of conferring a selective advantage to the hybrid cells in a mixed culture. The environment of the living organism is not likely to change so radically as to favor an accidentally produced hybrid somatic cell.

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References and Notes

1. G. Barski, S. Sorieul, F. Cornefert, *J. Nat. Cancer Inst.* **26**, 1269 (1961).
2. S. Sorieul and B. Ephrussi, *Nature* **190**, 653 (1961); J. W. Littlefield, *Cold Spring Harbor Symp. Quant. Biol.* **29**, 161 (1964); L. J. Scaletta and B. Ephrussi, *Nature* **205**, 1169 (1965); R. L. Davidson and B. Ephrussi, *ibid.*, p. 1170; B. Ephrussi and M. C. Weiss, *Proc. Nat. Acad. Sci. U.S.* **53**, 1040 (1965); R. L. Davidson, B. Ephrussi, K. Yamamoto, *ibid.* **56**, 1437 (1966); M. C. Weiss and B. Ephrussi, *Genetics* **54**, 1111 (1966); L. J. Scaletta, N. B. Rushforth, B. Ephrussi, *ibid.* **57**, 107 (1967); M. C. Weiss and H. Green, *Proc. Nat. Acad. Sci. U.S.* **58**, 1104 (1967).
3. W. H. Stone, J. Friedman, A. Fregin, *Proc. Nat. Acad. Sci.* **51**, 1036 (1964).
4. R. D. Owen, *Science* **102**, 400 (1945).
5. A second example of somatic cell mating in a dizygote cattle twin was recently recorded by W. H. Stone, R. G. Cragle, S. Bendel, J. Caulton [*Genetics* **60**, 73 (1968)].
6. Blood was cultured following the microculture procedure of R. L. Tips, G. S. Smith, D. L. Meyer, R. W. Ushijima [*Texas Rep. Biol. Med.* **21**, 581 (1963)], and the cells were harvested according to the method of P. S. Moorhead, P. C. Nowell, W. J. Mellman, D. M. Batipps, D. A. Hungerford [*Exp. Cell Res.* **20**, 613 (1960)].
7. E. P. Volpe and B. Gebhardt, *Science* **154**, 1197 (1966); E. P. Volpe and S. Curtis, *ibid.* **160**, 328 (1968).
8. E. P. Volpe, B. Gebhardt, S. Curtis, E. Earley, in *Biology of Amphibian Tumors*, M. Mizell, Ed. (Springer-Verlag, New York, 1969), p. 137.
9. C. L. Huskins, *J. Hered.* **39**, 310 (1948); ——— and L. N. Steinitz, *ibid.*, p. 34; C. L. Huskins and K. C. Cheng, *ibid.* **41**, 13 (1950).
10. A. K. Sinha, *Exp. Cell Res.* **47**, 443 (1967).
11. R. L. Teplitz, P. E. Gustafson, O. L. Pellett, *ibid.* **52**, 379 (1968).
12. W. Shumway, *Anat. Rec.* **78**, 139 (1940).
13. Supported by grant GM-11782 from the U.S. Public Health Service.

17 November 1969; revised 19 January 1970

Immune Responses of Inbred Mice to Repeated Low Doses of Antigen: Relationship to Histocompatibility (H-2) Type

Abstract. *Immunization of inbred strains of mice with repeated minute doses (0.1 to 1.0 microgram) of hapten-protein conjugates demonstrated wide differences in the magnitude of their antibody responses, which were related to the histocompatibility (H-2) type of the strains. Immunization with a single high dose (100 micrograms) of antigen failed to demonstrate these differences.*

