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VARIATIONS IN INTRAUTERINE pH WITHIN A CIRCADIAN RHYTHM (Gallus Domesticus)

C. M. Winget, C. A. Mepham, and E. G. Averkin

Environmental Biology Division
Instrumentation Division
NASA, Ames Research Center
Moffett Field, California

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Mailing address for Proofs: C. M. Winget
Environmental Biology Division
Ames Research Center
NASA
Moffett Field, California

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ABSTRACT

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Data from Gallus Domesticus are presented showing the relationship between uterine pH and time post-oviposition. The data suggest that uterine pH is associated with certain periods of uterine activity. A Fourier analysis, a periodogram, and a correlogram all indicate a cyclic phenomenon; furthermore, the correlogram indicates the pH cycle to be endodiurnal.

Author

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VARIATIONS OF INTRAUTERINE pH WITHIN A CIRCADIAN RHYTHM (Gallus Domesticus)

C. M. Winget,* C. A. Mepham, and E. G. Averkin**

Many physiological rhythms of 24-hour duration have been established in class Aves. Activity rhythms (1) and oxygen consumption rhythms (2) for both the adult and the developing embryo have been reported. Simpson and Galbraith (24) have shown that the body temperature rhythm normally follows the activity rhythm. Heart rate (15), erythrocyte number (8), rate of feather growth (12) and mitosis in the testes all exhibit a 24-hour cyclic pattern in Gallus Domesticus.

The cycle of ovulation to oviposition in the domestic fowl is the best established of all Circadian rhythms in birds (18). Both ovulation and oviposition take place during the light phase of the cycle while the release of the ovulatory hormone (LH) occurs during the hours of darkness. A neurohumoral system with afferent neurons originating in the reproductive tract is known to inhibit the release of LH (10). Other neural stimuli (i.e., time of feeding, temperature, and sound levels) may have regulatory effects on this Circadian rhythm. The influence of light on avian gonadal activity and other hormones is also discussed in detail by Wolfson (33).

The present study was conducted to evaluate pH changes in the uterus of the female reproductive tract at specific times during an established Circadian rhythm and to correlate these changes to known neural and endocrine activities.

*Environmental Biology Division
**Instrumentation Division

METHODS

S. C. white leghorn females approximately 14 months of age were used in these experiments. The animals were kept in individual cages equipped with an oviposition timing mechanism (Fig. 1). The birds were maintained at a constant environmental temperature (18° C ±0.5°) on 14 hours of light, and received feed and water ad libitum.

The pH of the uterus was measured by means of a specially adapted electrode (Radiometer GK2021C) to fit into a plastic egg (Fig. 2). A second set of electrodes (Radiometer K400 and G200C) was used to standardize the equipment at specific time periods. All pH values were obtained on a Radiometer, pH Meter 22, with external meter, type PHA621 which enables direct readings of better than 0.005 pH units.

The placement of the electrodes into the uterus was accomplished with relative ease. The orifice was first located and palpated with the right forefinger, followed by emplacement of the plastic egg and electrodes. To maintain proper electrode positioning the bird was restrained. In those cases where the electrode was forced out of the uterus by the bird or calcium was deposited over the electrode, the recorded readings were omitted.

Observations were made throughout the ovulatory Circadian rhythm at 15-minute intervals starting at the time of oviposition. Hourly averages and variances were determined. The data were further evaluated by the Fourier series, the Schuster periodogram, and the correlogram methods. The Fourier series was used as a method for variable separation (7, 3).

A periodogram using Schuster's (22) technique was constructed from the data. Serial correlations were calculated for the 24-hour data (11). The pH term was used in all computations rather than hydrogen ion concentration since the former has been shown to be most reliable and useful (31, 23).

RESULTS

The changes in the pH of the avian uteri studied are indicated by the circled points in Fig. 3 and the data in Table 1. The pH changes were recorded for a 24-hour period immediately after oviposition. These are the results of 32 experiments in which the pH changes following oviposition were observed. The data in Table 1 consist of the mean pH values per hour post-oviposition. A 12-term Fourier series passing through the 24 mean hourly observations was calculated. See Fig. 3 (broken line) and Table 5 for a listing of the estimated coefficients. A Fourier series consisting of the first six successive terms in Table 5 was evaluated and plotted (see Fig. 3, smooth line). The mean square error resulting from this approximation was of the order of 0.01 pH units. The 24 mean hourly observations were further reduced to 12 values, each representing a 2-hour period (Table 2).

