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.

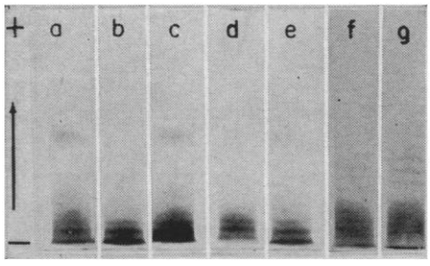

Fig. 1. Electrophoretic patterns of 400 μg of heavy chains in 4 percent acrylamide gel: (*a*) H-chain of antibody to DNP; (*b*) H-chain of antibody to arsanilic acid; (*c*) 1 : 1 mixture of (*a*+*b*); (*d*) nonspecific H-chain; (*e*) 1 : 1 mixture of (*a*+*d*); (*f*) H-chain of antibody to glucose hapten; (*g*) H-chain of antibody to galactose hapten; (*f*) and (*g*) were subjected to electrophoresis for 4 hours at 2.5 ma per tube in a 6 percent acrylamide gel.

existed between the preparations, mixtures would be expected to contain more bands or broader bands than individual preparations. The electrophoretic similarity of the individual preparations is thus demonstrated by their resemblance to the patterns of the mixtures. The limited quantity of heavy-chain preparations of antibody to glucose and galactose hapten unfortunately prohibited "mixture" experiments, and the apparent differences in mobility may actually have resulted from the slightly different conditions used for the electrophoresis of these two preparations (Fig. 1, legend). It is also possible to see fast-moving bands similar to light-chain patterns in these preparations (Fig. 1, *f* and *g*) which were fractionated by gel filtration in propionic acid (*4*) but not in the patterns (Fig. 1, *a* to *e*) of preparations fractionated in guanidine hydrochloride (*3*).

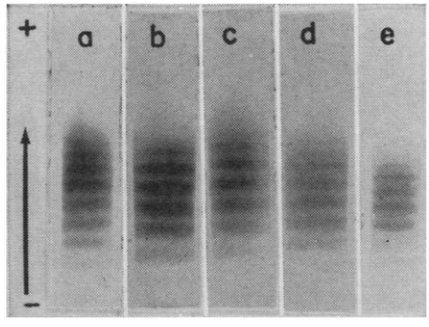

Fig. 2. Electrophoretic patterns of 300 μg of light chains in 4 percent acrylamide gel: (*a*) L-chain of nonspecific immunoglobulin; (*b*) L-chain of antibody to arsanilic acid; (*c*) 1 : 1 mixture of (*a*+*b*); (*d*) L-chain of antibody to DNP; (*e*) 1 : 1 mixture of (*b*+*d*).

Electrophoretic patterns of light chains from antibodies to Ars and DNP haptens and from nonspecific γG-immunoglobulin (Fig. 2) show no apparent differences. This is consistent with the findings of Cohen and Porter (*8*), who analyzed guinea pig antibodies to protein antigens in starch-urea gel at neutral and acid *p*H. Edelman *et al.* (*9*) found that the electrophoretic patterns of light chains from guinea pig antibody to hapten, observed in starch gel at acid *p*H, differed for antibodies of different specificities. Nussenzweig and Benacerraf (*10*), employing starch-gel electrophoresis at acid and alkaline *p*H and acrylamide gel electrophoresis at alkaline *p*H, found that reduced and alkylated guinea pig antibodies to hapten and normal guinea pig γG-globulins gave different electrophoretic patterns.

We have shown that it is possible to resolve electrophoretically both nonspecific and specific antibody heavy polypeptide chains into bands and that the patterns of the respective chains are quite similar. For several reasons we believe that these electrophoretic bands are not artifacts. It was possible to cut out bands and subject them again to electrophoresis under identical conditions and thereby obtain single electrophoretic components with essentially the mobility observed in the original pattern (Fig. 3). The banding was not due to polymerization of the chains, since high-speed sedimentation-equilibrium analyses (*11*) of similar preparations of rabbit and human polypeptide chains in 8*M* urea showed no evidence of mass heterogeneity (*12, 13*). Furthermore, the banding did not seem to be related to differences in alkylation since other extensively reduced but not alkylated heavy-chain preparations have shown banding similar to that observed for the extensively reduced and alkylated material (*13*).

