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Is the Manoilov Reaction a Better Test for Metabolic Level Than for Sex?

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In preliminary work with the Manoilov sex test we noted that a wrong or "reversed" reaction was rather frequently obtained when the test was applied to two blood samples from an adult pair of reproducing pigeons. These reversals then seemed to occur more frequently in those pairs which were *incubating eggs* at the time the test was made, and it was therefore decided to make a special study of the conditions attending these reversals. We designate as "reversals" those cases in which the comparison of the bloods from the two donor birds indicated the opposite of their actual sex. Conducting the study along these lines, in an animal in which many important details of the current reproductive states and processes are easily known, we have obtained results which we conclude are a contribution not only to this little understood reaction, but to the general problem of the nature of sexual difference.

When male and female mates are studied at a "resting stage" of the reproductive cycle (when no eggs are about to be laid, and when the pair is not incubating eggs) the Manoilov test gives a correct indication of the sex of the two donor birds in about 85 per cent of the tests (table 1). If, however, the blood be taken from the birds at or near the time eggs are ready to be released from the ovary, only 29 per cent of the tests give a correct answer. Also, while the birds are incubating eggs 2 to 12 days only 35 per cent of males and females are correctly placed by the test. Thus, under these two conditions, more males are diagnosed as females than as males, and *vice versa.* At the very end of incubation about 73 per cent of the cases are correctly placed by the test.

TABLE I.
Percentage of correct and reversed tests at various stages of the reproductive cycle.

Description	Resting stage		Ovulation period		2-12 days incubation		13-18 days incubation	
Ring doves								
Normal (♂ lighter than ♀)	85.0	17	27.8	5	40.0	8	81.8	9
Equal	——	—	11.1	2	——	—	9.1	1
Reversed (♂ darker than ♀)	15.0	3	61.1	11	60.0	12	9.1	1
Common pigeons								
Normal (♂ lighter than ♀)	84.6	11	30.8	4	28.6	4	50.0	2
Equal	——	—	7.7	1	7.1	1	25.0	1
Reversed (♂ darker than ♀)	15.4	2	61.5	8	64.3	9	25.0	1
Average (all pairs)								
Normal	84.8	28	29.0	9	35.3	12	73.3	11
Equal	——	—	9.7	3	3.0	1	13.3	2
Reversed	15.2	5	61.3	19	61.7	21	13.3	2

An important part of our method of study rests upon the utilization of a series of color standards (we used various dilutions of nicotine, to each of which the Manoilov reagents were added), such as would enable us to assign a definite value to the grade of color found (grades 1 to 14; 14 being colorless) in every blood test. The values thus obtained permit us to know whether it is the blood of the male or of the female, or of both, that undergoes change during incubation, egg-laying, etc. The figures found (table 2) show that for ring doves at the "resting" stage the average male stands at 10.5 in our scale, the female at 8.9. During "egg laying" and "incubation," however, the male figure drops to 9.2 and 9.0, while the female then becomes 9.7 and 8.4. At 13 to 18 days of incubation the values for male and female are lowest, 8.9 and 7.4, respectively. Less extensive data for common pigeons, a different zoological family, essentially confirm these findings in ring doves.

TABLE II.
Average values found for males and females in terms of a standard nicotine solution (N value).

Sex	Resting stage		Ovulation period		2-12 days incubation		13-18 days incubation	
	N value	No. tests	N value	No. tests	N value	No. tests	N value	No. tests
Ring doves								
Male	10.5	41	9.2	26	9.0	29	8.9	8
Female	8.9	37	9.7	30	8.4	28	7.4	10
Common pigeons								
Male	7.2	22	6.0	11	6.4	13	7.2	5
Female	6.4	16	7.4	12	7.4	14	5.8	3

The available facts suggest that the above values and periodic changes are best understood on the basis of known or probable metabolic levels, and periodic metabolic changes, in the sexes during the reproductive cycle. There is much evidence that among pigeons (as in at least most other organisms) the metabolic rate is higher in males than in females; and we may take our normally higher values (less coloration) for males, as an expression of this difference. Males become relatively inactive while incubating (the male pigeon nests during the daytime and is always inactive at night). This is therefore a period in which the current metabolic rate or level probably decreases in the male. The copulatory exhaustion of the male, at or before "ovulation" in the mate, would also probably lead to a decrease in his metabolic rate at that period. At both of these periods (ovulation; incubation) such a decrease, certainly a change toward the normal female level, is actually found.

The changes in the female may be explained as follows: Earlier studies by Riddle[1] have shown that when a female pigeon approaches the ovulation period the suprarenals temporarily enlarge; and Riddle and Honeywell[2] found evidence of increased adrenin secretion at this period as indicated by an increase of the blood sugar. Such increased adrenin secretion in the female should cause an increase in her metabolism at this period, and our figures for this period actually show an approach to normal male values. During incubation the female also, though she incubates chiefly at night, is probably less active and probably attains a lower metabolic level than during normal periods; the values found here fall below the normal. Our data thus suggest that the Manoilov "sex test" is really a better test of metabolic rate or level. According to this view it becomes a relatively good sex test also, in certain unmodified sexual periods, because metabolism and sex are intimately related in the way long urged by the senior author.

The amount of color remaining at the conclusion of the oxidation-reduction processes involved in the Manoilov test undoubtedly rests upon the amount of certain *substances*—probably reducing substances—in the substrate. Our hypothesis is that such substances tend to vary parallel with metabolic rate. Studies of a different nature with this reaction, to be described later, in part strongly and definitely support the above explanation; another part of those results are less easily interpreted.

A further fact is made clear by the data presented here. No sex-specific substance is involved in the Manoilov test, but the differential end-points of color rest plainly and purely upon a *quantitative* basis. We are anticipated in the publication of this conclusion by Alsterberg and Häkannson[3], whom we therefore confirm. It should be noted that this quantitative, not qualitative, character of the test conforms perfectly with our identification of the test as, primarily, one for metabolic levels. The metabolism of males and females is qualitatively essentially similar; it is normally quantitatively different in the sexes, but metabolism may suffer wide temporary fluctuations in either sex while the differentiated structures declaring the actual sexuality remain relatively fixed.

On the basis of the view developed here it is thought possible to explain the numbers, and the variable numbers, of failures of the test as these are recorded by all who have used the Manoilov reaction as a sex test. Investigators have frequently indicated the several physiological similarities (age, position of leaves on trees, etc.) that must exist in the male and female tissue compared. Such differences plainly involve, or at least suggest, metabolic differences.

The results indicate that the Manoilov sex test is a better test of metabolic level than of sex. From individual pairs of pigeons, on which repeated tests were made, the sex was usually correctly indicated by the test in ordinary life stages, but much more often incorrectly indicated at those particular reproductive stages in which metabolic change is known or probable.

The test rests purely upon a quantitative, not a sex-specific qualitative basis. In this we confirm Alsterberg and Häkannson. This fact, however, is necessary to and is in conformity with our association of the test with metabolic state or level.

The few or numerous failures of the test as a sex test are explainable in terms of the view developed here.

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[1] Riddle, O., *Am. J. Physiol.*, 1923, lxvi, 322.

[2] Riddle, O., and Honeywell, H. E., *Am. J. Physiol.*, 1923, lxvi, 240.

[3] Alsterberg, G., and Häkannson, A., *Biochem. Z.*, 1926, clxxvi, 251.