freemartin state is due to a purely humoral influence of the male foetus upon its synchorial female twin.

The experiments described in this article will in due course be reported on in full.

Summary

(1) Mice and chickens never develop, or develop to only a limited degree, the power to react immunologically against foreign homologous tissue cells with which they have been inoculated in foetal life. Animals so treated are tolerant not only of the foreign cells of the original inoculum, but also of skin grafts freshly transplanted in adult life from the original donor or from a donor of the same antigenic constitution.

(2) Acquired tolerance is immunologically specific: mice and chickens made tolerant of homografts from one donor retain the power to react against grafts transplanted from donors of different antigenic constitutions.

(3) Acquired tolerance is due to a specific failure of the host's immunological response. The antigenic properties of a homograft are not altered by residence in a tolerant host, and the host itself retains the power to give effect to a passively acquired immunity directed against a homograft which has until then been tolerated by it.

(4) The fertility of tolerant mice is unimpaired.

REFERENCES

1. Cf. Rawles, M. E., *Physiol. Rev.*, **28,** 383 (1948).
2. Felton, L. D., *J. Immunol.*, **61,** 107 (1949).
3. Owen, R. D., *Science*, **102,** 400 (1945). Owen, R. D., Davis, H. P., and Morgan, R. F., *J. Hered.*, 37, 291 (1946). Stone, W., Stormont, C., and Irwin, M. R., *J. An. Sci.*, **11**, 744 (1952).
4. Dunsford, I., Bowley, C. C., Hutchison, A. M., Thompson, J. S., Sanger, R., and Race, R. R., *Brit. Med. J.*, ii, 81 (1953).
5. Anderson, D., Billingham, R. E., Lampkin, G. H., and Medawar, P. B., *Heredity*, **5**, 379 (1951). Billingham, R. E., Lampkin, G. H., Medawar, P. B., and Williams, H. Ll., *Heredity*, **6,** 201 (1952).
6. Cannon, J. A., and Longmire, W. P., *Ann. Surg.*, **135,** 60 (1952).
7. Billingham, R. E., Brent, L., Medawar, P. B., and Sparrow, E. M. (unpublished work).
8. Billingham, R. E., Brent, L., and Medawar, P. B. (unpublished work). The passive transfer of transplantation immunity was first demonstrated by Mitchison, N. A., *Nature*, **171,** 267 (1953); *Proc. Roy. Soc.*, B (in the press).
9. The principle underlying this test was formulated by Chase, M. W., Abstr. 49th Gen. Meeting Soc. Amer. Bacteriol., 75 (1949).
10. Billingham, R. E., and Medawar, P. B., *J. Exp. Biol.*, **29**, 454 (1952).
11. Burnet, F. M., and Fenner, F., "The Production of Antibodies" (Melbourne, 1949).
12. Burnet, F. M., Stone, J. D., and Edney, M., *Aust. J. Exp. Biol. Med. Sci.*, **28,** 291 (1950).
13. Chase, M. W., *Proc. Soc. Exp. Biol. Med.*, N.Y., **61,** 257 (1946).
14. Mitchison (ref. 8). Medawar, P. B., Colloquia of the Ciba Foundation (in the press).
15. Summarized and reviewed by Snell, G. D., *Cancer Res.*, **12,** 543 (1952).

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VEGETATIVE HYBRIDIZATION OF ANIMALS BY JOINT BLOOD CIRCULATION DURING EMBRYONAL DEVELOPMENT[1]

MILAN HAŠEK

The question of vegetative hybridization holds a prominent place in Michurinist genetics. The concept of a vegetative hybrid, that is, a cross obtained by nonsexual mating, is not new at all: It was used by Darwin to specify grafted plants in which the two traits were joined in an asexual way.

The study of vegetative hybridization may become the means for explaining the substance of the most hidden and intimate life process, the fundamentals of heredity and its variability, which the Weissmanist-Morganists have addressed with a nonscientific, gene-based explanation. Extensive evidence for vegetative hybridization in plants was provided by I. V. Mičurin, T. D. Lysenko, and their followers. However, it has been argued that the method of vegetative hybridization cannot be used in animals, even in the historical discussion about the basic orientation of biology in 1948 by the opponents of the Michurinist direction. Zavadovskij stated in the discussion, "Hardly anybody could guess, that the Michurinist direction may be applied to animal organisms and particularly some vegetative hybridization, which is performed by Lysenko! Nobody has yet demonstrated the vegetative hybridization of species except chimeras—butterflies with multicolored wings. Give us concrete instructions and proposals, how to employ the vegetative hybridization mating method for animal species" (translated from a Russian original, 1949). However, since the time of this discussion about Michurinism, a number of authors have presented positive results on changing heredity in animals by vegetative hybridization.

For our experimental material, we used stabilized flocks of White Leghorn and Rhode Island Red chickens and Peking ducks, which we have bred under our control for three generations. Whenever possible, we used the progeny of the

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same parents as controls. The joining of blood circulation was performed by transplanting a piece of blastoderm tissue from eggs (incubated 20–40 hr) between the allantochorial vessels of two embryos at a more advanced stage of incubation (8–12-day-old chick embryos and 8–14-day-old duck embryos). The blastoderm piece acted as a mediator of coalescence. This method was described in detail in a previous publication (Hašek, 1953). Coalescence took place within 2 days, and the parabionts separated from extraembryonic membranes by normal rejection of umbilical cords during hatching (Fig. 1).

We believe that success in vegetative hybridization of animals depends particularly on the choice of a suitable method. The advantage of this method of vegetative hybridization of animals is that it leads to an intensive exchange of blood (i.e., of the general source of nutrition) at a time when the developing embryo is unable to react antagonistically to foreign proteins by production of antibodies. We believe that this time offers the best conditions for the mutual assimilation of two distant metabolisms.

It has been generally accepted that avian and mammalian embryos are unable to produce antibodies. This is conceivable from the aspect of historical adaptation, because both mammalian and avian embryos are well protected against the entry of foreign material; therefore, there is no biologic necessity for the generation of immunologic reactivity. Before we approached the parabiosis between ducks and chickens, we performed a series of immunizations of chick embryos to ascertain the inability to produce antibodies against antigens.

We injected 0.7 mL of duck serum into the yolk sack of chick embryos or 0.08 mL of duck blood intravenously. We tested the antibody response by precipitation reaction. The tests were performed until the chicks were 4 to 10 weeks old and no antibodies were detectable.