There are significant differences in the immune responsiveness of different mouse strains to complex multideterminant antigens, such as heterologous red cells, proteins, and hapten-protein conjugates (*1*). When poor-responder or good-responder animals were selectively bred, in a few generations homogeneously low or high levels of immune responsiveness were attained (*2*). These findings suggest that a relatively small number of genes may control the immune responses to a variety of antigenically complex immunogens. When substances with a restricted degree of heterogeneity, such as synthetic amino acid polymers, were used as immunogens, it was often observed that the ability to make immune responses was under control of single autosomal dominant genes (*3*). In the immune responsiveness to a homologous series of multibranched polypeptides this single gene was found to be closely linked to the major histocompatibility locus (H-2) of the species (*4*). The experiments described herein indicate that the magnitude of the antibody responses of mice to repeated injections of small doses of a complex immunogen, such as hapten-protein conjugates, was also associated with the H-2 type of the strains. This association with the H-2 locus was not observed when the animals were immunized with a single large dose (100 μg) of antigen.

Benzylpenicilloyl (BPO) conjugates of bovine gamma globulin ($BPO_{25}BGG$), hen's ovomucoid (BPO_4OM), bovine pancreatic ribonuclease (BPO_4RNAse) and a dinitrophenyl (DNP) conjugate of BGG ($DNP_{42}BGG$) were used as immunogens. Repeated 0.1-μg or 1.0-μg doses, mixed with 0.1 mg or 1.0 mg of an $Al(OH)_3$ gel adjuvant, were injected intraperitoneally into groups of three mice every 4 to 5 weeks. The animals were bled serially from the orbital sinus at various intervals after the antigen injections, over a period of 20 weeks after the primary immunization. Hapten-specific antibody titers were determined by passive hemagglutination (HA) or hapten-reacted human red cells (*5*) and by passive cutaneous anaphylaxis (PCA) in the mouse. The PCA tests in the mouse were done with sensitization periods of 2 hours and 48 hours, in order to assay respectively IgG_1 and reaginic antibodies (*6*). These methods have been described extensively elsewhere (*5*, *6*).

The results of immunization of mice of different strains with a single high dose (100 μg) of antigen are shown in Table 1, A columns. Primary antibody responses were elicited in all strains. There were moderate variations in the magnitude and duration of the antibody responses among the strains, but these were not related to their H-2 type. By contrast, immunization with repeated 1.0-μg doses of $BPO_{25}BGG$ plus 1.0 mg of $Al(OH)_3$ revealed marked differences among the strains. No significant antibody responses were detectable at 14 or 28 days after the first antigen injection. However, striking differences were seen in the magnitude of the secondary antibody responses evoked by a second injection of antigen given 4 weeks later. The antibody titers measured 7 days after the secondary dose are shown in Table 1, B columns. There was a clear relationship between the H-2 type of the strains and the magnitude of their secondary responses. No antibodies were detectable in H-2^b, H-2^d, or H-2^q strains or in SWR mice (*7*). On the other hand, quite vigorous antibody responses were seen in A/He mice (H-2^a) and in six out of nine H-2^k strains. Three other H-2^k strains (C57BR/cdj, MA/J, RF/J) and SJL mice (H-2^s) made weaker, although easily detectable, responses.

Eight strains of mice were also tested with repeated 0.1-μg or 1.0-μg doses of BPO_4OM, BPO_4RNAse, $DNP_{42}BGG$, or $BPO_{25}BGG$. When repeated 1.0-μg doses were used, H-2^a and H-2^k strains were the better-responding strains to each of the four different immunogens, as shown in Table 2. There was evidence, however, that certain strains responded better to certain immunogens. Table 2 shows that SJL mice responded moderately well to three of the antigens, but failed to respond to BPO_4RNAse; similarly, SWR mice also responded to three of the antigens, but failed to respond to $BPO_{25}BGG$. The two H-2^b strains tested (C57BL/6J and C57L/J) failed to respond significantly to any of the four immunogens. Similar patterns of immune responsiveness were seen when repeated 0.1-μg doses were used, although the antibody titers were lower in all strains.

