RATE OF CO_2 AND C^{14} EXHALATION IN LAYING HENS RESTING AND DURING EGG-SHELL MINERALIZATION AFTER A SINGLE INJECTION OF $NaHC^{14}O_3$

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INTRODUCTION

From the results of MONGIN and LACASSAGNE (1964, 1965, 1966 *a*, 1966 *b*) it can be seen that the mineralization of the hen's egg-shell is related to a fall in pH pCO_2 and bicarbonate. Blood acid base parameters were restored to normal values at or soon before oviposition.

The authors concluded that the acidosis with partial respiratory compensation was due to a decrease in blood bicarbonate resulting from the probable use of the bicarbonate by the uterus, thus corresponding with the theory of GUTOWSKA and MITCHELL (1945).

The primary variations in the metabolic component of the acid base equilibrium and the secondary change in alveolar ventilation, however, could as well be a result of a process in which protons are gained (SIMKISS-DIAMANTSTEIN hypotheses).

The problem now is to differentiate whether the hypobasemia observed during egg shell formation is due to a removal of HCO_3^- or due to a gain of H^+.

We attempted to investigate this alternative by measuring the CO_2 and C^{14} exhalation rates in resting hens and during egg shell formation after i.m. application of a single dose of C^{14} labelled sodium bicarbonate since quantitative information about the effect of egg shell deposition on CO_2 expiration are not yet available.

EXPERIMENTAL

9 laying hens (HNL), 8 months old, were used to determine the CO_2 and C^{14} expiration after i. m. application of 5 μCi C^{14} labelled $NaHC^*O_3$ (specif. activity 41,4 mCi/mM).