The first three successive terms of the Fourier were evaluated separately and plotted (Fig. 4; the smooth line representing the first term, the broken line, the second term, and the dotted, the third term). The range of the first Fourier term was approximately 0.13 pH units and it achieved its maximum at approximately 14.7 hours post-oviposition.

Next, the 24 mean hourly observations and also the resultant 12 two-hour-period points were analyzed utilizing a periodogram constructed by the method of Schuster (22). See Fig. 5 for the periodogram utilizing the 24 mean hourly values (solid line) and for the periodogram utilizing the 12 two-hour period values (broken line). Both periodograms indicate a period with an approximate length of 21 hours and an amplitude of about 0.07 pH units (see Table 3 for the results of the computations).

The data were analyzed in a third manner using serial correlation (see Fig. 6 and Table 4). It appears that this is a correlogram of a series of harmonic terms, since it does not vanish nor is it dampened.

The absolute pH values (7.2 to 7.6) reported herein agree with the values for the egg albumen from the uterus mucosa (7.3 to 7.5) reported by Buckner and Martin (5). These values are only slightly lower than those recently reported by Ogasawara (19). However, there is poor agreement with the uterine mucosal scrapings (pH 5.8 to 6.4) reported by Buckner and Martin. The lower pH values in the latter case may be due to a release of intracellular fluids during the mucosal preparations. The uterus was observed to be basic throughout the experimental period reported herein; whereas, Sturkie (25) states that the uterus of sexually mature females is in the vicinity of pH 5.8.

DISCUSSION

The developing egg enters the uterus approximately 4 hours after oviposition. During the early parts of this period, water is transferred to the egg; after 5 hours shell mineralization starts and proceeds at a

constant rate (4). During this time the pH was observed to have three maxima and three minima. The first of these three maximal pH values coincides with: 1) the onset of calcium deposition in the uterus (6); 2) maximum plasma calcium levels (32); 3) minimum plasma phosphate levels (unpublished data); and 4) approximate time of LH release (28). The final maximum pH occurs approximately when blood calcium is at a minimum (32). Minimal pH values during mineralization are observed to coincide with the time when Tanaka (26) has reported cholinesterase activity to be at maximum in the diencephalon. The data do not, however, establish a causal relationship between uterine pH and the mechanisms mentioned, but they do indicate a dynamic hydrogen ion concentration in the uterus of the bird. The biological implications of this diurnal variation in uterine pH, as a function of time post-oviposition, are by no means clear.

Furthermore, a constant pH is not maintained during that period (0 - 4 hours post-oviposition) prior to the movement of the egg into the uterus. Again, the mechanisms responsible for these changes are not obvious from the data presented herein; however, the maximum pH does coincide with time of egg engulfment and low diencephalon cholinesterase activities as reported by Tanaka (27). This period of metabolic activity required to move large amounts of salt and water into the uterus could result in a lowered pH.

From these results a mechanism more complex than a simple pH shift appears to be responsible for the movement of calcium across the uterine membranes. The three pH changes which have been shown in the same time period that Burmester (6) demonstrated constant calcium deposition;

therefore, this would indicate that pH shift has a minimal effect on the movement of calcium across the uterine wall. A previous study (32) on the efficiency of calcium removal also suggests a mechanism for calcium transport more involved than simple pH shifts. In other complex systems, such as the gut, where calcium moves across several cellular barriers at approximately equal efficiency to that of the uterus it is reported (17) that pH shifts have only a slight effect on the movement of calcium across the gut wall.

A close relationship between decreasing pH values and possible sperm viability in the female is suggested. It is noteworthy that the pH remains at a minimum during the period when sperm are most likely to be traversing the uterus. This observed minimum pH (7.25) is very close to the values of cock semen reported by others (20, 29). Therefore, the data suggest optimal uterine pH for the depositing of the semen. The data of Wilcox and Shaffner (30) would indicate that approximately 25 percent of the cock sperm will have lost their motility at this pH. In rats, it has been shown that matings occur immediately before or after maximum vaginal pH values (13).