Intensive Exchange of Blood Between Parabionts

We previously proved the passage of fluorescein, which was injected into the extraembryonic vessel of one parabiont and then into the blood of the second partner. Many authors have also confirmed the joint blood circulation in parabionts during postembryonal development in similar experiments. Injection of fluorescein was used by Borjačok-Nižnik (1951) in his parabionts obtained by the joining of young rabbits. Other authors have evaluated the functional connection between partners using the injection of various dyes. The first to write about parabiosis was Paul Bert (1862, 1864, 1866), from the laboratory of Claude Bernard, who succeeded in surgically joining two rats. He demonstrated physiologic anastomosis by showing that an injection of belladonna into one partner resulted in dilatation of pupils in the other animal within 20 to 30 min. Capillary connection between parabionts was demonstrated by Furth and colleagues (1940). They found that rat and chicken erythrocytes, after injection into one mouse parabiont partner, were found in the uninjected parabiont in small amounts after 20 min and in large amounts after 2 hours. Van Dyke and colleagues (1948) demonstrated the transfer of injected Fe59-labeled red blood cells from one animal to another. Paul Bert, the first to write about parabiosis in young animals, attempted to exploit this method for vegetative hybridization to achieve distant transplantations. However, later authors adopted the Weismanist views and investigated narrow questions of hormonal influence in parabionts. Other authors examined the influence of resistance to transplanted tumors, the so-called parabiotic intoxication, the effect of radiography, and some allergic reactions. Borjačoka-Nižnika used parabiosis for vegetative hybridization for the first time and obtained positive results.

In our research, we exploited the possibility of joining the phylogenetically distant animals (e.g., ducks and chickens) in parabiosis during embryonal development. Parabiosis was performed using chick partners and ducks at approximately 12 and 13 days of incubation, respectively. So far, we have obtained 10 pairs of duck and chicken parabionts, which still live in our breed. The joint blood circulation was confirmed immunologically together with Haškova by the use of specific antisera against duck serum to detect duck-specific serum components in the chicken parabiont partners.

Preparation of Antisera for Testing of the Parabionts

We immunized chickens with duck sera to obtain specific antisera. We immunized every second day using increasing doses (0.2, 0.4, 0.6, 0.8, 1.0, and 1.5 mL). Reimmunizations were performed with the same dosage. The series of immunizations were performed in 3- to 4-week intervals, and the blood for the antiserum was obtained from the wing vein 8 to 12 days after the last inoculation.

Testing was performed in small test tubes with an internal diameter of 2.5 to 3.5 mm. The same volume of the test antigen (titrated by dilution) was layered onto the antiserum. Formation of a precipitin ring was read after a 10-min incubation at room temperature.

We will report examples of testing chicken sera from chicken and duck parabionts. Detailed results will be published at a later date (V. Hašková, unpublished data).

Parabiosis Between Duck and Chick Embryo 44 from March 24, 1953

The duck egg from the Peking breed (family 3) at 12 days of incubation was joined using the described method with a 10-day incubated chick embryo of Leghorn breed (hen 98). On the beginning of the 20th day of the parabiont chick egg's incubation, the chick egg was separated from the duck egg, and the joining section was closed and sealed with paraffin. The duck egg was further normally incubated, and the duckling hatched after 4 days. Immediately after separation from the duck partner, the chick embryo was used to harvest the blood and test the serum for the presence of duck serum constituents.

Parabiosis Between Duck and Chick Embryo 41 from March 23, 1953

The duck egg from the Peking breed (family 1) at 14 days of incubation was joined with an 8-day incubated chick egg (hen 21). After 11 days (i.e., on the 19th day of the chick egg's incubation), the eggs were separated and closed with paraffin. (a) The first sample of blood was taken from the chick parabiont. (b) The next day, the second sample was taken from allantochorial vessels located under the shell. (c) The next day, the chicken parabiont hatched, and the third sample was taken from the wing vein. (d) The fourth sample was taken on the following day (Table 1).

The results of the tests are shown in Table 1. To determine the specificity of the reactions, the following controls were performed in all tests: saline greater than the antiserum and

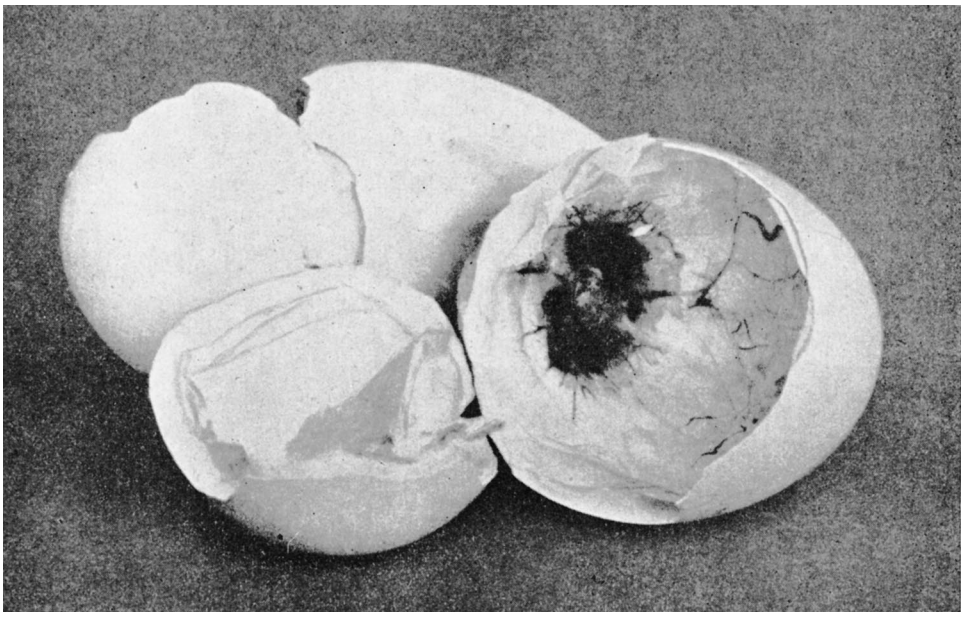


FIGURE 1. The remaining shells after the hatching of a pair of parabionts (Rhode Island and Leghorn breeds). The massive concrescence of allantochoria in the position where the eggs were joined (allantochoria had already dried out) is apparent.

test antigen greater than normal chicken serum. These tests were always negative and therefore are not shown. The titers of antisera against normal duck serum were always monitored, and normal chicken serum was used as a negative control.

