significant bradycardia (decrease of 10 to 20 beats per minute), and hypertension (increase of 20 to 40 mm systolic pressure; 10 to 30 mm diastolic pressure). However, this degree of hypertension is common in patients with pheochromocytoma, and therefore the hyperglycemia and glycosuria observed in such patients (*10*) may reflect, in part, insulin inhibition from either epinephrine or norepinephrine. Indeed, in one such patient with an abnormal oral glucose tolerance test, there was no rise in IRI until 120 minutes after glucose. This early inhibition was no longer present during a later glucose tolerance test, repeated after surgical removal of the pheochromocytoma (*11*). This inhibitory effect of infused norepinephrine on plasma insulin responses should raise doubts about the validity of any conclusions drawn from experiments in which norepinephrine is given to study the effects of an increased free fatty acid concentration upon glucose turnover. Although Nestel *et al.* have suggested that blockage of FFA mobilization during norepinephrine infusions by nicotinic acid reversed the effects of norepinephrine on glucose disposal (*12*), we have found no reversal of epinephrine inhibition of plasma IRI despite complete nicotinic acid blockade of epinephrine-induced lipolysis (*1*).

Although little is known of the local concentration of norepinephrine at sympathetic effector sites after stimulation of sympathetic nerves, conceivably these concentrations are similar to the concentrations of norepinephrine in the plasma achieved by our infusion, and therefore the sympathetic nervous system may play a tonic role in the regulation of insulin release. Certainly the pancreas has been shown to be innervated by sympathetic nerve fibers (*13*).

There was a prompt return of plasma IRI to normal concentrations 30 minutes after tolbutamide was given alone. When tolbutamide was given during a norepinephrine infusion there was a definite secondary peak in IRI although the tolbutamide had been given 60 minutes before. This "rebound" of serum insulin suggests a prolonged effect of the tolbutamide since plasma glucose was already at or below the values prior to infusion. This prolonged effect is not surprising in view of the 4-hour half-life of tolbutamide (*14*), and suggests that the prompt decline of the insulin concentrations in the course of a tolbutamide tolerance test in normal subjects may reflect counter regulation by epinephrine or norepinephrine secretion rather than lack of effective tolbutamide levels at 30 minutes. Compatible with this suggestion is the rise in heart rate of 10 to 20 heart beats per minute, 20 to 30 minutes after tolbutamide administration, which occurred in each subject. Therefore, lack of such counter regulation, rather than excessive drug response, may be responsible for part of the abnormal insulin response to tolbutamide noted in patients with organic hyperinsulinism.

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Chimeric and Ex-Parabiotic Frogs (Rana pipiens): Specificity of Tolerance

Abstract. *Rejection of orthotopic neural-fold transplants may be prevented by either embryonic parabiosis or reciprocal exchange of presumptive blood. These observations form the basis of the no blood–no tolerance hypothesis, which states that persistent tolerance by the host requires that the somatic transplant be accompanied by presumptive blood from the donor. Regarding parabionts and chimeras as special cases of transplantation of whole or partial animals, I found that ex-parabionts accept subsequent skin exchanges only from the homologous ex-parabiont; reciprocal chimeras are compatible provided each animal portion contains a primary blood source, and will accept transplants as frogs only from the homologous recombinant. Chimeric recombinations made anterior to the heart field prove incompatible and fail to survive to maturity. Successful chimeras as well as ex-parabionts survive to maturity and are apparently normal in every respect.*

When single neural folds are exchanged between embryos of *Rana pipiens* they are incorporated into the embryos, but the pigment cells derived from the donor neural crest are selectively rejected from the host skin during middle life of the larva. Rejection is accompanied by simultaneous repair from host sources, resulting in a host-pigment pattern at metamorphosis. In exceptional instances, repair may be incomplete by metamorphosis, at which time the pattern stabilizes, leaving permanent pigment-free areas in the skin of the frog (*1*). The incompatibility of tissue exchanges between sibling animals supports the findings of Hildemann and Haas (*2*) that skin transplants among some 600 larvae of *R. catesbeiana* were invariably rejected, indicating a large degree of variability in histocompatibility in the wild populations of these animals. Rejection has been avoided by two methods: After the exchange of neural folds, the animals were joined in parabiosis in tail-bud stages; or exchange of neural folds was followed by exchange of portions of the ventral belly region, to include cells destined to give rise to the blood of the animal (blood island). The second method amounts in effect to a surgical exchange of blood, comparable to parabiosis, as indicated by the observa-

tion that each of these methods prevents the rejection of donor neural crest. By these means it was demonstrated that the burnsi locus (dominant spot-inhibiting gene) and the kandiyohi locus (dominant gene introducing mottlings into the unspotted regions of the skin) act through alterations of the pigment cells derived from the neural crest, rather than by affecting either the skin proper or hormonal factors (*1*).