These results which show a decrease in pH followed by a rapid increase (10 - 24 hours) have been found to proceed at fairly uniform rates. These pH changes may well be the stimuli that initiate uterine muscle contractions which cause rotation of the egg in the uterus as shown by others (21, 4). It is well known that the length of muscle is dependent on pH (16) and that in the rabbit, uterine muscle tone is maximal between pH 7 and 8 (14). Furthermore, Horne (9) has shown that sperm migration in the human female is dependent on cornual musculature contraction.

The harmonics of the Fourier response curve do not suggest an easily explainable timing mechanism. For example, harmonics 2 and 3 (see Table 2 and Fig. 4) do not indicate a clear timing mechanism as far as uterine pH is concerned. However, harmonic 1 does suggest a timing mechanism of approximately 24-hour duration. Harmonic 1 appears to reflect fairly closely the movement of the ovum down the oviduct. The data suggest that Fourier analysis can be used as a tool for studying the mechanisms of biologic responses.

Although the periodogram and correlogram are often useful in data interpretation, in this instance they did not indicate any new mechanism. The correlogram does indicate that the Ph of the uterus is cyclic and endodiurnal. The periodogram confirms that the uterine pH is cyclic and suggests a major cycle with at least one and possibly two minor cycles.

SUMMARY

The intrauterine pH of the sexually mature bird appears to fluctuate diurnally. The changes in pH during mineralization would suggest a more complex mechanism responsible for the movement of calcium from the plasma to the uterus. The increase in intrauterine pH during the period of active salt and water transport may indicate pH is important in this mechanism.

The Fourier series, periodogram, and correlogram methods all indicate that intrauterine pH is cyclic. Furthermore, the correlogram suggests that the cycle is endodiurnal.

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Table 1.- Uterine pH values at specific times post-oviposition

Time since oviposition, hr	Uterus	
	Number of birds	pH ± standard error
1	13	7.40 ± 0.01
2	11	7.47 ± 0.02
3	9	7.33 ± 0.04
4	8	7.29 ± 0.04
5	10	7.25 ± 0.02
6	9	7.25 ± 0.02
7	11	7.29 ± 0.03
8	12	7.33 ± 0.04
9	10	7.35 ± 0.02
10	10	7.56 ± 0.02
11	12	7.52 ± 0.04
12	12	7.48 ± 0.05
13	12	7.47 ± 0.02
14	12	7.42 ± 0.02
15	12	7.34 ± 0.01
16	10	7.55 ± 0.03
17	8	7.53 ± 0.04
18	9	7.38 ± 0.03
19	5	7.39 ± 0.05
20	8	7.37 ± 0.05
21	8	7.56 ± 0.02
22	7	7.35 ± 0.04
23	7	7.37 ± 0.03
24	6	7.38 ± 0.03

Table 2.- Mean pH values of the two hour sampling periods

Period number	Mean pH value
1	7.44
2	7.31
3	7.25
4	7.31
5	7.46
6	7.50
7	7.44
8	7.44
9	7.46
10	7.38
11	7.46
12	7.38

Table 3.- Table of data obtained by Schuster's periodogram analysis of mean uterine pH data. Maximum intensities are indicated by bold-faced figures.

Length of period = τ	Amplitude	
	24 points	12 points
1	0.0025	0.0050
2	.0025	.0050
3	.0177	.0153
4	.0268	**.0317**
5	**.0476**	.0302
6	.0238	.0153
7	.0323	.0300
8	.0468	.0364
9	**.0560**	.0493
10	.0558	.0566
11	.0528	.0584
12	.0523	.0584
13	.0552	.0588
14	.0599	.0601
15	.0648	.0620
16	.0689	.0641
17	.0721	.0660
18	.0743	.0675
19	.0756	.0685
20	.0762	.0692
21	**.0763**	**.06944**
22	.0760	.06940
23	.0753	.0691
24	.0744	.0686
25*	.0733	.0679

*Values for $\tau > 25$ are monotonically decreasing.

