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SEROLOGICAL EXAMINATION OF THE BLOOD-RELATIONSHIP BETWEEN WILD AND DOMESTIC DUCKS[1]

Kiyotsuna SASAKI

I. INTRODUCTION

It was first observed by BORDET (6) that the anti-fowl serum obtained from a rabbit treated with fowl blood serum acquired precipitating power for fowl serum; since this was conformed by UHLENHUTH (19) the serological examination of the blood-relationship amongst birds, especially poultry, has been investigated by NUTTAL (14) and many others (2, 9, 18). On the other hand, the subject of the interrelationship between poultry and their wild forms, which has special importance in regard to the origin of domestic animals, has been treated of from the morphological or genetical point of view, but so far as the writer is aware there is no serological research in this field. Hence the blood relationship between wild and domestic ducks is examined by the writer by means of precipitin reaction, and the results are set forth in this paper.

II. MATERIAL AND METHODS

A. Material.

The stocks used in the experiment were some waterfowls belonging to *Anatidae* (1,7); they are as follows: (Plates 4, 5)

[1] From The Physiological and Zootechnical Institute, Kyushu Imperial University, Fukuoka, Japan.

a) Domestic ducks

1 *Anas domestica erecta* (Japanese duck)
2 *Cairina moschata L.* (Muscovy duck)

b) Wild ducks

1 *Anas boschas L.* (Wild form of Japanese duck)
2 *Mareca penelope L.*
3 *Eunetta falcata* (Georgi)
4 *Nettion formosum* (Georgi)
5 *Nettion crecca crecca L.*

c) Domestic goose

1 *Anser cygnoides Gm.* (Chinese goose)

B. Methods

In these experiments the blood serum which was obtained from each stock mentioned above was employed as the antigen. Before proceeding to bleed the stock was starved for about 24 hours to prevent the serum from clouding; bleeding was accomplished by plucking off the feathers inside the wing along the humerus, sterilizing these parts with alcohol, making a small cut in a vein along the humerus, and allowing the drops of blood which flow out to fall into the sterile test-tubes. They were then placed in the thermostat 37°C for about 4 hours, and within this length of time the serum was expressed; this was pipetted off into small sterile test-tubes, and the supernatants centrifugalized from the serum. Thence the clear serum was transferred to the sterile sealed tubes and kept in the refrigator.

For the production of precipitating antisera 3-4 c.c. of avian serum from each of these stocks was intravenously injected five times into each of two or three rabbits whose live-weight is about 2 k.g. respectively at intervals of three days between injections. When seven days had elapsed after the last injection a small quantity of blood was drawn off carefully from the marginal ear-vein to test it for the strength of the precipitating power; if it proved insufficiently powerful injections were repeatedly continued, whereas if it showed power enough the rabbits were bled by isolating and making a small cut into a carotid. By treating the blood in the same manner as described before the antisera were obtained, transfered to sterile ampullae, and kept pure in the refrigator until used.

As NUTTAL (14) has previously shown, the degree of the precipitation depends upon the protein content as well as on the qualitative difference of precipitinogen (blood serum); the errors caused by the amount of

protein in the precipitinogen should be excluded as much as possible, when the precipitin reaction is applied in order to examine the blood-relationship amongst animals. The protein content in the blood serum used by the writer as the precipinogen varies not only with the individual but also with the age, etc., therefore the amount of the protein in each blood serum was first determined by AUFRECHT's method (5), and the standard solution of each serum was diluted with physiological NaCl solution so as to contain 2 % protein

In experimenting with the precipitin reaction FORNET's "Ring Probe" (8) was employed. The degree of precipitation can well be expressed numerically by the number of dilutions of the blood serum, (when the number is large the precipitation with the anti-avian serum is hardly shown) the standard solution of the blood serum (protein content 2%) being 1, double diluted 2, four times diluted 3, eight times 4, and so on. A series of little test-tubes were put on the rack, anti-avian serum 0.1 c.c. was transfered to them, to which each dilution 0.3 c.c. above mentioned was carefully added along the walls of the tubes; then the reaction occurs on the zone of contact. As the strength of the precipitating power varies in the course of time the speed of reaction was also taken into consideration; the reaction was observed at a room temperature of 15-25°C, three times namely 15 m, 30 m and 90 m after the diluted solution was added to it. In order to simplify the record only the result of the last observation is shown in this paper. The controls (1) immune serum 0.1 c.c. + physiological NaCl solution 0.3 c.c., (2) normal rabbit's serum 0.1 c.c. + standard solution 0.3 c c. were tested in each experiment, but always a negative reaction was observed in each case; therefore these records are omitted in the following tables. On the other hand, similar experiments were repeated with each of the same kind of the immune sera, one immune serum being, of course, tested at least twice, but the results agreed with each other in the majority of cases. Only one result, therefore, is here described.

