Serum Protein and Calcium of Pigeons During The Reproductive Cycle.† (27021)

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Advantages in the use of pigeons for certain hormonal and metabolic studies(1) make desirable additional information concerning the serum proteins of this species, especially as related to sex and reproduction. Plasma or serum proteins have been studied electrophoretically during the menstrual cycles of the baboon(2) and of women(3). However, inasmuch as this technique has not been used in following such changes in other species, its application to studies of the reproductive cycle of the female pigeon(4-6) seemed desirable. To this end, serum electrophoretic patterns of both male and female birds have been obtained, together with the accompanying values for serum total protein and calcium, in the course of the egg-laying cycle.

Materials and Methods. Adult white Carneau pigeons, labeled as to sex, were purchased from the Palmetto Pigeon Plant, Sumter, S. C. They were housed in separate pairs, male and female, in outdoor cages. The birds were fed and watered twice a day, with supplies available *ad libitum*. The feedings, separated by an interval of 4 hours, consisted of a diet of a 1:1 mixture of scratch (wheat, corn, oats), and peas, with a mineral mixture supplement. Eight pairs of mated males and females were used. At various points of the reproductive cycle, which were determined as outlined by McDonald and Riddle(6) and noted below, 8 to 12 ml samples of blood were withdrawn from a wing vein as quickly as possible just prior to feeding. The blood was allowed to clot, and centrifuged. The separated serum was then kept at refrigerator temperature for use in analyses. Total nitrogen was determined gasometrically, according to Van Slyke and Kugel (7). Calcium was determined according to Sendroy by ashing(8), and spectrophotometric analysis at 400 mμ(9). For electrophoretic analysis, 1 ml samples of serum were diluted with 2 ml of a buffer solution of 0.02 M PO_4 (1:5 of NaH_2PO_4, and Na_2HPO_4, at pH 7.4) and 0.15 M NaCl(10). Following overnight dialysis against 500-1000 ml of the same buffer solution, analysis was carried out in a Perkin-Elmer apparatus (schlieren scanning, in a standard 2 ml two channel cell, 15 mm path, 2 mm width, 18.0-18.7 ma current) at 2°C. Although both ascending and descending patterns were obtained, only the latter were used for evaluation of the distribution of the proteins by the areametric method of Sendroy and Cecchini (11).

From each of the 16 birds, 5 samples of serum, corresponding to points designated in the following outline of a reproductive cycle, were obtained. The first day of a cycle was marked by the laying of the first egg of a clutch. Sample 1 was taken on the following day, preceding the laying of the second egg on the third day. Sample 2 was taken 3 days after appearance of the second egg. Sample 3 was taken 5 days before the estimated hatching date, or about the 14th day of the cycle. Sample 4 represented a point in the cycle 2 days after the first hatching (when crop milk formation was maximal). Sample 5 was taken 11 days later (when crop milk production was considerably decreased), several days prior to onset of a new cycle. The cycles reported here were observed throughout the month of November.

Results. As representative of changes common to all of the birds studied, Fig. 1 gives electrophoretic patterns of the sera of a mated pair of pigeons at various points in the reproductive cycle. The female patterns shown are (A) at ovulation (Sample 1), (B) in the subsequent incubation period prior to crop gland enlargement (Sample 2), and (C) at approximate hatching date (Sample 4). The male patterns (approximately the same for all 5 samples) shown are (D) for Sample 1, and (E) for Sample 4.

Average mobilities of the several serum components for male and female pigeons are given

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TABLE I. Mobilities and Percentage Distribution of Proteins from Electrophoretic Analysis of Sera of Pigeons at Selected Points of Reproductive Cycle.

