

STUDIES OF THE AVIAN SHELL GLAND DURING EGG FORMATION

I. — THE EXTRACELLULAR SPACE OF LAYING HENS

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SUMMARY

Radiosulphate spaces were measured over a period of 240 minutes following injection of carrier free $\text{Na}_2^{35}\text{SO}_4$ in laying hens at three different physiological stages of egg formation. Just after oviposition and during shell calcification, radiosulphate spaces reach an equilibrium. The volumes of distribution measured were 267 ± 7.5 and 232 ± 5.0 ml/kg body weight respectively. The elapsed times after isotope injection corresponding to these estimates of extracellular volume were 71 ± 6 and 58 ± 5 minutes respectively. Unexpectedly, and without apparent reason, the volume of distribution of $^{35}\text{SO}_4^-$ during albumen plumping never reached equilibrium. However, if 70 minutes past injection was arbitrarily taken as a correct time for estimate of extracellular volume, the value obtained was 243 ± 10 ml/kg body weight. When all data are pooled, regardless of the physiologic state of egg formation, the volume of distribution of $^{35}\text{SO}_4^-$ at 70 ± 4 minutes after isotope injection was found to be 248.5 ± 4.2 ml/kg body weight.

In the determination of intracellular electrolyte composition, a precise estimate of the extracellular volume of the tissue is of great importance. Previously, both inulin and chloride ion have been commonly used as extracellular markers. Perhaps their use in mammalian tissues can sometime be questioned, but in avian tissue

their use as a volume marker appears definitely unwise. In the bird, inulin space expressed as a percentage of body weight never reaches an equilibrium with time whether the inulin is given as a single injection or as a constant infusion (HYDEN and KNUTSSON, 1959). Moreover, inulin apparently enters the cells of the chicken coprodeum when injected into the lumen of the lower part of the gut (BRAYSHER and GREEN, 1972).

On the other hand, chloride spaces require that the intracellular concentration of Cl^- in tissues is not only very small, but essentially constant. Although these conditions may pertain in an average sense for the total body, it is to be expected that some tissues might, within their normal physiologic functions, have wide swings in intracellular chloride concentrations due either to active chloride transport or as a result of significant changes in trans-membrane potential. Such would appear to be the conditions within the shell gland mucosa. For this reason neither Cl^- nor Br^- would appear to be reliable extracellular markers for this tissue. On the other hand, a usually nontransported anion with a valence of -2 (thus being relatively less effected by changes in membrane potential) might serve as a more reliable extracellular marker.

For these reasons, the use of isotopic sulfur as $^{35}\text{SO}_4^-$ was explored (WALSER *et al.*, 1954; BARRAT and WALSER, 1968). We present data for sulphate space in the laying hen during three different physiological stages of the egg formation cycle, *i.e.*; 1) just after oviposition, 2) during albumen plumping, and 3) during egg shell calcification.

METHODS

Non-fasting, single comb, white Leghorn laying hens between the ages of 10 to 12 months were placed in individual cages. The birds were kept in a windowless, air conditioned room where light was automatically timed on for 14 continuous hours per day. Food and water were given *ad libitum*. The time of oviposition was automatically recorded and 5 birds were selected for each of three time periods of egg formation studied.

At the appropriate time, each bird was anesthetized by an intravenous injection of pentobarbital. The left wing vein was cannulated for injection of barbiturate, as required, and for the injection of the radioactive sulfate. The right wing artery was likewise cannulated and used for the collection of blood samples. The ureters were bilaterally cannulated and urine collected for the determination of $^{35}\text{SO}_4^-$.

In collecting blood from the wing cannula, exactly 16 drops were allowed to flow prior to the collection in order to empty the cannula of old blood. The consequent loss of radioactivity by this technique was measured and calculated to give an estimated error in extracellular volume of less than 0.22 p. 100.

