BLOOD pH AND CATION LEVELS
IN RELATION TO EGG-SHELL FORMATION

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INTRODUCTION

The final processes of egg formation, the deposition of the albumen and shel around the yolk, take place while the egg is passing down the oviduct. Normally the time taken between the ovulation of the yolk and the oviposition of the finished egg is about 26 hours and during the greater portion of this time, approximately 20 hours, the egg remains in the shell gland. Two basic processes are carried out whilst the egg is in the shell gland : firstly, the greater part of the plumping of the albumen and secondly, the formation of the shell. These two processes require consi-derable quantities of ions, particularly the calcium which as calcium carbonate forms about 95 p. 100 of the shell, but also other ions involved in the plumping process such as potassium and sodium. As the shell gland has little or no capacity for the storage of these substances, most of the ions which are incorporated into the egg have to be derived from the shell gland blood supply when the gland is active. The only exceptions to this are the bicarbonate ions which go to form the shell carbonate and these are almost certainly derived mainly from the shell gland metabolic carbon dioxide (HODGES and LÖRCHER, 1967).

The purpose of the present series of experiments was to obtain a comprehensive picture of the fluctuations of several of the more important ions associated with plum-ping and shell formation both in systemic and shell gland blood and during the whole of the shell formation cycle. Although a number of investigations of this nature have been performed in the past, most of them only measured calcium levels and this only on systemic blood (CHARLES and HOGBEN, 1933 ; POLIN and STURKIE, 1957 ; WINGET and SMITH, 1958 ; HERTELENDY and TAYLOR, 1961). If shell gland blood also was sampled it was done only on a very limited scale (KNOWLES, HART and HAL-PIN, 1935 ; WINGET, SMITH and HOOVER, 1958 ; HUNSAKER and STURKIE, 1961). In this series the cations calcium, potassium and sodium have been measured and at the same time, in order to obtain a measure of the blood acid-base fluctuations which occur during shell formation, the blood pH was determined.

MATERIALS AND METHODS

The birds used in the experiments were actively-laying hybrid hens of the *White Leghorn* strain (*Sterling White Links* and *Shaver 288*'s). Each bird was anæsthetized with sodium penta-barbitone and ether and a plastic cannula was inserted into the posterior end of the inferior ovi-ducal vein (HODGES, 1965) using the technique described by HODGES (1966). Cannulae were also inserted into the sciatic artery and vein in the upper left leg. From these three cannulae it was possible to take samples of systemic arterial and venous blood and of shell gland venous blood. Since the main artery supplying the shell gland, the hypogastric artery, branches off from the femoral artery at the level of the left kidney (FREEDMAN and STURKIE, 1963) the blood being sampled from the sciatic artery was of similar composition to that entering the shell gland.

After the operation the birds were kept lightly anæsthetized with ether and were heparinized. Hourly blood samples were taken throughout the period of each experiment and the timing of sampling to coincide with regular hourly periods within the shell formation cycle was performed by timing the laying of the previous egg or by the oviposition or the entry of an egg into the shell gland during the experimental period. Blood samples for pH determination were taken into capil-lary tubes (80 µl) and the pH values were measured with a Radiometer pH Micro-Electrode and pH-Meter 27. At the same time samples of 0.75 ml were taken into centrifuge tubes and the plasma was used for the cation measurements. Plasma sodium and potassium were measured with an EEL flame photometer (WOOTON, 1964) using 0.2 ml of plasma and plasma calcium was measured by means of Trinder's method (TRINDER, 1960) using 0.1 ml of plasma.

The experimental series was controlled by using unanæsthetized, unrestrained birds. Can-nulae were inserted into the wing veins of six birds and samples were taken every two hours over a period of approximately 24 hours.

The time scale of the tables is divided into two parts. Firstly, there is the period of shell gland quiescence between the point of lay (Egg Laid) and the time when another egg entered the shell gland (Egg Entered Shell Gland). Secondly, there is the period of shell formation lasting for 20 hours.

RESULTS

pH. (Control values — table 1 ; experimental values — table 2)

The control pH values were rather variable, but did show a regular fluctuation throughout the shell formation period, with a maximum level of about 7.38 at six hours after the egg entered the shell gland and a minimum value of 7.32 at 16 hours of shell formation. This pattern was somewhat obscured by a secondary fall in pH which occurred between four and six hours after lay.

In the experimental birds the pattern of the arterial results was basically simi-lar to that of the controls, a maximum of 7.51 two hours after the egg entered the shell gland and a minimum of 7.43 at thirteen hours of shell formation, but these points occurred about three hours earlier in the experimental birds than in the con-trols.

The shell gland venous values, in general, closely followed the pattern of the arterial values at a rather more acid level. However, whereas this arterio-venous difference was minimal during the period of shell gland quiescence (0.015 pH units four hours after lay), during the following period of shell formation the difference gradually increased to reach a maximum of 0.079 pH units at the fifteenth hour of shell formation (table 3). From then on the arterio-venous difference decreased until the egg was laid.