Several antiserums were analyzed for the presence of antibodies specific for determinants of the carrier protein molecules, by using solutions of native BGG, OM, or ribonuclease to elicit PCA reactions. Both IgG_1 and reaginic

Table 1. BPO-specific antibody responses of inbred strains of mice following immunization with either a single high dose or repeated small doses of $BPO_{25}BGG$. (A) columns: Antibody titers at 9 or 14 days after a single injection of 100 μg of $BPO_{25}BGG$ plus 20 mg of $Al(OH)_3$ gel intraperitoneally; serum pools of three mice. (B) columns: Antibody titers at 7 days after a secondary injection of 1.0 μg of $BPO_{25}BGG$ plus 1.0 mg of $Al(OH)_3$ gel intraperitoneally; serum pools of three mice. Abbreviation: n.t., not tested.

H-2 type	Mouse strains	Immunization with					
		(A) 100 μg, once			(B) 1.0 μg, twice		
		HA	IgG_1	Reagin	HA	IgG_1	Reagin
a	A/HeJ	8*	160†	40†	128	320	160
b	C57BL/6J	1	20	10	2	0	0
b	C57L/J	4	160	40	4	0	0
b	LP/J	n.t.	n.t.	n.t.	0	0	0
b	129/J	n.t.	n.t.	n.t.	0	0	0
d	BALB/cJ	16	40	10	0	0	0
d	DBA/2J	16	80	20	0	0	0
k	AKR/J	16	160	5	256	320	0
k	CBA/J	8	80	20	64	160	80
k	CE/J	n.t.	n.t.	n.t.	128	320	80
k	C3H/HeJ	64	160	160	128	320	80
k	C57BR/cdj	32	160	80	4	40	10
k	C58/J	n.t.	n.t.	n.t.	64	160	40
k	MA/J	n.t.	n.t.	n.t.	4	20	0
k	RF/J	16	160	160	4	20	5
k	ST/bJ	n.t.	n.t.	n.t.	512	320	5
q	BUB/BNJ	n.t.	n.t.	n.t.	0	0	0
q	DBA/1J	n.t.	n.t.	n.t.	0	0	0
s	SJL/J	128	320	5	16	10	0
?	SWR/J	32	640	40	0	0	0

* Hemagglutination titers (reciprocal $\times 10^{-3}$); HA tests negative at 1 : 2000 are shown as zero. † IgG_1 and reaginic antibody reciprocal titers, as measured by PCA tests in CFW recipient mice with sensitization periods of 2 or 48 hours, respectively; PCA reactions at 1 : 5 are shown as zero.

antibodies specific for the carrier protein were found in serums from the responder strains. Neither IgG_1 nor reagins were detected in serums from the poorly responding strains. Representative results are shown in Table 3, which compares the responses of A/He and SWR mice to either $BPO_{25}BGG$ or BPO_4RNAse. Table 3 also shows that there was an antigenic preference in strain responsiveness to the different antigens: A/He mice responded to repeated 0.1-μg doses of $BPO_{25}BGG$ and virtually not at all to BPO_4RNAse, whereas SWR mice responded to BPO_4RNAse but not to $BPO_{25}BGG$.

The magnitude of the immune responses induced in different strains by a single high dose of antigen (Table 1, A columns) as opposed to repeated small doses, did not correlate with their H-2 type. At present, there is no precise explanation for this dosage effect, but two possibilities are being considered. One possibility is that the factor determining the immune responsiveness to low doses of antigen might be the binding avidity of cellular receptors for a given type of immunogen molecule, and these receptors might vary in different strains. At high doses of antigen, even cell receptors with low binding avidities might bind sufficient antigen to initiate the immune response. Another possibility is that lowering the dose of the immunogen restricts its heterogeneity by reducing the concentration of minor immunogenic constituents to subimmunogenic concentrations.