A similar examination of these blood sera was carried out with the specialized immune sera. In specializing the immune sera WEICHHARDT's "Absättigungsverfahren" modified by MISAO (13) was used. The immune sera were specialized so that they would react only with the homologous blood serum and not with that of the heterologous. The antiserum was added to the heterologous blood serum so that a rich precipitum occured, then the precipitum was centrifugalized. For this purpose first the proportion of antiserum to blood serum which cause

maximum precipitum was estimated quantitatively with the precipitometer. Next the blood serum was added to the antiserum in this proportion in the majority of cases, more blood serum being added in only a few cases. The specialized immune serum obtained in this way was absolutely specific to the homologous blood serum.

III. EXPERIMENTAL RESULTS

i) Japanese duck and Chinese goose

Table I.

Immune serum		Anti-Jap Duck S.		Anti-Ch. Goose S.	
Test-tube	Precipitinogen / Dilution	Duck	Goose	Duck	Goose
1	1:1 (2 %)	+++	+++	+++	+++
2	1:2	++	++	++	+++
3	1:4	++	++	++	++
4	1:8	++	++	++	++
5	1:16	++	++	+	++
6	1:32	++	+	+	+
7	1:64	+	+	+	+
8	1:128	+	+	+	+
9	1:256	+	+	+	+
10	1:512	+	+	+	+
11	1:1024	+	+	+	+
12	1:2048	+	+	−	+
13	1:4096	+	−	−	−
14	1:8192	−	−	−	−

Table 2

Specialized Immune serum		Anti-Jap. Duck S. + Ch.-Goose S. (1:1)		Anti-Ch. Goose S. + Jap.-Duck S. (1:1)	
Test tube	Precipitinogen / Dilution	Duck	Goose	Duck	Goose
1	1:1 (2 %)	++	−	−	++
2	1:2	++	−	−	+
3	1:4	+	−	−	+
4	1:8	+	−	−	+
5	1:16	+	−	−	+
6	1:32	+	−	−	+
7	1:64	+	−	−	+
8	1:128	+	−	−	−
9	1:256	−	−	−	−

It will be seen from Table 1 that the anti-serum for the Japanese duck reacts as far as 13 with the homologous blood serum i.e. with the Japanese duck serum, but as far as 12 with the Chinese goose serum; on the contrary, the anti-serum for the Chinese goose reacts as far as 12 with the homologous blood serum, and as far as 11 with that of heterologous. Therefore the blood sera of these two kinds of stocks can be clearly distinguished serologically from each other. The tests with each of the specialized immune sera for these two stocks are given in Table 2. The specialized immune sera for the Japanese duck, which is obtained by saturating the immune serum with the blood serum of the Chinese goose in proportion of equivalent volume and centrifugalizing the precipitum, shows marked positive reaction with the blood serum of the Japanese duck, but no trace of reaction with that of the Chinese goose. The reverse results were obtained with the specialized immune serum of the Chinese goose.

ii) Japanese duck and Muscovy duck

The *Anas* serum as well as the *Cairina* serum react positively in the same degree with the anti-*Anas* serum. It is the same also with the anti-*Cairina* serum, so that these two kinds of waterfowls can not

be distinguished serologically with their corresponding anti-sera i.e. they stand right close to each other. These two can however be clearly distinguished from each other with the specialized anti-*Anas* serum and anti-*Cairina* serum; for the *Anas* serum reacts positively as far as 7, *Cairina* serum not at all with the former; while the *Cairina* serum reacts positively as far as 8, and the *Anas* serum not at all with the latter, as is shown in Table 3.

