TRANSFER OF CONTINUOUSLY I. V. INFUSED $NaHC^{14}O_3$ AND $Ca^{47}Cl_2$ TO THE HEN'S EGG-SHELL (1)

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INTRODUCTION

The mechanism of egg shell carbonate production still represents a much discussed problem. GUTOWSKA and MITCHELL (1945) suggested that the shell gland removes bicarbonate from the blood. On the other hand, SIMKISS (1961) and DIAMANTSTEIN (1966) postulated that egg-shell carbonate may be formed by the uterus from carbon dioxide or its hydration products which are present in the blood (table. 1).

TABLE I

Theories of the mechanism of production of the egg-shell carbonate fraction

Hypothesis of Gutowska and Mitchell :	
Blood Shell Gland $2HCO_3^- \rightarrow 2HCO_3^- \rightarrow H_2CO_3 + CO_3^{--}$ C. A. $\uparrow\downarrow$ $CO_2 + H_2O$	production of 100 meq CO_3^{--} implies removal of 100 meq HCO_3^- gain of 50 mM CO_2
Hypothesis of Simkiss :	
endogenous carbon dioxide $2CO_2 + 2H_2O \rightarrow 2H_2CO_3 \rightarrow 2H^+ + 2HCO_3^- \rightarrow$ *a)* $2H^+ + 2CO_3^{--}$ *b)* $\downarrow$ $CO_3^{--} + CO_2 + H_2O$	
Production of 100 meq CO_3^{--} implies : *a)* consumption of 50 mM CO_2, gain of 50 + 50 = 100 meq H^+ *b)* consumption of 100 mM CO_2 gain of 50 mM CO_2 net consumption of 50 mM CO_2, gain of 100 meq H^+.	

There is little evidence in favour of the hypothesis of GUTOWSKA and MITCHELL, because after a single i.v. injection of C^{14} labelled bicarbonate, arterio-shell gland

(1) This paper is a part of Ch. ZSCHEILE's DMV Thesis

venous C^{14} differences during egg-shell formation were not significantly different from those obtained in resting hens (HODGES and LÖRCHER, 1967).

To support these results and to provide further evidence against the theory that blood bicarbonate is removed by the uterus, the following experiment was designed : after continuous i.v. infusion of $Ca^{47}Cl_2$ and/or $NaHC^{14}O_3$ to achieve constant activity of either nuclide in the blood, there should be a transfer of correspondent amounts of both, Ca^{47} and C^{14} to the egg shell if GUTOWSKA and MITCHELL's theory is correct.

MATERIALS AND METHODS

17 HNL layers, 2 years old, were used during egg-shell formation in this trial. The wing veins of either side were canulated by inserting plastic tubes (Braun-Melsungen N° P_2).

The experimental procedure is demonstrated schematically in a diagramm (fig. 1).

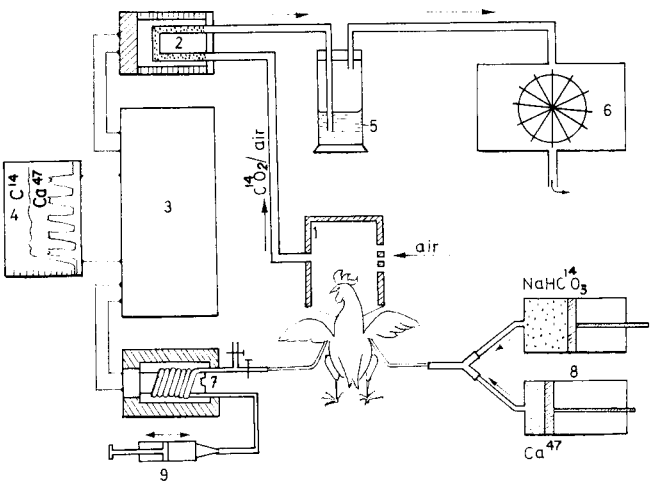

FIG. 1. — *Experimental design*

1 helmet-mask ; 2 Anthracene packed gas flow cell (Packard model 3 041) ; 3 2 channel scintillation spectrometer (Packard model 3022) ; 4 dual pen recorder ; 5 alkali ; 6 exhaust air pump ; 7 Naj crystal (Packard model 5052) ; 8 infusion pumps 9 syringe.

For constant input of $NaHC^{14}O_3$ and/or $Ca^{47}Cl_2$ infusion pumps were used (Braun-Melsungen, Unita II *b*).

Following an initial dose, 6 birds received $NaHC^{14}O_3$,
6 birds received $Ca^{47}Cl_2$
5 birds received either nuclide simultaneously.
i. v. at constant rates as summarized below :

	(1) $NaHC^{14}O_2$ specif. activity 41.4 mCi/mM	(2) $Ca^{47}Cl_2$ specif. activity 150 mCi/g Ca
constant rates of input (ml/min) ..... :	0.075	0.02
concentration of isoton. solution pH 7,5 (μCi/ml)	10	2

(1) (2) Source of supply : The Radiochemical Centre, Amersham/England. Duration of infusion 120 mn in all birds.