Measurements, running for 90 minutes, were made with all birds

a) during the resting period, between the 1st and 3rd hour after oviposition,

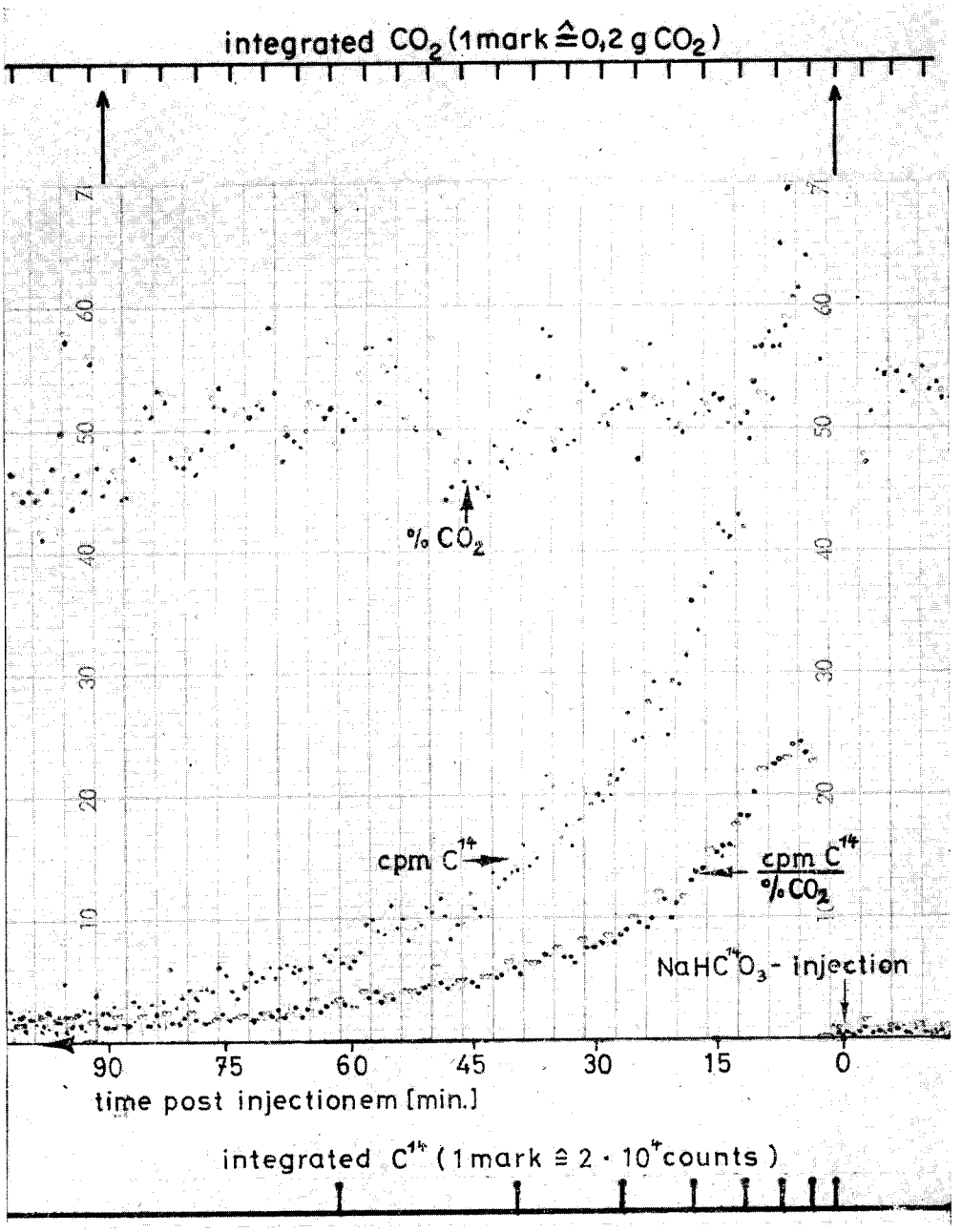

FIG. 1. — *CO_2 and C^{14} exhalation after a single injection of $NaHC^{14}O_3$ in hens.*

b) during egg-shell formation (digital control) between the 8th and 16th hour after oviposition of the previous egg.

The rates of expired CO_2 and C^{14} were registered and integrated continuously and simultaneously by a CO_2-C^{14} exhalation flow analyzer (Frieseke u. Hoepfner, model FHT-50).

The recordings of a triple pen recorder are presented in figure 1.

C^{14} expiration rate is plotted by pen 1

CO_2 expiration rate is plotted by pen 2

C^{14}/CO_2 expiration rate continuously computed by a calculator, is plotted by pen 3.

The dots at the top of the chart represent the integrated CO_2 exhalation rate (1 mark = 0,2 g CO_2).

The lines at the bottom of the chart represent the integrated C^{14} expiration rate (1 mark = 2×10^4 cpm C^{14}).

RESULTS AND DISCUSSION

CO_2 and C^{14} exhalation rates measured are summarized in table 1. The CO_2 exhalation is expressed in mM per 90 mn. The amount of C^{14} expired in 90 mn is presented as a percentage of injected C^{14} dose.

From the data it can be seen that during egg shell formation (between the 8th and 16th hour after oviposition of the previous egg) there is a significant increase ($P < 0{,}01$, WILCOXON range test) of both, CO_2 and C^{14} exhalation rates compared with the corresponding values obtained during the resting period of the hens.

Similar results have been obtained towards the end of egg-shell carbonate production (18th-21st hour after oviposition of the previous egg). The differences in CO_2 exhalation rates, however, became smaller than those observed during the preceding period (8th-16th hour after oviposition).

In figure 2, non expired C^{14}-activity (injected C^{14}-dose minus p. 100 of dose exhaled) is plotted on a log. scale versus time, showing a straight line. This indicates that accumulation in the exhalation phase was not yet saturated at the end of the experiment, otherwise the non-exhaled C^{14} activity would have a curvilinear shape. Although there may exist other channels through which blood bicarbonate disappears, removal by other organs beside the lungs, however, is very small compared to the amount exhaled ; measuring only the most predominant phase not yet saturated we were not able to detect those other channels.

From the slope of the lines in fig. 2 one may find the approximate exponential rate constant ($k = 2{,}3 \times$ slope) for removal of C^{14} from the blood as well as the approximate turnover time ($1/k$) of HCO_3^-. The latter was found to be ca. 20-25 min. during egg shell mineralization.

The *accumulated* specific activity of the C^{14} exhaled within 90 mn. during egg-shell formation is roughly 1 (p. 100 of C^{14}-dose exhaled/mM CO_2/90 mn). If egg-shell carbonate is formed from blood bicarbonate removed by the uterus, corresponding to the hypothesis of GUTOWSKA and MITCHELL, specific activity of the egg-shell carbonate deposited during the experimental period is expected to be not smaller than that of the C^{14} exhaled.