Table 3

Specialized Immune serum		Anti-Jap. Duck S. + Mus.-Duck S. (1:3)		Anti.-Mus. Duck S. + Jap.-Duck S. (1:1)	
Test-tube	Precipitinogen / Dilution	Jap.	Mus.	Jap.	Mus.
1	1:1 (2 %)	++	—	—	+++
2	1:2	+	—	—	+++
3	1:4	+	—	—	+++
4	1:8	+	—	—	++
5	1:16	+	—	—	+
6	1:32	+	—	—	+
7	1:64	+	—	—	+
8	1:128	—	—	—	+
9	1:256	—	—	—	—

iii) Wild ducks

A *Anas boschas* and *Nettion crecca*
B *Nettion formosum* and *Eunetta falcata*
C *Nettion formosum* and *Nettion crecca*

Although these three pairs of wild ducks are not distinguishable from each other with the corresponding antisera they can be clearly distinguished with the corresponding specialized antisera, as is shown in Table 4.

Table 4 (A)

Specialized Immune serum		Anti-*Anas bosches* S. + *Nettion crecca* S. (1:1)		Anti-*Nettion crecca* S. + *Anas bosches* S. (1:2)	
Test-tube	Precipitinogen / Dilution	*Anas bos.*	*Nettion cr.*	*Anas bos.*	*Nettion cr.*
1	1:1 (2 %)	+	—	—	+
2	1:2	+	—	—	+
3	1:4	+	—	—	+
4	1:8	+	—	—	+
5	1:16	+	—	—	—
6	1:32	+	—	—	—
7	1:64	+	—	—	—
8	1:128	—	—	—	—
9	1:256	—	—	—	—

Table 4 (B)

Specialized Immune serum		Anti-*Nettion formosum* S. + *Eunetta falcata* S. (1:2)		Anti-*Eunetta falcata* S. + *Nettion formosum* S. (1:3)	
Test-tube	Precipitinogen / Dilution	*Nettion*	*Eunetta*	*Nettion*	*Eunetta*
1	1:1 (2 %)	+	—	—	+
2	1:2	+	—	—	+
3	1:4	+	—	—	+
4	1:8	+	—	—	+
5	1:16	+	—	—	+
6	1:32	—	—	—	+
7	1:64	—	—	—	+
8	1:128	—	—	—	+
9	1:256	—	—	—	—

Table 4 (C)

Specialized Immune serum		Anti-*Nettion formosum* S.+ *Nettion crecca* S. (1:3)		Anti-*Nettion crecca* S. + *Nettion formosum* S. (1:3)	
Test-tube	Precipitinogen / Dilution	*Formosum*	*Crecca*	*Formosum*	*Crecca*
1	1:1 (2 %)	+	—	—	+
2	1:2	+	—	—	+
3	1:4	+	—	—	+
4	1:8	+	—	—	+
5	1:16	+	—	—	—
6	1:32	—	—	—	—
7	1:64	—	—	—	—
8	1:128	—	—	—	—
8	1:256	—	—	—	—

iv) Wild and domestic ducks

A *Anas domestica* and *Eunetta falcata*
B *Anas domestica* and *Marecca penelope*
C *Anas domestica* and *Nettion crecca*
D *Cairina moschata* and *Anas boschas*
E *Anas domestica* and *Anas boschas*

Japanese duck (*Anas domestica*) can not be distinguished serologically from the wild ducks (*Anas boschas, Eunetta falcata*) with the corresponding antisera as is shown in Table 5. By means of the specialized antisera, however, Japanese duck can be clearly distinguished from *Eunetta*, *Marecca* as well as *Nettion crecca*, and the Muscovy duck (*Cairina moschata*) from *Anas boschas* (cf. Table 6.)

It will be observed from Table 6 (E) that although the Japanese duck can be distinguished from *Anas boschas* with the anti-*Anas dom.* serum, which is specialized by addition of the *Anas boschas* serum, it is impossible with the specialized anti-*Anas boschas* serum, as the latter

reacts always in the same degree both with the *Anas boschas* serum and with that of the Japanese duck in any grade of saturation, and it ceases to react with both sera simultaneously in the strong saturation.

Table 5

Immune serum		Anti-Jap. Duck S.			Anti-*Anas boschas* S.			Anti-*Eunetta* S.		
Test-tube	Precipitinogen / Dilution	Jap.	*Anas*	*Eunetta*	Jap.	*Anas*	*Eunetta*	Jap.	*Anas*	*Eunetta*
9	1:256	++	++	++	++	++	++	++	++	++
10	1:512	++	++	++	++	++	+	++	++	++
11	1:1024	+	+	+	+	+	+	+	+	+
12	1:2048	+	+	+	+	+	+	+	+	+
13	1:4096	±	±	±	±	±	±	±	±	+
14	1:8192	—	—	—	—	—	—	—	—	—

Table 6 (A)