The conferring of tolerance, by either parabiosis or the surgical exchange of blood, raises a point of considerable theoretical interest. Can tolerance persist in the absence of donor-blood derivatives to accompany the somatic transplant? Stated simply, the view may be called the no blood–no tolerance hypothesis.

Any generalizations concerning the success of homoplastic exchanges must also recognize two special cases of transplantation. The first of these, parabiosis, may be regarded as the transplantation of one individual to another; the second is that of recombination of whole-animal halves (chimeras), or the transplantation of complementary whole-animal parts.

I now present further evidence that tolerance can be achieved only when the donor somatic transplant is accompanied by donor-blood derivatives, and that the tolerance thus achieved is specific to the individuals involved.

Of special interest is a new means of parabiosis that results in spontaneous separation of the parabionts after metamorphosis (*1*): it consists in joining tailbud embryos by the gill *anlagen* (Fig. 1, oval area). Methods of operation are standard and have been described (*1, 3*). A curiosity of the gill method is the observation that the left-hand animal develops with external gills; the operculum fails to close. Figures 2 and 3 represent a pair of burnsi gill parabionts in dorsal and ventral aspect respectively; despite the external gills of the left-hand animal, both ex-parabionts survive separation at metamorphosis. Figure 4 represents a pair of burnsi animals in the first stages of separation, which involve stretching of the skin joining them after resorption of the gills; within a few more days the common skin becomes necrotic as a result of mechanical twisting, and the animals separate; several pairs have been maintained following separation. Ex-parabionts accept skin transplants only from the homologous ex-parabiont, rejecting skin from both unoperated controls and nonhomologous ex-parabionts in the usual way (8 days at 22°C). The tolerance is specific and persists for at least 2 years, the longest test period between separation and skin exchange. Figures 5 and 6 represent a pair of ex-parabionts: on the left, a double mutant (burnsi-kandiyohi); on the right, a kandiyohi mutant. Six weeks after separation, white belly skin was reciprocally transplanted to the back, and was accepted and retained throughout life. One should note that there is no question that formed blood elements freely exchange between the parabionts, as was directly observed in anastomosed gill capillaries.

Chimeric combinations are of special interest, since results may be interpreted in terms of what is known concerning the embryonic distribution of blood primordia (*4*). In Fig. 1, the vertical lines represent the levels at which recombinations were made in tailbud embryos, the plane of the cuts being perpendicular to the long axis of the animal: Level *1* bisects the blood island ventrally into approximately equal portions; level *2* passes through the heart field, anterior to the blood island; and level *3* passes through the eye and gil *anlagen* anterior to both the heart and blood-island fields.

On the basis of what is described concerning the origin of definitive blood strictly from the blood island (definitely posterior to level *2*), it was predicted that, while *1*-level chimeras would develop normally, *2*- and *3*-level chimeras would both exhibit incompatibility, the posterior animal regarding the head as foreign. However, such is not the case: chimeras produced at levels *1* and *2* each develop normally, never exhibiting any sign of tissue incompatibility.

Adherence to the no blood–no tolerance hypothesis requires a primary source of blood anterior to the *2* level. Convincing proof that this is indeed the case comes from a study of meroplasts (isolated parts) produced at the *2* level in early tailbud stages. Anterior *2*-level meroplasts develop with a very abnormal and reduced circulation that remains free of formed blood elements until very late (until corresponding feeding stages). Meroplasts surviving until this time produce cellular blood elements apparently directly from the endothelium of the heart. This observation is of incidental interest since it does not support the view that definitive blood cells are derived strictly from the embryonic blood island.