TABLE I

Wing vein blood pH and plasma total calcium values from the control birds.
Samples taken at 2-hourly intervals throughout the shell formation cycle

Point of time in shell cycle	Mean pH ± S. E. (No. of measurements)	Mean plasma calcium ± S. E. (No. of measurements)
Egg laid	7.347 ± .016 (5)	23.6 ± 2.53 (5)
+ 2 hours	7.356 ± .028 (5)	23.4 ± 2.52 (5)
+ 4 —	7.321 ± .026 (5)	26.6 ± 1.11 (5)
+ 6 —	7.320 ± .037 (5)	27.6 ± 1.4 (5) a
Egg entered shell gland	7.370 ± .017 (6)	24.0 ± 1.7 (5)
+ 2 —	7.346 ± .028 (5)	23.8 ± 1.2 (5)
+ 4 —	7.379 ± .020 (5)	21.8 ± 0.7 (5)
+ 6 —	7.386 ± .018 (5)	21.4 ± 0.8 (5)
+ 8 —	7.363 ± .026 (4)	20.7 ± 1.01 (4)
+ 10 —	7.328 ± .022 (4)	20.0 ± 1.12 (4) a
+ 12 —	7.349 ± .034 (4)	20.5 ± 1.75 (4)
+ 14 —	7.321 ± .026 (4)	20.25 ± 2.3 (4)
+ 16 —	7.318 ± .053 (4)	21.3 ± 2.79 (4)
+ 18 —	7.323 ± .037 (4)	21.5 ± 3.55 (4)
Egg laid	7.347 ± .016 (5)	23.6 ± 2.53 (5)

a. These values are not significantly different (P > 0.05).

Calcium. (Control values — table 1; experimental values — table 4)

The fluctuations in plasma total calcium which were found in the control birds appeared to be closely associated with shell formation. A maximum level of 27.6 mg p. 100 occurred at six hours after lay and a minimum level of approximately 20.2 mg p. 100 occurred between the tenth to fourteenth hours of the next shell formation period. The difference of 7.2 mg p. 100 between these maximum and minimum points was not, however, significant (P > 0.05).

In the experimental birds the sciatic arterial and venous values were very similar to each other throughout the whole of the experimental period. Cyclical fluctuations were again found during the shell formation period but they were not identical to those found in the controls. Maximum levels of 20.3 mg p. 100 occurred at oviposition and again during the first hour of the shell formation period, and the minimum level of 16.2 mg p. 100 occurred at ten hours of shell formation. The difference of 4.5 mg p. 100 between the maximum and minimum points was not significant (P > 0.05). During the second half of the shell formation period the plasma calcium level slowly recovered. It was noticeable that the experimental birds had an average systemic plasma calcium level at least 3 mg p. 100 below that of the controls.

The arterio-shell gland venous differences were very small during the first six hours of the shell gland quiescent period (average difference = 0.21 mg p. 100). Between the sixth hour of quiescence and the second hour of shell formation this difference had increased slightly (average difference = 0.85 mg p. 100) and by the third

TABLE 2

Systemic arterial (A) and venous (V) and shell gland venous (S) blood pH values measured at hourly intervals throughout the shell formation cycle in anaesthetised birds

The figures in brackets following each mean ± S. E. relate to the numbers of readings included in the mean.

Point of time in shell cycle	Vessel	Mean ± S. E.	Point of time in shell cycle	Vessel	Mean ± S. E.	Point of time in shell cycle	Vessel	Mean ± S. E.
Egg Laid	A	7.462 ± .026 (30)	+ 2 hours	A	7.514 ± .011 (12)	+ 11 hours	A	7.450 ± .015 (8)
	V	7.345 ± .036 (30)		V	7.360 ± .020 (12)		V	7.302 ± .021 (8)
	S	7.428 ± .024 (30)		S	7.471 ± .009 (12)		S	7.399 ± .019 (7)
+ 1 hour	A	7.458 ± .012 (24)	+ 3 hours	A	7.492 ± .016 (12)	+ 12 hours	A	7.451 ± .020 (4)
	V	7.330 ± .017 (24)		V	7.342 ± .024 (12)		V	7.374 ± .019 (4)
	S	7.436 ± .012 (24)		S	7.450 ± .011 (12)		S	7.398 ± .026 (4)
+ 2 hours	A	7.470 ± .014 (20)	+ 4 hours	A	7.497 ± .016 (12)	+ 13 hours	A	7.426 ± .029 (6)
	V	7.352 ± .015 (20)		V	7.364 ± .028 (12)		V	7.340 ± .013 (6)
	S	7.433 ± .012 (20)		S	7.422 ± .016 (11)		S	7.385 ± .025 (6)
+ 3 hours	A	7.458 ± .017 (18)	+ 5 hours	A	7.493 ± .013 (15)	+ 14 hours	A	7.437 ± .025 (7)
	V	7.337 ± .024 (18)		V	7.243 ± .026 (15)		V	7.350 ± .017 (7)
	S	7.437 ± .020 (18)		S	7.437 ± .013 (15)		S	7.393 ± .026 (6)
+ 4 hours	A	7.470 ± .015 (14)	+ 6 hours	A	7.475 ± .016 (12)	+ 15 hours	A	7.469 ± .018 (11)
	V	7.362 ± .019 (14)		V	7.304 ± .023 (12)		V	7.364 ± .017 (11)
	S	7.455 ± .031 (14)		S	7.417 ± .016 (12)		S	7.386 ± .020 (10)
+ 5 hours	A	7.472 ± .014 (15)	+ 7 hours	A	7.477 ± .024 (10)	+ 16 hours	A	7.458 ± .016 (14)
	V	7.417 ± .019 (15)		V	7.305 ± .028 (10)		V	7.407 ± .015 (14)
	S	7.453 ± .012 (15)		S	7.418 ± .026 (9)		S	7.387 ± .019 (13)
+ 6 hours	A	7.488 ± .010 (15)	+ 8 hours	A	7.479 ± .016 (10)	+ 17 hours	A	7.474 ± .007 (10)
	V	7.365 ± .014 (15)		V	7.321 ± .016 (10)		V	7.338 ± .024 (10)
	S	7.464 ± .009 (15)		S	7.417 ± .019 (9)		S	7.415 ± .009 (10)
Egg entered shell gland	A	7.502 ± .015 (13)	+ 9 hours	A	7.493 ± .014 (9)	+ 18 hours	A	7.456 ± .010 (20)
	V	7.376 ± .014 (13)		V	7.362 ± .019 (9)		V	7.352 ± .014 (20)
	S	7.461 ± .014 (13)		S	7.435 ± .010 (8)		S	7.404 ± .012 (18)
+ 1 hour	A	7.503 ± .015 (12)	+ 10 hours	A	7.506 ± .013 (5)	+ 19 hours	A	7.464 ± .008 (23)
	V	7.375 ± .018 (12)		V	7.293 ± .009 (5)		V	7.345 ± .013 (23)
	S	7.468 ± .013 (12)		S	7.448 ± .013 (5)		S	7.419 ± .008 (22)