The nature of the relationship between histocompatibility loci and the genetic control of the immune response is unknown. A close linkage between histocompatibility loci and immune responsiveness to certain antigens has been demonstrated in two independent systems. McDevitt *et al.* (*4*) demonstrated that the intensity of the immune responses of mice to a series of multibranched polypeptide antigens is under the control of a single autosomal dominant gene, which is closely linked with the H-2 locus. Gasser (*8*) described a linkage between the agouti locus and the ability of inbred mice to form antibodies against mouse erythrocyte antigens ($Ea\text{-}1^{a,b}$) present only in red cells from wild-type *Mus musculus*; the linkage with the agouti locus implies also a linkage with histocompatibility loci (H-3, H-6).

The present results show that there are also striking genetic influences on the immune responsiveness of mice to low doses of hapten-protein conjugates, which are correlated with the H-2 type of the strains. Of interest, the magnitude of the immune responses of eight mouse strains to conjugates of proteins of widely different origins, such

Table 2. Hapten-specific antibody responses of inbred strains of mice following immunization with repeated 1.0 μg doses of different hapten-protein conjugates.

Immunogen	Antibody response (titers)*	(a) A/He	(k) CBA	(k) AKR	(k) C3H	(b) C57BL	(b) C57L	(?) SWR	(s) SJL
$BPO_{25}BGG$	HA†	256	64	64	64	0	0	0	64
$BPO_{25}BGG$	IgG_1‡	640	160	320	160	0	0	0	40
$BPO_{25}BGG$	Reagin‡	80	80	10	80	0	0	0	10
BPO_4OM	HA	32	64	64	32	0	0	16	8
BPO_4OM	IgG_1	320	160	320	160	0	0	5	20
BPO_4OM	Reagin	80	40	10	40	0	0	0	40
BPO_4RNAse	HA	32	16	32	16	0	0	16	0
BPO_4RNAse	IgG_1	320	20	320	160	5	5	80	0
BPO_4RNAse	Reagin	80	10	10	10	0	0	0	10
$DNP_{42}BGG$	HA	128	32	16	64	8	4	16	16
$DNP_{42}BGG$	IgG_1	320	160	160	160	0	0	10	10
$DNP_{42}BGG$	Reagin	160	40	0	20	0	0	10	0

* Antibody titers measured 7 days after a third injection of 1.0 μg of immunogen plus 1.0 mg of $Al(OH)_3$ gel, made 9 weeks after primary immunization. † Hemagglutination titers of hapten coated cells, in reciprocals $\times 10^{-3}$; HA tests negative at 1 : 2000 are shown as zero. ‡ IgG_1 and reagin titers as determined by PCA reactions in CFW recipient mice with 2-hour or 48-hour sensitization periods, respectively; PCA reactions negative at 1 : 5 are shown as zero. Letters in parentheses over mouse strains represent the H-2 type of the strains.

Table 3. Hapten-specific and carrier protein-specific antibody responses of A/He and SWR mice to immunization with repeated 0.1-μg doses of hapten-protein conjugates. Values are PCA titers in CFW recipient mice, with sensitization periods of 2 or 48 hours; PCA reactions negative at 1 : 5 are shown as zero.

Antibody response*		Mouse strains	
Type	Specificity	A/He	SWR
	Immunogen: $BPO_{25}BGG$		
IgG_1	Anti-BPO	160	0
IgG_1	Anti-BGG	160	0
Reagin	Anti-BPO	160	0
Reagin	Anti-BGG	80	0
	Immunogen: BPO_4RNAse		
IgG_1	Anti-BPO	5	80
IgG_1	Anti-RNAse	5	320
Reagin	Anti-BPO	0	10
Reagin	Anti-RNAse	10	40

* Antibody titers 7 days after a third injection of 0.1 μg of antigen plus 1.0 mg $Al(OH)_3$, made 9 weeks after primary immunization.