The results indicate that duck serum proteins are present in relatively high titer both during chick and duck parabiosis and after hatching. The existence of duck proteins in the chicken and vice versa introduces a number of questions that we are presently analyzing and will refer to later.

We can present the following facts about the detection of the exchange of blood between the parabionts and its use by the partner. During parabiosis, a pronounced "tug" of body weight in favor of one of the partners is occasionally encountered. Thus, in one case of duck and duck parabiosis, the partners originating from eggs of equal weight differed considerably in the size of the egg's air bubble. This difference became even greater until hatching. The air bubble of one egg constituted more than one third of the egg, whereas the difference was negligible in the second egg. The hatched ducklings then differed by one third of the body weight. Furthermore, in one duck and chick parabiosis, there was a major shift of weight from the duck embryo in favor of the chicken. This is notable because it indicates greater use of duck blood by the chicken embryo.

Parabiosis Between Duck and Chick Egg 12 from March 11, 1953

The following is extracted from the experimental protocol: The duck egg weighed 82 g, and the chick egg weighed 62 g before incubation. The duck and chick eggs were put in the incubator on February 25 and 27, 1953, respectively. Parabiosis using a chick blastoderm piece was performed on March 11. The coalescence of allantochoria of partners was easily discernible. The chicken hatched on March 20 (chicken 676), and the duckling hatched on April 23 (duckling 688). The duckling weighed 55 g immediately after hatching (27 g less than its egg), and the chicken weighed 54 g (8 g less than its egg and 9 g more, i.e., 63 g, with the residual shell).

The usual loss of body weight represents 12% to 14% of the original egg weight after 18 days of incubation, and the hatched chicken weighs approximately two thirds of the original weight of the egg. However, our parabiont with its shell weighed 1 g more than the egg from which it originated.

Manifestations of Vegetative Hybridization in Parabionts

We did not find morphologic changes in 10 chickens that hatched from parabiosis with duck embryos with firmly confirmed massive concretion or pronounced morphologic changes in ducklings parabiotic with chickens. These findings concern very young animals; thus, we cannot draw any firm conclusions.

Parabionts between chickens of different breeds were obtained between red Rhode Island and white Leghorns. We did not find morphologic changes in five cases, but one chicken had a conspicuous change in the color of feathers. This change was apparent on the head and neck when compared with control red Rhode Island progeny from the same hen (Fig. 2). This chicken had a bright yellow head and neck with a lemon tinge, characteristic of white Leghorns, in contrast with the typically brown color of all controls (at least 20) (Fig. 2). This change was not found in further red Rhode Island (chicken 20) and white Leghorn parabionts, whereas another pair died on the eighteenth day of incubation (Rhode Island) or after hatching (Leghorn). After the eggs hatched, the Leghorn was well developed with feathers of normal color, whereas the Rhode Island was small (10.66 g in weight, 4.5-mm beak, and 14-mm third finger, thus corresponding to an age of 15 days). The coloration was also different: white feathers on the head, neck, and upper part of the back, and brown lower back (Figs. 3 and 4). We consider the change in color to be notable when compared with the controls, although we are fully aware of the small size of the material. A complicating factor might be that the extent of blood change is not between the individual parabionts.

We did not find any change in color in white Leghorns parabiotic with red Rhode Islands.

TABLE 1. Results of precipitation reactions; sera in different dilutions were tested with the chicken antiserum against duck serum

Antigens	Dilution of the antigen										
	1/2	4	8	16	32	64	128	256	512	1024	2048
Duck serum	+	+	+	+	+	+	+	+	+	±	—
Chicken serum	—	—	—	—	—	—	—	—	—	*)	*)
Parabiosis no 44. Serum of the chick parabiont	*)	*)	+	+	+	+	+	+	—	—	—
Parabiosis no. 41 Serum of the chick parabiont											
Sample: a	+	+	+	+	+	*)	+	±	—	—	—
b	*)	*)	+	+	+	+	—	—	—	*)	*)
c	+	+	+	+	+	+	+	+	—	—	—
d	+	+	+	+	+	+	+	+	—	—	—

*) These samples were not tested.

We observed enhanced vitality in some individual parabionts in the increase of body weight and growth development. Record body weight increment was recorded in Leghorn hen 553, parabiont with Rhode Island. This was determined during winter conditions of maintenance compared with controls in the same conditions (Table 2).

Hen 553 grew faster compared with its two Leghorn cock partners (551 and 556). In addition, cocks have greater body weight increments than hens. Hen 553 also grew faster than the sexual Leghorn-Rhode Island hen 555. Hen 553 matched the body weight increments of the sexual Leghorn-Rhode Island cock 552. In sexual hybrids (Leghorn × Rhode Island), the heterosis is regularly apparent by higher body weight increments than seen in Leghorns and the more massive Rhode Island.

The superior weight of this hen is prominent in comparison with the standard body increment curves in Table 3. Weight increments of Leghorn and Leghorn × Rhode Island hybrids were compiled according to Nikitina (1948), Smetněva (1948), and our data. It should be emphasized that these are mean values from both sexes, that is, the weight of hens is lower and hen 553 was weaned during winter. Nevertheless, parabiont 553 exceeded both the standard increments of Leghorns and the heterosis in sexual hybrids Leghorn × Rhode Island.

Compared with a larger number of control Leghorn hens of our breed, Hen 553 is remarkable. The values of control hens are shown in Table 4.

Vegetative hybrid hen 553 weighed 620 g on day 55, whereas control hens on the corresponding day weighed an average of 405.3 g. On day 75, hen 553 weighed 805 g, and the mean control weight was 603.88. This comparison indicates an exceptional weight gain in the experimental hen.

Hen 553 reached constitutional values at the age of 2.5 months (control measurements were not performed in sufficient numbers) (Table 5).

These data indicate that parabionts could be advantageous material for further breeding work. Their follow-up in further generations will be interesting. This justifies the possible exploitation of parabiosis for practical breeding, because the method itself, when skillfully performed, is not so traumatic for the embryo as is the partial exchange of the egg white. We obtained an 80% rate of hatching from good-quality starting material and even 100% in some experiments.

We believe that the inheritance in vegetative hybrid parabionts became unbalanced. We believe that higher vitality also results from unbalanced inheritance, similar to sexual hybridization (heterosis).

However, profound results were derived from the immunologic observations of parabionts. Compelling facts about the profound influence on parabionts in a vegetative manner were obtained by the analysis of their blood cell antigens. A number of authors (Thomsen, 1934, and Boyd and Otis, 1940), who investigated the blood groups in chickens, concluded that isoagglutinins are not present in hens and that a larger (~30) number of group agglutinogens occur.