hour of shell formation a considerable difference had developed. At the fifth hour a statistically significant difference (P < 0.05) of 3.9 mg p. 100 was apparent and thereafter an arterio-venous difference of this magnitude (2 to 4 mg p. 100) was maintained until the sixteenth hour of shell formation. Between this latter point and the time of lay the difference gradually decreased.

TABLE 3

Systemic arterial-shell gland venous differences in blood pH.
Mean values from the data in Table 2

Point of time in shell cycle	Mean A-S difference (No. of values)	Point of time in shell cycle	Mean A-S difference (No. of values)
Egg laid	0.034 ± .008 (30)	+ 7 hours	0.063 ± .005 (9)
+ 1 hour	0.021 ± .007 (24)	+ 8 —	0.064 ± .007 (9)
+ 2 hours	0.037 ± .009 (20)	+ 9 —	0.051 ± .004 (8)
+ 3 —	0.021 ± .008 (18)	+ 10 —	0.066 ± .007 (5)
+ 4 — *a*	0.015 ± .007 (13)	+ 11 ——	0.048 ± .007 (7)
+ 5 —	0.019 ± .007 (15)	+ 12 —	0.053 ± .009 (4)
+ 6 —	0.024 ± .005 (15)	+ 13 —	· 0.061 ± .017 (6)
E. E. S. G.............	0.030 ± .008 (13)	+ 14 —	0.036 ± .010 (6)
+ 1 hour	0.035 ± .007 (12)	+ 15 — *a*	0.079 ± .008 (10)
+ 2 hours	0.043 ± .006 (12)	+ 16 —	0.071 ± .009 (13)
+ 3 —	0.042 ± .009 (12)	+ 17 —	0.060 ± .008 (10)
+ 4 —	0.064 ± .013 (11)	+ 18 —	0.047 ± .006 (18)
+ 5 —	0.056 ± .006 (15)	+ 19 —	0.042 ± .006 (22)
+ 6 —	0.058 ± .007 (12)	Egg laid	0.034 ± .008 (30)

a. Values are significantly different at the 1 % level (P < 0.01).

Potassium. (Control values — table 5 ; experimental values — table 6)

In both the control and experimental birds a cyclical fluctuation of plasma potassium values was found to occur in association with the shell formation cycle. In the controls the maximum level (17.1 mg p. 100) occurred six hours after the previous egg was laid and the minimum value of 11.6 mg p. 100 was found at the tenth hour of shell formation. Between this latter point and the point of lay it recovered to 14.5 mg p. 100. The difference of 5.5 mg p..100 between the maximum and minimum points was statistically significant (P < 0.05).

In the experimental birds maximum potassium levels occurred during the quiescent period (17 mg p. 100) and minimum values between the fourth and eighth hours of shell formation (12 mg p. 100). The difference of 4.5 mg p. 100 between the combined arterial and shell gland venous figures at four hours after lay and at eight hours of shell formation was statistically significant (P < 0.05). The minimum values in the experimental birds occurred somewhat earlier than in the controls. It was evident at nearly every point throughout the experimental cycle that the systemic venous potassium values were greater than the systemic arterial values (average difference 0.8 mg p. 100 ; greatest difference 1.9 mg p. 100). These differences were

TABLE 4. — *Systemic arterial (A) and venous (V) and shell gland venous (S) plasma total calcium values measured at hourly intervals throughout the shell formation cycle in anaesthetised birds.*
Each value in the table represents the mean ± the standard error of the numbers of readings given in brackets