Control of « constant » activity in the blood

1. $HC^{14}O_3^-$.

a) Within 2 hours 5 plasma samples were taken from the canula at regular intervals ; C^{14} activity of deproteinized (methanol) plasma was determined in a liquid scintillation spectrometer (Packard Instr. Comp. Incorp. model 4312).

b) Continuous survey of $C^{14}O_2$ exhalation (see fig. 1) exhausted through an anthracene packed flow cell, which was connected to a scintillation spectrometer (Packard Instr. Comp. Incorp., model 3241).

2. *Ca^{47} pulse height analysis.*

About 3 ml blood samples were drawn at regular intervals (see fig. 1) from canulated vein into plastic tube wound along a 2 × 2 NaJ cristal for Ca^{47} survey by a pulse height ratemeter (Packard Instr. Comp. Incorp., model 3241 and 5052). Thereafter, 2 ml of blood were reinjected into the vein and 1 ml left for counting Ca^{47} activity precisely in a gamma spectrometer (Packard Autogamma system).

In double traced birds $C^{14}O_2$ and Ca^{47} survey was done alternatively, using a beta-gamma control, which incorporates a separate HV supply, enabling beta-gamma switching to be accomplished without interrupting the heigh voltage.

Analysis of egg-shell activity

1. *C^{14} :* 0,5 g of powdered egg-shell were analyzed in a liquid scintillation spectrometer, using an aerosil Gel scintillator.

2. *Ca^{47} :* 1 g of ground egg-shell was analyzed in a gamma spectrometer.

3. In double traced egg-shells Ca^{47} activity was determined by gamma spectrometry, whereas the C^{14} content was obtained after the decay of Ca^{47} or by discrimination from the beta countrate of Ca^{47}/Sc^{47}.

RESULTS AND DISCUSSION

The average egg-shell incorporation of continuously infused Ca^{47} came out to be 34.3 ± 5 p. 100 of the total dose infused. On the other hand, only 1.5 ± 1 p. 100 of the C^{14} dose infused as $HC^{14}O_3^-$ were analyzed in the egg shells.

To exclude different rates of calcification which may have influenced the values of the single traced birds, 5 hens received either nuclide simultaneously. The result of this trial is given in table 2. Both, Ca^{47} and C^{14} activities analyzed in the blood are rather constant. Ca^{47} and C^{14} amounts found in the egg-shells are not significantly different from those obtained in single traced birds. Thus, a ratio between

$$\frac{Ca^{47}\ \text{egg shell}}{Ca^{47}\ \text{infused}} : \frac{C^{14}\ \text{egg shell}}{HC^{14}O_3^-\ \text{infused}}$$

of approximately 25-30 : 1 was observed.

This result is against the hypothesis of GUTOWSKA and MITCHELL for the following reasons :

1) The calculated ratio $\frac{Ca^{47}\ \text{egg shell}\ (C^{14}/HCO_3^-)\ \text{blood}}{C^{14}\ \text{egg shell}\ (Ca^{47}/Ca)\ \text{blood}}$ was less than unity (0,3).

2) If

k = constant rate input to achieve constant blood activity,

Ca^{47} blood = constant

$HC^{14}O_3^-$ blood = constant

Ca blood = 5 mM/l = const.
HCO_3^- blood = 30 mM/l = const.
} neglecting the decrease of calcium and bicarbonate in the blood during egg shell mineralization,

TABLE 2

Incorporation of Ca^{47} and C^{14} into the hen's egg-shell during « constant » blood levels of either nuclide for 2 hours achieved by continuous i. v. infusion of $Ca^{47}Cl_2$ and $NaHC^{14}O_3$

Bird No.	Average activity of 5 blood samples (cpm/ml plasma)		Egg-shell weight (g)	Incorporation into egg shell (% of i. v. infused dose)	
	Ca^{47} (1)	C^{14}		Ca^{47}	C^{14}
8	1,286 ± 47	10,270 ± 549	3.571	30.75	0.94
9	680 ± 31	8,031 ± 542	4.868	37.87	0.78
18	609 ± 47	10,389 ± 516	4.736	37.35	1.35
25	386 ± 16	15,900 ± 682	3.302	32.48	1.28
17	324 ± 23	12,731 ± 95	4.160	56.70	1.87
			$\bar{x}$	39.02	1.24
			s	± 10.34	± 0.42

(1) Ca^{47} activity measured, not corrected for decay among various birds.

then

$$\frac{Ca^{47}}{Ca}\ \text{blood} = \text{constant}; \quad \sum_{t=0}^{t} \Delta\ Ca^{47}\ \text{blood} = ktCa^{47}$$

$$\frac{HC^{14}O_3^-}{HCO_3}\ \text{blood} = \text{constant}; \quad \sum_{t=0}^{t} \Delta\ HC^{14}O_3^-\ \text{blood} = ktHC^{14}O_3^-$$

If egg-shell carbonate is produced from blood bicarbonate removed by the uterus, 30 parts of blood Ca^{47} and at least 5 parts of blood $HC^{14}O_3$ would be necessary to form equimolar amounts of egg-shell carbonate ($Ca^*C^*O_3$);
thus,

$$\frac{Ca^{47}\ \text{egg shell}}{ktCa^{47}} : \frac{C^{14}\ \text{egg shell}}{ktHC^{14}O_3^-}\ \text{should be approx. 6 : 1 or less,}$$

GUTOWSKA and MITCHELL's theory being correct.