Chimeric combinations at level *3*, anterior to both the heart and blood-island fields, invariably demonstrate incompatibility in the form of necrosis along the boundary marking the original zone of contact of the recombined halves (between the eyes, dorsally). The initial incompatibility, expressed in midlife of larva, has a variable subsequent expression: In most cases the original evidence of necrosis disappears, animals that complete metamorphosis developing with the genetic spotting pattern characteristic of each of the respective portions; in other instances the necrotic boundary may proceed forward into the head before repair from host (body) sources, the animal metamorphosing with the genetic pattern characteristic of the body genotype. Figure 7 shows a larva in which the necrotic boundary, originally between the eyes, has proceeded forward onto the snout, the line of rejection and repair being marked by the evident diagonal line. Whatever the history of the incompatibility response, the animals always die before, during, or shortly after the completion of metamorphosis, in many instances exhibiting before death a second resurgence of tissue incompatibility. In *3*-level meroplasts, only the posterior fragment develops formed blood elements, which facts supports the view that self-recognition requires the presence of donor-blood derivatives to accompany the donor somatic transplant.

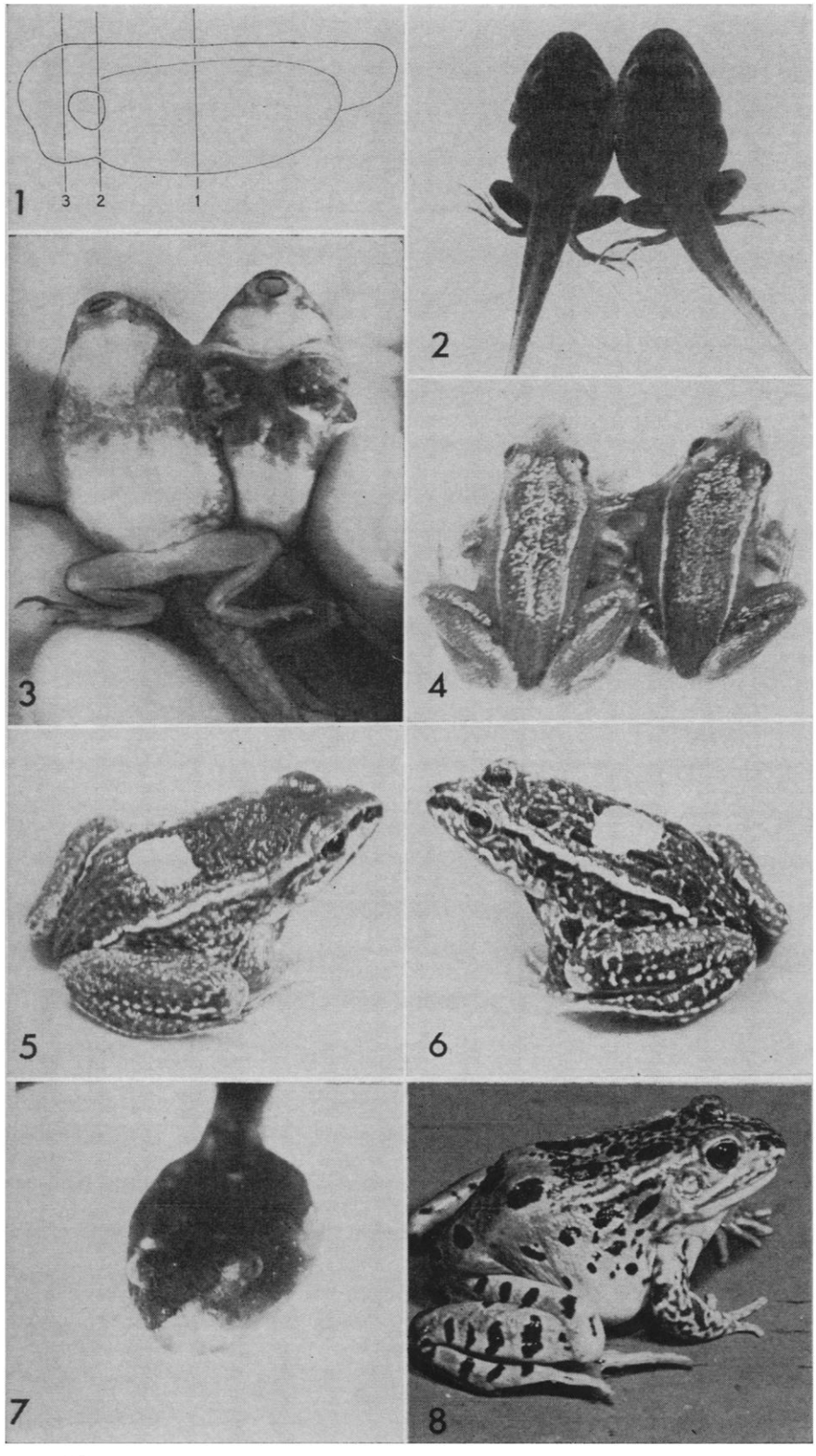
The arrest of necrosis in *3*-level meroplasts probably represents a transient immunological paralysis, since the animals never survive to maturity. I must emphasize that failure to proceed with the rejection of donor tissue does not in itself indicate compatibility, since the host's system of immunity may be neutralized by sufficiently large doses of donor antigen. Thus Volpe and Gebhardt (*5*), while supporting Davison (*1*) in observation of rejection of single neural-fold transplants, have found that when both neural folds are transplanted the donor cells persist (in some instances but not in all) through metamorphosis. Such animals may be comparable with *3*-level chimeras (also representing massive doses of donor tissue) in which rejection is sometimes arrested but apparently only temporarily.