Table 4.- Serial Correlations of the mean hourly pH data

Order of correlation k	r_k
1	+0.443
2	+0.197
3	+0.062
4	+0.010
5	-0.097
6	-0.251
7	-0.292
8	-0.182
9	-0.066
10	-0.119
11	-0.054
12	-0.403
13	-0.329
14	+0.056
15	+0.411
16	+0.009
17	-0.056
18	+0.031
19	+0.740
20	+0.106
21	-0.328
22	+1.000

Table 5.- Successive coefficients of the 12-term Fourier series fitted to the 24 mean hourly pH values

i	a_i*	b_i**
0	7.4025	---
1	-0.0518	-0.0426
2	0.0399	-0.0204
3	0.0152	0.0381
4	-0.0141	-0.0096
5	-0.0107	0.0301
6	0.0048	0.0205
7	0.0031	-0.0033
8	-0.0007	0.0090
9	-0.0008	-0.0005
10	-0.0024	-0.0003
11	-0.0021	0.0014
12	-0.0010	0.0011

*Coefficient of cos(ix)

**Coefficient of sin(ix)

where $x = 2\pi n/k$,

where $n = 0, 1, 2, \ldots,$

$k - 1$ where $k = 24$;

hence, $pH = a_0 + a_1 \cos(x)$,

$+ b_1 \sin(x)$

$+ a_2 \cos(2x)$

$+ \ldots$

FIGURE TITLES

Fig. 1. Diagram of the oviposition timing mechanism.

Fig. 2. Schematic illustration of the electrode (Radiometer GK202k) fitted into the plastic egg for the purpose of measuring uterine pH.

Fig. 3. Plot of mean hourly uterine pH (circles) 12-term Fourier (broken line) and 6-term Fourier (smooth line) at specific times post-oviposition.

above: a, period ovum is in anterior magnum; b, period ovum is in posterior magnum; c, period ovum is in isthmus; d, period of active water and salt transport; e, period of active calcium deposition; d and e, period ovum is in uterus; f, time of oviposition; g, time of ovulation. Middle: h, low cholinestarase activity; i, minimal acid phosphatase, activity j, high cholinesterase activity; k, low inorganic phosphatase; l, maximal calcium level; m, maximal cholinesterase activity; n, maximal inorganic phosphate and maximal acid phosphatase activity; o, minimal calcium level; p, maximal cholinesterase activity. Refer to text for explanation.

Fig. 4. The first three successive harmonics of the 12-term Fourier series in Fig. 3. The smooth line represents the first term, the broken line the second, and the crosshatched the third.

Fig. 5. Two periodograms computed from the same mean uterine pH data. In one case (broken line) Dt = 2 (12 two-hour period observations), while in the other case (solid line) Dt = 1 (24 mean hourly observations).

Fig. 6. Correlogram of data presented in Table 4.