Protein	Globulin γ-		β-		α-		Albumin		"F"	
Sex	F	M	F	M	F	M	F	M	F	M
	Mobility, x 10^{-5} $cm^2Volt^{-1}sec^{-1}$									
No. of Sera	33	20	34	21	33	21	34	21	26	11
Mean	.5	.4	2.1	2.1	3.6	3.5	4.4	4.4	5.3	5.1
	% of Total Protein									
Sample 1										
No. of Birds	8	6	8	6	8	6	8	6	7	2
Mean	14	11	40	16	11	14	30	50	6	9
Sample 2										
No. of Birds	8	3	8	3	8	3	8	3	3	1
Mean	15	15	21	20	9	10	44	43	11	12
Sample 3										
No. of Birds	7	7	7	7	7	6	7	7	7	3
Mean	12	10	13	18	12	10	33	49	30	14
Sample 4										
No. of Birds	7	3	7	3	7	3	7	3	6	3
Mean	16	14	14	16	13	11	34	32	23	28
Sample 5										
No. of Birds	5	4	5	4	5	4	5	4	4	3
Mean	13	16	22	15	9	13	31	34	25	23

in Table I. The mobilities of 0.5, 2.1, 3.6, 4.4, and $5.2 \times 10^{-5} cm^2Volt^{-1}sec^{-1}$, respectively, obtained for γ-, β-, and α-globulins, albumin, and an "F" component (discussed below), are in good agreement with the data for a single sample of pigeon serum in pH 7.4 phosphate given by Moore(10). Also shown in Table I are averages of the percentage distributions of the components for the 5 points of the cycle, arbitrarily identified and classified on the basis of their mobilities. Table II gives averages of serum total protein values for the cycle points, with corresponding values for β-globulin and total calcium.

Discussion. Visual comparison of the patterns (Fig. 1) indicates a marked change in protein composition of the female pigeon serum during the reproductive cycle, with relatively little change in the male pattern. The quantitative accounting of the distribution in Table I indicates relatively unchanged γ- and α-globulin throughout all samples, and a large increase in β-globulins (also reflected in total protein values of Table II), of female serum at time of ovulation (Sample 1). For the post-ovulation Sample 2, however, the female pattern (B) and distribution values closely resembled those of the normal male (D). Matching of the patterns of the sexes also occurred (with small but notable change from normal in the male serum (E)) of Sample 4. Present in many of the samples, at all 5 cycle points for both female and male, was a component which moved faster than the albumin. This component "F" is a pre-albumin or a protein possibly related to the "f" component observed in the serum of several species of birds(10,12), and especially of the chicken(13-15), by workers who did not follow changes throughout a cycle. The component "F" was increased in relative concentration in Samples 3, 4 and 5 for the female, and in Samples 4 and 5 for the male, apparently at the expense of a lowered albumin in each case. That this component is lipoprotein in nature has already been suggested(13). Its appearance in both female and male sera, and at increased levels during periods of crop gland enlargement, indicates a possible relationship to the mechanism of pigeon milk formation, which takes place in both sexes of this species(1).

In addition to increases in protein and lipids, extraordinarily high levels of phosphorus and calcium have previously been found in avian plasma at time of ovulation (5,6,16,). Clegg and Hein(17) showed that in chickens, the increased (and non-diffusible) serum calcium bore no relationship to the albumin.

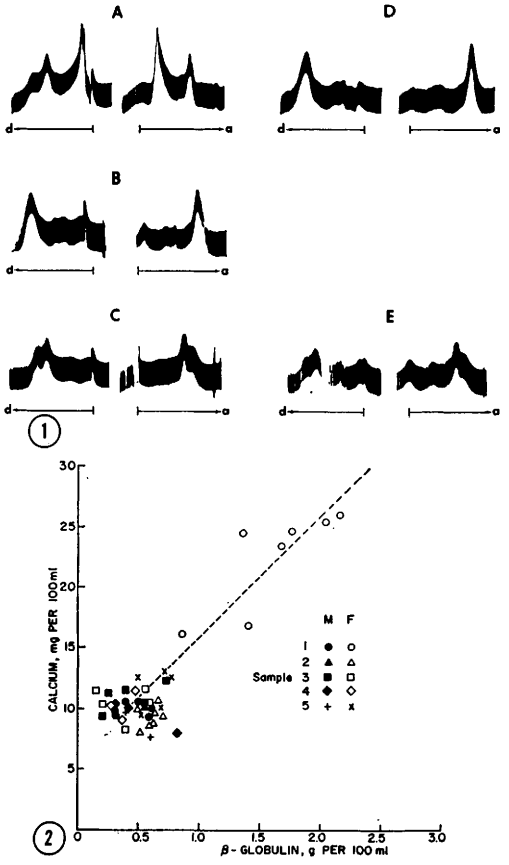

FIG. 1. Electrophoretic patterns of sera of a pair of mated pigeons, at selected points (see text) in reproductive cycle. Ascending (a) and descending (d) patterns A, B, and C are shown for female Samples 1, 2, and 4, respectively; patterns D and E are for male Samples 1 and 4, respectively.

