

# NORMAL AND EXPERIMENTAL VARIATIONS IN ACID EXCRETION BY THE LAYING HEN

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## INTRODUCTION

In the fowl, as in other animals, the maintenance of acid-base equilibrium within normal limits is dependent on chemical and physiological compensatory mechanisms. Unlike other animals, however, the laying fowl must compensate for the regular daily occurrence of an acute metabolic acidosis. This acidosis coincides with the period of shell calcification (MONGIN and LACASSAGNE, 1964) and, apart from the immediate contribution of the blood buffers to the maintenance of homeostasis, there is evidence of both respiratory and renal compensation (MONGIN and LACASSAGNE, 1965; ANDERSON, 1967; TAYLOR and KIRKLEY, 1967).

If acid-base balance is further altered by experimental means, the amount of calcium carbonate which is deposited on the shell membrane may be increased or decreased (HUNT and AITKEN, 1962; HELBACKA, CASTERLINE and SMITH, 1963; FRANK and BURGER, 1965; HOWES, 1966). HUNT and AITKEN (1962) suggested that, since total plasma calcium level was not altered in the laying bird during ammonium chloride acidosis, the reduction in shell thickness which was observed was due to a limitation in the formation of carbonate radicals rather than to the reduced availability of calcium for shell formation. A marked increase in urinary calcium excretion during ammonium chloride acidosis nevertheless occurs in man (MARTIN and JONES, 1961), thus an effect of ammonium chloride on calcium metabolism in the fowl cannot be discounted.

There is, therefore, substantial evidence that shell calcification and acid-base status of the fowl are interrelated (see MONGIN, 1968). Much, however, remains to be learned of this relationship and how it may be exploited to provide optimum dietary and environmental conditions for the production of eggs of adequate shell thickness and strength.

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This paper deals with some of the changes in acid-base excretion associated with egg formation in the intact and colostomized fowl under normal conditions and during ammonium chloride acidosis. Some of the observations have previously been reported in brief form (ANDERSON, 1967).

## METHODS

Light hybrid hens (*Thornber 606* and *Shaver 288*) were used. Pre-lay birds were 19-20 weeks of age and laying birds were 24-31 weeks of age. They were kept in individual cages at a controlled temperature (16-20°C) with 12 hour artificial light per day and received a complete layers ration (No. 1 Complete Specialist Layers Chips or Mash-B. O. C. M.) and water *ad lib*. The ammonium chloride ration was prepared by mixing 2 p. 100 of ammonium chloride with the control ration.

Some birds were colostomized using a stainless steel cannula (HILL and ANDERSON, unpublished) and others using a modification of the technique described by FUSSELL (1960). Urine was collected in a light polythene bag held in place over the vent by a light harness. Droppings and urine pH measurements were made with a direct reading pH meter (Model 23 A, E. I. L.) and a spear glass electrode, and urinary total CO<sub>2</sub> was measured with a microgasometer (NATELSON, 1951). Urine bicarbonate concentration was calculated by substitution of the measured pH and total CO<sub>2</sub> values in the Henderson-Hasselbach equation using a value of 6.1 for pK' and 0.0309 as the solubility factor. Urinary sodium and calcium estimations were made by atomic absorption flame spectrophotometry (SP.90, Unicam).

Capillary blood samples were obtained by clipping a claw after immersion of the foot in warm water (50°C) for 2 minutes. Blood pH measurements were carried out on the Astrup Micro-equipment (Type AME 1, Radiometer). Bicarbonate concentration and pCO<sub>2</sub> was derived from the published nomogram (SIGAARD-ANDERSEN, 1962).

Shell thickness was calculated from the Specific Gravity of the whole egg (TYLER and GEAKE, 1961), using a hydrometer to obtain the weight of the egg in water (WELLS, 1967).