as bovine gamma globulin (BGG) and hen's ovomucoid (OM) varied similarly with the H-2 type. Furthermore, a remarkable coincidence exists between the pattern of immune responsiveness of mouse strains to low doses of hapten conjugates of BGG or OM, as presently described, and the responsiveness of the same strains to immunization with a synthetic amino acid polymer, (His, Glu)-Ala-Lys, as described by McDevitt and Chintz (*4*). The immune responsiveness to (His,Glu)-Ala-Lys was shown to be under control of a single gene which is closely linked to the H-2 locus (*4*). Segregation analysis will be necessary to establish whether a similar situation exists in the control of the immune response to low doses of hapten-protein conjugates. If this proves to be the case, it would mean that genetic differences at a single locus may influence the development of immune responses to a wide range of different antigens with, as yet, no obvious structural similarities or antigenic cross reactivity (*9*).

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References and Notes

1. M. A. Fink and V. A. Quinn, *J. Immunol.* **70**, 61 (1953); W. F. Barth, C. L. McLaughlin, J. L. Fahey, *ibid.* **95**, 781 (1965); N. L. Warner, N. M. Vaz, Z. Ovary, *Immunology* **14**, 725 (1968); N. M. Vaz and A. Prouvost-Danon, *Progr. Allergy* **13**, 111 (1969).
2. W. R. Sobey, J. M. Magrath, A. H. Reisner, *J. Immunol.* **96**, 511 (1966); D. Hardy and D. Rowley, *Immunology* **14**, 401 (1968); G. Biozzi, C. Stiffel, D. Mouton, *Ann. Inst. Pasteur* **115**, 965 (1968).
3. H. O. McDevitt and M. Sela, *J. Exp. Med.* **122**, 517 (1965); B. B. Levine and B. Benacerraf, *Science* **147**, 517 (1965); P. H. Maurer and P. Pinchuck, *Immunity Cancer and Chemotherapy* (Academic Press, New York, 1967), p. 319.
4. H. O. McDevitt and A. Chinitz, *Science* **163**, 1207 (1969); H. O. McDevitt and B. Benacerraf, *Advan. Immunol.* **11**, 31 (1969).
5. B. B. Levine, M. J. Fellner, V. Levytska, *J. Immunol.* **96**, 707 (1966); B. B. Levine and V. Levytska, *ibid.* **98**, 648 (1967).
6. I. Mota and J. M. Peixoto, *Life Sci.* **5**, 1723 (1966); N. M. Vaz and Z. Ovary, *J. Immunol.* **100**, 169 (1968); B. B. Levine and N. M. Vaz, *Int. Arch. Allergy Appl. Immunol.*, in press.
7. These animals were later challenged with 100 μg of BPO-BGG in alumina gel, and made antibody responses similar to the responses of normal control mice.
8. D. L. Gasser, *J. Immunol.* **103**, 66 (1969).
9. Experiments testing for cross reactivity between BGG and OM conjugates were done and were negative.
10. Supported by contract No. DADA 17-67-7119 from the U.S. Army Medical Research and Development Command. N.M.V. is a visiting scientist from the Instituto Nacional do Cancer, Rio de Janeiro, Brazil. B.B.L. is a career investigator of the Health Research Council of the City of New York. We acknowledge the excellent technical assistance of Henry Chang, Jr. We thank Dr. Victor Nussenzweig, Department of Pathology, New York University School of Medicine, for his stimulating interest in this work.

9 January 1970

Catecholamine Biosynthesis in Brains of Rats Treated with Morphine

Abstract. *In the brains of rats without tolerance to morphine, the accumulation of [^{14}C]dopamine formed from [^{14}C]tyrosine injected intracisternally is increased, reaching a maximum in the hypothalamus and striatum 1 hour after administration of morphine. In tolerant rats, the rate of incorporation of carbon-14 into dopamine and into norepinephrine in these areas is more than twice that in animals that have received only one injection of morphine.*