Ten μCi of carrier-free $\text{Na}_2^{35}\text{SO}_4$ (32 P free) were injected intravenously. Zero time corresponds to $^{35}\text{SO}_4^-$ injection and at fixed intervals thereafter blood and urine samples were collected up to 240 minutes (fig. 1). The radioactivity was measured by liquid scintillation counting (Packard Model 2420) on an aliquot of 0.1 ml of plasma or urine. Extracellular fluid (ECF) volume was calculated as follows :

$$\frac{\text{c.p.m. (injected)} - \text{c.p.m. (in urine)}}{\text{SA/in plasma}} \text{ in ml/kg BW.}$$

Standard statistical methods were used and results with P values < 0.05 were considered significant.

Electrolyte determinations and measurement of blood pH and pCO_2 were carried out by methods previously reported used in this laboratory (CUNNINGHAM *et al.*, 1971).

RESULTS

After oviposition (fig. 1)

At this physiological stage, sulphate injection occurred on the average 1.5 hours after the previously recorded oviposition and the last blood collection took place 5.5 hours after oviposition; in other words while the forming egg goes through magnum and isthmus.

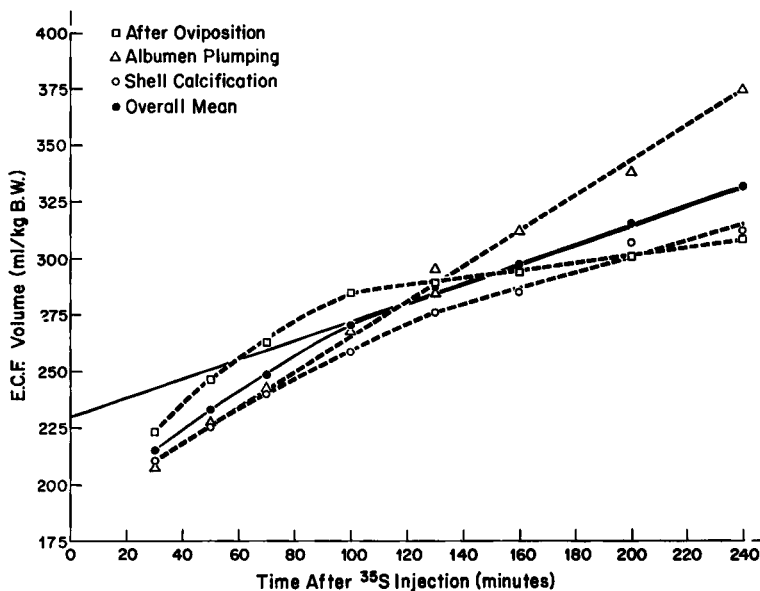


FIG. 1. — Plot of volume distribution (expressed as ml/kg body weight), of radioactive sulfate in laying hen against time

From 130 minutes after injection up to 240 minutes, the radiosulphate space is a linear function of the time.

$$\text{ECF volume (ml/kg)} = 0.176 \times \text{time (mn)} + 267.0; \quad n = 23; \quad r = 0.87.$$

Between zero and 130 minutes, spaces are a parabolic function of the time.

$$\text{ECF volume} = 153.7 + 2.25 t - 0.0095 t^2; \quad n = 27; \quad r = 0.92.$$

Assuming that the increase of the apparent ECF volume after 130 minutes represents only a diffusion of sulphate into pools other than the extracellular fluid, the extrapolation of the linear curve to zero time gave an ECF volume of 267 ± 7.5 ml/kg. This volume is that measured at 71 ± 6 minutes after injection.

During albumen plumping

Since albumen plumping is mainly a transfer of water to the egg it might be expected that there would be little change in the ECF volume from previous physiological state.

On the average injections occurred 7 hours and 20 minutes after oviposition or 2 hours and 20 minutes after the beginning of plumping and the last sampling was 11 hours and 20 minutes after oviposition of 40 minutes before plumping was achieved.

The results in 5 chickens were uniform, but differed significantly from the previous group (after oviposition) in that all curves were perfectly straight lines from 30 minutes up to 320 minutes after injection.

For all the birds in this period of study the regression line is :

$$\text{ECF volume} = 0.715 t + 188.2 ; n = 40 ; r = 0.95.$$

It was not possible to use the same calculations as before because of the characteristics of the curve. However, assuming that 70 minutes was the correct time to estimate the ECF volume, the results in these animals gave a volume of 243 ± 10 ml/kg, slightly lower than the value of 267 ± 7.5 ml/kg noted above.