Our observations suggest that there are electrophoretically distinct subpopulations of heavy chains similar to those already established for light chains (*14*). The antibody preparations we have investigated show no apparent relation of antibody specificity to electrophoretic heterogeneity of either heavy or light chains. Presumably, the inability to relate the electrophoretic patterns of light and heavy polypeptide chains to antibody specificity results from the great heterogeneity inherent in antibody molecules. This heterogeneity has been demonstrated by Doolittle and

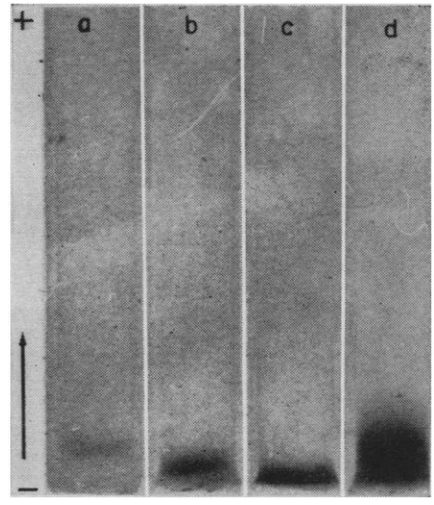

Fig. 3. Patterns of H-chain of antibody to arsanilic acid obtained by subjecting electrophoretic bands to re-electrophoresis: (*a*) component 3; (*b*) component 2; (*c*) component 1; (*d*) 400 μg of H-chain of antibody to arsanilic acid. An individual gel was sliced with a gel cutter, and the slices were again subjected to electrophoresis at *p*H 9.4.

Singer (*15*), who showed that the amino acid sequence in or near the active site region of both chains varies from one antibody molecule to another within a given population of molecules of antibody to DNP. Although the heterogeneity of specific antibody chains may be less than that of normal γG-immunoglobulin polypeptide chains, this heterogeneity may still be so great that it is not possible to distinguish specific antibodies to haptens from γG-immunoglobulins on the basis of the net charge of their polypeptide chains (*16*).

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16. It is possible that a different immunization procedure (for example, a single injection of alum-precipitated antigen, followed by a single bleeding) might give more homogeneous antibody. However, comparable electrophoretic patterns of heavy and light chains have been obtained from purified antibody to arsanilic acid isolated from a single bleeding of a rabbit immunized with edestin–arsanilic acid coated on acrylamide beads. Antigen (3.3 mg) was injected intravenously on day zero, and additional antigen (2.2 mg) was given by the same route on days 5, 10, and 15, and the rabbit was bled on day 21.
17. We thank James Boone and June Hyepock for the excellent technical assistance.

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Transformation of Auxotrophic Mutants of Group H Streptococci

Abstract. *Stable auxotrophic mutants of a group H streptococcus (strain Challis) were isolated on a modified Mickelson defined medium after exposure to* N-*methyl*-N′-*nitro*-N-*nitrosoguanidine. Such mutants were transformed to both prototrophy and streptomycin-resistance and hence may be used as nutritional markers in the study of streptococcal genetics.*

A number of investigators have reported the transformation of some streptococci to streptomycin-resistance (*1*) and have described conditions for competence (*2*), but to our knowledge nutritional markers have not been used for transformation studies in bacteria of this genus. By use of the chemical mutagenic agent *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (NTG) (*3*) in a procedure not yet reported (*4*), we have succeeded in isolating auxotrophic mutants from cultures of a group H streptococcus, strain Challis (*5*). In brief, rapidly growing cultures of Challis H were exposed to NTG (100, 250, and 500 μg/ml) for 30 minutes and were then plated on brain-heart infusion agar (Difco). After 3 days of incubation at 37°C, the smallest colonies were isolated and checked for nutritional deficiencies in a chemically defined medium. This medium, designated MS9 by us, is a modification of the one described by Mickelson (*6*) and is shown in Table 1. It supports excellent growth (more than 10^9 cells per milliliter) of the parent Challis H strain, which was initially adapted to grow rapidly in this basal medium after 10 to 15 daily transfers.