## RESULTS

### *I. Acid-base changes in the excreta on a standard diet*

#### *Normal hens.*

*Droppings pH.* — Measurement of droppings pH at intervals before and after oviposition was carried out on 17 birds. The mean post-oviposition pH was higher than the mean pre-oviposition pH in 45 out of 49 observations. (mean pH difference =  $0.69 \pm 0.38$  S. D.). In 23 of the observations, no egg was laid on the following day, but the increase in the droppings pH of this group after oviposition was not significantly different from that of the group in which an egg was laid on the following day.

In a more detailed study, droppings were collected at hourly intervals throughout each 12 hour light period for 4 days from 3 laying and 3 non-laying birds. The droppings evacuated during 12 hours of darkness were also collected and the pH measured, after it had been established that the change in the pH of droppings kept at room temperature over 12 hours was negligible. The pH changes and their relationship to oviposition are shown in figure. 1. The mean value from the 3 non-laying birds ( $8.02 \pm 0.25$  S. D.) was significantly higher ( $p < 0.001$ ) than that from the 3 laying birds ( $6.51 \pm 0.78$  S. D.). Throughout the experimental period, the hourly variation in the droppings pH of the non-laying birds was less than that of the laying birds.

There was no consistent over-all pattern in the dropping pH values of the 3 laying birds, although bird 5/1896 showed changes apparently related to oviposition — the laying of each egg being preceded by a period when the droppings pH was low and being followed by a marked rise in pH at oviposition. Low pH values (approximately 6.0) were recorded for approximately 10 hours before the first egg was laid, the main decrease having occurred during the 12 hours of darkness immediately preceding the beginning of this period. After the post-oviposition rise in pH, values decreased gradually to about 6.0 some 20 hours before laying of the second egg.

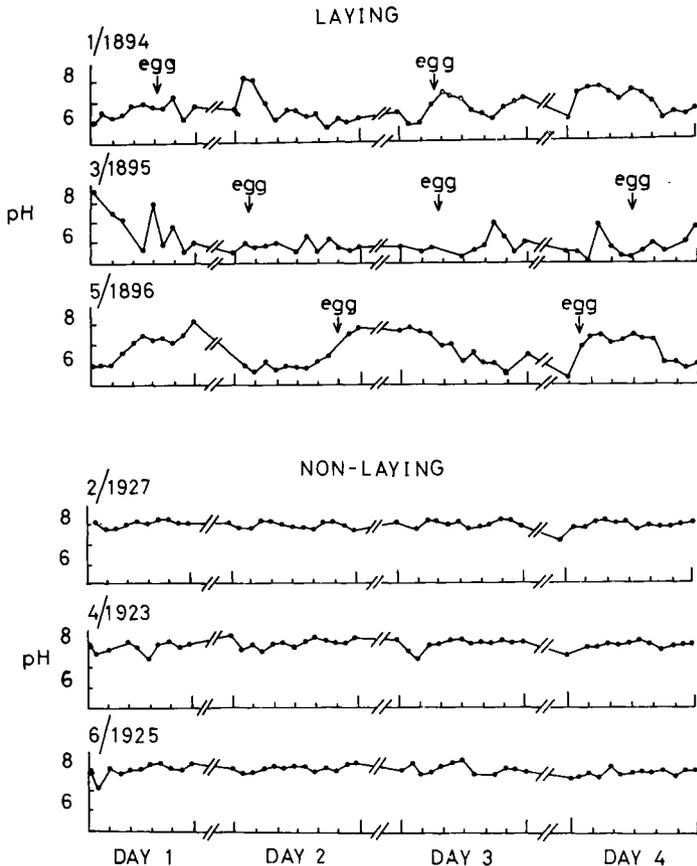


FIG. 1. — The pH values of droppings collected at hourly intervals from 3 laying and 3 non-laying hens. The first value on each day, except day 1, is from droppings excreted during the preceding 12 hour period of darkness. The subsequent values are from droppings excreted during the 12 hour period of light.