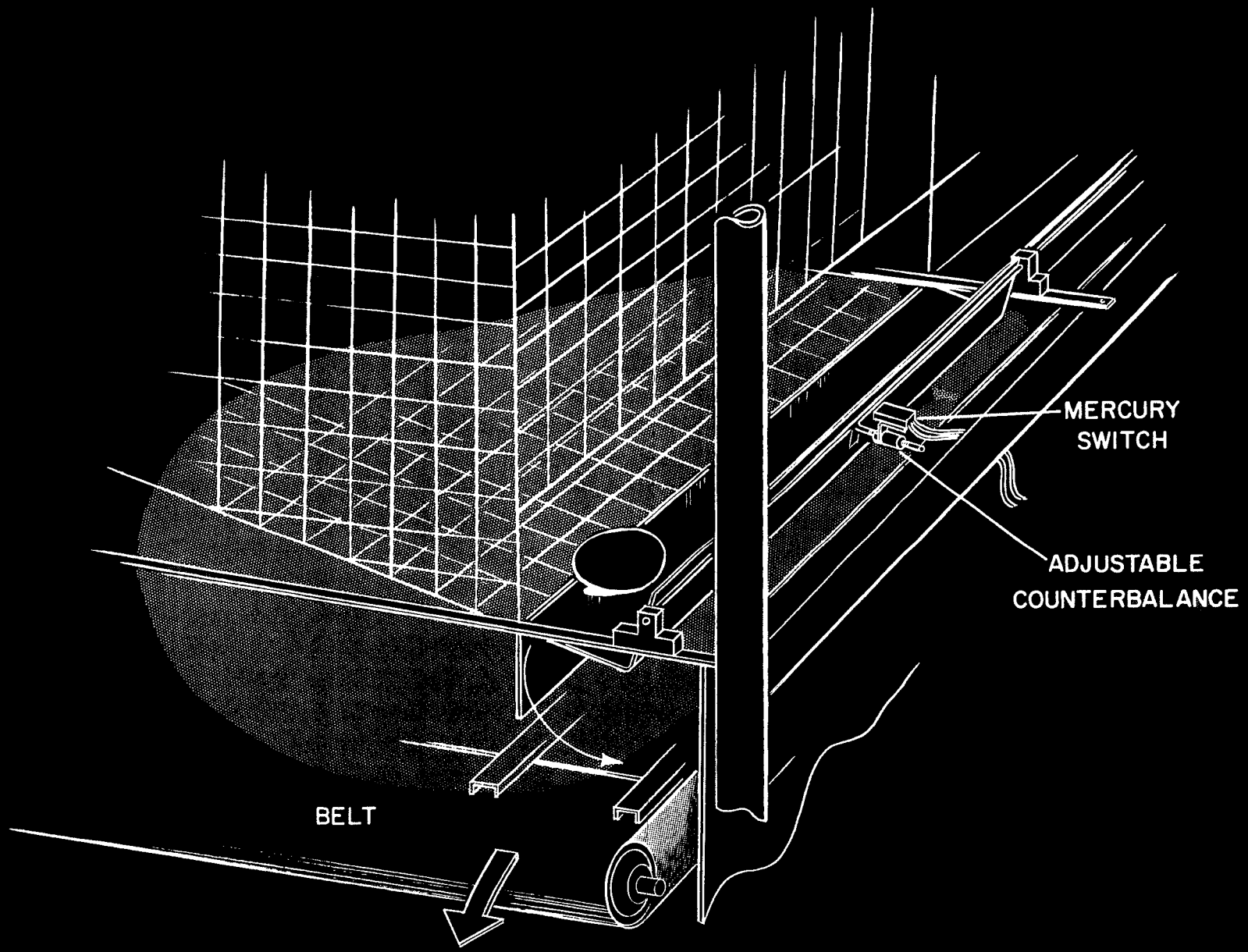

Figure 1.

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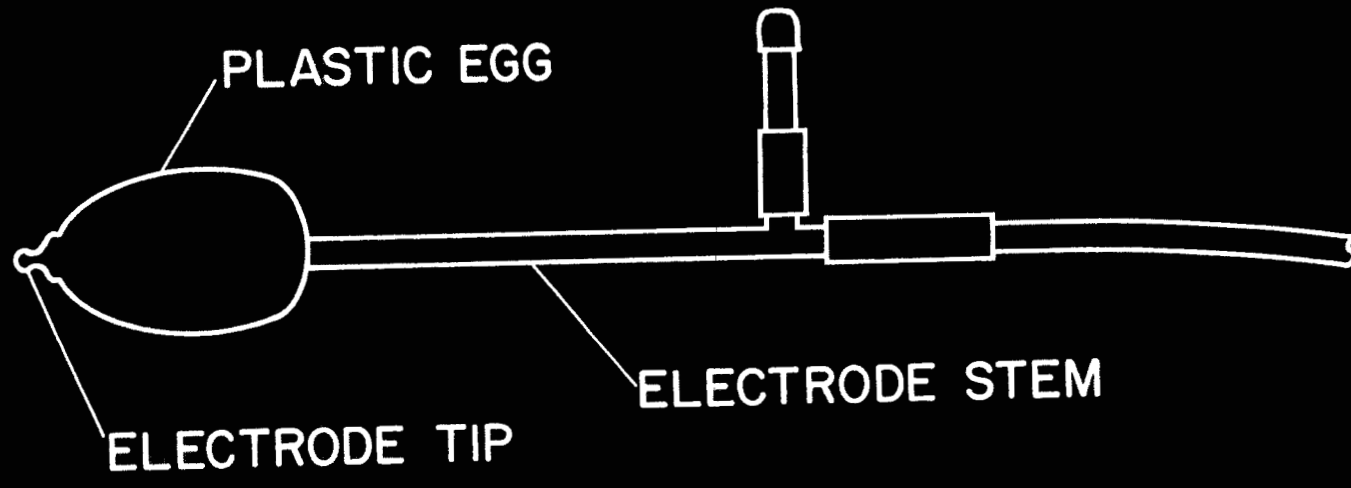

