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A failure to induce heritable changes in four generations of the White Leghorn chicken by inter- and intra-specific blood transfusion*†

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1. INTRODUCTION

Reports of the success achieved by Soviet biologists in inducing hereditary changes in the domestic chicken by blood transfusion have received considerable attention among geneticists. What appears to be the first summary of this work made to an international audience was presented in 1956 at the International Genetics Symposium held in Japan (Kushner, 1957). Two years later Kushner elaborated on this matter at the Xth International Congress of Genetics in Montreal (Kushner, 1958*a*). Almost concurrently, a report on essentially the same subject was made at the XIth World's Poultry Congress in Mexico City (Sopikov, 1962). A paper by Stroun *et al.* (1958) from Switzerland read at the 1958 Genetics Congress, in general, corroborated the results achieved by Soviet investigators. The most recent report on the success of the blood transfusion procedure in inducing heritable changes in chickens is that of Leroy (1962).

In addition to the above cited studies, there exists extensive Soviet literature concerning the effectiveness of the blood transfusion techniques for inducing directed heritable modifications in poultry. The salient points of these earlier Russian language papers are contained in the reports of Kushner and Sopikov cited above. Furthermore, several annual progress reports of the Institute of Genetics, Academy of Science, U.S.S.R., and of the All-Union Poultry Breeding Institute (U.S.S.R.) also summarized the experimental work on blood transfusions in progress at the respective institutes (e.g. Tolokonnikova & Moiseeva, 1958; Sopikov, 1958; Penionshkevich, 1961).

In essence, the procedure as first used by Soviet and, later, by Swiss and French investigators involved long-term injections of whole blood obtained from several species of poultry into chicken recipients, usually White Leghorns. More recently, however, some Soviet investigators have used either whole blood or plasma only

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for injections (Kushner *et al.*, 1959; Tolokonnikova *et al.*, 1961), and have utilized the Russian White, a Soviet breed, as one of the recipients (Tolokonnikova *et al. loc. cit.*). These injections for each recipient group, being limited to a single donor species, were continued from the time the recipients were a few days old to sexual maturity.

Among the donors used by this long series of investigators have been several breeds of chickens with coloured plumage, e.g. New Hampshires and Rhode Island Reds (reddish-brown), Australorps (black), Bronze turkeys or, less frequently, the guinea fowl. Observations usually have been limited to easily identifiable phenotypes of traits of both the original 'parental' generation (the first to receive injections) and of the subsequent generations. As a rule, the latter continued to receive injections either of whole blood or of blood plasma of the type infused into their parents. The traits most frequently observed have been plumage colour, egg shell colour and body-weight.

According to the reported results of such studies, both the phenotype and the genotype of blood-injected stock frequently were found to be modified in the direction of the blood-donor organism. This result has been interpreted by numerous Soviet biologists (e.g. Sopikov, 1959; Kushner, 1958*b*; Penionshkevich & Michin, 1959; Gromov & Feoktistov, 1956; Gromov, 1959) as a further corroboration of one of the basic tenets of 'Michurinist biology', according to which the *heredity* of an organism can be rather easily directed into a *desired* channel by environmental means. Perhaps the most authoritative and complete exposition of the views on this matter in the U.S.S.R., together with the supporting experimental data are contained in the two-volume proceedings of the Conference on Biology, held in 1957 in Moscow in honour of the 40th Anniversary of the October Revolution (Nuzhdin, 1959).

The obvious implications that the concept of 'directed heredity' has for genetics, as illustrated by the reported success of the blood infusion technique in poultry, led to the initiation of the present study in 1959 at this laboratory. The aim of this study was to verify the reputed effectiveness of the method in inducing hereditary changes in the chicken. The criteria used included, in addition to such gross traits as plumage colour, egg shell colour, and reproductive efficiency, also antigenic and protein specificity as revealed by immunological, electrophoretic and chromatographic examination. The results of this study form the subject of this paper.*

2. GENERAL METHODS

Two hundred Single Comb White Leghorn chicks, hatched on March 24, 1959, formed the initial *Po.* population for this study. The chicks came from the strain of pure-bred White Leghorns which has been maintained for experimental purposes

* Preliminary reports of this study were presented at the 30th Annual Meeting of the Genetics Society of America, Purdue University, Lafayette, Indiana, August 28–30, 1961,and 5th Annual Meeting of the Northwest Section of the Society for Experimental Biology and Medicine, Biology Laboratory, Hanford Laboratories, Richland, Washington, November 17–18, 1961.

at WSU since 1947. The strain, homozygous for dominant white and other genetically verifiable morphological traits, had been propagated by the mass mating of birds, selected for a number of 'utility' points. Among these were: egg size, egg shape and egg shell colour in females, 'masculinity' in males, breed 'type' and general vitality in both sexes. Except for an infrequent occurrence of such morphological 'defects' as stubs on shanks, side-sprigs on the comb, 'salmon' colouration on the breast, or yellowish-creamy cast over the shoulders (the former colouration being more frequent in females, the latter in males), the WSU stock has been free of deviations from the accepted breed standard for the 'utility-type' White Leghorns.

Immediately on hatching, the 1959 hatched generation, *Po.*, was divided at random into the experimental *Po. ext* and the control *Po. ct* halves. On March 27, 1959, chicks in the former group began to receive intra-peritoneal injections of whole blood obtained from a group of 15 mature Broad Breasted Bronze turkey male donors. The same donors provided blood for the entire series of injections into *Po. ext* birds. The blood contained 10%, by volume, of a 5% aqueous solution of sodium citrate. Except for few unavoidable deviations from the injection schedule, involving at most a delay of one day, the injections were repeated at 4-day intervals. Initially, each chick received 2 ml. of blood per injection. Later, when the chicks were 3 weeks old, the dosage was increased to 3 ml., a month later to 4 ml., and finally, when they reached the age of 12 weeks, to 5 ml. By the end of the blood injection treatment, on August 14, 1959, each bird had received 143 ml. of blood. The control chicks, *Po. ct*, received no injections. Both groups were reared together. All birds were periodically examined for any evidence of gross deviation in the plumage colour.

On November 5, 1959, the P generation birds were mated in five single-sire breeding pens according to the scheme shown in Table 1. The hens were trap-nested to permit the pedigreeing of eggs. Shell colour, shape and size of eggs laid by individual hens were also noted, although the number of birds involved was admittedly small to permit a critical study of this aspect of the problem.

All F_1 chicks arising from *Po.* parents, were carefully examined at hatching for morphological deviations. This was periodically repeated for as long as the birds were kept. Space limitations prevented the raising to maturity of all the offspring of the *Po.* generation. However, *all* chicks were kept at least until they were 10 weeks old. The same plan, i.e. keeping all of the chicks under observation at least until the 10th week of their life, prevailed in subsequent generations.

Of the F_1 generation chicks hatched over the next several months, those arising from matings A and D (Table 1) were particularly important for the purpose of this study. Type A chicks, being the first generation offspring of the blood-injected parents provided two sub-lines: positive controls, $F_1(ext)$, i.e. the offspring of blood-injected parents, which themselves, however were not to be injected, and a second generation of White Leghorns, $F_1 ext$, to be injected with turkey blood. The $F_1(ext)$ birds provided a critical test on the appearance of plumage deviation from the normal white colour, following only one blood-injected generation, *Po. ext.* In this

they were to be compared with F_1ct chicks, the negative controls, which were the offspring of the original *Po. ct* non-injected stock.

Type B matings (Table 1) was to test for the possible existence of maternal effect, in which the dam was the injected organism. Type C mating was the reciprocal of type B.

Table 1. *Experimental and control matings*

Parents	Mating code	No. of pens	Total No. of hens	No. of eggs set	% Fertile	% Fertile eggs hatched	No. of chicks examined at hatching	No. of chicks deviating in plumage colour from White Leghorn type	
								At hatching	At 10 weeks of age
Po. ext	A	2	25	642	75	62	301	None	None
Po. ext ♂ *Po. ct* ♀	B	1	4	109	78	63	83	,,	,,
Po. ct ♂ *Po. ext* ♀	C	1	4	80	84	57	38	,,	,,
Po. ct	D	1	6	197	76	69	104	,,	,,
F_1 *ext*	E	2	17	1335	77	78	796	None	None
F_1 *ct*	F	2	21	1525	75	82	937	,,	,,
Po. exnh	G	2	9	634	75	81	384	,,	,,
F_2 *ext*	H	2	26	1265	95	89	1063	None	1*
F_2 *ct*	I	1	10	759	70	79	427	,,	None
F_1 *exnh*	J	2	20	703	87	84	513	,,	,,

* One male with reddish-brown feathers over the shoulders. See text for details.