#### *Colostomized hens.*

*Urine pH and bicarbonate concentration.* — In order to ascertain whether the urinary or the alimentary component of the droppings was primarily responsible for the pH changes observed, experiments were carried out on colostomized birds. Urine pH and bicarbonate measurements were made at consecutive 2 hour intervals on laying colostomized birds for periods up to 3 days. Urine pH decreased from values around

8.0 20 hours before oviposition to about 5.5 14 hours before oviposition (fig. 2). These low values were maintained until oviposition, when within 2 hours, there was a rapid increase to pH 8.0. Urine bicarbonate concentration was high (20-40 meq /l),

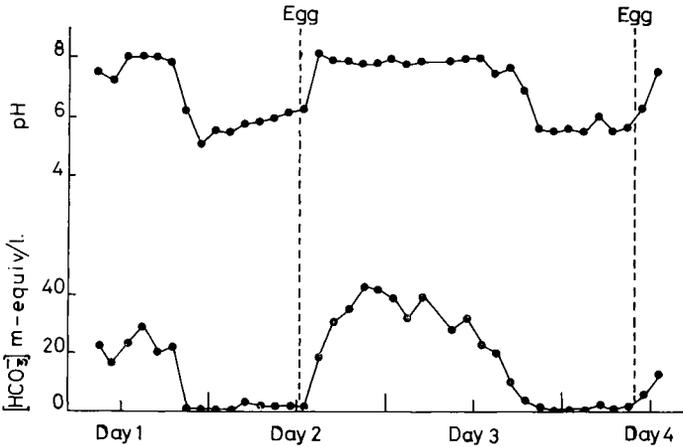


FIG. 2. — The pH and bicarbonate values of urine samples collected from a colostomized hen at 2 hour intervals over a period of four days

until 20 or more hours before oviposition but little or no bicarbonate was present during the last 14 hours of shell formation. The pH of the faeces collected from the stoma remained consistently high (about 8.0) during all these observations. Similar pH changes were observed during the formation and oviposition of 8 eggs (fig. 3).

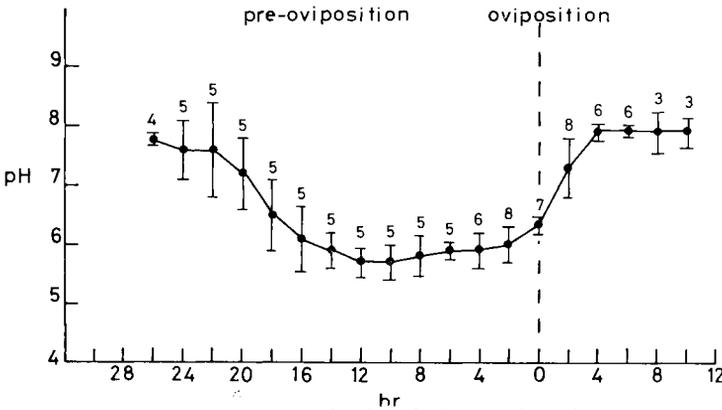


FIG. 3. — Urine pH values during egg formation  
Mean and standard deviation of values obtained from experiments on four colostomized hens. The number of individual observations is shown above each point on the graph

2. Acid-base changes during ammonium chloride acidosis

Normal hens.

Six out of twelve 26 week old Shaver 288 hens (Group A) were given the control ration throughout the three periods of the experiment. The remaining six birds

(Group B) received the control ration for 10 days (Period 1), the 2 p. 100 ammonium chloride ration for 21 days (Period 2) and the control ration for a further 21 days (Period 3) (table 1).

TABLE 1

*Design of the experiment to establish the effect of ammonium chloride acidosis on normal laying hens (Shaver 288).*

	Period 1 (10 days)	Period 2 (21 days)	Period 3 (21 days)
Group A <sup>(1)</sup> (control)	Control diet	Control diet	Control diet
Group B <sup>(1)</sup> (test)	Control diet	Test diet (2 % NH <sub>4</sub> Cl)	Control diet

<sup>(1)</sup> 6 hens in each group.