Figure 2.

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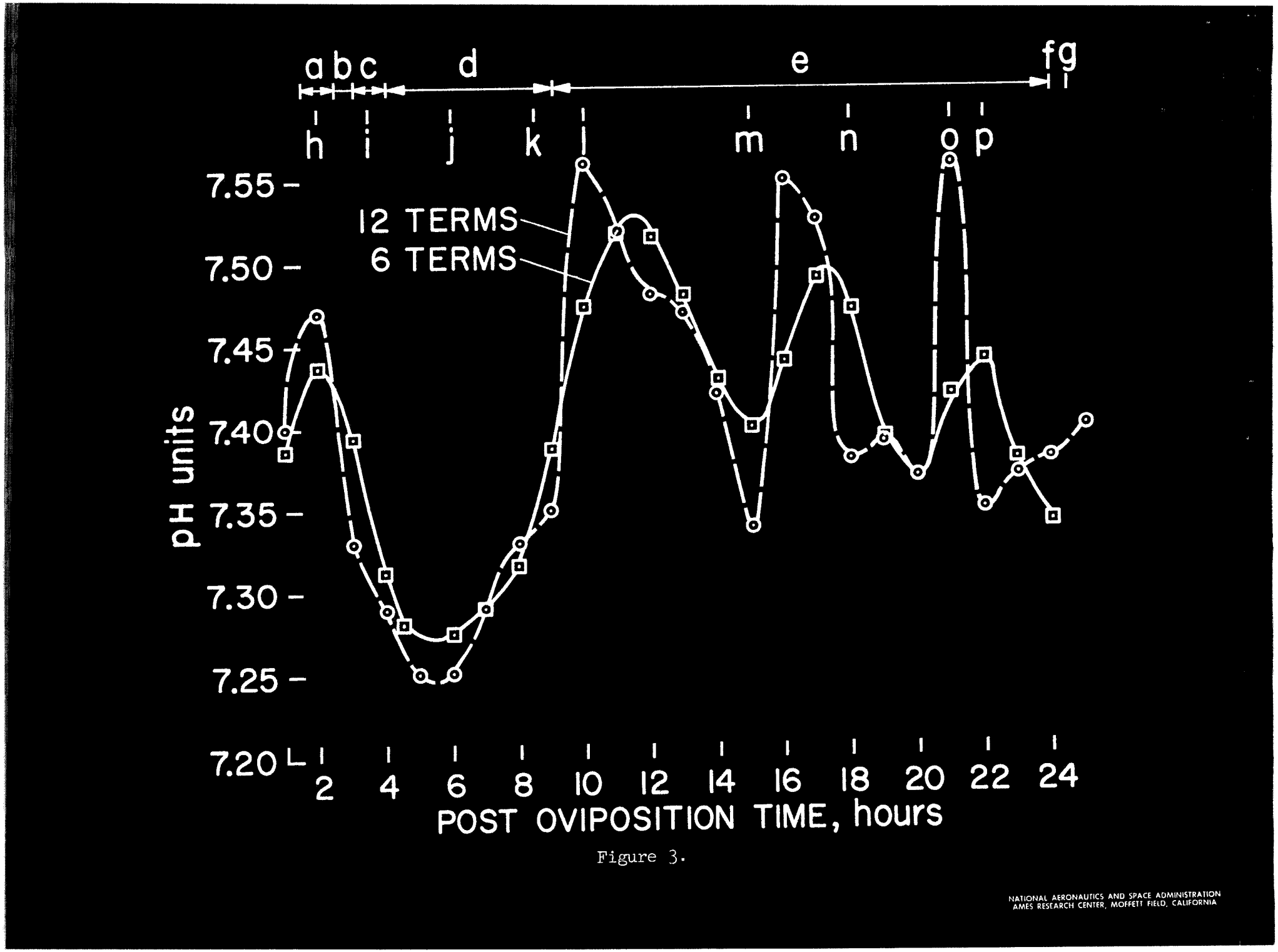

Figure 3.