Injections of F_1ext chicks with the turkey blood started on February 5, 1960, when they were 3 days old. In all, 67 chicks, their sex not ascertained at the start of the treatment, were involved in that group. The plan of injections followed closely that already described for *Po. ext* birds. Again, the blood was provided by a group of Broad Breasted Bronze mature males, set aside specifically for the purpose. By the time the injections were stopped on June 28, 1960, each bird had received 161 ml. of blood. The corresponding controls, F_1ct, of which there were 50 chicks, received no injections.

Concurrently with the F_1ext series of injections, another series, *Po. exnh*, was instituted. In this series, whole blood obtained from a group of pure-bred New Hampshire male chicken donors was injected into 50 WSU-strain White Leghorn chicks of both sexes. The handling of the blood, the dosage and the timing of injections were same as that for the F_1ext series. The F_1ct chicks served as controls.

On July 10, 1960, the birds were placed in six single-sire pens according to the plan outlined in Table 1. The next series of blood injections into the chicks resulting from these matings was begun on November 16, 1960, and continued through March 30, 1961. The chicks were 5 days old at the time of the initial injection.

During the treatment period, each injected chick received 155 ml. of an appropriate-type blood in 29 injections. The treated groups, each consisting of 50 chicks of both sexes, were: F_2ext (the offspring of F_1ext) and F_1exnh (the offspring of *Po. exnh*), i.e. chicks receiving turkey and New Hampshire blood, respectively. Fifty of F_2ct chicks served as non-injected controls for both treated lots.

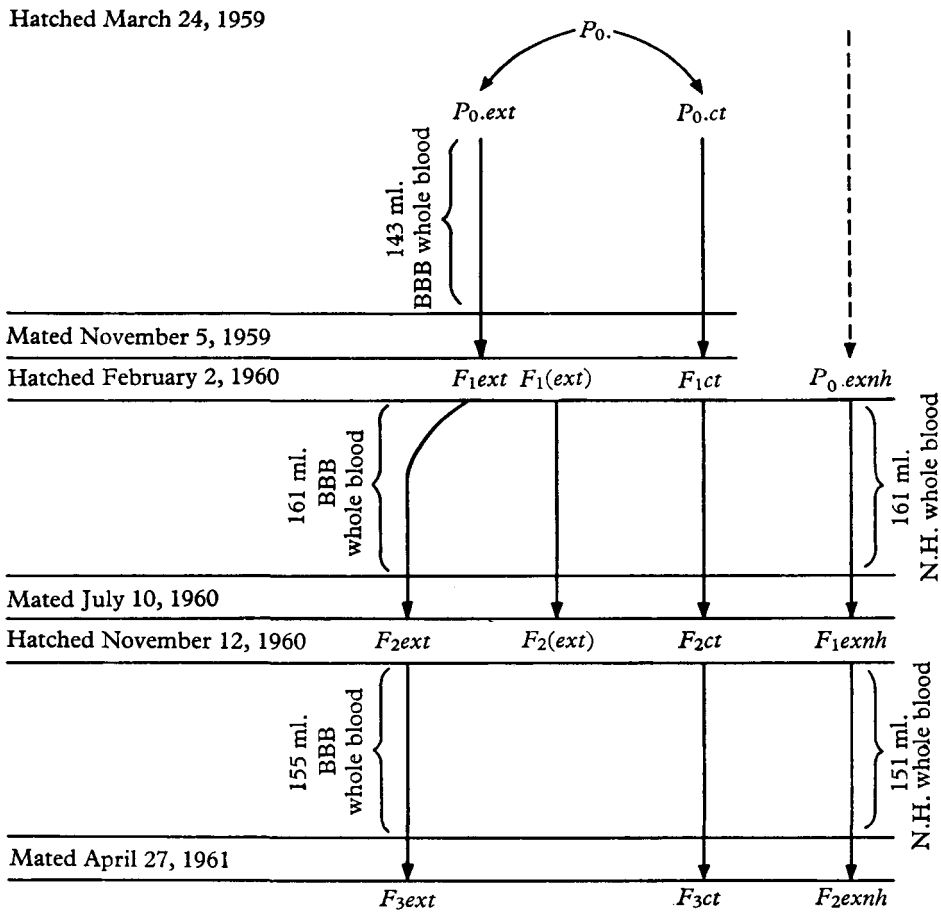

Fig. 1. The scheme of matings and of injections.

An additional group of 50 non-sexed chicks, picked at random from a large population of WSU-strain White Leghorns, was assigned to receive injections of turkey blood. Both the source of the blood, the general methodology applied to the group, as well as the age of the chicks were the same as that used in injecting the F_2ext group. The new series of injections was initiated in order to test for the possible existence in the F_2ext stock of immunological tolerance to turkey blood injections. Sopikov (1958) and Gromov (1959) observed such a shift in the direction of a higher degree of immunological tolerance among the offspring of White Leghorns receiving turkey blood injections.

On April 27, 1961 birds of the F_2ext, F_2ct and F_1exnh groups were mated, to produce the third and last 'experimental' generation of chicks observed in this study. In all, 2003 chicks of that generation were raised to at least 10 weeks of age. The mating scheme is outlined in Table 1. The overall plan of matings involved in the study is summarized in Fig. 1.

3. MORPHOLOGY AND REPRODUCTION

(i) *Methods*

The experimental and control material, used to determine the effect of heterologous whole blood injections on traits amenable to gross examination has already been described under the heading of General Methods. It should suffice to say here that observations on plumage colouration were supplemented, where appropriate, by growth rate data and by the performance records of mature birds in mating pens.

It is to be recalled that according to the Soviet reports already cited (e.g. Sopikov, 1962; Tolokonnikova *et al.*, 1961) plumage colour was considered in such studies to be the most important *single* criterion in judging the gross effect of blood infusion.

It is well recognized that data bearing on such traits as reproductive performance and growth rate show considerable variability. This fact necessitates the use of large numbers of experimental animals per experimental group to permit a critical evaluation of different treatments involving these traits. This point should be kept in mind in evaluating the results of some Soviet investigators (e.g. Ovenko, 1958; Penionshkevich, 1961), who have concluded, on the basis of what appears to be limited data, that heterologous blood injections lead to greater vitality, faster growth and improved reproductive performance. The requirement for large numbers was not met to the satisfaction of the present authors by the experimental material available in this study. Therefore, the relevant information which bears on production traits has been presented here merely as a matter of record, without any attempt at detailed analysis.

(ii) *Results*

None of the 3178 chicks from blood-injected parents showed at hatching any deviation in the colour of down from the normal White Leghorn colouration. With chick mortality averaging below 3% for the first 10 weeks, over 3000 of these chicks were available for colour check of the juvenile feathers at the end of that period. Again, no deviations from normality were observed. It will be recalled that the offspring of treated parents in each generation which were intended for replacement purposes also received blood injections. This group, therefore, was subjected to whatever effects might have been imposed on heredity by whole blood injection, both through their parents and through blood injections administered to them directly. Up to 10 weeks of age, however, such chicks did not differ in feather colouration either from their full sibs, which received no direct treatment themselves (but only through their parents), or their respective control counterparts. One male

bird from mating H (Table 1), showed a reddish-brown colouration over the shoulders as it neared sexual maturity; by that time it acquired, of course, the adult-type feathering. Acid methanol extraction of the pigment in the feathers of the involved area yielded a colour similar to that obtained from the corresponding feathers of normal New Hampshires. However, type H mating involved birds which received injections of blood from Broad Breasted Bronze turkey donors. Unfortunately the male bird in question died before it could produce any progeny.

The relatively low fertility of the matings involving F_2ct (mating I, Table 1) was attributable to the sire. Earlier in the mating season, the reproductive performance of this pen compared favourably with other pens of the same generation birds (matings H and J, Table 1). The apparently failing fertilizing capacity of the sire was not discovered until it was too late to make appropriate substitutions.

No shifts in the shell colour of standard white that could be attributed to blood injections were observed in the experimental groups. Eggs with slightly tinted shells appeared occasionally among those laid by hens both in experimental and control groups. This was limited to a few pullets just coming into egg lay, a not unusual occurrence in White Leghorns. The incidence of such eggs in the present study was below 1%. The frequency of their appearance was comparable between control and experimental lines.

Table 2. *Body-weight of chicks hatched on June* 11, 1961

	Offspring			
			Body-weight (g.)	
Parents	Sex	*N*	1st day	43rd day
F_3ext	♂ and ♀	71	33	
	♂	28		490
	♀	32		451
F_3ct	♂ and ♀	71	33	
	♂	25		509
	♀	32		457
F_2exnh	♂ and ♀	71	34	
	♂	26		478
	♀	30		430

Limited data were gathered on the body-weight of F_2exnh and F_3t. This material, summarized in Table 2, indicates that at least up to the age of 43 days, the offspring of control (non-injected) parents compared favourably in body-weight with their counterparts from the injected parents.