Since the rate of egg-shell mineralization $\simeq$ 100 meq CO_3^{--}/15 h
$\simeq$ 10 meq CO_3^{--}/90 mn
(5 mM CO_3^{--}/90 mn)
at least 5 meq HCO_3^{--}/90 mn
(5 mM HCO_3^{--}/90 mn)

would be needed to form 10 meq of egg shell carbonate.

TABLE I

CO_2 and C^{14} expiration per 90 min in laying hens resting and during egg-shell formation after a single injection of $NaHC^{14}O_3$

No. of bird	*a* = no shell formation 1st-3rd hr after oviposition		*b* = during shell formation 8th-16th hr after oviposition of previous egg		*b* — *a* = relative difference (%)	
	C^{14} expiration % of dose inject.	CO_2 expiration (mM)	C^{14} expiration % of dose inject.	CO_2 expiration (mM)	C^{14} expiration	CO_2 expiration
3	88.84	88.61	90.80	95.08	+ 2.21	+ 7.30
7	86.50	84.06	98.42	88.61	+ 13.78	+ 5.41
27	85.71	90.89	88.76	99.95	+ 3.56	+ 9.97
18	88.62	86.33	98.37	86.74	+ 11.00	+ 0.47
1	89.29	83.12	91.78	95.40	+ 2.79	+ 14.77
6	88.61	84.15	93.34	88.61	+ 5.34	+ 5.30
9	90.13	70.89	89.09	70.40	— 1.15	— 0.69
17	74.08	68.17	79.54	99.06	+ 7.37	+ 14.96
28	77.89	82.67	83.49	84.95	+ 7.19	+ 2.76
$\bar{x}$	85.52	82.10	90.40	89.87	± 5.79	+ 6.69
s	± 5.65	± 7.60	± 6.21	± 9.08	± 4.62	± 5.67

The expected C^{14}-content of the egg shell, therefore, should be at least 5 p. 100 of the injected dose, thus having a specific activity of 1 (p. 100 of C^{14} dose injected/mM egg shell carbonate/90 mn).

After a single injection of C^{14} labelled bicarbonate the amount of C^{14} analyzed

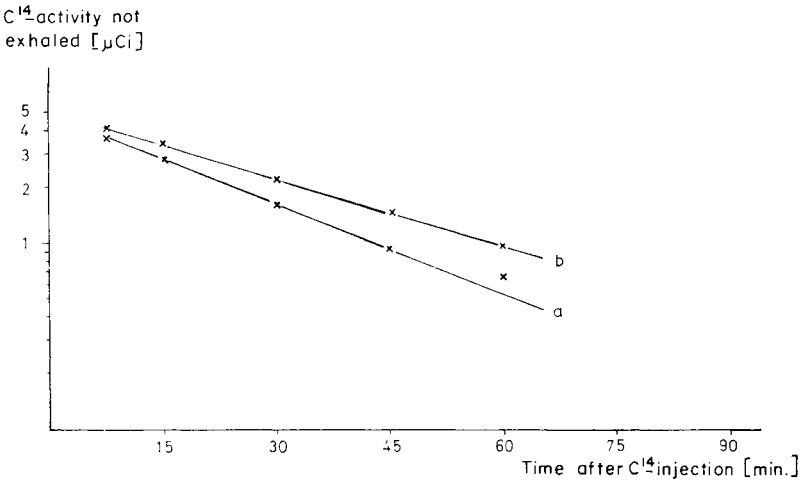

FIG. 2. — *C-activity not exhaled after i. m. injection of 5μCi $NaHC^{14}O_3$ versus time.*
a) during egg-shell formation
b) during a period when no carbonate is produced in the uterus.

TABLE 2

Buffer reactions in blood counteracting a gain of strong acid or a loss of bicarbonate

I — Gain of strong acid consumes HCO_3^- and Buf^-, generates CO_2

$$\text{Gain of } H^+ \begin{cases} + HCO_3^- \rightarrow H_2CO_3 \rightarrow CO_2 + H_2O \\ + Buf^- \rightarrow HBuf \end{cases}$$

HCO_3^- + Buf^- would decrease after addition of strong acid by an amount equal to the amount of acid added.