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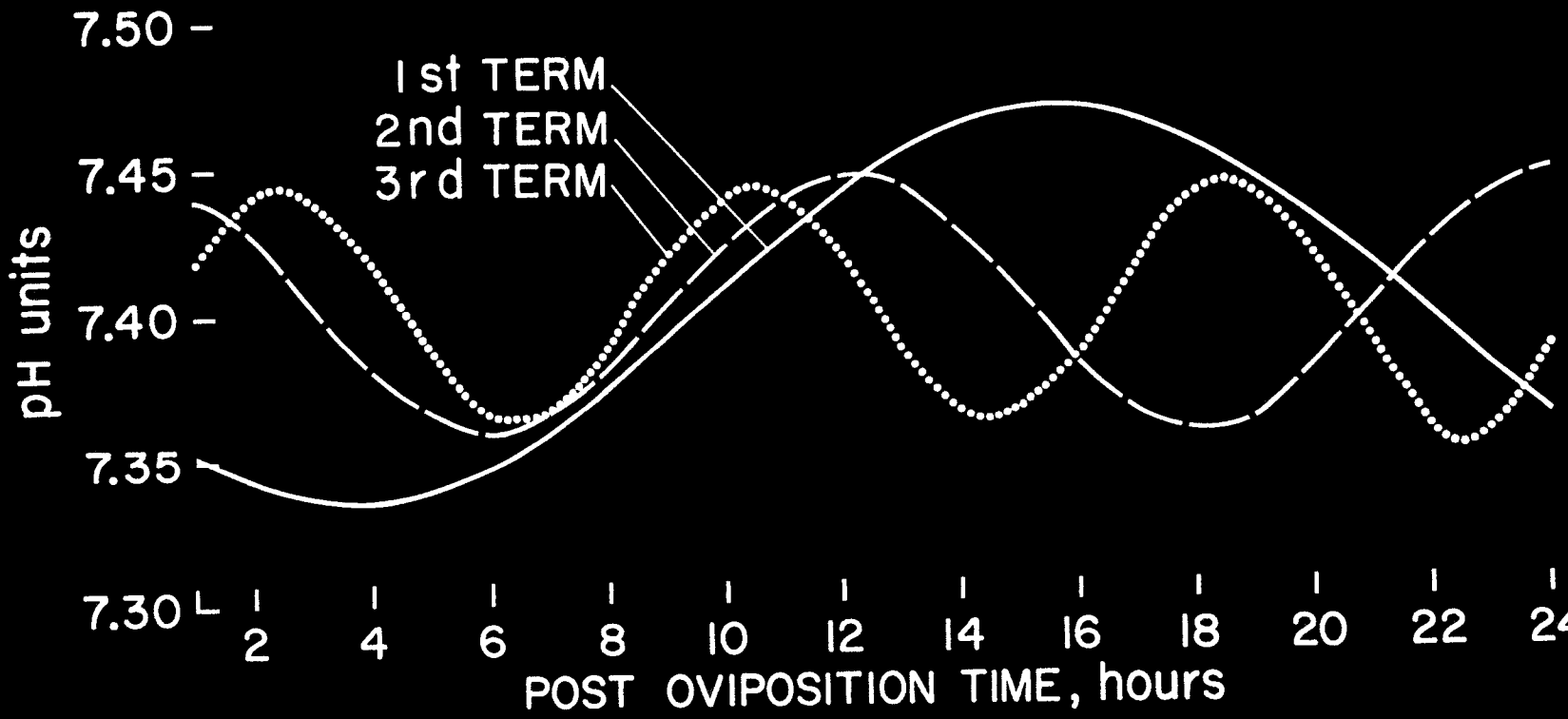

Figure 4.