Specialized Immune serum		Anti-Jap. Duck S. + *Eunetta* S. (1:2)		Anti-*Eunetta* S. + Jap. Duck S. (1:2)	
Test-tube	Precipitinogen / Dilution	Jap.	*Eunetta*	Jap.	*Eunetta*
1	1:1 (2 %)	++	—	—	++
2	1:2	++	—	±	++
3	1:4	++	—	—	+
4	1:8	+	—	—	+
5	1:16	+	—	—	+
6	1:32	+	—	—	+
7	1:64	+	—	—	±
8	1:128	+	—	—	—
9	1:256	—	—	—	—

Table 6 (B)

Specialized Immune serum		Anti-Jap. Duck S. + *Mareca* S. (1:3)		Anti-*Mareca* S. + Jap. Duck S. (1:4)	
Test-tube	Precipitinogen / Dilution	Jap.	*Mareca*	Jap.	*Mareca*
1	1:1 (2 %)	+	—	—	+
2	1:2	+	—	—	+
3	1:4	+	—	—	+
4	1:8	+	—	—	+
5	1:16	+	—	—	+
6	1:32	—	—	—	+
7	1:74	—	—	—	—
8	1:128	—	—	—	—
9	1:256	—	—	—	—

Table 6 (C)

Specialized Immune serum		Anti-Jap. Duck S. + *Nettion* S. (1:3)		Anti-*Nettion* S. + Jap.-Duck S. (1:1)	
Test-tube	Precipitinogen / Dilution	Jap.	*Nettion*	Jap.	*Nettion*
1	1:1 (2 %)	++	—	—	++
2	1:2	++	—	—	+
3	1:4	++	—	—	+
4	1:8	++	—	—	+
5	1:16	+	—	—	+
6	1:32	+	—	—	±
7	1:64	+	—	—	—
8	1:128	±	—	—	—
9	1:256	—	—	—	—

Table 6 (D)

Specialized Immune serum		Anti-Mus. Duck S. + *Anas boschas* S. (1:4)		Anti-*Anas boschas* S. + Mus-Duck S. (1:2)	
Test-tube	Precipitinogen / Dilution	Mus.	*Anas*	Mus.	*Anas*
1	1:1 (2 %)	++	—	—	+
2	1:2	++	—	—	+
3	1:4	++	—	—	+
4	1:8	+	—	—	+
5	1:16	+	—	—	+
6	1:32	+	—	—	+
7	1:64	+	—	—	+
8	1:128	+	—	—	+
9	1:256	—	—	—	—

Table 6 (E)

Specialized Immune serum		Anti-Jap. Duck S. + *Anas boschas* S. (1:2)		Anti-*Anas boschas* S. + Jap.-Duck S. (2:1)		(1:1)	
Test-tube	Precipitinogen / Dilution	Jap.	*Anas*	Jap.	*Anas*	Jap.	*Anas*
1	1:1 (2 %)	++	—	+	+	—	—
2	1:2	++	—	+	+	—	—
3	1:4	+	—	+	+	—	—
4	1:8	+	—	+	+	—	—
5	1:16	+	—	—	—	—	—
6	1:32	+	—	—	—	—	—
7	1:64	+	—	—	—	—	—
8	1:128	+	—	—	—	—	—
9	1:256	—	—	—	—	—	—

IV. DISCUSSION

1. The Japanese duck can be clearly distinguished by the precipitation method from the Chinese goose with the corresponding antisera, but not from many kinds of wild ducks; this shows that the Japanese duck is more closely related to the wild duck than to the Chinese goose. Therefore the blood-relationship amongst these three kinds of stocks examined from the serological point of view is in accordance with the classification from the morphological standpoint.

2. It is reported by AKAMATSU (2) that poultry whose relation is closer than that of genus can not be distinguished from each other by means of precipitin reaction as well as agglutination. In the writer's case also similar results are obtained, but by specialization of immune sera *Nettion formosum* can be clearly distinguished (absolute specificity being given) from *Nettion crecca*; Japanese duck from Muscovy duck, *Eunetta Marecca* as well as *Nettion crecca*; and Muscovy duck from *Anas boschas*.

3. Although the Japanese duck (*Anas dom. erecta*) can be distinguished from the wild duck (*Anas boschas*) with the specialized anti-*Anas domestica* serum it is impossible with the specialized anti-*Anas boschas* serum. The wild duck, *Anas boschas*, is regarded as the wild form of the domestic duck by REINHARDT (16) and YATSU (20) from the morphological standpoint; so the above findings may have some relationship with the fact that the Japanese duck aboriginated from *Anas boschas*.