*Droppings pH.* — The mean pH of the droppings was significantly decreased during the feeding of ammonium chloride (table 2). The droppings pH of both groups increased inexplicably during Period 3 when all birds received the control ration.

TABLE 2

*The pH of droppings of during control and test periods. The values given represent the mean pH of all droppings evacuated during each period*

	Period 1	Period 2	Period 3
Group A (control)	5.74	5.86	6.56
Group B (NH <sub>4</sub> Cl in Period 2)	5.82	5.57 (*)	6.55

(\*) Significant difference from Group A ( $p < 0.05$ ).

*Shell thickness, blood pH and other parameters.* — Eggs from the test group (Group B) showed a significant decrease in shell thickness during Period 2, but egg production and weight were not affected (table 3).

There was a significant decrease in the pH and bicarbonate concentration (but not the pCO<sub>2</sub>) of the capillary blood during ammonium chloride acidosis (table 4).

The food intake of both groups increased throughout the three periods of the

experiment and was not affected by the inclusion of ammonium chloride in the ration. Water intake, however, showed a highly significant increase when the test ration was fed, returning to its previous level on resumption of the control ration (table 5) Droppings moisture and the volume of water excreted in the droppings also showed a large increase in association with the feeding of the ammonium chloride ration.

TABLE 3

*Shell thickness ( $\mu$ ) derived from Specific Gravity measurements.  
Mean values from all eggs collected during each period*

	Period 1	Period 2	Period 3
Group A (control)	330	331	347
Group B (NH <sub>4</sub> Cl in Period 2)	330	308 (*)	335

(\*) Significant difference from Group A ( $p < 0.05$ ).

#### *Colostomized hens.*

Two colostomized laying hens were fed the 2 p. 100 ammonium chloride ration for three days following a control period of six days. The pattern of urinary changes was similar in both birds, thus the findings from one bird only are reported.

*Urine and faeces output.* — There was a marked diuresis when the ammonium chloride diet was fed; urine flow increased from an average rate of 2.4 ml/h during the control period to 4.8 ml/h during ammonium chloride acidosis. (fig. 4). There was little variation in the total daily output of faeces nor in the faecal moisture content during the experiment.

*Calcium excretion.* — An egg was laid on each day of the experiment except Day 5. (fig. 5). On the preceding day (Day 4) — the only day on which egg formation did not occur — there was a marked calciuria. There was no increase in urinary calcium excretion on days 7, 8 and 9 when the ammonium chloride ration was fed, though there was a marked diuresis. As in the previous experiment with intact birds, there was a reduction in shell thickness during this period.

The faecal excretion of calcium showed a two-fold increase on Day 4 (the non-egg-forming day) but, unlike the urinary pattern, excretion remained relatively high on days 6, 7 and 8, returning on Day 9 to approximately the same level as on the first three days of the control diet. The total output of faeces remained fairly constant throughout the experiment.

The total (faecal and urinary) calcium excretion during ammonium chloride administration was not substantially different from that of the control period.

TABLE 4

The pH, bicarbonate concentration and  $p\text{CO}_2$  of capillary blood collected during control and test periods. Each hen was sampled twice during each period. The average value for each hen was used in obtaining the group average.

	Period 1			Period 2			Period 3		
	pH	$\text{HCO}_3$ (m-equiv/l)	$p\text{CO}_2$ (mm Hg)	pH	$\text{HCO}_3$ (m-equiv/l)	$p\text{CO}_2$ (mm Hg)	pH	$\text{HCO}_3$ (m-equiv/l)	$p\text{CO}_2$ (mm Hg)
Group A:									
Mean .....	7.46	25.2	35.2	7.45	24.5	35.4	7.47	24.6	33.3
S. D. ....	0.01	1.3	2.1	0.02	1.4	5.0	0.04	0.8	4.0
Group B:									
Mean .....	7.45	23.0	33.1	7.34 <sup>(1)</sup>	18.4 <sup>(1)</sup>	32.9	7.48	24.5	32.4
S. D. ....	0.04	1.5	3.1	0.04	1.7	4.7	0.03	1.1	3.7

<sup>(1)</sup> Significant difference from Group A ( $p < 0.001$ ).