Because of the enormous number of possible combinations of agglutinogens, each hen essentially has a different blood group. We can talk about a certain "biochemical individuality" of hen blood when considering that each antigen has a chemical individual. In addition, once agglutinogen formation during an animal's development is better understood, the currently acceptable concept will probably be confirmed: An agglutinogen, like every other antigen, is an indicator of metabolism and protein specificity. With this point of view, we approach the application of immunologic analysis in vegetative hybrids.

Considering the quoted situation in blood groups in hens, immunization with a randomly selected hen results in an antiserum with a more or less complex composition of agglutinins produced at a higher titer against the different injected agglutinogens. The complexity of such antiserum is reflected by the fact that the contained multiple agglutinins react with practically all randomly selected erythrocytes. Boyd and Otis reported that the antisera against the erythrocytes from one donor never failed to react with erythrocytes from any animal. Thomsen, as well as Boyd, then tried to narrow the number of agglutinins by absorption of the sera with erythrocytes from other animals. Such absorptions can narrow the agglutination of randomly selected animals, and further absorptions can theoretically lead to the isolation of a single agglutinin. Thus, Boyd and Otis prepared five antisera by multiple absorptions that nearly became single agglutinins.

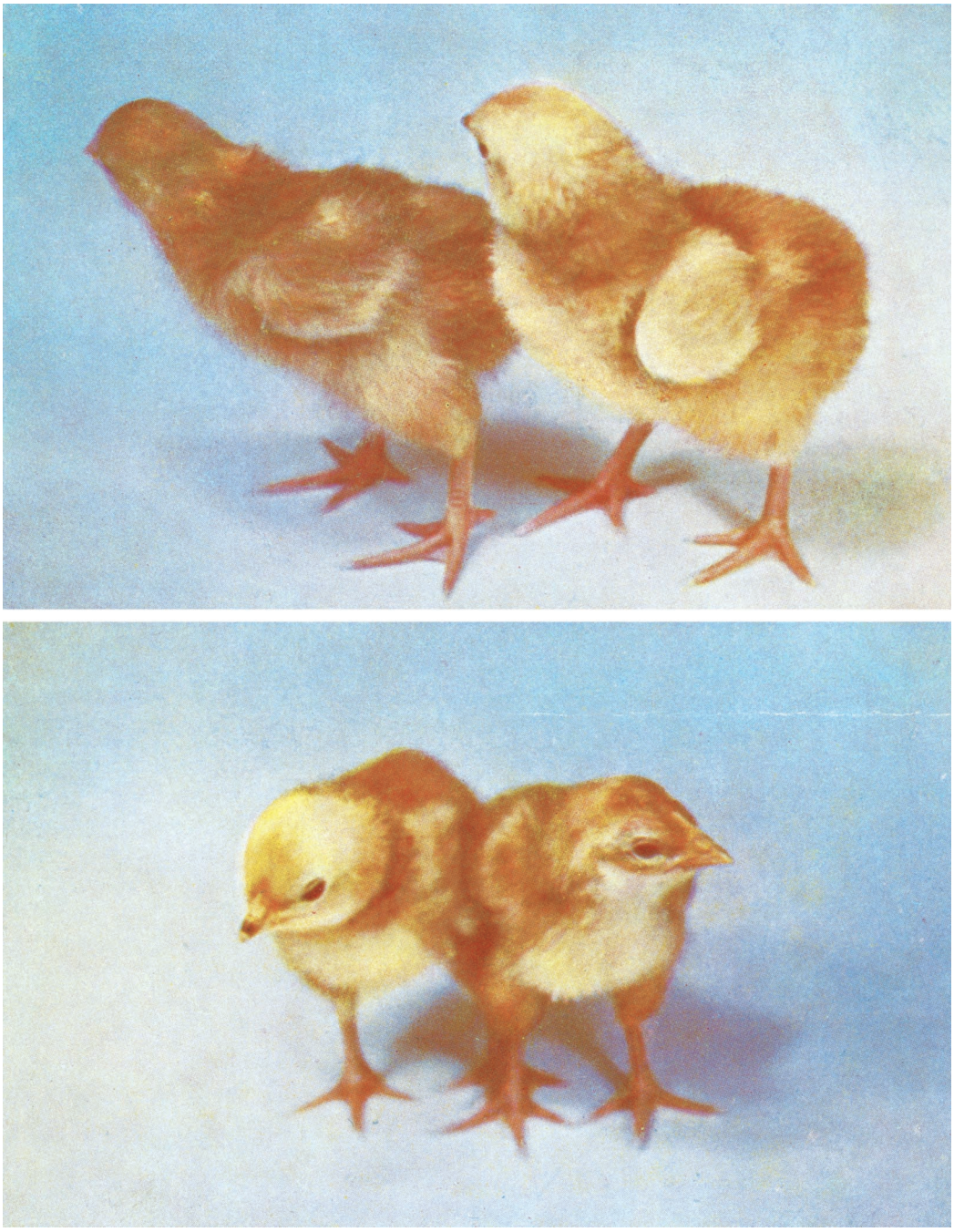


FIGURE 2. Rhode Island red chicken, parabiont with white leghorn (top right, bottom left). Rhode Island red control (top left, bottom right).

Reciprocal immunization with blood cells between vegetative hybrid parabionts originating from two different hens produced the following results. As reported in the literature, reciprocal immunization between two hens leads to antibody formation against different agglutinogens. We obtained complex antisera in all seven control reciprocal immunizations. B. Frenzl, our collaborator in investigating blood groups in chickens, has also been attempting to isolate the individual agglutinins. He performed 16 reciprocal immunizations in our experimental breeds and each time obtained positive antisera. However, two parabiont hens that had a joint blood circulation during embryogenesis failed to produce any antibodies after reciprocal immunization. We verified this fact by a further reimmunization of this pair of parabionts, and we performed the reciprocal immunization in two more pairs of parabionts. Here again, the agglutination test with sera from the immunized parabionts were negative, that is, the inoculations of blood did not lead to antibody formation. We presume that after reciprocal immunization between two animals, the agglutinins are being produced against the foreign agglutinogens, which are missing in the immunized animal. No antibodies are formed against injected agglutinogens that are shared with the agglutinogens of the host. Thus, in our case, the outcome from reciprocal immunization between parabionts indicates that the injected blood behaves as the blood of the immunized recipient.

