viewed at 400× magnification by transmitted light. Fig. 3 shows the altered appearance of the surface glands in a whole mount of a dehydrated chick middle concha, and threads of mucus may be seen adhering to the surface. The threads show also in the histological section through the concha. The mucous blanket was apparently fixed while in the act of tugging toward the direction of ciliary propulsion, but was anchored to the mucosa by fine strands stretching tautly from the surface to the blanket. Such strands may evidently stretch until they snap "like a rubber band," as Proetz has described strands of mucus in another situation(3).

Some of the kinds of anchoring, stretching,

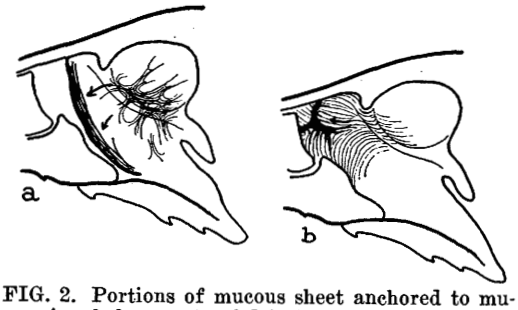

FIG. 2. Portions of mucous sheet anchored to mucosa in whole mounts of dehydrated chicks. a. Septum of a chick water-deprived for 24 hr, showing matted mucus adherent to inner rim of olfactory impression and mid-septum, and a ridge of mucus which had apparently begun to pile up along the forward line of advance of this portion of the sheet. Threads of mucus hold the blanket attached to the surface. b. Septum of a chick water-deprived for 72 hr, showing a band of mucous blanket fixed at a time that it was apparently being propelled by the cilia while anchored to several rows of glands or cilia.

and piling up of mucus seen in these preparations are shown in Fig. 2. Septum "a" shows that a thin portion of the sheet may remain partly stuck while the remainder is carried forward; in this case the forward moving part then piled up in a ridge along the line of advance. Septum "b" shows a segment of blanket anchored to the neighboring rows of glands. The size of the strands is greatly exaggerated in the drawing; in reality they are exceedingly delicate. The fine lines in the diagram represent the pattern of lineation of the glands in the immediate area of the anchored portion of the sheet.

Lucas and Douglas(4) have described the mucous sheet as consisting of an outer viscous and an inner serous portion. A situation which seemed to act this concept was seen in one of the chicks which had been water deprived for 72 hours. When India ink was spread along the posterior rim of the lateral half of the chamber in the usual way, most of it lay immobile on the surface, and a few wisps sank down through the thick mucus and lay on the mucosal surface. Quite suddenly, one of these underlying small puddles began to streak forward underneath the still immobile surface, climbing over the curve of the maxillary concha in 8 or 9 parallel lines, the ink particles apparently propelled along contiguous rows of the cilia. Within a few seconds, the heavy over-lying sheet began slowly to move in the same direction, so that a deep and a surface burden of ink could clearly be seen moving simultaneously at different rates.

To think of the mucous sheet in terms of layers may not be as accurate as the concept of a continuing secretion of the mucous cells which is variable in character (thin and fluid, thick and viscous, stringy, or lumpy) and which overlies a separate fluid secretion.

Discussion. Environmental humidity of less than 50% has been shown to stop the activity of cilia in trachea of rats within 8 to 10 minutes(5). In several species of animals and in monkeys and man, it has been noted that the path of the principal air currents between nares and choanae is the main factor determining rate of ciliary beat: motion is more rapid in the sheltered areas, slower along the paths of rapid airflow(6). This is true also in gulls and chickens, a maximum rate consistently clearing the olfactory chambers and maxillary sinuses and a visibly slower rate obtaining on the mid-septum. It was especially apparent in the chickens that after deprivation the motion of the blanket, though decelerated, persisted in these sequestered areas after it had effectively halted on the mid-septum. Histological sections of chickens and gulls show that the glands in the olfactory border area and around the sinus ostium are much deeper than elsewhere in the chamber. Whether the mucous secretion is intrinsically different in given areas, or whether changes of external environment or internal water balance induce area-specific changes in viscosity is not known. Ciliary action is known to be less affected by certain kinds of environmental changes than is the mucous blanket, neither accelerating nor decelerating under conditions which retard, speed up, or immobilize the overlying blanket(5,6). That cilia continued to beat under immobilized mucus in water deprived gulls and chickens was perfectly clear. Acute and chronic dehydration are known to have severe effects on systemic and cellular fluid and electrolyte balances(7).