TABLE 5  
*The water intake, droppings moisture and the water excreted during control and test periods. Average values from measurements made throughout each period. Droppings moisture and water output was not measured in Period 1*

	Water intake (ml/day)			Droppings moisture (%)			Water excreted (ml/day)		
	1	2	3	1	2	3	1	2	3
Group A: Control . . . . .	227	235	270		84	78		126	104
Group B: NH <sub>4</sub> Cl in Pe- riod 2 . . . . .	256	467 (1)	247		92 (1)	81		268 (1)	126

(1) Significant difference from Group A ( $p < 0.001$ ).

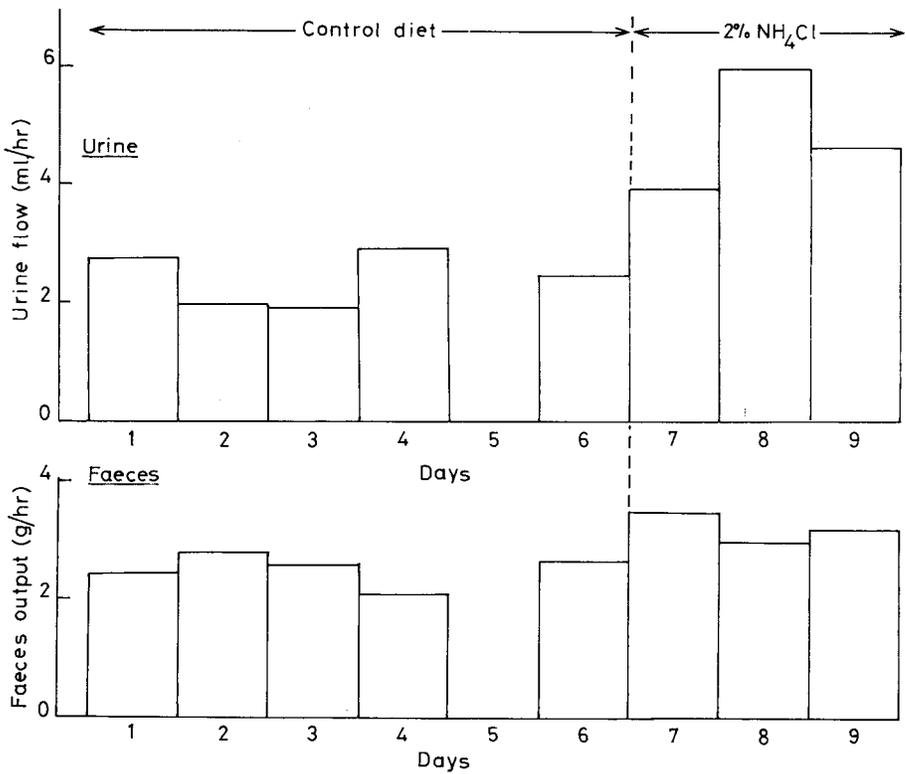


FIG. 4. — The average rate of urine and faeces output by a colostomized laying hen receiving the control diet (days 1-6) and a diet containing 2 p. 100 ammonium chloride (days 7-9).  
Collection was incomplete on day 5