The amount of bicarbonate removed from the blood to form egg-shell carbonate may be computed, if we take HCO_3^- turnovertime to be at least ca. 20 mn during egg-shell formation, an approximate value we have determined in other trials.

The average egg-shell calcification rate $\approx$ 50 mM CO_3^{--}/15 hours
$\approx$ 6,66 mM CO_3^{--}/ 2 hours

If HCO_3^- pool 15 mM const.,

turnover of HCO_3^- in 2 hours $\approx \frac{120}{20} \times 15 \approx 90$ mM

$HC^{14}O_3^-$ infused in 120 mn thus represents ca. 90 mM HCO_3^-.

If GUTOWSKA and MITCHELL's theory is correct, C^{14} activity of the egg-shells should be ca. 6-7 p. 100 of the infused C^{14} dose, since this amount of C^{14} would correspondent to ca. 6,6 mM HCO_3^-.

From the results actually obtained, however, we may conclude that this hypothesis is no longer tenable.

SUMMARY

To decide whether or not blood bicarbonate is removed by the uterus as a source of egg-shell carbonate — corresponding to the hypothesis of GUTOWSKA and MITCHELL — the following experiment was carried out: After continuous i.v. infusion of $Ca^{47}Cl_2$ and/or $NaHC^{14}O_3$ for 2 hours to achieve constant activity of either nuclide in the blood the transfer of Ca^{47} and/or C^{14} to the egg shell was determined in 17 HNL layers. Control of constant activity in the blood was effected by continuous survey of $C^{14}O_2$ exhalation as well as by blood analyses at regular intervals.

With respect to a (HCO_3^-) / (Ca) ratio in the blood of approx. 30 mM/5 mM per liter, 30 parts of blood Ca^{47} and 5 parts of blood $HC^{14}O_3^-$ would produce equimolar amounts of egg carbonate ($Ca^*C^*O_3$), if the shell gland removes bicarbonate from the blood. The ratio between

$$\frac{Ca^{47}\ \text{egg shell}}{Ca^{47}\ \text{infused}} : \frac{C^{14}\ \text{egg shell}}{HC^{14}O_3^-\ \text{infused}}$$

actually determined, however, was 25-30 : 1.

Furthermore, the ratio $\frac{Ca^{47}\ \text{egg shell}\ (C^{14}/HCO_3^-)\ \text{blood}}{C^{14}\ \text{egg shell}\ (Ca^{47}/Ca)\ \text{blood}}$ was calculated to be 0.2-0.3.

Therefore, GUTOWSKA and MITCHELL's theory is suggested to be no longer tenable.

RÉSUMÉ

TRANSFERT DE C^{14} ET Ca^{47} A LA COQUILLE DURANT UNE PERFUSION CONTINUE DE $NaHC^{14}O_3$ ET $Ca^{47}Cl_2$

Afin de savoir si les bicarbonates du sang sont ou non mobilisés par l'utérus comme sources des carbonates de la coquille de l'œuf — selon l'hypothèse de GUTOWSKA et MITCHELL — l'expérience suivante a été entreprise : après une perfusion intraveineuse en continu de $Ca^{47}Cl_2$ et/ou de $NaHC^{14}O_3$ durant 2 heures, afin d'obtenir une activité constante de chacun des nuclides dans le sang, le transfert de Ca^{47} et/ou de C^{14} à la coquille de l'œuf a été déterminé chez 17 pondeuses HNL. Le contrôle de la constance de l'activité sanguine a été fait par l'examen en continu de l'expiration de $C^{14}O_2$ ainsi que par des analyses de sang à intervalles réguliers.

Compte tenu d'un rapport $(HCO_3^-)/(Ca)$ dans le sang d'environ 30 mM/5 mM par litre, 30 parties de Ca^{47} sanguin et 5 parties de $HC^{14}O_3^-$ sanguin produiraient des quantités équimolaires de carbonate de la coquille $(Ca^*C^*O_3)$, si l'utérus mobilisait des bicarbonates du sang.

En fait, le rapport réellement trouvé entre :

$$\frac{Ca^{47} \text{ de la coquille}}{Ca^{47} \text{ perfusé}} : \frac{C^{14} \text{ de la coquille}}{HC^{14}O_3^- \text{ perfusé}} \text{ était de } 25\text{-}30 : 1$$

En outre, le rapport $\dfrac{Ca^{47} \text{ coquille } (C^{14}/HCO_3^-) \text{ sang}}{C^{14} \text{ coquille } (Ca^{47}/Ca) \text{ sang}}$ calculé était de 0,3.

Ainsi, il est suggéré que la théorie de GUTOWSKA et MITCHELL n'est pas soutenable plus longtemps.

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