Although degree of dehydration might be

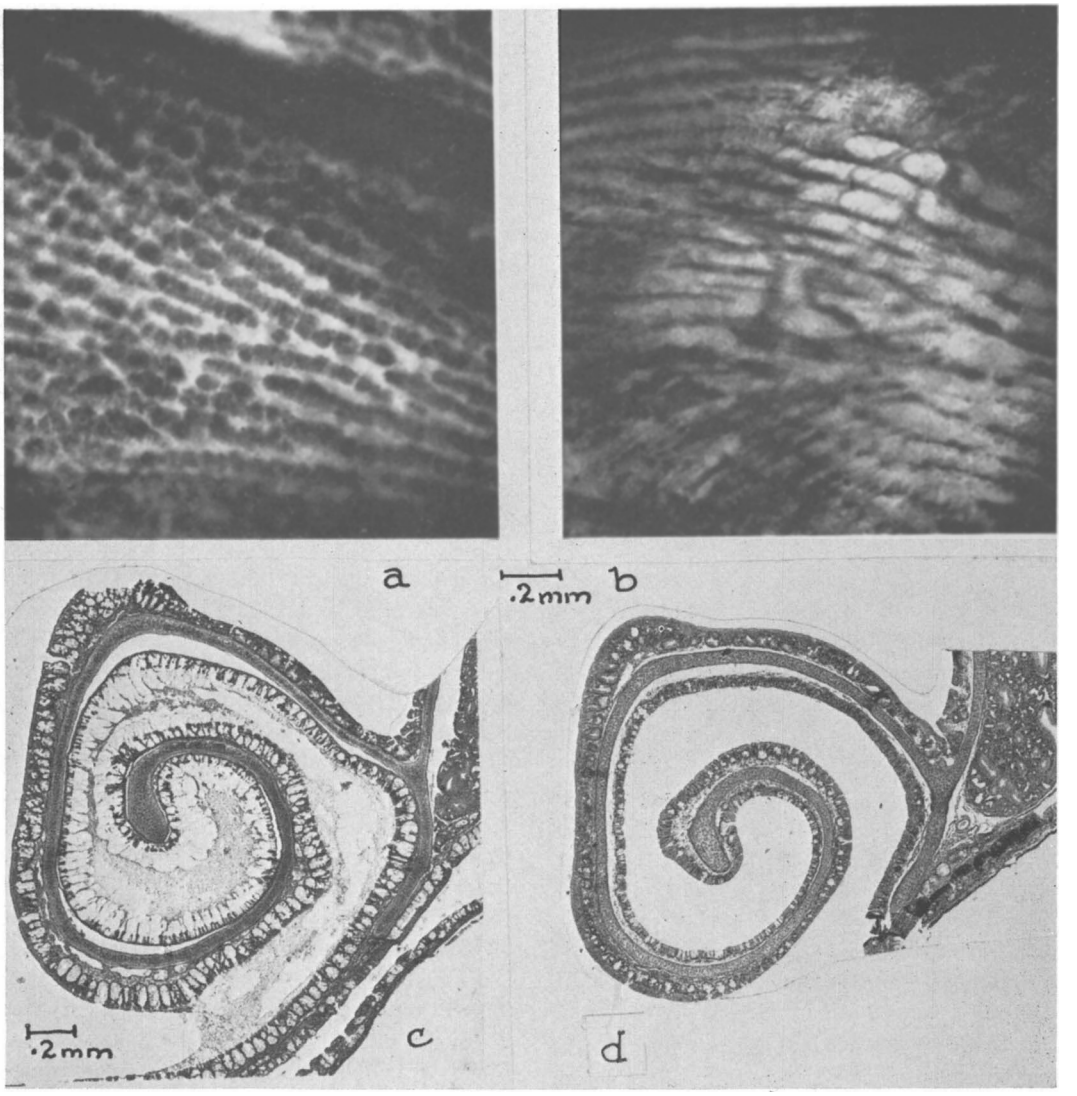

FIG. 3. Photographs of PAS-stained whole mounts showing inner base area of maxillary concha of: a. A normally hydrated chick. Mucus is in rows of glands. b. A chick deprived of water for 72 hr. Mucus does not show in glands, but seems held in or between rows. c. Section through the middle of middle concha of a chick deprived for 72 hr; threads of mucus stretch from mucous sheet to mucosa. H and E. d. Section through same area of a normally hydrated chick. H and E. Note difference in appearance of mucous glands in dehydrated and normal chick. Separation of mucosa from cartilage in "c" and "d" are artifacts of preparation.

expected to have a direct effect on viscosity of the mucus secreted, we have been unable to find references to the effect of internal hydration on the function of the respiratory tract. It is not surprising that water deprivation, alone or in combination with emotional stress, should be reflected in some way in production or transport of nasal mucus. It seems apparent in these crude but suggestive observations on herring gulls and chickens that systemic dehydration may have a pronounced effect on transportation of mucus in the upper respiratory tract. It has been reported that dehydration has less effect on pharyngeal mucous secretion in dogs and man than on parotid secretions(8).

Whether the nasal mucus of birds may be especially responsive to water deprivation is

not known, but preliminary experiments with 21-day-old mice indicate that the blanket motion is markedly inhibited in certain areas in these mammals after deprivation.

Summary. A striking depression in rate of flow of the nasal mucous blanket was observed in 4 water-deprived herring gulls, in contrast to 10 normally hydrated gulls. The observation was repeated in a series of 20 water-deprived and 10 normally hydrated chickens. In both species normal mean rate of flow was about 10 mm/minute. Two gulls and 2 chickens that were deprived then rehydrated showed a normal rate of motion of the blanket. Anchoring of the mucous sheet to the mucosal surface by fine strands of mucus was seen in whole mounts and histological sections of conchae of dehydrated baby chicks.