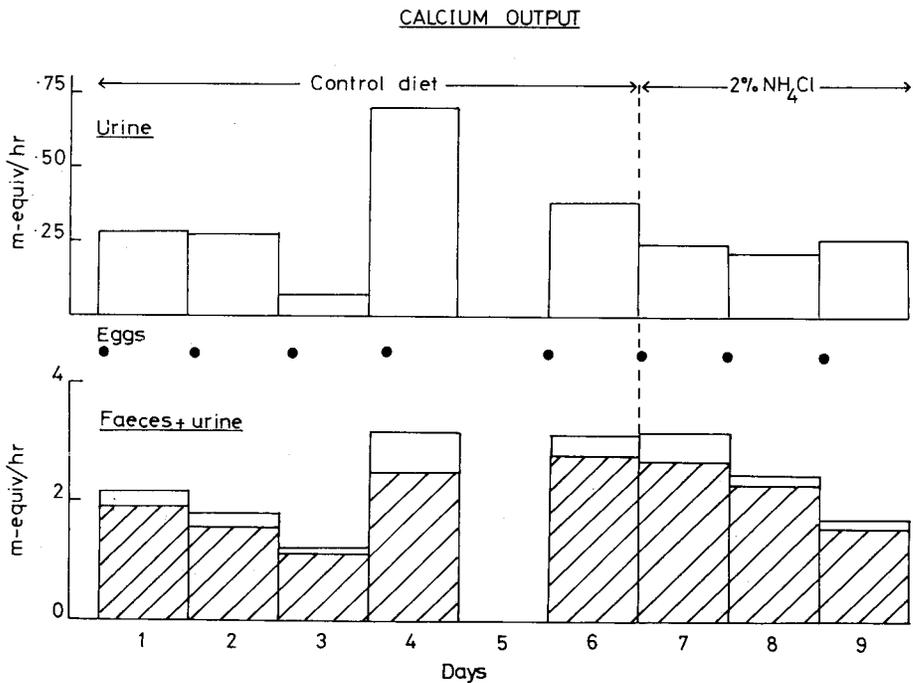


FIG. 5. — The average rate of urinary and faecal calcium output by a colostomized laying hen receiving the control diet followed by the 2 p. 100 ammonium chloride diet  
Collection was incomplete on day 5. The hatched areas represent faecal output

*Sodium excretion.* — Urinary sodium output remained fairly constant during the control period, but a very marked natriuresis occurred when the ammonium chloride ration was fed (fig. 6) reaching a maximum rate on Day 8.

