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Blood calcium in relation to sex in pigeons.

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Some years ago Reach¹ made comparisons of the amount of calcium recoverable from ashing the entire bodies of male and female white mice. His examination included fair numbers of normal males and females, and of other males and females which had been castrated 42-108 days. He obtained higher percentages of CaO from both groups of females (1.283 normal; 1.275 castrated) and lower percentages from the two groups of males (1.180 and 1.005). Regarding the utilization of calcium, Reach drew the conclusion that "we have here a *secondary sexual character*—the females of these animals are richer in calcium than the males." Lawrence and Riddle² later showed that there is more phosphorus in the *blood serum* of female than of male fowls; and that still larger amounts of phosphorus are found in the blood of the laying hens than of non-laying hens.

In view of the known relationships of the calcium and phosphorus of the blood, both of the preceding studies suggest that the sexes—at least in birds—may show differences in their calcium metabolism. Because of its bearing on the problem of sexuality in pigeons the present study was undertaken. Present methods of blood calcium determination probably warrant the assumption that a sufficient number of determinations made on normal carefully controlled birds will reveal differences, if any very notable sexual differences exist. This report, however, does not represent an adequate study; it deals with the data obtained in a first attack on the problem.

The Kramer and Tisdall method was used. Our determinations therefore are for calcium plus appreciable but unknown amounts of magnesium. Along with each serum sample a check determination was made on oxalate solution of known strength. Adult birds of three kinds were taken for study; most of the pairs

¹ Reach, F., *Biochem. Zeitschr.*, 1912, xvii, 59.

² Lawrence, J. V., and Riddle, O., *Am. J. Physiol.*, 1916, xii, 430.

(except "family hybrids") were in a more or less active state of reproduction. The age and the obvious reproductive history of all birds were exactly known. All birds—at least the male and female of each pair studied—had been subject to closely similar conditions of diet, light, confinement and parentage. Some of the birds proved at autopsy to be infested with intestinal worms (*Ascaridia*) or were otherwise not entirely healthy. The members of a pair were usually not of identical age. An attempt was made—only partially successful—to obtain two determinations from each bird at intervals of about 10 days. It was thus necessary to draw the first sample from a wing-vein, and this often failed to yield 2.0 cc. serum. The second sample was taken from the decapitated bird. No anticoagulant was used, and in a few cases even the serum coagulated on standing and prevented calcium estimation. It now seems probable that the drawing of samples from the wing-vein was unwise since failure to autopsy the bird immediately led to occasional error in judging its reproductive state.

TABLE I.
Calcium in blood serum of male and female pigeons.

Kind and sex of pigeon	First determination		Second determination		Range of values found		
	No. of birds	Calcium (mg. per 100 cc.)	No. of birds	Calcium (mg. per 100 cc.)	No.	Values	
Common pigeons	♂	7	9.81	9	10.00	16	9.1-10.9
	♀	6	12.24	8	11.27	14	8.9-18.9
Ring doves	♂	2	9.05	5	10.58	7	8.2-12.0
	♀	4	12.41	7	12.78	11	9.5-17.3
Family* hybrids (♂)		8	10.86	12	10.43	20	8.7-12.5

Other determinations made on samples of less than 1 cc. serum.						
Kind and sex of pigeon	No. of birds	Calcium (mg. per 100 cc.)	No. of birds	Calcium (mg. per 100 cc.)	No.	Values
Common pigeons	♂	3	12.83		3	9.0-15.0
	♀	2	15.60		2	12.0-19.2
Ring doves	♂	3	10.35		3	9.4-11.6
	♀	3	15.81		3	12.4-18.8
Family* hybrids (♂)		5	11.82		5	8.5-16.6

*From a cross of ♂ common pigeon x ♀ ring dove. All these hybrids were producing sperm, but some of them were probably zygotic females; two of the 13 had left oviducts.

The results are recorded in Table 1. If individual, instead of group or average, determinations were tabulated they would show a greater diversity than we have been able to indicate in the summary given here. The two determinations for the same bird—even in the case of the males—sometimes completely fail to check, and this divergence is pronounced in the case of females. The determinations made on samples of less than 1.0 cc. serum are known to be untrustworthy and are usually discarded. Averages of these are given in order that the whole of our data may be presented; they show both higher values and a wider variation than were obtained from adequate samples.

Altogether the table offers a comparison of values obtained from six groups of males with six groups of females. Considered as groups, the males show the lower, females the higher, calcium-magnesium values in every case. If one attempts to group the individual data on the basis of health, age, or body weight, no significant correlation is obtained. Only on the basis of sex have we been able to arrange a consistent grouping of the values found.

The "family hybrids" require further mention. Common pigeons (*Columba*) and ring doves (*Streptopelia*) belong to different zoological families, and when crossed their offspring are males only. The 13 birds of this origin used here resulted from such a cross and were of the same fraternity. Other data to be published elsewhere supply evidence that, although they all produced sperm, some of them were probably zygotic (chromosomal) females. It will be noted that these hybrids, considered as a group, had a blood calcium value intermediate to the values found for the normal males and females of the two parental species. It can not be said that the particular individuals otherwise suspected of being zygotic females showed the higher calcium values. The two most marked cases (presence of oviducts) of relative "femininity" among this group, however, do give a higher average ($4=11.16$; $16=10.45$), and one of these two showed the highest value obtained for any of the 13 birds.

In connection with the studies earlier mentioned in this paper, it should further be noted that in the femur and humerus of rats, earlier thyroparathyroidectomized at 100 days, Hammett³ found the amount of calcium unchanged in the males, but somewhat

³ Hammett, F. S., *J. Biol. Chem.*, 1923, lvii, 285.

less than normal in the females. This case again suggests some difference in the calcium metabolism of the two sexes.

It remains to point out that the data here reported may be unduly influenced by the inclusion of females actively producing eggs, and thus with specially active calcium glands in the oviducts of some of the females. Data since obtained, and to be reported later by Riddle and Reinhart, indicate that this is the case. We here report no data on juvenile birds, nor on virgin females. In the present data, and on the type of adult pigeons used by us, a difference in the calcium metabolism of the sexes is found; the various groups of females show notably higher amounts of calcium in the blood.

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A spasm-inciting substance in the sputum during asthmatic attacks.

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Studies were carried out on eight cases of bronchial asthma whose ages varied between 25 and 56. Six of these cases had been found to be sensitive to one or more foreign proteins. Special care was taken to collect the sputa of every patient at the height of the attack, for it was thought that that would be the most favorable time to obtain the spasm-producing substance, if any such was present. The sputa thus obtained were extracted in alcohol, with the exception of two instances in which studies were made in the fresh state after being dissolved in Tyrode solution. The precipitate obtained with alcohol was also dissolved in Tyrode solution before using. The material obtained in this manner was tested on isolated strips of smooth muscle from cat's intestine, suspended in Tyrode solution. Prior to the actual experiment the constant contractility of the muscle to pilocarpine was first established.

As a result of these investigations it was found that the sputum of the asthmatics studied contained a substance which stimulated smooth muscle to contract. In most of our cases the contraction