4. IMMUNOLOGY

Serology has been potentially one of the more promising tools in systematics ever since the first demonstration of its utility at the turn of the century (Nuttall, 1901). In 1953, Boyden in a critical review of the 50 years of progress achieved in determining genetic relationships among vertebrate species through the use of this method,

concluded that serological reactions permit one to study the biochemical constitution of organisms. DeFalco (1942), Mainardi (1959), among others, have successfully used serology to establish the immunogenetic relationships of several species of the domestic fowl.

The reported hereditary shifts following several generations of blood injection treatment could have been expected, therefore, to be detectable as immunological shifts in the descendants of treated birds. Accordingly, an appropriate series of immunological analyses was undertaken in the present study involving F_1, F_2 and F_3 generations.

(i) *Methods*

All immunological comparisons were based on birds which were hatched on the same date. The basic procedure involved standard agglutination and precipitation techniques, as well as Ouchterlony's agar diffusion method.

In the agglutination test, blood plasma (source of the antibodies) for each test was obtained by centrifuging citrated blood from a group of three male donors. The red blood cells (source of the antigens) came from pooled samples of citrated blood obtained from another group of appropriate male donors. Before they were used, the RBC were washed several times in a 0·9% physiological saline solution.

In carrying out the agglutination tests, 0·5 ml. of a 2·5% saline suspension of RBC was added to a series of 6 tubes containing graded dilutions of the plasma. The lowest dilution was 1/20. The agglutination titres were determined microscopically after the antigen and antibody complex was first incubated for 1 hr. at 37° C., and then placed overnight in a refrigerator at 4–5° C. The blood plasma used in the precipitation tests was prepared from aliquots used in the agglutination tests referred to above, involving the blood of F_1ext and F_1ct birds injected with turkey blood. The plasma which carried the induced antibodies was diluted 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} with a 0·9% physiological saline solution. The appearance of the precipitation ring was the end point used in the test. Parallel series of non-injected birds from the same two groups that were referred to above served as negative controls.

The medium for the Ouchterlony's agar diffusion method consisted of 1·25% Difco Agar, 0·01% Trypan Blue, and 0·01% merthiolate, made up with 8% sodium chloride–phosphate buffer (pH 7·2–7·4). Either rabbit or chicken anti-turkey blood plasma, placed in the centre well, served as the antibody carrier.

The antigenic configuration of soluble protein fractions, obtained from blood plasma extracts, was tested against the already-mentioned antibody carriers. For the inhibition test, chicken blood plasma was incorporated into the agar base.

(ii) *Results*

Figure 2 shows that on the basis of tube agglutination tests, the time of the first detection of induced anti-turkey antibodies, as well as the titres obtained, were identical in F_2ext and F_2ct chicks when both were subjected to a sustained series of turkey blood injections. The second peak, which appeared for a short time about

four months after the initiation of injections, coincided with the first detectable evidence for the presence of natural antibodies in the uninjected F_2 progeny of both experimental (i.e. turkey-blood injected) and control parents. These observations duplicated those made earlier on F_1 birds. Furthermore, a comparison between F_1 and F_2 generations revealed that the 40-plus day interval between the time of the first detection of induced antibodies and of natural antibodies remained essentially unaltered, for the two generations, in both treatment lines.

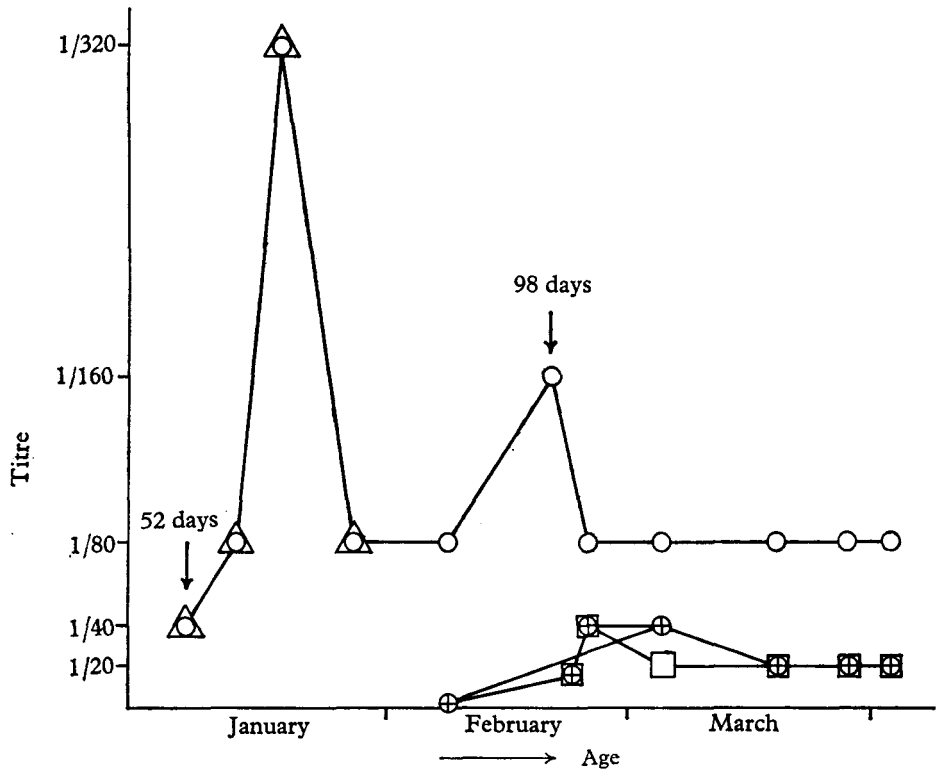

Fig. 2. Results of agglutination tests in 1961. ○ F_2ext, △ F_2ct; both were injected with turkey whole blood. $+F_2ct$, non-injected. □ F_1exnh, injected with New Hampshire whole blood.

Results obtained from precipitin tests applied to F_1ext and F_1ct birds showed that the highest precipitin titre of 10^{-3} was recorded in F_1ext. No natural precipitins could be detected in the F_1ct group.

The rapid drop in the antibody titre just before sexual maturity was observed in all of these birds, irrespective of the treatment received by their parents. This drop occurred both in the agglutination and precipitin test series. In the former, the titre became stabilized at a constant level of 1/80, while in the latter the titre readings dropped to 0.

The use of the agar-diffusion procedure (Ouchterlony's) showed that a similar complex of anti-turkey antibodies was evoked both in F_2ext and F_2ct by a long-term

series of injections of the turkey blood. This can be seen from the antibody–antigen diffusion patterns illustrated in Figs. 3 and 4. The reactions illustrated in these figures occurred when the induced antibody titres were at the highest point in F_2ext and F_2ct, as revealed by the agglutinin reactions referred to earlier (see Fig. 2). The plasma of F_2ext and F_2ct birds which had been induced to develop anti-turkey antibodies reacted in a similar fashion with the antigens provided by the plasma of adult turkeys. At the same time, no reaction with these antibodies was evoked by the plasma of non-injected F_3ext and F_3ct.

The agar-diffusion method also showed that, antigenically, the chicken and the turkey have much in common. Both chicken and turkey plasma, as carriers of antigens, were tested against the serum of rabbits which had been conditioned to develop high titres of anti-turkey blood antibodies. The sample plate, illustrated in Fig. 5, shows a maze of common lines, linking the chicken and the turkey antigen donors. It also demonstrates the existence of an 'extra' reaction line which is specific for the turkey (see the line that subtends the area marked out by wells 1–3). The area subtended by wells 4 and 6, containing plasma from non-injected F_3ext, and by well 5, containing plasma from non-injected F_3ct, lacks this 'extra' line. Its presence was also demonstrated (Fig. 6) when the antigen was provided by turkey embryos (saline extracts of whole embryo homogenates) and young poults (blood plasma), but not when the plasma came from chickens. No explanation, based on experimental evidence, is available to us for the differences shown by Fig. 6 in the intensity of 'extra' line precipitin reactions involving well no. 1 (plasma from mature turkey males) and well no. 4 (plasma from turkey poults at hatching). Possibly the question of optimal antigen-antibody ratio is involved here, the ratio being more favourable for well 4 than for well 1.

Furthermore, the agar diffusion method demonstrated that the injection of turkey proteins, as whole blood, into F_2ct and F_2ext did not lead to the presence of detectable 'foreign protein' fractions in the recipients. The latter at that time ranged in age between 86 and 136 days and had been receiving injections of turkey blood regularly twice a week from the time they were 2 days old. These results are shown in Table 3.