Similar results were obtained by ISHIHARA and MISAO in the carp, crucian and goldfish. The carp can be clearly digtinguished from crucian as well as goldfish with the corresponding specialized antisera, and the serological relation between goldfish and crucian is just as between Japanese duck and *Anas boschas*.

4. As is shown above, the serum-proteins of some waterfowls are not distinguishable from each other with corresponding antisera, but they can be clearly distinguished with specialized antisera. This serological relation, for example, between *Nettion formosum* and *Nettion crecca* may be explained as follows, as ISHIHARA suggested in the case of carp and crucian.

First assume that the structure of the serum-proteins (precipitinogens) of *Formosum* and *Crecca* have A in common and a side-chain K for the former as well as L for the latter peculiar to each, i.e. the protein-molecules of *Formosum* as A K and these of *Crecca* as A L; next that

injecting the serum of each stock into each rabbit a part of serum-protein is decomposed into A and K resp L, consequently two sorts of precipitins will be produced in each anti-serum, one being common and the others peculiar to each i.e. *a*, *ak* for *Formosum* and *a*, *al* for *Crecca*, and that the common precipitin *a* combines with the precipitinogen AK or AL, but the precipitin *ak* or *al* only with the corresponding precipitinogen AK or AL.

By saturating the anti-*Formosum* serum with the *Crecca* serum, the precipitin *a* unites with the precipitinogen AL, consequently the precipitum AL *a* occurs, and centrifugalizing this precipitum the precipitin *ak* remains in this saturated immune serum. Therefore adding the *Formosum* serum i.e. the precipitinogen AK to this saturated immune serum, the precipitum AK *ak* occurs; in this case the reaction is positive; but the reaction is negative when the *Crecca* serum i.e. the precipitinogen AL is added to it, for there is no precipitin to unite therewith. A similar explanation may be given in the case in which the anti-*Crecca* serum which has the precipitins *al* as well as *a* is saturated with the *Formosum* serum i.e. the precipitinogen AK. This interelation between *Formosum* and *Crecca* may be represented diagrammatically as follows.

	Formosum		*Crecca*	
Precipitinogen (Blood serum)	AK		AL	
Precipitin (Antiserum)	$\overbrace{ak,\ a}$		$\overbrace{al,\ a}$	
	+ AL		+ AK	
Precipitum (To centrifuge) ...	= AL *a*		= AK *a*	
Precipitin (Remained)	*ak*		*al*	
Precipitinogen...	+ AK	+ AL	+ AK	+ AL
Precipitum	AK *ak*	no	no	AL *al*
Reaction	+	—	—	+

5. Muscovy duck being distinguishable from Japanese duck as well as *Anas boschas* with their corresponding specialized antisera, almost in the same degree as *Formosum* from *Crecca*; accepting the above dia-

grammatic representation, then the serum-protein of *Anas boschas* would be represented by AX, that of Muscovy duck AY, and that of Japanese duck AZ.

The fact that in the combination of Japanese duck and *Anas boschas* only the immune serum for the former is acquired the absolute specificity by saturation should be considered as follows, as ISHIHARA suggested in the case of goldfish and crucian: the serum-protein molecules of these two stocks have A as well as the side-chain X specific to *Anas boschas* in common, and Japanese duck has the further specific component M, i.e. the side-chain of protein molecules of Japanese duck Z is composed of X and M This relation may also be represented diagrammatically.

	Anas domestica		*Anas boschas*	
Precipitinogen (Blood serum)	AXM		AX	
Precipitin (Antiserum)	$axm, \overbrace{ax, a}$ + AX		$\overbrace{ax, a}$ + AXM	
Precipitum (to centrifuge) ...	= AX *ax*, AX *a*		= AXM *ax*, AXM *a*	
Precipitin (remained)	*axm*		no	no
Precipitinogen...	+ AXM	+ AX		
Precipitum	AXM *axm*	no		
Reaction	+	—	—	—

V. SUMMARY

Treating the rabbits with each of the blood sera of the domestic ducks, wild ducks and domestic geese belonging to *Anatidae*, the blood-relationship amongst these stocks were examined by means of precipitin reaction, and the results are as follows:

1. The Japanese duck is distinguishable from the Chinese goose with the corresponding antisera.
2. The Japanese duck and the Muscovy duck, are not distinguishable from each other with the corresponding specialized antisera.