Point of time in shell cycle	Vessel	Mean value ± S. E. mg %	Point of time in shell cycle	Vessel	Mean value ± S. E. mg %	Point of time in shell cycle	Vessel	Mean value ± S. E. mg %
Egg Laid	A	20.4 ± 1.02 (17) (1)	+ 1 hour	A	20.3 ± 1.06 (9)	+ 10 hours	A	16.2 ± 1.58 (5) (1)
	V	19.9 ± 1.1 (17)		V	19.6 ± 1.20 (9)		V	16.6 ± 1.45 (5)
	S	21.4 ± 0.7 (13)		S	19.4 ± 1.27 (9)		S	14.2 ± 1.02 (5)
+ 1 hour	A	20.8 ± 0.69 (16)	+ 2 hours	A	20.3 ± 0.96 (10)	+ 11 hours	A	17.3 ± 1.43 (7)
	V	20.5 ± 0.66 (16)		V	20.2 ± 0.97 (10)		V	17.5 ± 1.49 (7)
	S	20.5 ± 0.67 (15)		S	19.5 ± 1.13 (10)		S	15.2 ± 1.57 (6)
+ 2 hours	A	20.7 ± 0.69 (15)	+ 3 hours	A	19.4 ± 1.28 (8)	+ 12 hours	A	18.7 ± 2.27 (4)
	V	20.1 ± 0.79 (15)		V	19.6 ± 1.14 (8)		V	18.9 ± 2.20 (4)
	S	20.7 ± 0.64 (15)		S	17.6 ± 1.44 (8)		S	15.7 ± 2.66 (4)
+ 3 hours	A	19.8 ± 0.67 (15)	+ 4 hours	A	17.5 ± 1.39 (8)	+ 13 hours	A	19.1 ± 1.66 (5)
	V	19.3 ± 0.67 (15)		V	17.5 ± 1.49 (8)		V	19.7 ± 1.70 (5)
	S	19.8 ± 0.92 (14)		S	14.9 ± 1.76 (8)		S	16.5 ± 2.00 (5)
+ 4 hours	A	19.2 ± 0.76 (15)	+ 5 hours	A	17.9 ± 1.30 (10) (2)	+ 14 hours	A	18.5 ± 1.5 (4)
	V	18.9 ± 0.78 (15)		V	18.0 ± 1.08 (10)		V	19.2 ± 1.26 (4)
	S	18.7 ± 1.02 (15)		S	14.8 ± 1.33 (10) (2)		S	14.3 ± 0.91 (3)
+ 5 hours	A	18.8 ± 0.58 (13)	+ 6 hours	A	18.3 ± 1.42 (10)	+ 15 hours	A	19.3 ± 0.92 (10)
	V	18.9 ± 0.86 (13)		V	17.7 ± 1.22 (10)		V	19.3 ± 0.91 (10)
	S	18.6 ± 0.78 (13)		S	15.6 ± 1.49 (9)		S	15.6 ± 0.98 (8)
+ 6 hours	A	19.5 ± 0.74 (9)	+ 7 hours	A	17.2 ± 1.11 (9)	+ 16 hours	A	18.7 ± 0.82 (9)
	V	19.0 ± 0.79 (9)		V	17.1 ± 1.10 (9)		V	19.3 ± 0.67 (9)
	S	19.2 ± 0.85 (9)		S	15.1 ± 1.05 (8)		S	16.1 ± 0.86 (7)
+ 7 hours	A	19.2 ± 0.07 (11)	+ 8 hours	A	17.7 ± 1.34 (9)	+ 17 hours	A	20.0 ± 1.00 (11)
	V	19.5 ± 0.67 (11)		V	17.8 ± 1.47 (9)		V	20.3 ± 0.92 (11)
	S	18.2 ± 0.86 (11)		S	15.7 ± 1.15 (8)		S	17.8 ± 0.90 (9)
Egg entered shell gland	A	19.8 ± 0.81 (11)	+ 9 hours	A	16.4 ± 1.49 (7)	+ 18 hours	A	19.1 ± 0.78 (17)
	V	19.8 ± 0.88 (11)		V	16.4 ± 1.54 (7)		V	20.0 ± 0.78 (17)
	S	19.1 ± 1.00 (11)		S	14.8 ± 1.00 (6)		S	18.0 ± 0.73 (15)
						+ 19 hours	A	20.2 ± 1.02 (16)
							V	19.9 ± 0.94 (16)
							S	19.1 ± 0.84 (13)

(1) The difference between these two values was not significant (P > 0.05).
(2) The difference between these two values was significant (P < 0.05).

TABLE 5

Wing vein plasma potassium and plasma sodium values from the control birds. Samples taken at 2-hourly intervals throughout the shell formation cycle.

Point of time in shell cycle	Mean plasma potassium ± S. E. (No. of measurements) mg %	Mean plasma sodium ± S. E. (No. of measurements) mg %
Egg laid	14.5 ± 0.90 (5)	312 ± 10.4 (5)
+ 2 hours	15.5 ± 0.98 (5)	323 ± 13.4 (5)
+ 4 —	16.3 ± 0.89 (5)	314 ± 9.0 (5)
+ 6 —	17.1 ± 1.17 (5) *a*	303 ± 8.7 (5) *b*
Egg entered shell gland	15.7 ± 0.77 (6)	311 ± 7.1 (6)
+ 2 hours	16.2 ± 1.28 (5)	308 ± 14.0 (5)
+ 4 —	13.9 ± 1.48 (5)	308 ± 11.4 (5)
+ 6 —	12.9 ± 1.05 (5)	304 ± 22.5 (5)
+ 8 —	12.6 ± 0.50 (4)	304 ± 15.7 (4)
+ 10 —	11.6 ± 0.66 (4) *a*	304 ± 19.6 (4)
+ 12 —	12.9 ± 1.98 (4)	309 ± 15.8 (4)
+ 14 —	12.0 ± 0.97 (4)	309 ± 23.2 (4)
+ 16 —	13.1 ± 1.24 (4)	326 ± 5.6 (4)
+ 18 —	14.2 ± 0.89 (4)	326 ± 7.4 (4) *b*
Egg laid	14.5 ± 0.90 (5)	312 ± 10.4 (5)

a. Values are significantly different at the 5 p. 100 level (P < 0.05).
b. These values are not significantly different (P > 0.05).

greatest during the first fourteen hours of shell formation. On the other hand no definite and consistently positive arterio-shell gland venous differences were found within the period of shell formation except during the last three hours, when differences of 1.0 to 2.1 mg p. 100 were measured.

Sodium. (Control values — table 5 ; experimental values — table 7)

In the control birds the plasma sodium levels remained fairly constant and no significant fluctuations were measured at any point during the shell formation cycle. Similarly in the experimental birds no overall pattern of fluctuations could be detected and no consistent arterio-shell gland venous differences were demonstrated.

There was, however, one anomaly between the control and experimental birds ; the values for the former were approximately 40 mg p. 100 lower than those for the latter throughout the whole of the cycle.

DISCUSSION

pH

The control results were only partially in agreement with those of MONGIN and LACASSAGNE (1964), who also sampled wing vein blood. Their pH values were maximal when the egg entered the shell gland and were minimal at 17 hours of shell formation.