As with ex-parabionts, successful (levels *1* and *2*) reciprocal chimeras accept skin transplants only from the homologous recombinant; transplants to and from nonreciprocal recombinants are rejected. Successful chimeras

as well as ex-parabionts are normal in every respect, never indicating tissue incompatibility as either larvae or adults. Representative animals have been reared to sexual maturity. Figure 8 represents a *1*-level chimeric female adult in which the front half is kandiyohi while the rear half is wild type; the original level of recombination remains discrete throughout the life of the animal.

I do not support Volpe's (*6*) interpretation of interplay or other forms of interaction between mutant and wild-type pigment cells in chimeric frogs. However, Volpe has noted a curious apparent exception to the rejection of simple neural-fold transplants: When the neural fold of the donor is transplanted to the belly region of the host neurula, it persists to yield the donor pigment pattern in the belly of the host animal (*6*). I have repeated the experiment and confirmed this finding, employing triploid donor neural crest, triploid embryos being produced by the methods of Briggs (*7*). Of special significance to the argument that blood derivatives must accompany the transplant is the observation that diploid hosts may demonstrate a chimeric distribution of blood cells (erythrocytes), with a variable number of large cells of a size and shape indistinguishable from triploid control cells. Thus, transplantation of neural crest to the belly, and accordingly to the blood-island region, amounts to a simultaneous transplantation of presumptive pigment cells and blood. This result is not surprising, since it is well documented that cells derived from the neural crest may normally give rise to tissues usually regarded as mesodermal, notably mesenchyme and cartilage of the head (*8*). Thus the apparent exception supports the hypothesis that donor blood must accompany the somatic transplant.

It would be dangerous to apply the findings with *R. pipiens* to considerations of tissue immunity generally, since



Figs. 1–8. Fig. 1. Diagram of a tailbud embryo, indicating the levels of chimeric recombinations (vertical lines). The oval area represents the region by which embryos are joined in gill parabiosis. Fig. 2. Gill parabionts in dorsal aspect. Fig. 3. Gill parabionts in ventral aspect. Note the external gills of the left-hand animal (on the right). Fig. 4. A pair of *burnsi* gill parabionts undergoing spontaneous separation following metamorphosis. Fig. 5. Double-mutant ex-parabiont. Fig. 6. The homologous ex-parabiont, a kandiyohi animal. Six weeks after separation white belly skin was reciprocally transplanted to the back and is accepted. Fig. 7. A *3*-level chimeric larva exhibiting tissue incompatibility. Incompatibility appeared originally as a necrotic line between the eyes, dorsally. The necrotic region progressed forward onto the snout and is now evident as the diagonal line proceeding downward from the animal's left eye. Fig. 8. A 2-year-old, sexually mature, chimeric female produced at level *1*. The anterior half is kandiyohi; the posterior, wild-type.

in frogs the basic mechanisms may differ somewhat from those in higher animals. However, I would like to suggest that the term "eutolerence" be used to describe persistent compatibility produced by the methods I have described, to distinguish it from that produced by the familiar means of massive doses of antigen, radiation, and drugs.

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Electrophoretic Heterogeneity of Polypeptide Chains of Specific Antibodies

Abstract. *Heavy and light polypeptide chains isolated from different specific antibodies to haptens and from γG-immunoglobulin of normal rabbits have been resolved into distinct, multiple components by disc electrophoresis in polyacrylamide gels in the presence of urea. In spite of the resolution of these chains into multiple bands, different specific antibodies and normal rabbit γG-immunoglobulin were indistinguishable from each other by this method.*

In order to look for a possible relation between antibody specificity and electrophoretic behavior we examined heavy and light polypeptide chains of rabbit γG-immunoglobulin and specific antibodies to haptens by disc electrophoresis in polyacrylamide gels. Rabbit heavy polypeptide chains have not heretofore been resolved into distinct electrophoretic subgroups. We now report an electrophoretic resolution of heavy polypeptide chains isolated from different rabbit antibodies to hapten and show that there is a lack of correlation of this banding with antibody specificity.