3 The Japanese duck can not be distinguished from the wild ducks with the corresponding antisera. By means of the corresponding specialized antisera, however, it can be clearly distinguished from *Eunetta*, *Marecca* as well as *Nettion crecca*, and the Muscovy duck from *Anas boschas*.

4 The Japanese duck can be distinguished from its wild form (*Anas boschas*) with the specialized antiserum for the former, but not with that for the latter.

In conclusion, the writer desires to express his sincere thanks to Prof. M. ISHIHARA under whose direction this work was done, and to Prof. T. KUBO for selecting the material. He is also indebted to Prof. M. ITAGAKI for kind advice, and to Asst. Prof. T. MISAO for much valuable help.

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EXPLANATION OF PLATES

a) Domestic ducks (males)

1 *Anas domestica erecta* (Japanese duck)

2 *Cairina moschata* L. (Muscovy duck)

b) Wild ducks (males)

3 *Anas boschas* L. (Wild form of Japanese duck)

4 *Mareca penelope* L.

5 *Eunetta falcata* (Georgi)

6 *Nettion formosum* (Georgi)

7 *Nettion crecca crecca* L.

Fig. 2

Fig. 4

Fig. 1

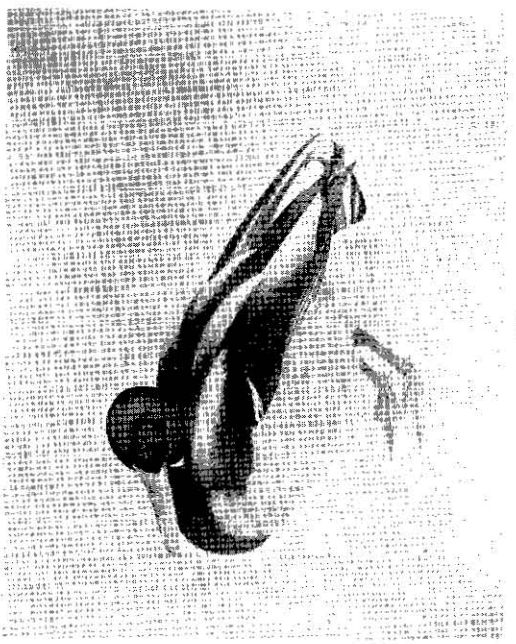

Fig. 3

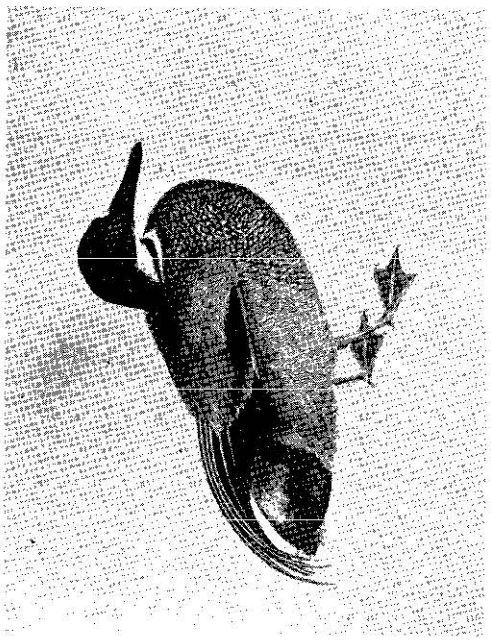

Fig. 5

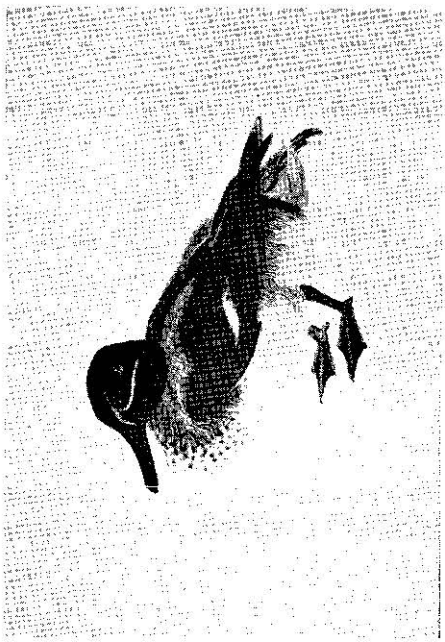

Fig. 7

Fig. 6