TABLE 6

Systemic arterial (A) and venous (V) and shell gland venous (S) plasma potassium values measured at hourly intervals throughout the shell formation cycle in anaesthetised birds.

Each value in the table represents the mean ± the standard error of the number of readings given in brackets.

Point of time in shell cycle	Vessel	Mean value ± S. E. mg %	Point of time in shell cycle	Vessel	Mean value ± S. E. mg %	Point of time in shell cycle	Vessel	Mean value ± S. E. mg %
Egg Laid	A	16.8 ± 0.95 (16)	+ 1 hour	A	13.8 ± 0.96 (10)	+ 10 hours	A	12.8 ± 0.46 (5)
	V	17.0 ± 0.92 (16)		V	15.4 ± 1.00 (10)		V	14.0 ± 0.63 (5)
	S	15.1 ± 1.00 (12)		S	14.7 ± 1.22 (10)		S	12.2 ± 0.75 (5)
+ 1 hour	A	15.2 ± 0.73 (16)	+ 2 hours	A	14.0 ± 1.41 (9)	+ 11 hours	A	13.2 ± 0.94 (7)
	V	16.1 ± 0.53 (16)		V	15.2 ± 1.43 (9)		V	14.8 ± 0.46 (7)
	S	16.3 ± 0.72 (14)		S	15.3 ± 1.27 (9)		S	12.4 ± 1.06 (6)
+ 2 hours	A	14.8 ± 0.78 (14)	+ 3 hours	A	13.2 ± 1.18 (9)	+ 12 hours	A	12.9 ± 0.96 (4)
	V	15.2 ± 0.83 (14)		V	13.4 ± 1.11 (9)		V	13.5 ± 0.71 (4)
	S	14.2 ± 0.55 (14)		S	12.7 ± 1.32 (9)		S	14.4 ± 1.19 (4)
+ 3 hours	A	15.2 ± 0.89 (14)	+ 4 hours	A	12.0 ± 0.96 (9)	+ 13 hours	A	13.2 ± 1.22 (5)
	V	15.6 ± 0.70 (14)		V	13.0 ± 1.21 (9)		V	13.8 ± 1.00 (5)
	S	15.6 ± 1.06 (13)		S	11.7 ± 1.15 (9)		S	13.5 ± 0.55 (5)
+ 4 hours	A	16.4 ± 0.68 (10) (¹)	+ 5 hours	A	11.9 ± 0.86 (12)	+ 14 hours	A	13.8 ± 0.63 (5)
	V	16.5 ± 0.75 (10)		V	12.7 ± 1.00 (12)		V	14.8 ± 1.02 (5)
	S	16.5 ± 0.48 (10)		S	11.8 ± 0.94 (12)		S	14.3 ± 0.87 (4)
+ 5 hours	A	15.2 ± 0.90 (10)	+ 6 hours	A	12.1 ± 0.94 (11)	+ 15 hours	A	14.0 ± 0.79 (9)
	V	17.1 ± 0.97 (10)		V	13.1 ± 1.24 (11)		V	14.4 ± 0.82 (9)
	S	16.4 ± 1.08 (9)		S	12.1 ± 1.28 (10)		S	13.6 ± 0.81 (7)
+ 6 hours	A	14.2 ± 1.36 (10)	+ 7 hours	A	11.7 ± 0.91 (9)	+ 16 hours	A	15.3 ± 0.85 (9)
	V	15.0 ± 1.16 (10)		V	13.1 ± 1.10 (9)		V	16.1 ± 0.39 (9)
	S	14.1 ± 0.86 (9)		S	12.2 ± 1.21 (8)		S	14.8 ± 0.77 (7)
+ 7 hours	A	16.7 ± 0.51 (11)	+ 8 hours	A	12.1 ± 0.89 (9)	+ 17 hours	A	15.7 ± 0.99 (11)
	V	16.3 ± 1.24 (11)		V	13.2 ± 1.02 (9)		V	15.5 ± 1.11 (11)
	S	15.1 ± 1.02 (11)		S	11.9 ± 1.00 (8)		S	14.7 ± 1.15 (9)
Egg entered shell gland	A	14.9 ± 1.08 (12)	+ 9 hours	A	12.7 ± 0.71 (9)	+ 18 hours	A	14.6 ± 0.74 (14)
	V	16.1 ± 1.25 (12)		V	13.9 ± 1.11 (9)		V	15.4 ± 0.78 (14)
	S	15.5 ± 1.06 (12)		S	12.5 ± 0.91 (8)		S	12.9 ± 0.87 (11)
						+ 19 hours	A	16.3 ± 1.02 (14)
							V	16.1 ± 0.98 (14)
							S	14.2 ± 0.78 (11)

(¹) The difference between these two values was significant (P < 0.05).

TABLE 7

Systemic arterial (A) and venous (V) and shell gland venous (S) plasma sodium values measured at hourly intervals
throughout the shell formation cycle in anaesthetised birds.
Each value in the table represents the mean ± the standard error of the number of readings given in brackets.