During shell calcification

Birds are injected with $^{35}\text{SO}_4^-$ at an average time of 19 hours after oviposition. In this group the last samples were taken at an average time of 23 hours after oviposition thus, measurements were made 9 hours after calcification began and until 1 hour after its completion.

The same curve was found as was seen for « after oviposition » and the equation for the straight part of the curve ($t > 130$ mn) is as follows :

$$\text{ECF volume} = 0.348 t + 232 ; n = 20 ; r = 0.90.$$

The extrapolated time for ECF volume estimation was 58 ± 5 minutes after sulphate injection. This value is not significantly different from that found for the after oviposition stage.

DISCUSSION

The results reported here suggests that the $^{35}\text{SO}_4^-$ method for estimating ECF volume in the chicken is simple and adequate for the purpose. Our results yield an average ECF volume of 248.5 ± 4.2 ml/kg at a time point of 70 ± 4 minutes after isotope injection.

These results compare favorably with those obtained by the use of antipyrine (MEDWAY and KARE, 1959) where the ECF volume was reported as 262 ml/kg. However, these values for ECF volume contrast markedly with the data of HYDEN and KNUTSSON where polyethylene glycol and inulin gave values of 115 and 80 ml/kg respectively (HYDEN and KNUTSSON, 1959).

The unexpected result of our study was the apparent total linearity of the $^{35}\text{SO}_4^-$ volume of distribution curve during albumen plumping (fig. 1). As noted, during this period, only water is added to the egg and no physiologic change in ECF volume, at least of significance, would be expected. Despite similar findings in all five birds studied, the possibility of some alteration in the physiologic status of the birds was

sought. No such derangement could be found. For instance, in three of the five birds, mean blood pH was found to be 7.54 with a mean $p\text{CO}_2$ of 42 mmHg ; entirely normal results.

That during albumen plumping there might be direct loss of $^{35}\text{SO}_4^-$ into the egg making process was examined, although it was difficult to see how this could lead to the type of volume-time curve described during this period.

Six different samples of egg albumen taken during the albumen plumping period, when examined for radioactivity, were found to have counts at the level of background only. In addition, similar results were obtained when the potassium hydroxide extract of egg membranes were studied

Along the oviduct, 8 samples of tissue, three in the magnum, two in the isthmus and three in the shell gland were taken. These were extracted either with 10 p. 100 acetic acid which represents activity in fluids or with N KOH which represents activity of SO_4^- incorporated into organic molecules. The total radioactivity may explain at most 20 ml of the apparent ECF volume found at time 240 minutes. But if we assume that the curve during albumin plumping should be the same as that after oviposition, the sulphate space at the end of the experiment exceeds this theoretical value by 58 ml/kg. Therefore the activity recovered in the oviduct and the egg explains only 35 p. 100 of the missing activity. Obviously the activity in the egg and oviduct cannot explain the discrepancy between plumping curves and those of the two other physiological stages.

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RÉSUMÉ

ÉTUDE DE LA GLANDE COQUILLIÈRE DURANT LA FORMATION DE L'ŒUF.

I. — MESURE DE L'ESPACE EXTRACELLULAIRE CHEZ LA PONDEUSE

L'espace de diffusion du radiosulfate (S^{35}) a été mesuré durant 240 minutes après injection d'une dose unique chez la Poule pondeuse à trois stades physiologiques différents de la formation de l'œuf.

Juste après l'oviposition et durant la calcification de l'œuf, l'espace sulfate atteint un état d'équilibre. Les volumes extracellulaires (ECF) ainsi mesurés étaient de $267 \pm 7,5$ et 232 ± 5 ml/kg de poids vif respectivement. Ces valeurs étaient atteintes respectivement 71 ± 6 et 58 ± 5 minutes après l'injection.

Durant l'hydratation de l'albumen, l'espace sulfate n'a jamais atteint un état d'équilibre. Toutefois au bout de 70 minutes le volume estimé était de 243 ± 10 ml/kg.

Quand tous ces résultats sont regroupés indépendamment du stade physiologique le volume extracellulaire est alors de $248,5 \pm 4,2$ ml/kg à 70 ± 4 minutes après l'injection de radiosulfate.

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