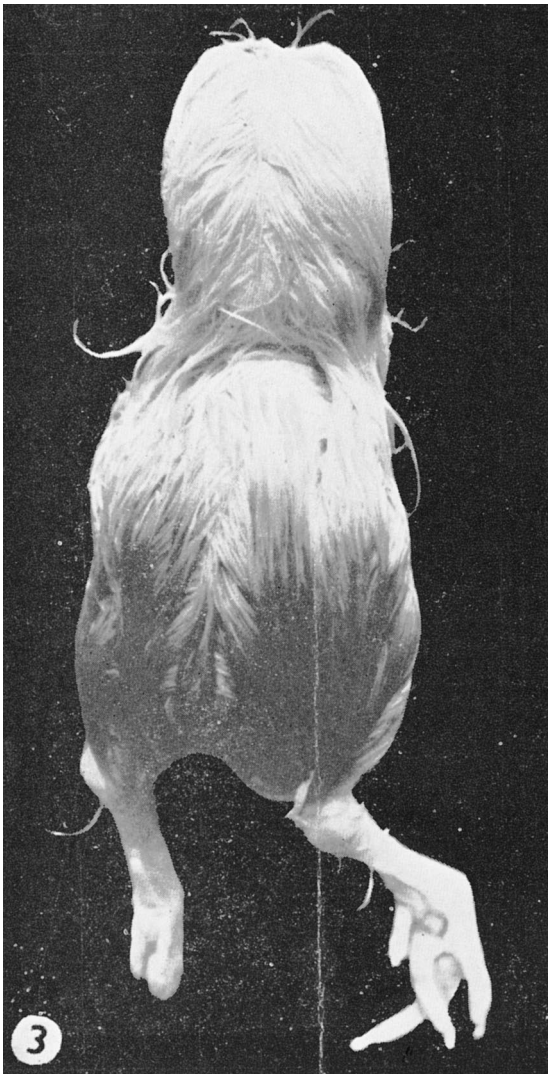


FIGURE 3. Rhode Island red chicken parabiont with white Leghorn.

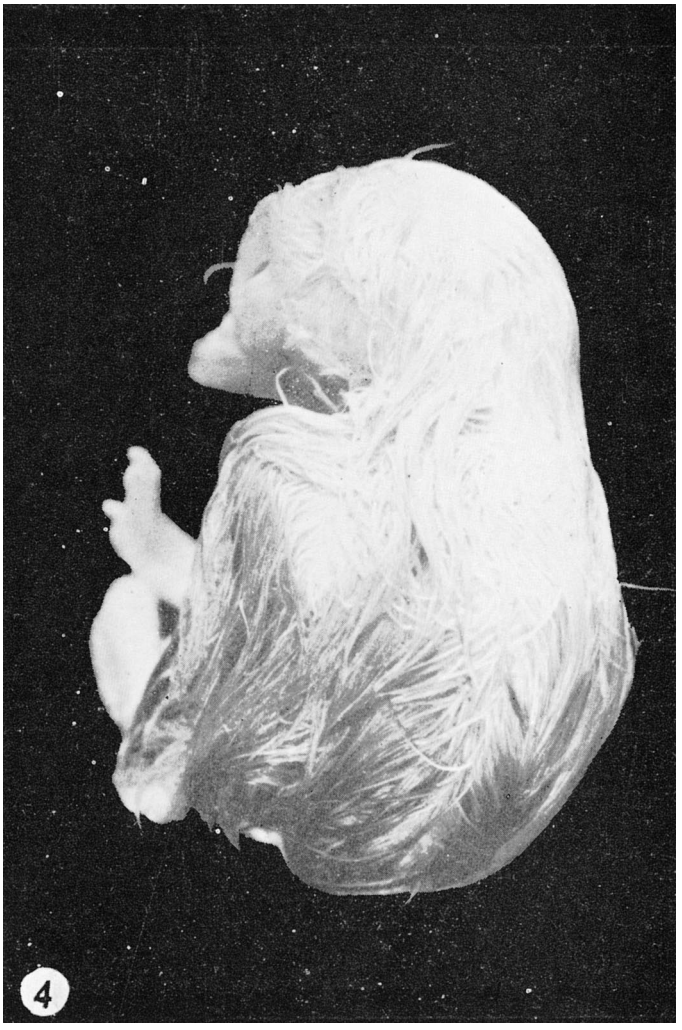


FIGURE 4. Rhode Island red chicken parabiont with white Leghorn.

Immunization Procedure

We performed immunizations with washed erythrocytes every second day with doses of 1.0, 1.0, 1.5, 1.5, 2.0, and 2.0 mL into the wing vein. For agglutination assays, we used plasma obtained from the wing vein in a citrate solution 8 to 18 days after the last inoculation. Reimmunization was performed in equal doses 3 to 4 weeks after the last immunization.

MATERIALS

Parabiosis 1 (September 9, 1952)

Parabiosis was performed by joining two chicken eggs on the tenth day of incubation. Both eggs were of the white Leghorn breed. Coalescence through the blastoderm piece was apparent after hatching. The chickens were numbered 516 and 517.

The results of agglutination tests after reciprocal immunizations are presented in Table 6. Reimmunization was performed in animals 514, 515, 516, and 517.

The same analysis as in parabionts from parabiosis 1 from September 18, 1952 was performed in two more pairs of parabionts from the following two parabioses.

Parabiosis 1 (September 30, 1952)

Parabiosis was performed by joining two chicken eggs on the tenth day of incubation. One egg was of the Leghorn breed, and the other egg was of the Rhode Island breed. Coalescence was apparent after hatching.

Parabiosis 3 (September 30, 1952)

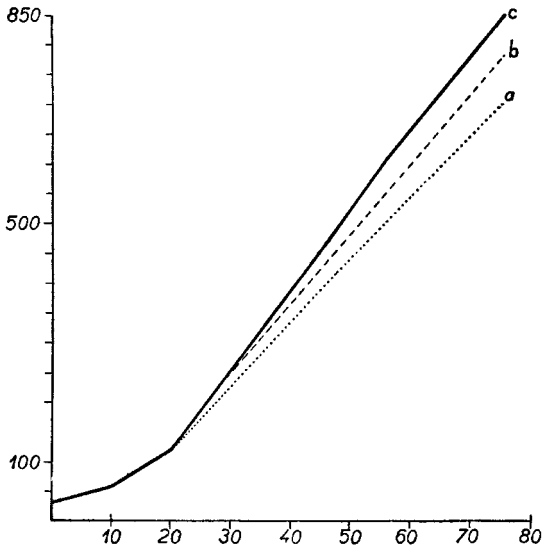
Parabiosis was performed by joining two chicken eggs on the tenth day of incubation. The joined eggs were Leghorn × (Leghorn × Rhode Island) F1. Coalescence was apparent after hatching, and blood communication between the partners was further verified on fresh allantochorial membranes by injection of one vein with a solution of methylene blue and its passage into the allantochorial veins of the other egg.