Point of time in shell cycle	Vessel	Mean value ± S. E. mg %	Point of time in shell cycle	Vessel	Mean value ± S. E. mg %	Point of time in shell cycle	Vessel	Mean value ± S. E. mg %
Egg Laid	A	355 ± 6.5 (16)	+ 1 hour	A	365 ± 9.0 (9)	+ 10 hours	A	346 ± 7.9 (5)
	V	362 ± 8.0 (16)		V	363 ± 7.0 (9)		V	346 ± 9.6 (5)
	S	352 ± 7.4 (12)		S	355 ± 6.5 (9)		S	347 ± 7.6 (5)
+ 1 hour	A	353 ± 6.6 (16)	+ 2 hours	A	361 ± 7.3 (9)	+ 11 hours	A	359 ± 13.1 (7)
	V	352 ± 7.1 (16)		V	365 ± 10.0 (9)		V	347 ± 10.0 (7)
	S	359 ± 6.9 (14)		S	366 ± 6.1 (9)		S	346 ± 7.4 (6)
+ 2 hours	A	341 ± 5.6 (14)	+ 3 hours	A	363 ± 8.0 (9)	+ 12 hours	A	342 ± 4.3 (4)
	V	344 ± 6.8 (14)		V	358 ± 7.3 (9)		V	343 ± 4.0 (4)
	S	342 ± 4.5 (14)		S	358 ± 6.3 (9)		S	347 ± 9.6 (4)
+ 3 hours	A	336 ± 4.3 (14)	+ 4 hours	A	353 ± 8.0 (9)	+ 13 hours	A	341 ± 3.5 (5)
	V	346 ± 6.2 (14)		V	348 ± 6.1 (9)		V	332 ± 8.7 (5)
	S	347 ± 5.4 (13)		S	359 ± 9.5 (9)		S	333 ± 14.9 (5)
+ 4 hours	A	340 ± 5.7 (11)	+ 5 hours	A	355 ± 4.1 (12)	+ 14 hours	A	332 ± 3.5 (5)
	V	346 ± 5.3 (11)		V	359 ± 4.0 (12)		V	338 ± 12.2 (5)
	S	350 ± 6.3 (11)		S	357 ± 5.4 (12)		S	342 ± 15.2 (4)
+ 5 hours	A	340 ± 4.7 (11)	+ 6 hours	A	364 ± 4.5 (11)	+ 15 hours	A	357 ± 11.6 (9)
	V	347 ± 7.3 (11)		V	367 ± 5.7 (11)		V	348 ± 4.8 (8)
	S	346 ± 6.3 (10)		S	366 ± 6.7 (10)		S	348 ± 9.1 (7)
+ 6 hours	A	350 ± 7.5 (11)	+ 7 hours	A	364 ± 6.6 (10)	+ 16 hours	A	357 ± 9.8 (8)
	V	357 ± 9.0 (11)		V	373 ± 8.9 (10)		V	367 ± 7.4 (9)
	S	347 ± 7.0 (10)		S	362 ± 7.4 (9)		S	381 ± 5.8 (7)
+ 7 hours	A	357 ± 5.6 (11)	+ 8 hours	A	357 ± 7.2 (10)	+ 17 hours	A	359 ± 5.8 (10)
	V	362 ± 6.5 (11)		V	356 ± 8.1 (10)		V	357 ± 6.1 (11)
	S	356 ± 5.3 (11)		S	346 ± 6.9 (9)		S	355 ± 5.4 (9)
Egg entered shell gland	A	354 ± 5.3 (11)	+ 9 hours	A	360 ± 7.2 (8)	+ 18 hours	A	348 ± 3.9 (14)
	V	359 ± 5.1 (11)		V	358 ± 9.5 (8)		V	352 ± 4.4 (14)
	S	365 ± 6.2 (11)		S	350 ± 6.9 (7)		S	351 ± 4.3 (11)
						+ 19 hours	A	352 ± 5.8 (14)
							V	347 ± 5.0 (14)
							S	354 ± 4.3 (11)

The present results were overall at least 0.01 pH units lower than those of MONGIN and LACASSAGNE and, in the case of the maximum point, approximately 5 hours later in the shell formation cycle. However, it was not easy to relate their time scale to the present one. Another point of difference was that whereas during the shell gland quiescent period the present results were in the process of recovering from the minimal values which had occurred before the oviposition of the previous egg, MONGIN and LACASSAGNE's results were constant and maximal.

In discussing the experimental results it is necessary to acknowledge that the pH values would be adversely affected by the anaesthetic. Although the average arterial pH in these experiments (7.474) was slightly higher than that found for unanaesthetised birds of a similar strain (7.410), and thus respiratory depression due to the anaesthetic would not seem to have occured, other experiments have shown that birds with a similar arterial pH may have an unusually high pCO_2 and bicarbonate, showing that some respiratory disturbance had occured. This was minimised by keeping the birds only lightly anaesthetised after the actual operation.

The sciatic venous values were very irregular and did not follow the trends occurring in the sciatic arterial and wing vein blood. This may have been due to the fact that the venous cannulae were inserted well into the vein, thus drawing off blood from the region of the junction of the sciatic vein with the renal portal vein. It is possible that blood from the renal portal vein is not truly representative of systemic venous blood.

The arterial and shell gland venous results possessed maximum and minimum values which coincided closely with those of MONGIN and LACASSAGNE (1964), but the actual cyclical pattern of these results corresponded more closely with the present controls than with the values of MONGIN and LACASSAGNE. After oviposition pH values slowly increased to a maximum just after the next egg entered the shell gland and, as the next phase of shell formation was initiated, the pH gradually declined to the minimum at 13 hours of shell formation. Thereafter it recovered to about midway between minimum and maximum points at the time of lay.

Although the shell gland venous pH values in general followed the trends of the arterial values, the arterio-venous pH difference varied according to the phase of shell formation and thus appears to be closely associated with the process of egg shell formation. The difference was minimal during quiescence and at the beginning of shell formation, but steadily increased from then on until the fifteenth hour of shell formation (there was some irregularity in the results between 9 and 14 hours). After the fifteenth hour the difference decreased rapidly until the point of lay, indicating that the rate of shell formation may be decreasing during this period .