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Sensitivity to Estrogen of Uteri of Ovariectomized Mice in Relation to Age.* (26389)

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It has been reported(1,2) that sensitivity to gonadal hormones of accessory reproductive organs of gonadectomized male and female rats increases with advancing age reaching a peak at age when the animals, if intact, would have reached puberty. Sensitivity then decreased with age. The object of the present investigation was to determine sensitivity to estrogen of uteri of several strains of ovariectomized mice in relation to age.

Materials and methods. Forty mice selected from inbred strains and F_1 hybrids (A/Jax, C57BL/6, BALB/c, and BAF_1) served as intact controls to observe age at opening of vaginal orifice and at onset of estrous cycle.

Groups of mice from each strain were ovariectomized on day 21. A total dose of 0.5 μg estradiol benzoate in 0.1 ml sesame oil was divided into 2 equal portions which were injected subcutaneously at 24 and 16 hours prior to autopsy. Ovariectomized control mice were treated similarly with sesame oil alone. Autopsy was performed at 24, 34, 40, 46, 52, 58, 64, or 70 days of age. At autopsy uteri were weighed immediately after they were freed from mesometrium, vagina, and uterine tubes and were pressed gently between filter paper. Sensitivity to estrogen of uteri of ovariectomized mice were judged by percentage increase in weight of uteri above appropriate ovariectomized controls and by absolute uterine weight.

Results and discussion. Opening of vaginal orifice was found at about 37 days of age in A/Jax, C57BL/6, and BAF_1 mice and at about 28 days of age in BALB/c mice. Onset of estrous cycle in each of 4 strains of mice occurred about 10 days after opening of vaginal orifice.

Uteri of ovariectomized immature mice did not show prepubertal subnormal growth (Table I) as was seen in ovariectomized im-

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