The agglutination tests after reciprocal immunization were negative in both pairs of parabionts. This concerned parabiosis 1 from September 30, parabionts between Leghorn and Rhode Island, and parabiosis 3 from September 30 parabionts between Leghorn and (Leghorn × Rhode Island) F1 hybrid. This eliminates even more the possibility of accidental overlap of blood groups in these animals, which none of the previous authors observed and which was not found in 23 reciprocal immunizations performed in pairs from our breed.

TABLE 2. Body weights of the Leghorn hen 553 vegetative hybrid with Rhode Island; controls are Leghorns and sexual Leghorn×Rhode Island hybrids

Wing marking	551	552	553	555	5565
Breed	Leghorn	Sexual hybrid: Leghorn×Rhode Island	Vegetative hybrid of Leghorn and Rhode Island	Sexual hybrid: Leghorn×Rhode Island	Leghorn
Sex	Male	Male	Female	Female	Male
Body weight					
After hatching	36	32	Not tested	Not tested	32
5 day	39	42	40	35	Not tested
10 day	65	73	72	50	58
25 day	160	190	180	140	165
40 day	340	410	390	260	335
45 day	390	440	450	310	385
55 day	520	600	620	480	490
75 day	700	810	805	580	670

TABLE 3. Body weight increments. a, mean weights of male and female white Leghorn breed; b, mean weight of female and male sexual hybrids Leghorn×Rhode Island; c, weights of female vegetative hybrid Leghorn×Rhode Island (no.553). Horizonal scale: age in days; vertical scale: body weight in grams.



We are currently investigating whether the partner's agglutinogens persisted in the blood of the second parabiont after parabiosis and during embryogenesis or whether the reactivity of vegetatively influenced animals changed in such a way that the partner's agglutinogens during embryonal parabiosis led to the failure to produce antibodies in adult age.

These are manifestations of vegetative hybridization that we successfully recorded in parabiotic animals. The results indicate that this mode of vegetative hybridization affected both animals in a nonsexual manner.

DISCUSSION

We believe that the study of vegetative hybridization (i.e., the influence of nutrition in the broadest sense of the word) will bring about valuable results in animals as much as it already has in plants. At the same time, the main direction of this line of research is to seek practicable roads toward the regulation of heredity for enhancing the utility of farmed animals. Some of our results on vegetative hybridization in animals already confirm the profound findings of Soviet authors on the higher vitality of vegetatively hybridized animals. Undoubtedly, improving the vigor of farm animals is a key issue for enhancing their productivity. This aim was clearly postulated by Lysenko (1949) in the "Three-year plan for the development of animal production. .: To find such means for growing relatives of both plants and all species of animals that the vigor of their progeny following inbreeding should not decline and the heredity—the behavior of organisms, which provides the useful traits, should rapidly develop, establish and stabilize itself." This task is fully applicable to our breeds of Leghorn hens, which are of relatively low resistance and vigor.

Vegetative hybridization also indicates that it is possible to transfer natural resistance against diseases, thus significantly contributing to the generation of new production breeds along the way, which has been elaborated by Michurin in the study of plants. However, here we stand at the beginning of work.

It would be incorrect to extrapolate the knowledge from plants to animals mechanically. In particular, it is necessary to evaluate correctly our current knowledge and to separate all specific features and differences in the ontogenesis of animals from that of plants. Today, we do not have a theory concerning the individual development of animals that would correspond to the theory of staged development, which has been elaborated by Lysenkem. The bourgeois theories of individual development are dom-

TABLE 4.

Weight of Leghorn hens in g after hatching	n=number of cases	N=number of degrees of freedom	x=arithmetic mean	P=variance=etc.	V=variance of means etc.	S=mean error of the mean=etc.
55 day	28	27	405.3	1713.4	61.19	7,823
78 day	9	8	603.88	8379.8	931.08	30,51

TABLE 5. (figures in mm)

Length of the back	137
Width of the trunk	36
Depth of the trunk	95
Length of chest bone	84
Length of shoulder	76
Length of forearm	74
Length of thigh	93
Length of calf	112

inated by autogenesis, and the assumed rules of development are interpreted in isolation from the environment of the organism. Therefore, these theories are unfruitful for practical purposes and do not create a path toward the regulation of heredity in animals. The unity of an animal with the environment during ontogenesis reaches a more multifaceted new basis, enabling profound organ differentiation within the organism, and a special organ of irritation, the central nervous system, is taking over the whole organism in its unity with the environment.

Embryogenesis is a prominent special feature of animals, whereby the contact of the mammalian embryo with the environment is mediated by the maternal organism, protecting it from direct outside influences. Therefore, the health condition of the mother is of significance for the growth of the embryo. The maternal organisms have an enormous influence on the embryo in birds also, although the egg is more exposed to direct outside influences. We previously investigated the effects of changing the embryonal nutrition (egg white) in the embryo, and this article also concerns a change in embryonal nutrition. Of course in the broad sense of the word, it concerns a continual direct change of plastic compounds of the blood. At the same time, these methods enable one to work with the youngest organisms with an as yet unsettled heredity.

The manifestations of vegetative hybridization that were determined with the aid of parabiosis during embryonal development (i.e., a change in the color of feathers, greater body weight, and a reactive change of blood groups) confirm the regulated nature of the genetic change. Darwin proved unequivocally the change of live matter under natural conditions and in the hands of a breeder. However, Darwin could not comprehend the reasons for the genetic change formulated by Nuždin (1952): "Darwin did not reveal the substance of life and thus did not appreciate the whole meaning of metabolism as the basis of life and therefore could not fully understand the nature of variability and could not discover concrete ways leading to the individual changes." The correct basis and direction for resolving these questions were described only by Engels, when he characterized proteins as the materials of life and metabolism as their most fundamental function.

TABLE 6a.