It was originally considered that the egg shell carbonate was derived from bicarbonate ions removed from the blood stream by the shell gland (GUTOWSKA and MITCHELL, 1945), and the removal of such bicarbonate ions could account for changes in the pH of the shell gland venous blood. GUTOWSKA and MITCHELL's theory received support from the fluctuations of systemic bicarbonate found by MONGIN and LACASSAGNE (1964). More recently, however, HODGES and LÖRCHER (1967) have demonstrated that the egg shell carbonate is derived from the metabolic carbon dioxide of the shell gland and that during this process considerable amounts of hydrogen ions are produced. The development of the large A-V. difference would therefore appear to be mainly associated with the production of hydrogen ions by the shell gland and

the changes that occur in the systemic pH, pCO_2 and bicarbonate levels concurrently with this A-V. difference would appear to be due to the introduction of large amounts of hydrogen ions into the circulation and to the compensatory efforts of the birds regulatory mechanisms. In the present experimental data the systemic pH began a gradual recovery after the thirteenth hour of shell formation even though the process of shell formation was still actively occurring, thus indicating the initiation of a compensatory mechanism. From the data of MONGIN and LACASSAGNE (1964) it can be seen that a slight respiratory alkalosis develops during shell formation as a partial compensation for the increasing metabolic acidosis. However, this acidosis develops steadily until about 17 hours of shell formation (three hours before lay), and then the process is reversed along the line of a metabolic alkalosis indicating active compensation by the kidneys. On the other hand, ANDERSON (1967) has shown, by measuring the pH of the urine, that secretion of acid by the kidneys begins soon after the initiation of active shell formation (15-16 hours before the egg is laid), indicating that compensation by the kidneys has begun long before the reversal of the metabolic acidosis. This suggests that by 17 hours of shell formation shell secretion is decreasing and that the kidney, which before was unable to compensate for the production of hydrogen ions, can now reverse the metabolic acidosis. However, in the present data the reversal apparently began earlier, at the thirteenth hour of shell formation, suggesting that the kidneys of these birds were able to reverse the metabolic acidosis at an earlier stage. Without further acid-base data this interpretation can only be a suggestion.

Calcium

The plasma calcium results are in general agreement with previous work. WINGET and SMITH (1958) demonstrated similar fluctuations in total plasma calcium during the shell formation cycle and HERTELENDY and TAYLOR (1961) showed a similar overall drop in plasma calcium between shell gland quiescence and the period of active shell formation. However, the drop in systemic plasma calcium which was found in both the control and experimental birds in the present experiments was not found to be statistically significant. This may have been due to the very large variation that can occur between birds (HERTELENDY and TAYLOR, 1961).

This fall in systemic plasma calcium, in the present experimental birds, continued until the tenth hour of shell formation. Thereafter the trend was reversed and levels returned to normal just after oviposition, in spite of the fact that shell calcification appeared to continue at a steady rate across most of the period of shell formation. This reversal in the fall of plasma calcium may be associated with a rapid mobilisation of calcium from the medullary bone. FUSSEL (1966) has demonstrated that there is frequently a considerable increase in urinary phosphorus output during the last eight or ten hours of shell formation, indicating a rapid breakdown of bone mineral. The autoradiographic studies of TYLER (1954) have also shown that the calcium laid down during the last few hours of shell formation is mainly of skeletal origin. In the present series not all of the birds were early layers and thus the whole of the rise in plasma calcium cannot be ascribed to bone mobilisation; part of this rise, at least during the last four or five hours before lay, must be attributed to renewed absorption of calcium from the intestine.

The present data is also in general agreement with previous work on the rates

of calcification of the egg shell. The work of BURMESTER, SCOTT and CARD (1939), BURMESTER (1940) and BRADFIELD (1951) have indicated that during the first four hours that the egg spends in the shell gland the rate of calcification gradually increases and thereafter a rapid and constant rate of calcification occurs throughout the remaining sixteen hours before lay. In the present results a large arterio-venous difference had developed by the fourth hour after entry into the shell gland, and this continued for about twelve hours. However, after the sixteenth to seventeenth hours the difference rapidly decreased, indicating a gradual decrease in the rate of shell formation.

Potassium

Similar patterns of fluctuation were found in both the potassium and calcium levels even though potassium mobilisation is mainly related to plumping and calcium movement is related to shell formation. The process of plumping begins in the isthmus and whilst it and shell formation are progressing the potassium content of the albumen is approximately doubled (DRAPER, 1966). Accumulation of this ion even continues when a hard shell is formed over the entire surface (DRAPER, 1966). It can be seen from table 8 that the initial fall in plasma potassium approximately coincided with the beginning of the rapid uptake of potassium into the albumen. The reciprocal relationship between plasma and albumen potassium continued until about the tenth hour of shell formation. From then on, however, the trend in plasma potassium was reversed although there was no cessation of increase of albumen potassium.

TABLE 8

Comparison of potassium levels in blood plasma (wing vein),
shell gland fluid and egg albumen during plumping and shell formation

Egg albumen figures recalculated from DRAPER (1966) and the shell gland fluid figures recalculated from EL JACK and LAKE (1967)

Point of time in shell cycle	Plasma potassium (mg %)	Albumen potassium (mg %)	Shell gland fluid potassium (mg %)
Egg laid	14.5		
+ 2 hours	15.5		
+ 4 —	16.3	39.0	
+ 6 —	17.1	39.0	
Egg entered shell gland	15.7		
+ 1 hour		46.6	
+ 2 hours	16.2		
+ 3 —			62.0
+ 4 —	13.9		
+ 6 —	12.9		
+ 8 —	12.6	58.3	
+ 10 —	11.6		
+ 12 —	12.9		
+ 14 —	12.0		
+ 16 —	13.1		
+ 18 —	14.2		293
Egg laid	14.5	78.0	

No arterio-shell gland venous potassium differences were found throughout almost the whole of the plumping/shell formation period and this was to be expected as, on the basis of the amount of potassium transferred into the albumen over the period of twenty hours, a rough calculation only gives an average A-V. difference of 0.2 mg p. 100, which would not become evident in the results. However, a difference of about 1-2 mg p. 100 was measured during the last three hours of shell formation. This can probably be explained by some results of EL JACK and LAKE (1967) (table 8). They measured the ionic constituents of the shell gland fluid at two points, during plumping and shortly before the egg was laid. They found that the potassium level of plumping fluid was only 16 mmol/l. whilst that taken towards the end of shell formation was 75 mmol/l. If such an unusually high potassium level could be shown to occur only during the last three hours of shell formation, then this would account for the arterio-venous difference during this period.