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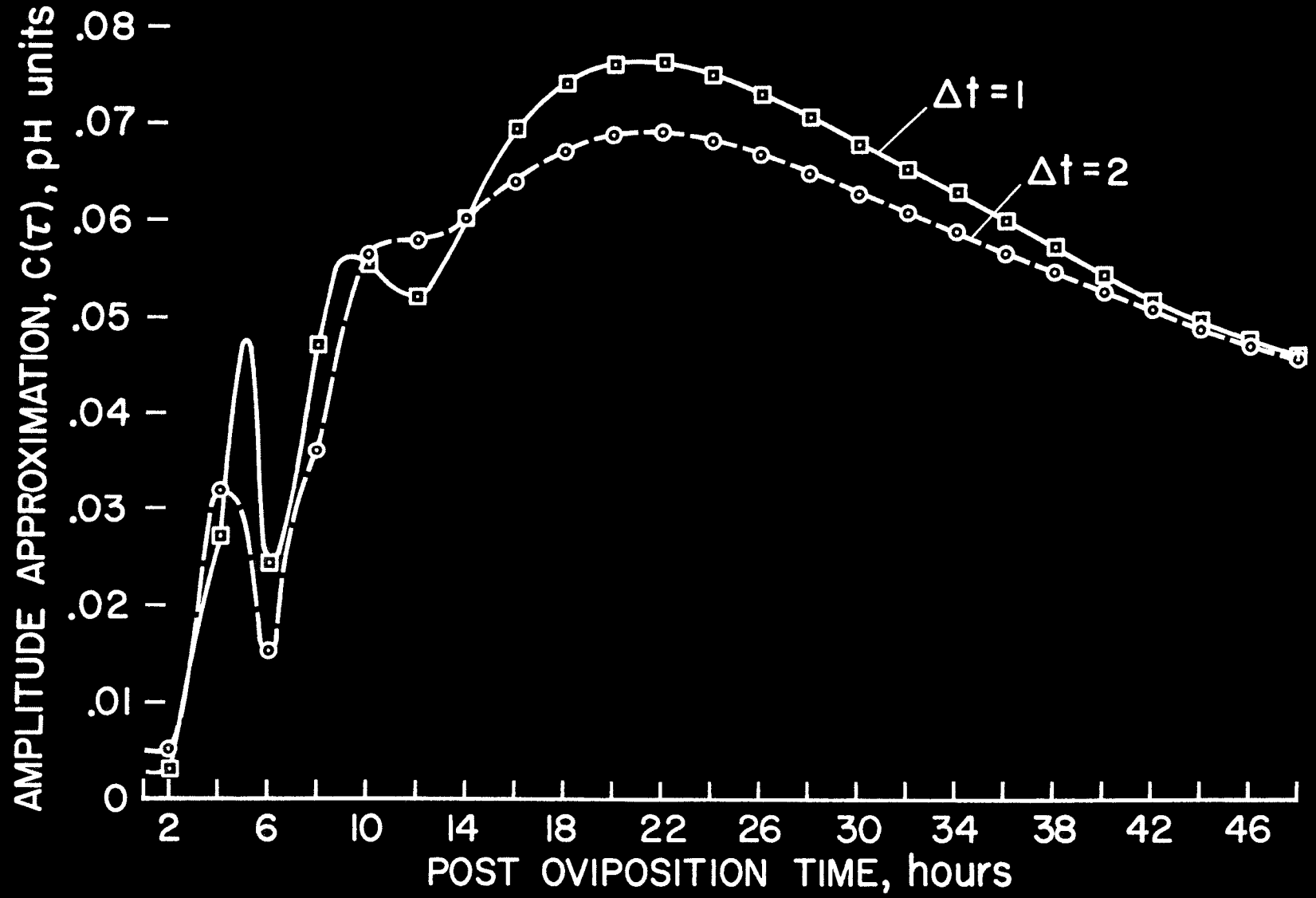

Figure 5.

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Figure 6.

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