It will be seen that neither the injected nor the non-injected F_2ct and F_2ext, when used as antigen sources, showed any reaction to the anti-turkey antibody complex induced in adult chicken males by the injection of turkey whole blood. At the same time, a strong positive reaction was repeatedly elicited when the antigenic source was turkey plasma (see test Nos. 1 and 2, Table 2). Moreover, the 'extra' line, revealing a specific interaction between turkey antigens and anti-turkey rabbit antibodies, was completely absent in F_2ct and F_2ext, even when the latter were injected with turkey blood (see tests, Nos. 3–10, Table 3).

The specificity of this extra line was demonstrated by means of an inhibition test, in which chicken plasma was incorporated into the agar medium. In such plates, all reactions were blocked, except those between the protein complex specific to the turkey, and the anti-turkey antibodies induced in the rabbit (see test Nos. 4 and 11, Table 3).

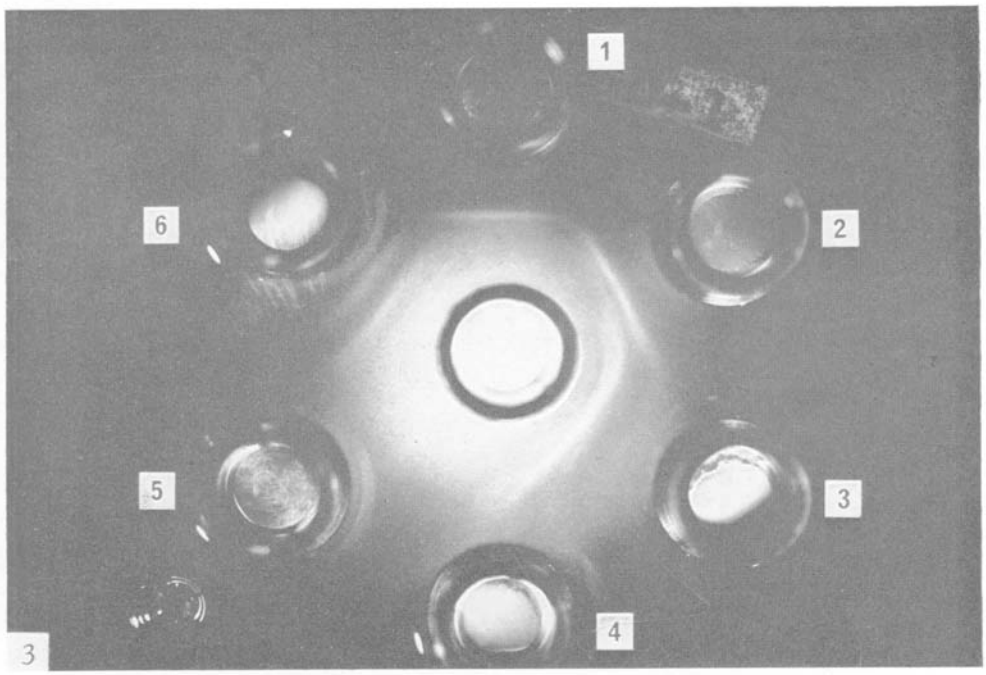

Fig. 3. An agar-diffusion test, showing absence of any reaction by the plasma of *non-injected* F_3ct and F_3ext birds to anti-turkey antibodies induced in an F_2ct subject. Centre well—anti-turkey F_2ct plasma; wells 1, 2 and 6—plasma from mature turkey males; well 3—plasma from 4-week-old turkey male; well 4—plasma from F_3ct; well 5—plasma from F_3ext.

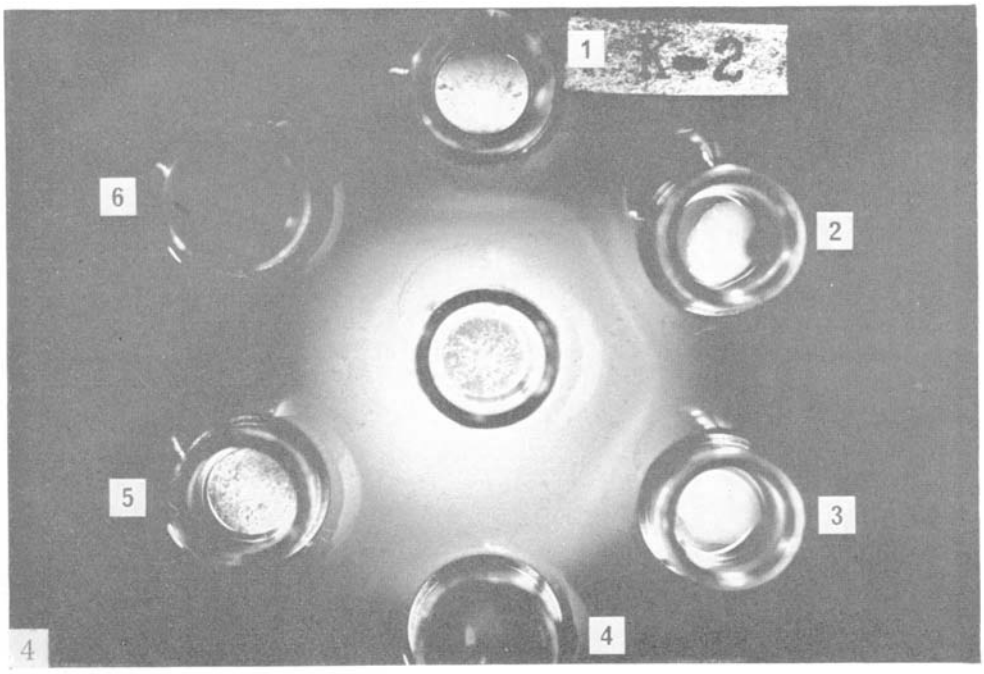

Fig. 4. An agar-diffusion test, showing the absence of any reaction by the plasma of *non-injected* F_3ct and F_3ext to anti-turkey antibodies induced in an F_2ext subject (compare this reaction with that shown in Fig. 3). Centre well—anti-turkey F_2ext plasma; wells 1, 2 and 3—plasma from mature turkey males; wells 4 and 6—plasma from F_3ext; well 5—plasma from F_3ct.

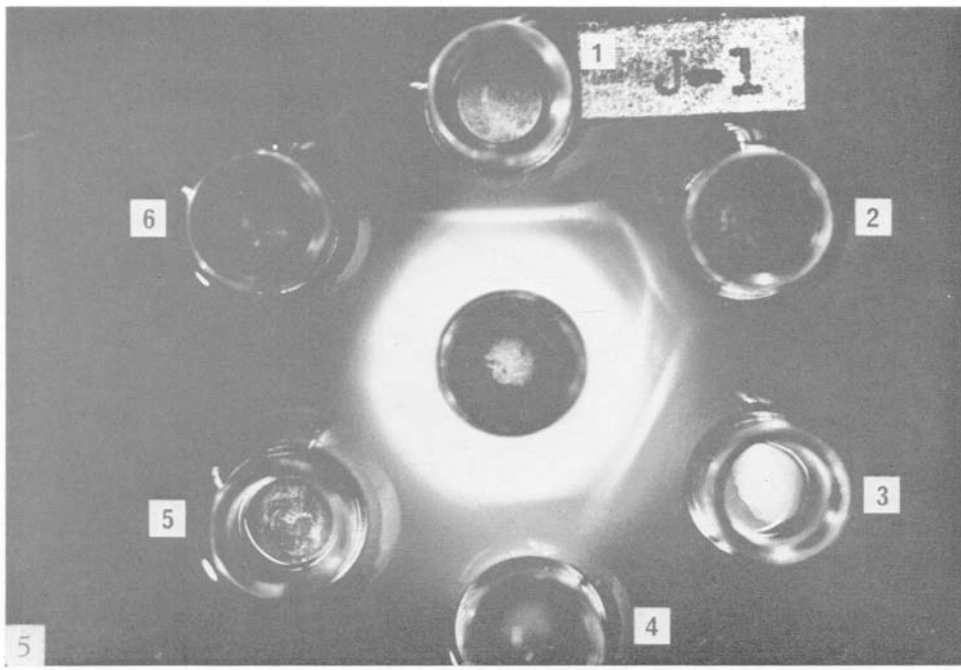

Fig. 5. An agar-diffusion test, showing the presence of an 'extra protein' line (probably complex) which differentiates chickens and turkeys. (The line subtends wells 1, 2 and 3.) The area immediately below this line, and surrounding the centre well is made up of numerous lines unresolved in the photograph. These lines are common both for the chicken and the turkey. Centre well—anti-turkey rabbit plasma; well 1—plasma from mature turkey males; well 2—plasma from whole turkey embryos; well 3—plasma from area vasculosa of the embryos used to provide plasma for well 2; wells 4 and 6—plasma from 1-month-old F_3ext; well 5—plasma from 1-month-old F_3ct.