II — Interaction reaction, hydration reaction and equilibrium of CO_2

$$\begin{array}{l} CO_2\ (g) \\ \uparrow\downarrow \\ CO_2\ (d) \\ \uparrow\downarrow \\ H_2CO_3 + Buf^- \rightleftarrows HBuf + HCO_3^- \end{array}$$

III — Loss of Bicarbonate consumes CO_2

$$\begin{array}{l} CO_2 + H_2O \\ \uparrow\downarrow \\ H_2CO_3 + Buf^- \rightarrow HBuf + HCO_3^- \rightarrow \text{loss of } HCO_3^-. \end{array}$$

in more than 20 egg shells being formed between the 8th and 16th h after oviposition of the previous laid egg, however, only came to 1 p. 100 of the injected C^{14} dose

on an average. This C^{14} activity detected in the egg shells is interpreted as being derived from recycling dissolved $C^{14}O_2$ of the blood going to the shell gland and being transferred to $C^{14}O_3^{--}$, corresponding to the theory of SIMKISS. From the above results we have reason to state the following :

1. Obviously, blood bicarbonate is not a significant source of egg shell carbonate thus little evidence in favour of the theory of GUTOWSKA and MITCHELL is present.

2. The acidemia (hypobicarbemia) observed during egg shell formation more likely is due to a gain of protons, partially counteracted by base consuming buffer reactions and partial respiratory compensation resulting in a negative CO_2 balance (table 2).

SUMMARY

In 9 laying hens CO_2 and C^{14} exhalation rates have been registered and integrated continuously by a CO_2-C^{14} flow analyzer (FHT-50) after a single i. m. injection of 5 μCi C^{14} labelled $NaHC^{14}O_3$.

The experiments ran for 90 minutes,

a) during a period when no carbonate was produced in the shell gland (1st-3rd hour after oviposition),

b) during egg-shell mineralization (8th-16th hour after oviposition of the previous egg).

There was a significant increase ($P < 0{,}01$) in both, CO_2 and C^{14} exhalation during egg-shell formation compared with the corresponding values obtained from the same birds when no egg shell carbonate was deposited in the uterus.

No removal of injected $HC^{14}O_3^-$ by the shell gland (adequate to the rate of egg-shell mineralization) was observed.

From the results the following conclusion was drawn :

The hypobasemia observed during egg-shell formation is more likely due to a gain of protons rather than to a removal of bicarbonate by the uterus. Thus little evidence in favour of the hypothesis of GUTOWSKA and MITCHELL is present.

RÉSUMÉ

VITESSE D'EXPIRATION DU CO_2 ET DU $C^{14}O_2$ CHEZ LA POULE AU REPOS ET DURANT LA CALCIFICATION DE LA COQUILLE APRÈS UNE INJECTION DE $NaHC^{14}O_3$

Les vitesses d'expiration de CO_2 et de ^{14}C ont été enregistrées et intégrées en continu à l'aide d'un analyseur à flux gazeux (FHT-50) après une injection intramusculaire unique de 5 μCi de $NaHC^{14}O_3$ à 9 poules pondeuses.

Les expériences ont duré 90 minutes :

a) durant une période où il n'y a pas de dépôt de carbonate dans l'utérus (1re à 3e heure après l'oviposition),

b) durant la minéralisation de la coquille de l'œuf (8e à 16e heure après oviposition de l'œuf précédent).

Par rapport aux valeurs obtenues chez les mêmes poules durant la période de repos de de l'utérus, l'expiration de CO_2 et de C^{14} est significativement ($P < 0{,}01$) accrue durant la formation de la coquille de l'œuf.

Il n'a pas été observé de mobilisation par l'utérus de $HC^{14}O_3^-$ injecté, compatible avec la vitesse de minéralisation de la coquille.

De ces résultats, nous concluons que l'hypobasémie observée durant la formation de la coquille de l'œuf est plus vraisemblablement due à un gain de protons qu'à un retrait de bicarbonate par l'utérus. Il n'y a donc que peu d'arguments en faveur de l'hypothèse de GUTOWSKA et MITCHELL.

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