There is evidence that biogenic amines in the central nervous system interact with morphine and other narcotic analgesic drugs in two ways: morphine alters amounts of amines in the nervous system, and changes in the amounts of amines alter the response to morphine. In most experimental animals, the administration of morphine causes release of dopamine and norepinephrine from the brain in the first hours after the initial injection of the drug (*1*). In animals made tolerant by long-term treatment with morphine, there is no depletion of amine (*2*). However, the rate at which tolerance develops may be changed by simultaneous administration of drugs that alter the amounts of biogenic amines in nervous tissue. Drugs such as reserpine lower amounts of amines; inhibitors of monoamine oxidase increase amounts of amines by inhibiting their catabolism (*3*). The exact relationship between biogenic amines and narcotic drugs may not be defined by measurements of the catecholamine content of nervous tissue since these reflect only gross changes and may not reveal local changes or alteration in the rate of turnover of amines. We have studied the rate of incorporation of ^{14}C from [^{14}C]tyrosine into dopamine and norepinephrine in whole brain and in regions of the brains of tolerant and nontolerant rats.

To measure accumulation of labeled catecholamine, we injected [^{14}C]tyrosine (uniformly labeled) into the fourth ventricle of rats 10 minutes before killing them. The rate of its conversion to [^{14}C]dopa (dihydroxyphenylalanine), and, in succession, to [^{14}C]dopamine and [^{14}C]norepinephrine was measured after the amines were adsorbed from an acidic extract of brain on alumina, and separated on Dowex-50 columns (*4*).

Thirty to 90 minutes after one injection of morphine (60 mg/kg body weight), the rate of accumulation of [^{14}C]dopamine in whole brain was significantly increased over that in controls injected with saline, reaching a maximum 60 minutes after the injection (Fig. 1). The rates of incorporation of ^{14}C into norepinephrine and into dopa were apparently unaltered, except at one time. Calculations of the rate of conversion of tyrosine to dopamine and norepinephrine in brain in vivo are complicated by the fact that

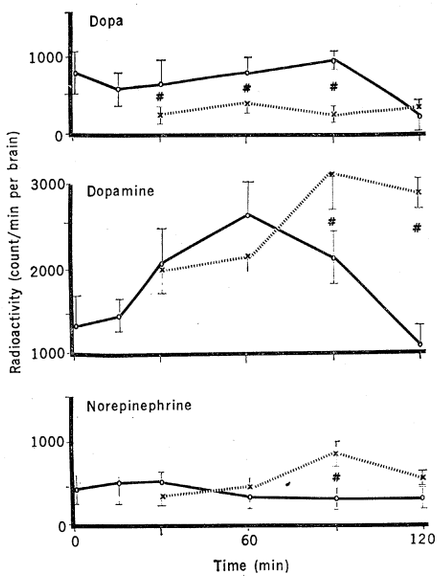

Fig. 1. The conversion of [^{14}C]tyrosine to [^{14}C]dopa, [^{14}C]dopamine, and [^{14}C]norepinephrine in rat brain. Each rat was injected intracisternally with 5 μc of [^{14}C]-tyrosine (uniformly labeled, 370 μc/μmole) and killed 10 minutes later. The brains were homogenized in 0.4*N* perchloric acid, the metabolites were separated on alumina and Dowex-50 columns, and the radioactivity was measured by scintillation spectrometry (*4*). The values for animals injected with saline are shown as zero-time values. All other rats were injected with morphine (60 mg/kg body weight) and killed at times from 15 to 120 minutes later; (———) rats injected once; (- - - - -) rats receiving a tenth daily injection of morphine (dose increasing from 20 to 60 mg/kg). The standard deviations are indicated by the perpendicular lines, and the starred values show significant differences ($P < .001$) between one and ten injections. At an average specific radioactivity of [^{14}C]tyrosine of 5.56 μc/μmole (calculated from time curves of [^{14}C]tyrosine remaining in brain during the 10-minute pulse), 478 count/min per brain (norepinephrine control value) represents a rate of synthesis of norepinephrine of 0.225 nmole/hour per gram of brain.