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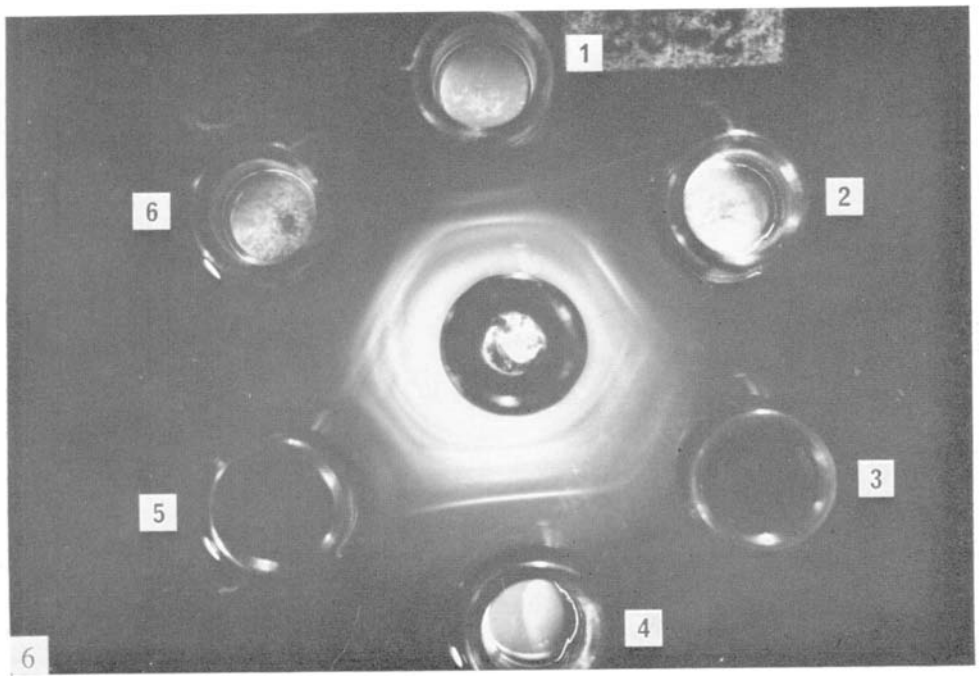

Fig. 6. An agar-diffusion test, showing the presence of an 'extra protein' line which differentiates the turkey from the chicken and from the Japanese quail. This line subtends well 1 and wells 3 and 4. For explanation of the area around the centre well, see the relevant section in the legend for Fig. 5. Centre well—anti-turkey rabbit plasma; well 1—plasma from mature turkey males; well 2—plasma from mature quail males; well 3—homogenate of 1-day-old turkey embryos; well 4—plasma from turkey poults at hatching; well 5—homogenate of 5-day-old chicken embryos; well 6—plasma from uninjected mature F_2ct.

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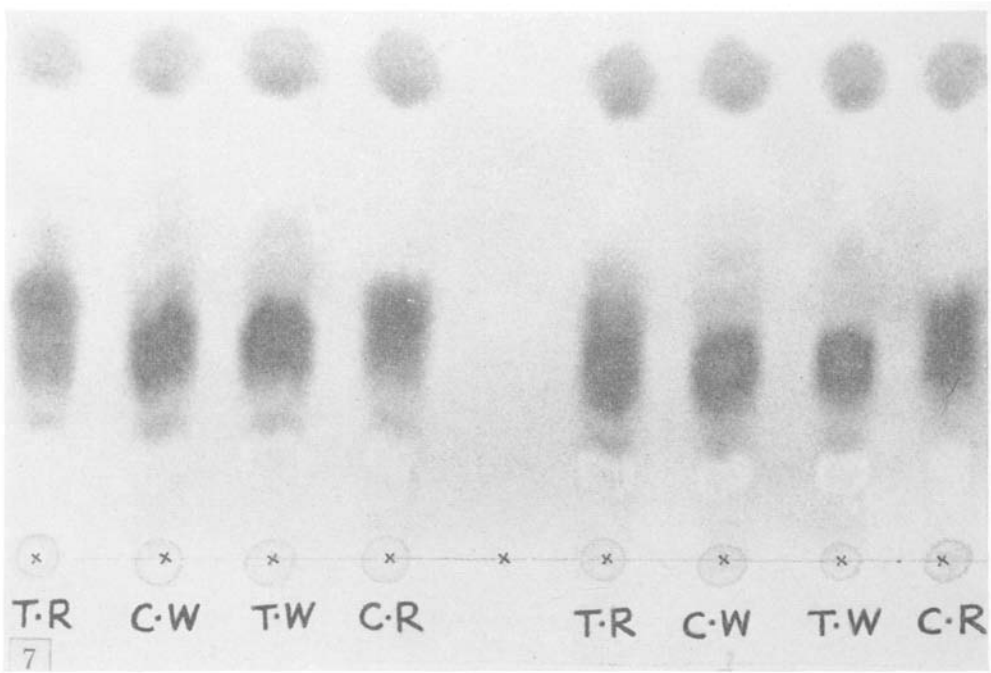

Fig. 7. Paper partition chromatography of white (W) and dark (R) muscles from turkey (T) and chicken (C) donors. Note the difference between the two types of muscles, regardless of the species.

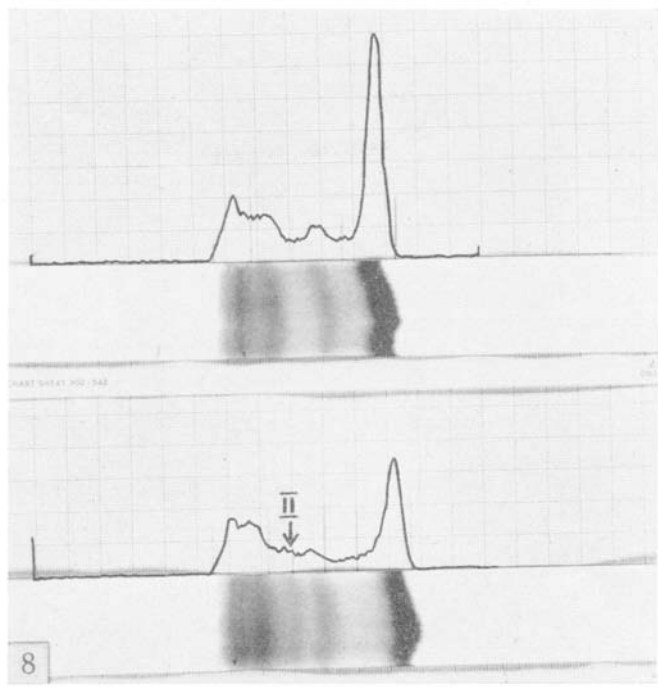

Fig. 8. An electrophoretic analysis of blood plasma from mature turkey donors (top curve) and injected mature F_2ext males (bottom curve). The latter was characteristic for White Leghorn males, of comparable age, in general.

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Table 3. *Results of immunological analysis by the agar-diffusion method*

Date tested, 1961		Antigens: Adult turkey male blood plasma	W.L. injected with turkey blood: 'Experimentals': $F_2 ct$	W.L. injected with turkey blood: 'Experimentals': $F_2 ext$	W.L. not injected: 'Controls': $F_2 ct$	W.L. not injected: 'Controls': $F_2 ext$	Antibody
			Males: 86 days old (3)†		Males: 1 month old (10)		
Feb. 9	1†	++*	—	—	—	—	Chicken anti-turkey blood plasma from 2 males
Feb. 10	2	++	—	—	—	—	Same as above
Feb. 14	3†	++	—	—	—	—	Rabbit anti-turkey plasma serum
		+++++**	+++++	+++++	+++++	+++++	
Feb. 14	4†	++	—	—	—	—	Same as above but inhibited by chicken plasma
		—	—	—	—	—	
Feb. 24	5†	++	—	—	—	—	Rabbit anti-turkey plasma
		+++++	+++++	+++++	+++++	+++++	
			Males: same as above‡		Males: 2 days old (10)		
Mar. 23	6†	++		—	—	—	Same as above
		+++++		+++++	+++++	+++++	
Mar. 23	7†	++	—	—	—	—	Same as above
		+++++	+++++	+++++	+++++	+++++	
Mar. 23	8†	++	—	—	—	—	Same as above
		+++++	+++++	+++++	+++++	+++++	
					1 month (5)		
Mar. 23	9†	++		—		—	Rabbit anti-turkey plasma
		+++++		+++++		+++++	
					14-day embryo (4)		
Mar. 23	10†	++	—	—	—	—	Same as above
		+++++	+++++	+++++	++	++	
			Males: 132 days old		Males: same as 6† (10)		
Mar. 27	11†	++	—	—	—	—	Same as above, but inhibited by chicken plasma

* Plus signs on this line indicate presence of an 'extra', species–specific line.