FIG. 2. Relationship of calcium to β-globulin concentration in serum of pigeons during reproductive cycle. Points represent individual analytical values. In units of concentration shown, Ca=10 β-Globulin +6.

Clegg, *et al.*(15) related such extra calcium-binding ability under hormonal influence[1], to the presence of a fast moving high phosphorus-containing electrophoretic component. On addition of *extra* calcium to such serum, this leading component was found to migrate in the area of β-globulin. The authors concluded that the fast moving component operated *in vivo* as a globulin, and that the calcium-binding ability was the property of a lipoprotein.

In the 2 comparisons available(10,12), it has been shown that the electrophoretic characteristics of pigeon serum are markedly different from those of the chicken. Nevertheless, the present work on the former species is generally in accord with the observations and conclusions drawn from the foregoing, and other previous work on the domestic fowl, cited by McIndoe(18). Table II shows that as the reproductive cycle is followed, the serum calcium of female pigeons undergoes a marked increase, *pari passu*, with the increase of β-globulin at, and only at, the time of egg laying. Values for calcium, even more so than for β-globulin, are steadily maintained in serum at normal levels in the female of the other points of the cycle, and at all times in the male. In a statistical test of average serum calcium values of Table II, the datum for Sample 1 of the female was found highly significantly different ($P = <0.001$) from the values for any other sample of either sex. The same was true for the β-globulin values. Apparently, no other serum component is involved in the increase of

[1] Electrophoretic patterns of the sera (in pH 8.6 borate buffer) of laying hens were found similar to those of diethylstilbestrol-treated cockerel(15).

TABLE II. Mean Concentrations of Total Calcium, Protein, and β-Globulin in Sera of Pigeons at Selected Points of Reproductive Cycle.

Sample No.	1		2		3		4		5	
Sex	F	M	F	M	F	M	F	M	F	M
					Total Calcium mg per 100 ml					
No. of Birds	7	8	8	7	5	5	5	6	6	7
Mean	22.6	9.9	9.4	8.5	10.4	10.9	9.7	10.6	11.7	9.2
S.D.	±4.2	± .6	± .9	±1.0	±1.3	±1.2	±1.3	±1.5	±1.5	±1.2
					β-Globulin, g per 100 ml					
No. of Birds	8	6	7	2	6	6	4	3	5	2
Mean*	1.59	.44	.60	.47	.35	.39	.39	.51	.64	.48
S.D.	± .41	± .11	± .07	± .13	± .19	± .22	± .09	± .28	± .12	± .17
					Total Protein, g per 100 ml					
No. of Birds	7	8	8	7	6	6	5	6	6	5
Mean	4.09	2.63	3.19	2.69	2.42	2.13	2.54	2.53	2.82	2.40

* Individual values obtained as product of individual total protein and β-globulin percentage values.

cyclic physiologically mobilized serum calcium[2].

Roepke and Hughes(19) first suggested that the phosphoprotein in the serum of laying hens was related to the vitellin of the egg yolk. An accumulation of evidence in favor of this view has been cited by McIndoe(18) who recently isolated such a lipoprotein complex from the plasma of laying hens. It is therefore of interest, if only as a coincidence, that the line of variation of β-globulin concentration with *extra* calcium (above the normal concentration) in serum of laying pigeons (Fig. 2 for individual values) yields a result of 10 mg calcium per g of β-globulin, in comparison with a value of 8.3 mg calcium per g of the calcium-protein complex (vitellin) of laying chicken plasma, calculated by Winget and Smith(20) from *in vitro* experiments. From *in vivo* relationships for phosphoprotein bound -calcium and protein phosphorus (serum vitellin), established by McDonald and Riddle (6) for pigeons under a wide variety of hormonal changes, the value of 7.7 mg calcium bound per g protein (vitellin) is found. Obviously, many factors, some beyond experimental control, affect the results of a biological study of this kind. The frequency and extent of bleeding, diurnal(21) and seasonal(16) variations in plasma constituent concentrations, age(14,17), diet, and extent of confinement of the birds(22), pH of the samples(15), and the buffer used for electrophoresis(10), are a few of the variables which would make impractical a more serious attempt to calculate, from the present work, the extent of *in vivo* serum calcium-protein binding.