Arsanilic acid (Ars) was conjugated with bovine γ-globulin (BGG) (*1*), and dinitrophenyl bovine γ-globulin (DNP-BGG) was also prepared (*2*). Two individual rabbits of known allotype ($a^1a^1b^4b^4$) were immunized with these conjugates as follows: an initial injection of 2.5 mg of antigen in complete Freund's adjuvant was followed in 2 weeks with an injection of 2.5 mg of antigen in incomplete adjuvant; and subsequently four doses of 1.0 mg each of antigen were administered intravenously over a period of 4 months. The serums from three to four bleedings were combined; these were taken over a period of 1 to 4 months after the onset of immunization from the rabbit immunized with the DNP-BGG, and an equal number of bleedings were collected and combined over a period of 3 to 4 months from the rabbit immunized with Ars-BGG.

Rabbit antibody to arsanilic acid (*1*) and rabbit antibody to DNP (*2*) were extensively reduced and alkylated in 7*M* guanidine hydrochloride (*3*), and heavy and light polypeptide chains of the γG-immunoglobulin, specific for DNP and arsanilic acid, were then isolated by gel filtration on Sephadex G-200 in 5*M* guanidine hydrochloride (*3*). After gel filtration, these preparations were dialyzed exhaustively against large volumes of distilled water to remove essentially all the guanidine hydrochloride. Samples of heavy polypeptide chains (isolated by the procedure of Fleischman *et al.*, *4*) from antibodies to glucose and galactose haptens were made available to us (*5*). We extensively reduced and alkylated (*3*) these preparations and subsequently dialyzed them to remove guanidine hydrochloride prior to electrophoresis.

Disc electrophoresis was performed in polyacrylamide gels (*6*) with a modification of the gel composition (*7*). The composition of the various solutions needed to prepare the 4-percent gels is shown in Table 1.

The buffer (*p*H 8.91) in the upper tray consisted of 5.16 g of tris, 3.48 g of glycine, 700 ml of 10*M* urea, and enough water to make 1 liter. The buffer (*p*H 8.07) in the lower tray consisted of 14.5 g of tris, 60 ml of 1*N* HCl, and water to make 1 liter.

The solutions indicated above were freshly prepared just prior to use and care was taken to deionize the urea solutions by passing them over a column of mixed-bed ion-exchange resin (Rexyn I-300). The conductivity of a 10*M* solution of urea was not allowed to exceed 3 to 5 μmho. Electrophoresis was performed at a constant current of 2.5 ma per tube over a period of 3 hours for heavy chains, and 2 hours for light chains.

All preparations had from four to seven components with comparable degrees of heterogeneity (Fig. 1). The one exception was the unique, fast-moving electrophoretic component in the preparation of antibody to DNP. This component proved to be a contaminant (albumin), as determined by immuno-electrophoresis, disc electrophoresis, and gel diffusion of the original preparation of antibody to DNP (not shown). Such contamination can be avoided by removal of the albumin (for example, by sodium sulfate precipitation) before isolation of the specific antibody essentially by the method of Farah (*2*).

To evaluate possible electrophoretic differences among some of the preparations, mixtures (1 : 1) were subjected to electrophoresis. If slight differences

Table 1. Polyacrylamide gel system, Bis: *N,N'*-methylenebisacrylamide; TEMED: *N',N,N'*-tetramethylethylenediamine.

No. of volumes	Components per 100 ml	
	Substance	Amount
	Lower gel	
1	Acrylamide	16 g
	Bis	0.8 g
	10*M* urea	to vol.
1 *	Tris	18.15 g
	1*N* NCl	24 ml
	TEMED	0.24 ml
	10*M* urea	to vol.
2	Ammonium persulfate	0.14 g
	10*M* urea	to vol.
	Upper gel	
1	Acrylamide	8 g
	Bis	0.8 g
	10*M* urea	to vol.
1 †	Tris	2.23 g
	1*M* H_3PO_4	12.8 ml
	TEMED	0.1 ml
	10*M* urea	to vol.
2	Riboflavin	1 mg
	Ammonium persulfate	0.04 g
	10*M* urea	to vol.

* *p*H 9.4, measured at 25°C without urea. † *p*H 6.74, measured at 25°C without urea.