** Plus signs on this line indicate presence of numerous lines of 'common proteins' between chicken and turkey plasmas.

† Number of birds tested.

‡ The plasma was tested 16 hours after the last injection of turkey whole blood.

5. CHROMATOGRAPHY AND ELECTROPHORESIS

Moore (1945) and Deutsch & Goodloe (1945) indicated that species differences can be recognized in a number of vertebrates, among them chickens, by the electrophoretic analysis of serum and plasma proteins. More recently, Sibley & Johnsgard (1959) have cautioned that the inherent variability of experimental material used in

the analyses of this type may lead to spurious results unless numbers of animals and of blood specimens are adequate.

The demonstration that chromatography may be a useful tool in taxonomic study is of more recent origin. Buzzatti-Traverso & Reichnitzer (1953) were among the first to apply, successfully, paper partition chromatography for this purpose. They used several species of fish as the experimental animals. Mainardi (1958) concluded that the method of ascending, one dimension paper chromatography enabled him to identify tissue homogenates of guinea fowl, turkey and chicken donors.

In the light of these observations, both the blood-injected birds and their offspring (those which were blood-injected and those which were not), together with appropriate controls, were subjected to the chromatographic and electrophoretic analyses. This was done with the full realization that neither of these two methods, as practised at the present time, is as sensitive in detecting inherent species differences as is the immunological procedure already described in Section 4.

(i) *Methods*

Blood and muscle tissue from the same generation birds as used for observations described in Sections 3 and 4 (studies on morphology and immunology, respectively) provided the necessary experimental material. As a general rule, sera and plasma for the chromatographic and electrophoretic tests came from the same aliquots of blood used in immunological tests. A few tests involved homogenates of 5-day embryos.

Pooled tissue homogenates, used in the paper partition chromatographic analyses, one- and two-dimensional, were in each test obtained from three donors. The homogenates prepared separately from white and red chicken breast muscle were extracted with 20% trichloracetic acid (TCA), and then analysed after being washed three times with ether. The analysis was carried out with the aid of Whatman No. 3 mm. filter paper. The reagent was one part of 1% ammonia plus 2 parts of absolute *n*-propanol. Five lambdas of plasma or of tissue extract to be compared were placed directly on the filter paper. Ninhydrine 0·2% ethanol solution was used for the development of both the tissue and the blood plasma patterns.

The electrophoretic analysis involved a Spinco Model R paper electrophoresis system provided with an integrator. Five lambdas of a blood plasma specimen were used in each analysis. The protein distribution patterns were recorded on filter paper strips 20 mm. wide. The Duostat was adjusted for 2·5 mA and 75 volts per cell. The strips were moistened with the veronal buffer (pH 8·6) prior to the application of the sample.

(ii) *Results*

Contrary to the observations reported by Mainardi (1959), efforts at this laboratory to distinguish chromatographically between turkey and chicken pectoral muscles in 10-week-old birds produced negative results. It was not surprising, therefore, that massive injections of 'foreign' blood into the experimental White Leghorns failed to induce recognizable differences either in one- or two-dimensional

chromatographs of plasma and muscle homogenates obtained from them. At the same time, the white and the red pectoral muscles from mature donors in either species could be easily identified on the basis of their characteristic partition patterns (Fig. 7). However, species-specific differences between the chicken (either control or blood-injected) and the turkey could be observed only with difficulty, especially in the white muscle tissue. As mentioned, a few tests were conducted in which homogenates of 5-day-old chicken embryos (less the extra-embryonic membranes) of *F*$_3$*ct*, *F*$_3$*ext* and *F*$_2$*exnh* types were analysed chromatographically. In the latter two cases, the parents had received their allotted dosage of heterologous blood. These tests also yielded negative results; i.e. no discernible differences among the three types of embryos could be detected.

Table 4. *Analysis of electrophoretic patterns*

Year	Type of bird		Globulin fractions I	II	III	IV	Albumin
1960	Adult males	White Leghorns (5)*	16·3†	11·5	26·0	15·3	30·7
		New Hampshires (5)	15·8	11·5	20·7	17·3	34·7
		BBB turkeys (5)	18·4	0	30·5	17·8	33·8
	F_1, 4-month-old males	W.L. injected with turkey blood (1)	11·6	10·6	28·3	18·7	30·8
		W.L. injected with N.H. blood (1)	11·7	8·1	31·3	11·7	37·2
1961	One-month-old male	White Leghorns (1)	10·7[a]	7·1	14·3	19·6	48·2
			(16·4)[b]	(0)	(18·2)	(20·0)	(45·5)
		New Hampshires (1)	8·8	6·3	13·9	21·7	49·3
			(14·4)	(0)	(20·5)	(14·5)	(50·6)
		BBB turkeys (1)	14·0	0	16·3	23·0	46·5
			(15·8)	(0)	(15·8)	(15·8)	(52·7)
	F_2, one-month-old males	W.L. injected with turkey blood (5)	10·3	6·0	14·7	23·5	45·5
			(18·1)	(0)	(17·0)	(14·9)	(50·0)

* Number of birds involved.
[b] Indicates that the same fraction as used in [a] was inactivated by heating for 30 min. at 56° C.
† Per cent of the total area.

The presence of a three-line globulin pattern in the blood plasma, both of *F*$_2$*ct* and *F*$_2$*ext* males was demonstrated by the electrophoretic analysis. One of these lines (line II) was characteristically absent in turkey male plasma (Fig. 8). The extra-chicken 'line' was eliminated when the plasma was either heated to 56° C. for 30 min. or subjected to a 5-day storage at 4–5° C. before the test. Results of the integrator analysis are summarized in Table 4. Essentially similar patterns of protein distribution were observed with the *F*$_3$*ct* and *F*$_3$*ext* stock. The latter test, repeated 5 times, involved 30 controls and 30 experimental one-month-old birds.

A detail, which is apparent from Table 4, is that White Leghorns which were undergoing a continuous series of injections with the turkey blood retained globulin line II.

6. DISCUSSION

The concept that the heredity of an animal can be altered in a *directed* way by the introduction of blood from another closely related animal, different from the first phenotypically and genotypically, was first tested by Francis Galton in 1871.* He was primarily concerned with testing the theory of pangenesis, according to which many types of 'gemmules', each type believed to be functional carriers of heredity specific for an individual organ within an organism, are present in the animal's blood. To quote Galton (*loc. cit.*): 'If Pangenesis were true . . ., the results would be startling in their novelty, and of no small practical use: for it would become possible to modify varieties of animals, by introducing slight dashes of new blood, in ways important to breeders.'

Accordingly, Galton performed a series of tests involving transfusion of blood in rabbits. The latter belonged to several distinct breeds. A total of 88 animals in 13 litters were obtained from treated parents. None showed any evidence of breed alteration. The idea that 'gemmules' in the blood of the donor could affect the heredity of the recipient was, therefore, found untenable by Galton.

More recently, in 1946, Lysenko suggested that the cellular complex of each organism carries 'granules' which embody the organism's hereditary capacities handed to it by its parents, but modified by environment to which the organism was subjected during its life. Lysenko has subsequently developed his original ideas further, applying them to speciation (Lysenko, 1956). This hypothesis, which implies a measure of environmental control over the genotype, led Sopikov (1950) to apply the principle of 'vegetative hybridization' to poultry improvement, utilizing the blood transfusion technique. His work has been followed by a long series of studies of a similar kind which have already been referred to in the Introduction.

Even though the early work of Sopikov (*loc. cit.*) and others in the U.S.S.R. followed the demonstration by Avery and his associates (1944) on the induction of transformation in certain bacteria by exogenous DNA extracted from other bacteria, no immediate attempts were made by Soviet investigators to regard the phenomenon of transformation to be a factor in their observations. Among the first to mention the possibility that DNA may be involved is the work of Sopikov (1954).

It is interesting to note that according to some of the most recent Soviet reports on 'transformation' in poultry (Kushner *et al.*, 1959, and Tolokonnikova *et al.*, 1961), the presence of DNA is not essential for the induction of heritable modifications by the transfusion technique. These workers have reported achieving 'transformation' in the chicken through the use of blood plasma only.

* The senior author is indebted to Dr R. M. Irwin, Dept. of Genetics, University of Wisconsin, for drawing his attention to Galton's study.