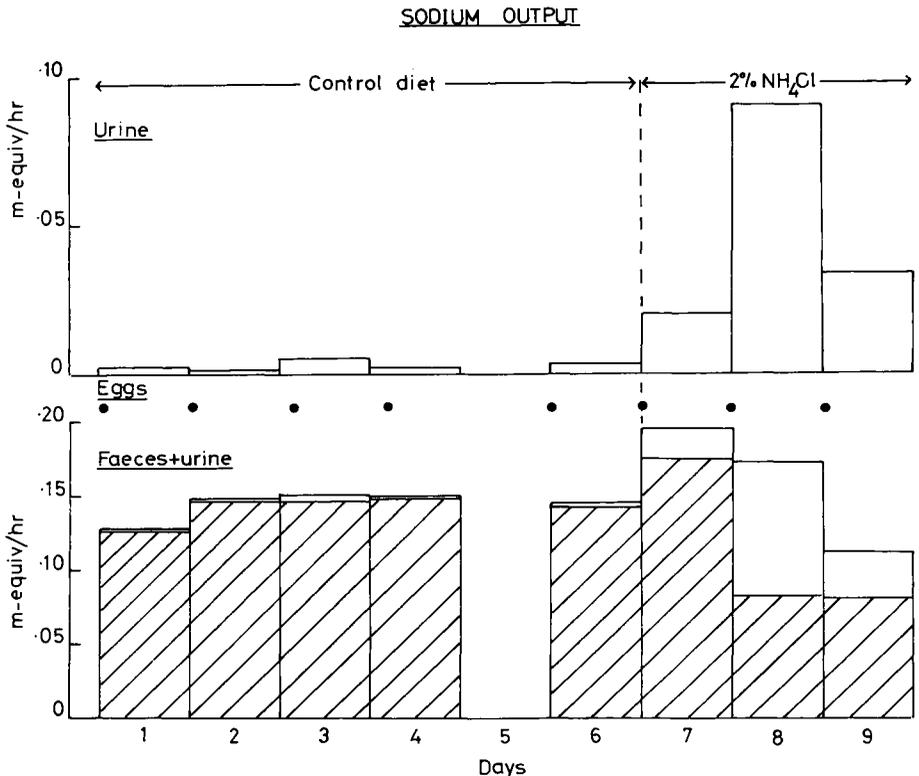


FIG. 6. — The average rate of urinary and faecal sodium output by a colostomised laying hen receiving the control diet followed by the 2 p. 100 ammonium chloride diet.

Collection was incomplete on day 5. The hatched areas represent faecal output

There was little daily variation in faecal sodium excretion during the control period and although a slight increase occurred on the first day of ammonium chloride administration, on Days 8 and 9 excretion decreased to a level below that of the control period.

The total (faecal and urinary) sodium excretion increased on Days 7 and 8, but returned to the control level on Day 9.

*Urine pH.* — Measurements of urine pH were not made at regular intervals but the values during the control period showed a similar relationship to egg formation to that seen in the previous experiment (fig. 7). On Days 7, 8 and 9, however, there was a downward trend in urinary pH values, reaching a lower limit of pH 5.1. The marked increase which occurred immediately after oviposition on Days 1-6 was almost abolished when the ammonium chloride diet was fed.

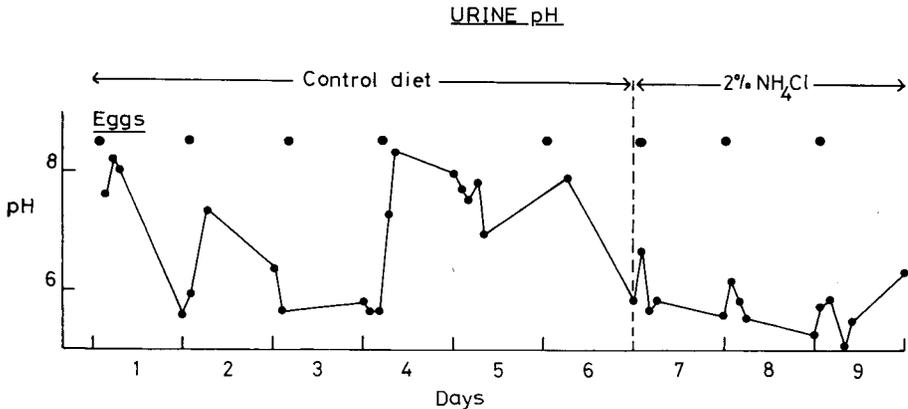


FIG. 7. — Urinary pH values from a colostomized laying hen receiving the control diet followed by a 2 p. 100 ammonium chloride diet.

#### DISCUSSION AND CONCLUSIONS

Since both laying and non-laying birds received the same ration in Experiment 1, the relatively greater acidity of the droppings from the laying birds may be assumed to be of metabolic rather than dietary origin. The clear relationship between the increased excretion of acid and the period of shell calcification which was seen in the urine of colostomized birds was not, however, so apparent in the excreta of the intact birds. The more irregular fluctuations in droppings pH are probably explained by the varying proportions of faeces and urine in the mixture of which the droppings are comprised. If, as seems probable from studies on colostomized birds, faecal material does not pass into the colon at a constant rate, then the droppings pH will vary according to the extent by which the urine is diluted and buffered by the faeces. Furthermore, there is now convincing evidence that post-renal urinary re-absorption occurs in the large bowel (SKADHAUGE, 1967) and the extent to which such re-absorption has occurred will further modify the final pH of the droppings. Whether or not post-renal urinary re-absorption plays a significant role in the maintenance of acid-base equilibrium is, however, open to question, since the acid-base status of the colostomized laying fowl does not appear to differ from that of the intact bird (ANDERSON, unpublished).