Cultures of auxotrophs were tested for transformability by the following method in liquid suspensions: 4.5 ml of brain-heart infusion broth, supplemented with 4 percent inactivated horse serum (heated for 30 minutes at 60°C) were inoculated with 0.5 ml from overnight cultures of the auxotrophs. The tubes were incubated from 60 to 90 minutes or until the original optical density doubled as measured with a Coleman Junior spectrophotometer set at 570 nm. As a rule 0.5 ml of these cultures (about 5×10^8 cells) was added to duplicate tubes containing 1.0 ml of brain-heart infusion broth supplemented with inactivated human serum. Deoxyribonucleic acid (20 μg) was added to one tube; a mixture of DNA and deoxyribonuclease was added to the other tube as a control. The DNA used was isolated by Marmur's method (*7*) from a wild-type culture of group H streptococci, strain SBE:12, resistant to 2 mg of dihydrostreptomycin per milliliter (*8*). The tubes were incubated for 60 minutes, diluted in physiological saline, and plated. All incubations were done at 37°C.

For the recovery of transformants to prototrophy the samples were plated on MS9 medium reinforced with 1 percent brain-heart infusion broth. After plating on MS9 medium the plates were overlaid with about 10 ml of plain Bacto agar. The transformants to either marker were counted after 2 or 3 days of incubation of the plates.

The conditions for transformation found to be optimum for the parent strain Challis H were used for all the auxotrophs, and no attempts were made to find the optimum conditions for each auxotrophic mutant. Table 2 shows the results obtained with several stable auxotrophs (stable after 10 to 15 serial transfers in complex medium)

Table 1. Components of chemically defined medium MS9. The medium was sterilized by filtration through a Millipore filter (0.45 μm) or by autoclaving at 2 atm for 15 minutes, glucose being added aseptically.

Component	Amount (g/liter)	Component	Amount (g/liter)	Component	Amount (g/liter)
L-Amino acids		*Purines and pyrimidines*		*Salts*	
Arginine	0.6	Adenine	0.01	K_2HPO_4	12.0
Methionine	0.2	Guanine	0.01	KH_2PO_4	5.2
Valine	0.2	Uracil	0.01	$(NH_4)_2SO_4$	2.0
Leucine	0.2			$MgSO_4 \cdot 7H_2O$	0.2
Isoleucine	0.2	*Vitamins*		$MnSO_4 \cdot H_2O$	0.01
Lysine	0.2	Niacinamide	0.01	$FeSO_4 \cdot 7H_2O$	0.01
Serine	0.2	Thiamine	0.01	Na-Acetate	10.0
Tyrosine	0.4	Riboflavin	0.01	Glucose	10.0
Cysteine	0.2	Ca-Pantothenate	0.01	Water (double distilled) to make 1 liter	
Histidine	0.2	Biotin	0.003		
Cystine	0.05	*p*-Aminobenzoic acid	0.002	*p*H	7.0
Phenylalanine	0.2	Pyridoxal·HCl	0.002		
Glycine	0.2	Pyridoxamine	0.002		
Tryptophan	0.2	Folic acid	0.002		
Glutamic acid	0.5				

Table 2. Transformation of group H streptococci to either prototrophy or streptomycin-resistance. Frequencies of transformation were obtained by dividing the number of transformants to either marker by the number of colony-forming units of the recipient cultures (usually 5×10^8). Control samples with a mixture of DNA and deoxyribonuclease gave rise to less than 100 colonies per milliliter of sample when placed on MS9 medium or brain-heart infusion agar containing dihydrostreptomycin (300 μg/ml).

Auxotrophic mutant	Frequency of transformation (%)	
	Streptomycin-resistance	Prototrophy
M-308 (asparagine⁻)	1.2	0.4
M-40 (proline⁻)	0.6	0.2
M-475 (alanine⁻)	2.1	0.6
M-37 (unidentified)	3.0	0.2
Challis H (wild type)	3.5	