Numerous attempts have been made by biologists to test the principle of transformation in animals. Among the earliest of these was the study of Hörstadius and his associates (1954), in which they sought to change the genotype of a species of the sea urchin, *Paracentrotus lividus*, by injecting it with DNA obtained from *Echinocardium cordatum*, also a sea urchin. All results were negative. The report of successful induction of heritable changes in the Pekin duck by DNA from the Khaki-Campbell duck (Benoit *et al.*, 1957) has stimulated further interest in this problem. Negative results, based on morphological observations, have been obtained in a number of such studies. The species involved, among others, were the silk worm (Astaurov *et al.*, 1960), the rat (Perry & Walker, 1958; Bearn & Kirby, 1959), the rat and the rabbit (Tigye *et al.*, 1959), the duck (Svoboda & Hašková, 1959) and the chicken (Burger *et al.*, 1961). At least one report of positive results has appeared in the literature, that by Gershenzon & Kiseleva (1958), who successfully induced mutations in *Drosophila melanogaster* by feeding larvae a medium containing DNA extracted from calf thymus. Gershenzon and Kiseleva did not claim, however, that they induced *directed* changes in the recipient stock.

There is some evidence pointing to the fact that foreign DNA can, under certain conditions, become incorporated in the DNA of the recipient animal. This was demonstrated on the cellular basis by Leuchtenberger *et al.* (1958), and Bensch & King in 1961. To bring the problem of DNA incorporation to the level of molecular biology, Novikov and his associates, 1961, used the molecular weight of DNA in the recipient ducks (after the latter received injections of foreign DNA) and of their offspring, as a criterion in measuring the extent of induced changes. These investigators observed shifts in the molecular weight of DNA when the Pekin and mallard ducks were treated with DNA on a reciprocal basis. Genetic tests will be needed, however, to provide critical evidence that true transformation, in the functional sense, can occur in multicellular organisms. Until such evidence is forthcoming, the question of the ability by the recipient multicellular animal to incorporate into its own DNA a foreign DNA, thus giving rise to a new functional genic complex, will have to be regarded as still another unsolved biological problem.

In the meantime, one cannot, of course, dismiss the possibility that unsuccessful attempts, in higher animals, to effect such integration of two or more DNA complexes may be attributable to one of several possibilities. It can be postulated, for example, that contrary to the rather simple situation in bacteria grown on a DNA-enriched medium, the known barriers to a successful incorporation of exogenous DNA by a multicellular animal are infinitely more formidable. The other, of course, is the possible loss of physiological activity by DNA due to the methodology involved in isolating the compound in the first place. Indeed, most of the studies reporting negative results on DNA incorporation point to this as a possible explanation of the failure.

If DNA-induced *directed* heritable changes in higher organisms can be at all realized, then the use of living nucleated cells, such as avian erythrocytes and white blood cells should permit one to overcome the difficulty imposed by a possible inactivation of extracted DNA prior to its introduction. However, the lack of

unanimity on the effectiveness of whole blood injections as a method for inducting *directed* heritable changes in poultry clearly contradicts this expectation. Results of the present study have been negative, both on the level of gross morphological observations as well as on the basis of more sensitive immunological tests, and are in agreement with those from Brazil (Buschinelli, 1961). In the latter case, the blood of *Crax fasciolata*, a gallinacous South American wild species, served as the donor of whole blood injected into White Leghorns. No recognizable gross changes were observed in the recipient birds following three generations of extensive observations. Lack of evidence in the present study of the occurrence of immunological shifts in the progeny of treated birds in the direction of the donor organism is difficult to reconcile with the affirmative results on this point reported by Sopikov (1958) and Gromov (1959). In the absence of other reasonable, scientifically tenable, explanations for this and other points of divergence in the results, one is forced to fall back on such patently unsatisfactorily alternatives as the existence of (1) 'subtle' differences in the techniques used, (2) genotypic differences in the stock available at the respective locations, and (3) unaccountable 'experimental' errors, to explain results obtained in the present study and those reported, for example, by Kushner (1958*a*). It should be pointed out in this respect that the senior author personally obtained from Prof. Kushner at the time of the Genetics Congress in Montreal details of the blood transfusion procedure used by him and his associates for inducing heritable changes in poultry.*

7. SUMMARY AND CONCLUSIONS

1. A three-year study was conducted to test the efficacy of inter- and intraspecific blood transfusions in domestic poultry for inducing heritable changes in the recipients. The latter were pure-bred White Leghorns. Pure-bred Broad Breasted Bronze turkeys and New Hampshire chickens served as blood donors to two distinct lines of recipients. All injections started when the recipient chicks were 2–5 days old. Altogether, more than 3000 chicks from blood injected lines were involved in the study, conducted between 1959 and 1961 and distributed between the parental and three subsequent generations. Each injected chick received a total of some 155 ml. of whole blood in the course of a five-month injection period. An adequate number of control (non-injected) birds was used throughout. Observations were made on plumage colour, body-weight, egg-weight, egg-shell colour, fertility and hatchability. Furthermore, blood plasma and muscle tissue of appropriate birds were subjected to immunological, chromatographic and electrophoretic analyses.

2. On the basis of all these criteria, no evidence of heritable shifts in the direction of the donor organism was discerned among birds belonging to either of the two treated lines.

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* Three papers reporting success with the blood transfusion method, published after this paper went to press, are listed at the end of the References.

References

Astaurov, B. L., Bedniakova, T. A., Gintsburg, G. I., Zbirskii, I. B. & Ramenskaia, G. P. (1960). Opyt polucheniia nasledstvennykh izmenenii u tutovogo shelkopriada (*Bombyx mori* L.) posredstvom mezhlineinykh in'ektsii desoksiribonukleinovoi kisloty. [An experiment on the acquisition of heritable changes in the silkworm (*Bombyx mori* L.) by inter-line injections of desoxyribonucleic acid.] *Dokl. Akad. Nauk S.S.S.R.* **134,** 449–452.

Avery, O. T., MacLeod, C. M. & McCarthy, M. (1944). Studies on the chemical nature of the substance inducing transformation of pneumococcal types. Induction of transformation by a desoxyribonucleic acid fraction isolated from pneumococcus type III. *J. exp. Med.* **79,** 137–159.

Bearn, J. G. & Kirby, K. S. (1959). Failure of deoxyribonucleic acid to produce pigment changes in the albino rat. *Exp. Cell. Res.* **17,** 547–549.

Benoit, J., Leroy, P., Vendrely, C. & Vendrely, R. (1957). Des mutations somatiques dirigées sont-elles possible chez les oiseaux? *C. R. Acad. Sci., Paris,* **244,** 2320–2321.

Bensch, K. G. & King, D. W. (1961). Incorporation of heterologous deoxyribonucleic acid into mammalian cells. *Science,* **133,** 381–382

Boyden, A. (1953). Fifty years of systematic serology. *Systematic Zool.* **2,** 19–30.

Burger, R. E., Shoffner, R. N. & Roberts, C. W. (1961). Treatment of fowl sperm and developing embryos with deoxyribonucleic acid extracts. *Poultry Sci.* **40,** 559–564.

Buschinelli, A. (1961). *Injeções periódicas de sangue em aves e suas implicações geneticas.* [*Periodic injections of blood into birds and their genetic implications.*] Tese para o doutoramento em Ciências. Facultade de Filisofia, Ciências e Letras da Universidade de Sâo Paulo, Brazil. 142 pp.

Buzatti-Traverso, A. A. & Reichnitzer, A. B. (1953). Paper partition chromatography in taxonomic studies. *Science,* **117,** 58–59.

DeFalco, R. J. (1942). A serological study of some avian relationships. *Biol. Bull., Wood's Hole,* **83,** 205–218.

Deutsch, H. F. and Goodloe, M. B. (1945). An electrophoretic survey of various animal plasmas. *J. biol. Chem.* **161,** 1–20.

Galton, F. (1871). Experiments in pangenesis, by breeding from rabbits of a pure variety, into whose circulation blood taken from other varieties had previously been largely transfused. *Proc. roy. Soc.* **19,** 393–410.

Gershenzon, S. M. & Kiseleva, I. A. (1958). Vyzyvanie napravlennykh nasledstvennykh izmeninii u *Drosophila melanogaster.* [Induction of directed hereditary changes in *Drosophila melanogaster.*] *Dokl. Akad. Nauk S.S.S.R.* **123,** 554–557.

Gromov, A. M. (1959). Izmeniniia u kur, voznikaiushchie pod vliianiem perelivaniia chuzherodnoi krovi. [Changes induced in chickens by the transfusion of foreign blood.] *Ptitsevodstvo,* **9,** 26–27.

Gromov, A. M. & Feoktistov, P. I. (1956). Izmenenie nasledstvennosti u kur posredstvom perelivaniia krovi. [Changes in the heredity of chickens by blood transfusion.] *Ptitsevodstvo,* **6,** 42–45.

Hörstadius, S., Lorch, I. J. & Chargaff, E. (1954). The effect of deoxyribonucleic acids extracted from sea urchin sperm on the development of sea urchin eggs. *Exp. Cell. Res.* **6,** 440–452.