The decrease in urinary bicarbonate and pH during the 18 hours preceding oviposition corroborates the observation that shell calcification is associated with a metabolic acidosis (MONGIN and LACASSAGNE, 1964), and the titratable acidity of the urine and hence its efficiency in eliminating acid is greatly enhanced by the phosphaturia which coincides with the period of shell calcification (FUSSELL, 1960). Renal excretion of acid thus contributes substantially to the maintenance of acid-base equilibrium in the laying hen, thus any impairment of renal function may readily affect shell calcification through failure to compensate for the associated acidosis.

The failure to demonstrate convincingly any marked increase in calcium excre-

tion in association with ammonium chloride acidosis appears to corroborate the contention of HUNT and AITKEN (1962) that the decrease in shell thickness under these conditions is not due to an effect on calcium metabolism. If, however, calcium absorption, excretion and total blood calcium remain unaltered, the decreased output of calcium in the egg shell must, presumably, be balanced by decreased mobilization of calcium from the tissues.

The natriuresis and diuresis which occurred during ammonium chloride acidosis is well recognized in other species (SMITH, 1951), but has not, apparently, been reported previously in the fowl. It may be that much of the urinary sodium excreted by the colostomized fowl during acidosis would have been re-absorbed in the intact fowl, but the very low rate of urinary sodium excretion found during the control period suggests that the margin of safety for the manipulation of potentially acid-forming raw materials in rations of low sodium content is rather narrow.

### SUMMARY

The pH of droppings from normal laying and non-laying hens and the faecal and urinary pH and urinary bicarbonate concentration from colostomized laying hens were measured at regular intervals. The pH of droppings from the non-laying hens was significantly higher than that from the laying hens, in which there was some evidence of a decrease in the pH of droppings excreted during shell calcification. Urine pH and bicarbonate concentration invariably decreased during shell calcification and increased after oviposition.

During acidosis induced by dietary ammonium chloride, there was a decrease in the pH of the droppings and in the pH and bicarbonate concentration of the capillary blood of normal hens. Urinary calcium excretion of colostomized hens did not increase, but there was a marked increase in urinary sodium excretion. The observations support the contention that the decrease in shell thickness associated with ammonium chloride acidosis is not due primarily to an effect on calcium metabolism.

### RÉSUMÉ

#### VARIATIONS NORMALES ET EXPÉRIMENTALES DE L'EXCRÉTION D'ACIDES CHEZ LA POULE PONDEUSE

Le pH des déjections de poules normales pondeuses et non pondeuses ainsi que le pH des fèces et de l'urine et la concentration des bicarbonates urinaires de poules pondeuses à anus artificiel ont été mesurés à intervalles réguliers. Le pH des déjections des poules non pondeuses est significativement plus élevé que celui des poules pondeuses, pour lesquelles nous avons remarqué une nette diminution du pH des déjections lors de la formation de la coquille. Le pH et la teneur en bicarbonates de l'urine diminuent invariablement pendant la calcification de la coquille et augmentent après la ponte de l'œuf.

Au cours d'une acidose produite par l'ingestion de chlorure d'ammonium, il apparaît chez les poules normales une diminution du pH des déjections ainsi que du pH et de la teneur en bicarbonates du sang capillaire. L'excrétion de calcium dans l'urine des poules à anus artificiel n'est pas augmentée mais il y a un accroissement sensible de l'excrétion urinaire de sodium. Ces observations permettent de prétendre que la diminution d'épaisseur de la coquille liée à l'acidose induite par le chlorure d'ammonium n'est pas due en premier lieu à un effet sur le métabolisme calcique.

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