	Agglutinins	Aggluninogens Number					
		514	515	516	517	518	519
Reciprocal immunization	No. 514 Leghorn hen control	—	+	+	+	+	+
		—	+	+	+	+	+
	No. 515 Leghorn cock control	+	—	+	+	+	+
		—	+	+	+	+	+
Reciprocal immunization	No. 516 Leghorn cock parabiont (in parabiosis with no. 517)	—	—	—	—	—	—
		—	—	—	—	—	—
	No. 517 Leghorn hen parabiont (in oparabiosis with no. 516)	—	—	—	—	—	—
		—	—	—	—	—	—
Reciprocal immunization	No. 518 cock sexual hybrid Leghorn×Rhode Island control	+	+	+	+	+	+
	No. 519 cock sexual hybrid Leghorn×Rhode Island control	+	+	+	+	+	+

TABLE 6b. Age and body weights of experimental animals at the time of the first immunization

Wing tags	Breed	Sex	Age (days) at first immunization	Weight (g) at first immunization
514	Leghorn	Hen	107	885
515	Leghorn	Cock	107	1000
516 (parabiont with 517)	Leghorn	Cock	107	1000
517 (parabiont with 516)	Leghorn	Hen	107	875
518	Sex hybrid Leghorn×Rhode Island	Cock	101	1100
519	Sex hybrid Leghorn×Rhode Island	Cock	97	1075

The described changes under the influence of parabiosis indicate a profound influence on the metabolism of both parabionts. In addition, we should be aware that we are still very limited in analytical methods for live objects. We see a certain way in the immunobiologic analysis. Its potentials for the monitoring of genetic changes were pointed out by Žukov-Verežnikov (1951): "It is very difficult to determine morphologically, how the life matter is born within the old one. Help may be derived from the immunological research method, which monitors the specific change in the biosynthesis of proteins and thus also the type of change at those steps of the process of change in the substance of the organism, which yet cannot be detected by morphology."

In our case of parabiosis during embryogenesis, one type of change occurs with another and leads to mutual changes in the metabolism of permanent nature. The immunologic monitoring of changes in the quality of proteins, and thus of specific changes in their biosynthesis, provides great opportunities to unravel the nature of the revival of a live protein, that is, of the fundamental and most intimate basis for the expression of heredity and variation. In this sense, vegetative hybridization not only disproves the absurdity of the gene theory but also guides one toward an evaluation of the very natural substance of life.

As proved by the theory of staged development, ontogenesis is not merely a process of predetermined qualities but a set of qualitatively different stages with different requirements on the environment. Clarification of the stages of animal development, comparable with the stages in plants, will require a study of all environmental influences on the developing organism. The response to foreign materials, such as microbes or foreign proteins, may be one such influence.

The immune reactivity is not necessary during embryogenesis as a result of historical adaptation during intrauterine life or in avian eggs. Therefore, no potential antigen will lead to production of antibodies during embryogenesis. However, we showed that vegetative hybridization during this stage has qualitatively different characteristics. The entry of blood cells from a different group type to a partner with another type produced a permanent change that persisted even after embryogenesis. The fundamental question remains to clarify the physiologic mechanism that enables the embryo to change from one form of relating with the environment (when it is incapable of immunogenesis) to a second form in postembryogenesis, characterized by the onset of immune reactivity. The latter stage in our experiments represented a qualitatively different permanent change.

Pavlov (1909) wrote: "The most substantial connection of animals with the surrounding environment is mediated by certain chemical substances which must all the time enter into the system of an organism, i.e., a connection through nutrition." From this point of view, we clearly see the difference between embryogenesis and postembryonal development. The work of Lepešinska (1952) proved that the nutrition in the egg has a trophic role that affects differentiation. The egg yolk, which has previously not been considered a source of differentiation, is the source of blood, one of the most important body constituents.

There is a substantial difference between the "phylogenetically determined" embryonal nutrition and direct nutrition from the environment. This subject has been misinterpreted by the Morganists, who considered that the egg yolk and embryonal nutrition are generally the same as when a chicken is fed by corn, that is, nutrition for an autogenetically developing system of genes.

Therefore, there is full justification in the experiments concerning the change in embryonal nutrition, such as the change in egg white demonstrated by Bogoljubsky and others or the change of blood during embryogenesis described in the present report.

SUMMARY

We used our method of parabiosis of eggs during embryogenesis (Hašek 1953) as an expression of vegetative hybridization in animals. In this way, we connected the blood circulation between the Rhode Island and Leghorn breeds of chickens and between ducks and chickens.

The advantage of joining animals during embryogenesis is that it results in an intensive exchange of blood at a time when the embryo is not able to react antagonistically by producing antibodies against foreign proteins, that is, at a time when there are suitable conditions for mutual assimilation. We confirmed the lack of antibody formation after immunization of chick embryos by inoculation of duck serum into the yolk sack or intravenously.

The connected blood circulation was confirmed by histological analysis and by the passage of dye that was injected into one parabiont and then into the partner. We also investigated the intensity of this exchange by immunobiological assays, using specific antibodies against the partner's blood proteins. We proved the presence of duck serum in the blood of chickens, parabiotic with ducks, during parabiosis and also shortly after hatching.

We also described examples of a "tug" of body weight between duck and chicken parabionts, in which the hatched chicken was much heavier than expected from its own nutrition. This proves that the chicken used duck materials for its development.

Vegetative hybridization was expressed by a change in the color of feathers, greater body weight, and, the permanent nature (as immunologically demonstrated). The parabionts after reciprocal immunization with the partner's erythrocytes did not form any antibodies. This outcome is extraordinary when considered in the light of previous findings of other authors and ourselves and indicates a permanent change caused by the exchange of blood during embryogenesis. We are presently investigating whether there is a permanent change in blood groups, that is, whether the partner's agglutinogens permanently persist after vegetative hybridization and the exchange of blood during embryogenesis or whether there is a permanent change in reactivity in the sense that the presence of the partner's agglutinogens during parabiosis leads to a lack of antibody responsiveness in adult age. In any case, the described effects indicate that vegetative hybridization has a profound and permanent metabolic influence on the vegetative hybrids.

This method has great advantages over previous attempts of inducing parabiosis, particularly in animals because there are different conditions for mutual assimilation of two metabolisms between the embryonal and postembryonal stages. Furthermore, the method is not traumatic for the embryo, whereas the surgical joining of adults limits their mobility and with different nutrition is not physiological and traumatizing for the animals. With our method, only

the extraembryonal blood circulation is joined, whereas the embryos retain unrestrained mobility. They come out of parabiosis in a natural way by rejecting the umbilical cords from the extraembryonal circuit at the time of hatching. Despite its profound metabolic influence, the very physiological nature of our method is indicated by the high hatching rate of parabionts, which is 80% with good-quality starting materials (corresponding to the generally observed hatching rate) or even 100% in some experiments.