Kushner, H. F. (1957). The influence of metabolic factors on the heredity of animals. *Proc. Internat. Genetics Symposia, Tokyo and Kyoto, Japan,* pp. 515–519.

Kushner, H. F. (1958*a*). Inheritance of changes in feathering pigmentation in fowls (hens) subjected to foreign blood transfusion. *Proc. Xth Internat. Congress of Genetics,* **2,** 155.

Kushner, Kh. F. (1958*b*). Nasledovanie u kur izmenenii v okraske opereniia, voznikshikh pod vliianiem perelivaniia chuzherodnoi krovi. [Inheritance of changes in the plumage colour of chickens subjected to foreign blood transfusion.] *Zh. Obshch. Biolog.* **19,** 357–368.

Kushner, Kh. F., Tolokonnikova, E. V. & Moiseeva, I. G. (1959). Izmenenie okraski opereniia u potomstva kur v resul'tate vlivaniia im razlichnykh komponentov chuzherodnoi krovi. [Changes in the plumage colour of the progeny of chickens as a result of their injection with different components of foreign blood.] *Dokl. Akad. Nauk S.S.S.R.* **129,** 674–677.

LEROY, P. (1962). Observations faites sur des poules 'Rhode Island Red' génétiquement contrôlées et sur leurs descendands de 1re et 2e générations après injections répétées de sang de pintade. *C. R. Acad. Sci., Paris*, **254**, 756–758.

LEUCHTENBERGER, C., LEUCHTENBERGER, R. & UYEKI, E. (1958). Cytological and cytochemical changes in livers of white mice following intraperitoneal injections of DNA preparations from breast cancers of agouti C_3H mice. *Proc. nat. Acad. Sci., Wash.*, **44**, 700–705.

LYSENKO, T. D. (1946). *Heredity and its Variability*. King's Crown Press, Morningside Heights, N.Y., pp. 65.

LYSENKO, T. D. (1956). O biologicheskom vide i vidoobrazovanii. [On species and speciation.] *Agrobiologiia*, No. 4, 3–30.

MAINARDI, D. (1958). Immunology and chromatography in taxonomic studies on gallinaceous birds. *Nature, Lond.*, **182**, 1388–1389.

MAINARDI, D. (1959). Immunological distances among some gallinaceous birds. *Nature, Lond.*, **184**, 913–914.

MOORE, D. H. (1945). Species differences in serum protein patterns. *J. biol. Chem.* **161**, 21–32.

NOVIKOV, B. G., CHEPINOGA, O. P. & LIUBARSKAIA, M. A. (1961). Effekt in'ektsii chuzherodnoi DNK u utok. [The effect of injecting foreign DNA in ducks.] *Zh. Obshch. Biolog.* **22**, 317–320.

NUTTALL, G. H. F. (1901). The new biological test for blood in relation to zoological classification. *Proc. roy. Soc.* **69**, 150–153.

NUZHDIN, N. E. edit. (1959). *Nasledstvennost' i Izmenchivost' Rastenii, Zhivotnykh i Mikroorganizmov.* [*Heredity and Variability in Plants, Animals and Microorganisms.*] Vols. 1 and 2. *Akad. Nauk S.S.S.R. Moscow.*

OVENKO, K. S. (1958). Vliiaet li perelivanie krovi na rost tsypliat? [Does the transfusion of blood affect the growth of chicks?] *Ptitsevodstvo*, **8**, 38–39.

PENIONZHKEVICH, E. E. (1961). Metody vyvedeniia i sovershenstvovaniia miaso-iaichnykh porod kur. [Methods of breeding and improving the dual-purpose breeds of chickens.] *Trudy Vsesoiuz. Nauchno-issled. Inst. Ptitsev.* **27**, 3–14.

PENIONZHKEVICH, E. E. & MISHIN, G. A. (1959). Izmenenie nasledstvennosti u Belykh Leghornov v resul'tate in'ektsii im krovi serykh tsesarok. [Changes in the heredity of White Leghorns as a result of injecting them with the blood of grey guinea-fowl.] *Ptitsevodstvo*, **8**, 32–33.

PERRY, T. L. & WALKER, D. (1958). Failure of deoxyribonucleic acid to effect somatic transformation in the rat. *Proc. Soc. exp. Biol., N.Y.*, **99**, 717–720.

SIBLEY, C. G. & JOHNSGARD, P. A. (1959). Variability in the electrophoretic patterns of avian serum proteins. *Condor*, **61**, 85–95.

SOPIKOV, P. M. (1950). Novyi metod vegetativnoi gibridizatsii posredstvom perelivaniia krovi. [A new method of vegetative hybridization by blood transfusion.] *Priroda*, **39**, 66.

SOPIKOV, P. M. (1954). Izmenenie nasledstvennosti putem parenteral'nogo vvedeniia krovi. [Changes in heredity by the parenteral administration of blood.] *Agrobiologiia*, **6**, 34–45.

SOPIKOV, P. M. (1958). Izmenenie nasledstvennosti i povyshenie zhiznennosti zhivotnykh putem parenteral'nykh vvedenii retsipientam krovi i drugikh tkanei. [Changes in heredity and increased vitality in animals by parenteral administration to recipients of blood and other tissues.] *Trudy Vsesoiuz. Nauchno-issled. Inst. Ptitsev.* **25**, 101–132.

SOPIKOV, P. M. (1959). Izmenenie nasledstvennosti u zhivotnykh posredstvom perelivaniia krovi, implantatsii tkanei i nasledovannie priobretennykh prisnakov i svoistv. [Changes in the heredity of animals by blood transfusion, tissue implantation and the inheritance of acquired characters and traits.] *Nasledstvennost' i Izmenchivost' Rastenii, Zhivotnykh i Mikroorganismov.* [*Heredity and variability in plants, animals and microorganisms.*] *Nuzhdin, N. E. edit. Acad. Nauk S.S.S.R., Moscow*, **1**, 554–560.

SOPIKOV, P. M. (1962). Alteración de ciertas propiedades y caracteres en las gallinas, por medio de transfusión parenteral de sangre y su fijación en als crías. [The alteration of certain properties and characters in fowls by means of parenteral transfusion of blood and their fixation in the offspring.] *Memorias del XI* (1958) *Congreso Mundial de Avicultura*, 109–116. (English summary on p. 748.)

STROUN, J. H., STROUN-GUTTIERRE, L. R., ROSSI, J. & STROUN, M. (1958). Modifications de la poule Leghorn traitée par de sang de pintade. *Proc. Xth Internat. Congress of Genetics*, **2**, 280.

SVOBODA, J. & HAŠKOVÁ, V. (1959). Failure to produce somatic changes between strains of ducks by means of specific DNA. *Folia biol.* (*Prague*), **5**, 402–404.

TIGYI, A., BENEDECZKY, I. & LISSÁK, K. (1959). Studies on the modifying effect of isolated desoxyribonucleic acid in mammals. *Acta biol.* (*Hungary*), **10**, 197–205.

TOLOKONNIKOVA, E. V. & MOISEEVA, I. G. (1958). Vliianie vvedeniia chuzherodnoi krovi kuram na ikh potomstvo. [Injection of foreign blood into chickens—the effect on their progeny.] *Trudy Inst. Genet., Akad. Nauk S.S.S.R.*, **24**, 272–384.

TOLOKONNIKOVA, E. V., MOISEEVA, I. G. & BOGATYREVA, S. A. (1961). Izmenenie okraski opereniia u kur v resul'tate vvedeniia im razlichnykh komponentov chuzherodnoi krovi. [Changing the plumage of chickens by injecting them with various components of foreign blood.] *Zh. Obshch. Biolog.* **21**, 66–73.

The following papers, all reporting success with the blood transfusion method of inducing heritable changes in the domestic chicken, appeared too late for discussion in the text:

STROUN, J., STROUN-GUTTIÈRES, LITZA, ROSSI, J. & STROUN, M. (1962). Modifications de la couleur du plumage provoquées chez la Poule Leghorn blanche par injections répétées de sang de pintade grise. Observations sur cinq générations. *C. R. Acad. Sci., Paris*, **255**, 781–783.

STROUN, J., STROUN–GUTTIÈRES, LITZA, ROSSI, J. & STROUN, M. (1962). Modifications de la couleur du plumage provoquées chez la Poule Leghorn blanche par injections répétées de sang de pintade grise. Caractère héréditaire de ces modifications. *C. R. Acad. Sci., Paris*, **255**, 1030–1032.

PENIONZHKEVICH, E. E., POLETSKY, V. A. & NICKOLSKY, B. S. (1962). Effect of Heterogeneous blood on recipient's organism under vegetative hybridization of farm poultry. *Proc. XIIth World's Poultry Congress*, 148–152.