Sodium

Whilst plumping is occurring the total amount of sodium in the albumen increases by 50 p. 100, from approximately 110 mmol. to approximately 160 mmol. (DRAPER, 1966). However, owing to the very large uptake of water, the concentration in the albumen drops from 127 mmol/l. in the magnum to about 60 mmol/l. when laid. These comparatively small amounts of sodium were apparently not large enough to show up as either an overall plasma variation or as an A-V. difference.

There was one rather anomalous factor present in the plasma sodium results. The control results were consistently about 40 mg p. 100 lower than the experimental results. There does not seem to be, at present, any satisfactory explanation for this.

In the cases of the pH, the calcium and the potassium results it was noted that a maximum level was found at about the time of entry of an egg into the shell gland and a minimum level occurred at, or just after, the mid-point of the period of shell formation. In all three cases the levels returned gradually towards normal after the minimum point was passed and thus, although shell formation continued steadily for several more hours, the trend in all these three parameters was reversed. Because of this close similarity in time and trend it seemed possible that there was some common cause or factor underlying all three. This common factor may be the activity (excretory or resorbtive) of the kidney, but it will not be possible to demonstrate this until the complete pattern of excretion during shell formation is known.

SUMMARY

1. Cannulations of the inferior oviducal vein, the sciatic artery and the sciatic vein were performed upon actively-laying, anaesthetised hens. A control series of unanæsthetized birds were cannulated only in the wing vein. All cannulae were sampled at hourly intervals during the shell formation cycle and blood pH, plasma total calcium, potassium and sodium were measured.

2. pH. Arterial pH was at a maximum of 7.51 two hours after the egg entered the shell gland and at a minimum of 7.43 at thirteen hours of shell formation. The shell gland venous blood in general followed the arterial trends at a more acid level but, during shell formation, an increasing A-V. difference developed, reaching a maximum of 0.079 pH units at 15 hours of shell formation.

3. Calcium. Cyclical fluctuations of plasma total calcium were found with a maximum level (20 mg p. 100) during the quiescent period and a minimum level (16 mg p. 100) at 10 hours of shell formation. During shell formation an A-V. difference of 2-4 mg p. 100 developed.

4. Potassium. A cyclical pattern of plasma potassium associated with the shell formation cycle was again found. Maximum levels (17 mg p. 100) occurred during the quiescent period and minimum values (12 mg p. 100) at 4 to 8 hours of shell formation. An A-V. potassium difference (1-2 mg p. 100) was only found during the last three hours before lay.

5. Sodium. No cyclical pattern of sodium values or consistent A-V. differences were found which could be associated with the shell formation cycle.

6. The significance of the results was discussed in relation to the processes of plumping and shell formation.

RÉSUMÉ

VARIATIONS DU pH ET DES CATIONS DU SANG
LORS DE LA FORMATION DE LA COQUILLE DE L'ŒUF CHEZ LA POULE

1. Des canules ont été insérées dans l'artère et la veine sciatique et dans la veine inférieure de l'oviducte de poules anesthésiées. Des poules témoins non anesthésiées furent seulement canulées dans la veine alaire. Des prélèvements de sang ont été effectués chaque heure pendant la fortion de la coquille et le pH du sang ainsi que la teneur en calcium total, potassium et sodium du plasma ont été mesurés.

2. pH. Les valeurs artérielles présentent un maximum de 7,51 deux heures après l'entrée dans la glande coquillière et un minimum de 7,45 treize heures après le début de la formation de la coquille. Le sang veineux de la glande coquillière tend en général à suivre les valeurs artérielles à un niveau plus acide mais, pendant la formation de la coquille, une différence artério-veineuse croissante se développe qui atteint un maximum de 0,079 unités pH au stade 15 heures de formation de la coquille.

3. Calcium. La teneur en calcium total du plasma présente des variations cycliques, le niveau maximal (20 mg p. 100) intervenant pendant la période de repos de la glande coquillière et le niveau minimal (16 mg p. 100) après 10 heures de formation de la coquille. Au cours du dépôt de la coquille, une différence artério-veineuse de 2 à 4 mg p. 100 se développe au niveau de la glande coquillière.

4. Potassium. Il apparaît également des fluctuations cycliques de la kaliémie liées à la formation de la coquille. Les valeurs maximales (17 mg p. 100) sont trouvées durant la période de repos et les valeurs minimales (12 mg p. 100) entre 4 et 8 heures de formation de la coquille. Il n'existe aucune différence entre les teneurs des artères et des veines de la glande coquillère sauf pendant les trois dernières heures précédant l'oviposition où un écart de 1 à 2 mg p. 100 est enregistré.

5. Sodium. Aucun comportement cyclique de la natrémie ni de différence artério-veineuse utérine ne sont trouvés qui puissent être associés au cycle de formation de la coquille.

6. La signification de ces résultats est discutée en rapport avec le « plumping » et la formation de la coquille de l'œuf.

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