The described results of vegetative hybridization after parabiosis corroborate our earlier results on the exchange of egg white (Hašek 1952, Hašková 1953, Vojtíšková and Hašek 1953) and indicate that vegetative hybridization can be accomplished in animals, thus enabling better analysis of genetic changes.

LITERATURA

Barynšnikov I. A., Zaks J. G., Pavlov I. P.: *Vliv mateřského organismu na zbarveni srsti potomstva po transplantaci vaječníků u králíků. (Čes. překl. Sovětská věda, biologie, 1951, str. 675–685). Izv. AN SSSR, ser. biol., 6:* 77, 1950.

Bert Paul: Ann. Sci. nat. (Ser. IV), *18:* 86, 1862; J. Anat. *1:* 69, 1864; C. R. Acad. Sci., *62:* 515, 1886.

Bogoljubskij S. I.: *Razvitije cypljat, vyvedenych iz jajc s zamenenym belkom.* Tr. Puškinskoj n.-i. laboratorii rasz. s. ch. životnych, *3:* 7–16, 1949.

Borjačok-Nižnik G. V.: *Opyty vegetativnoj gibridizacii životnych.* Žurn. obšč. biol., *12* (4): 233–251, 1951.

Boyd W. C., Otis E. A.: *Individual blood differences in Chickens.* J. Heredity *31* (3): 135–136, 1940.

Davidov S. G.: *Mičurinská nauka o vypěstování nových druhů.* Čes. překlad, Praha 1950.

Ferdinandov V. V.: *O methodach vegetatinvoj gibridizaciji ptic.* Priroda, č. 7: 57, 1951.

Ferdinandov V. V.: *Izyskanije metodov vegetativnoj gibridizaciji životnych.* Usp. sovr. biol., *34:* 2(5), 1952.

Furth, O. B., Barnes W. A., Brower A. B.: Arch. Path., *29:* 163, 1940.Hašek M.: *Zvýšeni životaschopnosti kachen, ovlivněných cizorodým bilkem.* Čsl. Biologie, *1* (2): 76–78, 1952.

Hašek M.: *Proměňlivost chromosomů slepic a jejich závislost na metabolismu.* Čsl. biologie, *1* (2): 79–84, 1952.

Hašek M.: *Parabiosa ptáků v embryonálním vývoji,* Čsl. biologie, *2* (1): 25, 1953.

Hašková V.: *Přispěvek k záměnám bilku u ptačích vajec* Čsl. biologie, *2* (3): 167, 1953.

Kvasnickij A. V., Mankovskaja M. N.: *O, "vegetativnoj gibridizaciji" v životnovodstve.* Priroda, *11:* 39–45, 1949.

Kvasnickij A. V.: *Opyt peresadki jajeckletok ot odnovo k drugomu.* Sovetskaja zootechnija, *1* 1949.

Kurbatov A. D.: *Změny dědičnosti a zvýšeni životnosti potomstva transplantaci vaječných buněk mezi plemeny krlíků.* Usp. sovr. biol. *31:* 2, 1951.

Lepešinskaja O. B.: *Vznik buněk ze živé hmoty a úloha živé hmoty v organismu.* Čes. překlad, Praha, 1952

Lopyrin A. I., Loginova N. V., Karpov P. P.: *Opyt mežporodnoj peresadki zarodyšej u ovec.* Sovetskaja zootechnija, *8,* 1950.

Lysenko T. D.: *Trechletnyj plan, razvitija obščestvennovo kolchoznovo i sovchoznovo produktivnovo životnovodstva i zadači selskochozjajstvennoji nauki.* Moskva 1949.

Maštaler G. A.: *Vlijanije čužerodnovo jajčnovo bělka na razvitie pticy.* DAN SSSR, *71* (3): 549–552, 1950

Nikitin V. P.: Pticevodstvo. Moskva 1948.

Nuždin N. I.: *Darwin i mičurinskaja biologia.* Izv. AN SSSR, ser. biol., *3*, 6–29, 1952.

O stavu současné biologie. (Český překlad). Stenografický zápis zasedáni VASCHNILu 1949.

Pavlov I. P.: *Přirodověda a mozek. Výbor ze spispů.* Čes. překlad vyd. Praha 1952.

Paspalev G. V., Bratanov K. C.: *Vliv některých faktorů na dědění znaků. u ptáků* Izv. na biol. inst., Bulgarska Akademia na Naukitě, T. I., str. 411–429, 1951.

Problémy proměnlivosti mikrobů. Zasedání presidia Akademie lékařských věd SSSR. (Český překlad). Sovětská věda-láekařství, č. 6–7: 632, 1951.

Smetněv C. I.: *Pticevodstvo.* Moskva 1948.

Sokolovskaja I. I.: *Vozmožna-li negetativnaja gibridizacija u životnych.* Žurnal, "Karakulevodstvo i zverovodstvo", 2, 1949.

Sopikov P. M.: *Novyj metod vegetativnoj gibridizacijii ptic posredstvom perelivanija krovi.* Priorida, č. 10, 66, 1950.

Thomsenn O.: Zschr. Rassenphysiol., *7*, 47, 1934.

Thomsoo O.: Hereditas, *19:* 243, 1934.

Van Dyke D. C., Huff R. L., Evans H. N., Stanford M. Bull. *6*, 271, 1938.

Vojtišková M., Hašek M.: *Úplná změna sleptičího vaječného bílku za kachní jako způsob vegetativuí hybridisace živočichů.* Čs. Biologie *2:* 227, 1953.

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THE 50TH ANNIVERSITY OF TOLERANCE

JOHN W. FABRE

The genesis of this special feature was a conversation on tolerance with Juraj Ivanyi, at a function unrelated to science. He mentioned that he was writing a review of Milan Hašek's contribution to the experimental and theoretical development of immunologic tolerance for *Nature Reviews: Immunology* (*1*). This was excellent news, because I had long wondered about Milan Hašek. His contribution to the momentous events of the early 1950s has been debated over the years (see Ivanyi (*1*) and Brent (*2*)), but his key article has remained inaccessible to all but a few scientists, because it was published in Czech (*3*).

Reviews and opinions are valuable, but there is nothing like letting Milan Hašek speak for himself. Juraj indicated he would be willing to translate Hašek's article into English—probably the first time this has formally been done. As a Ph.D. student at Hašek's Institute in the 1960s, Juraj was better placed than anyone to accomplish this task. The editors of *Transplantation*

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