Inasmuch as no elevated serum calcium or β-globulin value has been observed for male pigeons at any time in these experiments, the finding of an increased value for either constituent in a normal (untreated) pigeon is clearly indicative of the female sex. It is suggested that from the practical standpoint, microchemical calcium analysis would be the most unequivocal, and paper electrophoresis possibly the simplest, way of detecting the sex of live pigeons, a matter which otherwise is apparently fraught with considerable difficulty(1).

Summary. At times of egg laying the serum of female pigeons showed a marked, significant increase in β-globulin and total calcium over the normal values found at all other times throughout the reproductive cycle, and at all times for the male. The relationship of calcium bound to β-globulin concentration was roughly in agreement with similar values calculable from previous work done by other methods. A fast moving component of mobility greater than that of albumin was present in many samples, male and female, particularly at times of milk formation.

[2] In the present experiments, a new cycle was found to succeed the previous one within a span of 33 to 40 days. In 2 instances (when Sample 5 was taken at a time which subsequently proved to be too close to onset of a new cycle, a "spiky" β-globulin pattern was obtained for the female. The lipid content of these samples was too high for a meaningful measurement of the fractional areas of the electrophoretic patterns. These were the 2 samples (not included in the average for Sample 5) which showed the highest serum calcium (33.3 and 32.2 mg %) values.

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Occurrence and Titer of Isohemagglutinins in Secretions of the Human Uterine Cervix.*† (27022)

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Gershowitz, Behrman, and Neel(1) have demonstrated the presence of anti-A and anti-B agglutinins in the cervical secretions of normal women and suggested that this might play a role in the deficiency of type A offspring in incompatible marriages of blood type A fathers to type O mothers. An incompatible marriage is here defined as one in which the male possesses an ABO blood group antigen for which the female partner has the corresponding antibody. Behrman, et al.(2) found an excess of type O women as well as a greater than expected number of incompatible matings in a series of 102 childless couples studied, for whom no apparent cause for infertility could be found "by the most exhaustive gynecological and urological examinations possible," and who had been attempting to conceive for at least 5 years. Matsunaga(3) observed both a reduction in fertility and an almost 2-fold increase in the frequency of childless couples in an incompatibly-mated group compared with a compatibly-mated control group. These observations, together with the finding of antigenic dimorphism in human spermatozoa by Gullbring(4) and the demonstration of the presence of antibodies to sperm antigens in the serum of some women by Rao and Sadri (5), lend support to the hypothesis of an immunological selection mechanism operating at a preconception level.

The present study represents an exploration into the quantitative levels of ABO agglutinins in the cervical secretions of women representing the different ABO blood groups, the relationship of these titers to the day of the menstrual cycle, and to the level of agglutinins in the serum.

Materials and Methods. A total of 428 samples of cervical mucus was collected from 182 women. Three of these were postmenopausal while the remaining 179 were in the reproductive age group. Blood and saliva specimens were also obtained in most cases. These subjects were selected from several sources including the Outpatient Gynecology Clinic of University Hospital (largely patients with infertility problems), inpatients from the Ypsilanti State Psychiatric Hospital, volunteers from medical students' wives groups, student nurses, and patients attending the Ann Arbor Planned Parenthood Clinic. The representativeness of the first source may be questioned but in fact these individuals, who comprise about 60% of Group I, contribute about 50% of the positive cervical specimens in this group and thus do not appear to bias the sample.

The total of 182 women was divided into 2 groups—Group I, comprising 141 subjects, from whom only a single specimen of cervical mucus was obtained, and Group II, 41 subjects, from whom 2 or more specimens were taken at different times of the menstrual cycle.

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