THE MIXED LYMPHOCYTE REACTION: AN IN VITRO TEST FOR TOLERANCE*, ‡

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The demonstration that lymphocytes transform into immature appearing forms when exposed to genetically foreign lymphoid cells (1) raised the possibility that this mixed lymphocyte reaction (MLR) could serve as an in vitro histocompatability test (2, 3). Subsequent attempts to correlate the MLR with other known immune phenomena, however, produced variable results (4–7). In spite of the uncertainties encountered in these correlation attempts, it is generally accepted that if the cell donors for the MLR are either identical twins or members of the same inbred strain of animal, no greater transformation will be observed when the cells are mixed together than when they are cultured separately (1, 8). Since such donors are tolerant to each other by way of genetic similarity, it seemed desirable to determine if neonatally induced tolerance would also abrogate or diminish the MLR even though genetic similarity was not obtained. The present study demonstrated that tolerance, induced by the neonatal administration of hemopoietic cells, significantly suppressed the transformation of lymphocytes. This finding suggested that the MLR may be used as an in vitro test for tolerance.

Material and Methods

Animals—In this study, five types of rats were employed. These included the Lewis (Lew)[1] and Brown Norway (BN)[1] strains which differ at an Ag-B histocompatability locus (9, 10), the F_1 hybrid of the Lewis and BN, (F_1[BN])[1], the F_1 hybrid of the Lewis and Buffalo (Buf), (F_1[Buf])[1], and the outbred Long Evans (LE) rat.[2]

Induction of and Tests for Tolerance.—Tolerance was induced by the intravenous injection of newborn Lewis rats with 40–42 x 10^6 bone marrow cells from adult BN donors (11). Alternatively, newborn BN animals received injections of 18 x 10^6 bone marrow cells from adult Lewis donors. The bone marrow cells were suspended in a maximum of 0.15 cc Hank's balanced

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[1] Microbiological Associates, Bethesda, Md.

[2] Simonsen Laboratories, Gilroy, Calif.

salt solution (BSS)[1] and injected into the retroorbital veins of the recipients. In all cases, the injections were made within 24 hr of birth.

After 3–12 wk, the injected animals (hereafter referred to as transfused), as well as noninjected controls, were tested for tolerance by determining the ability of their lymph node cells to produce runt disease. This was accomplished by preparing suspensions of mesenteric lymph node cells as previously described (12). The cells were then washed twice in BSS, resuspended in a maximum of 0.15 cc BSS and injected into recipients via the retroorbital vein. The donor-recipient combinations tested may be seen on Table I. In all cases, injections were made within 24 hr of birth and usually between 6 and 12 hr of age. Daily weights were recorded and if death occurred, the average survival time (AST) was determined. When runting was not observed, the animals were followed for 3 months and then sacrificed. In this study, a total of five transfused Lewis animals and five transfused BN rats served as the "tolerant" donors. An equivalent number of noninjected rats of the same strains were employed as donors of nontolerant, control cells.

As a second test for tolerance, the ability of mesenteric lymph node cell suspensions from normal and from transfused donors to produce normal lymphocyte transfer reactions (NLT reactions) in F_1(BN) and F_1(Buf) hybrids was ascertained (13). In all, transfer reactions from 35 transfused and 35 noninjected rats were made. Following the preparation of cell suspensions from these donors as described previously (12), 2.5–50 million cells, in a maximum volume of 0.15 cc BSS were injected intradermally in two or three sites on the ventral abdominal wall of each recipient. Care was taken to place the cells from the transfused donor on one side of the animal while the suspensions of cells from noninjected animals were placed in corresponding locations on the opposite side. 4½ days after the injection of cells, each animal received an intravenous injection of a 1% normal saline solution of Evans Blue dye in a dose of 0.4 cc per 100 g of body weight. 12 hr after injecting the dye, each animal was sacrificed and the ventral abdominal skin minus the panniculus adiposus was removed and everted on a cork board. The maximum diameters of the reactions were then determined and photographs taken if desired. The donor-recipient combinations may be seen in Table II. In the case of the NLT reactions involving the injection of BN cells into F_1(Buf) hybrids, the recipient animals were irradiated with 500 R total-body irradiation from a ^{60}Co source 24 hr before cell transfer. The irradiation was administered in 5 min at a distance of 80 cm in a field of 20 cm^2. This procedure prevented the host animal from responding to the grafted cells.

Mixed Lymphocyte Reaction (MLR).—In this part of the study, attempts were made to determine whether the mixed lymphocyte reaction (MLR) was suppressed when the donor animals were tolerant. After tolerance had been defined by the two tests described above, suspensions of cells were prepared either from the thoracic duct lymph or from the thymus glands and lymph nodes of noninjected donor animals or donors which were transfused in an identical manner to those which had been judged to be tolerant. These included 32 each of the transfused and noninjected BN rats, 35 each of the transfused and noninjected Lewis rats, 10 each of the F_1(Buf) and F_1(BN) hybrids, and 20 Long Evans rats. Because the collection of lymph may have altered the composition of the cell population in the lymphoid organs and because the responses observed were qualitatively similar regardless of the tissue origin, thymus cells were routinely employed in the cultures. This allowed simultaneous NLT tests on F_1(BN) hybrids to be made with the lymph node cells from each Lewis and Brown Norway donor. Aliquots of the thymus cell suspensions were cultured according to two general schemes. In the first scheme, the cells were (*a*) cultured individually as nonstimulated controls, (*b*) exposed to pokeweed mitogen[3] as a nonspecific blastogenic control (14), (*c*) in the case of the Lewis and BN, added to cultures containing equal numbers of cells from Long Evans donors

[3] PWM—Grand Island Biological Company, Grand Island, N. Y.

and (*d*) mixed so that the cultures contained equal numbers of cells from the following: two nontolerant donors (Lew x BN); two tolerant donors (Lew_T x BN_T); and one tolerant and one nontolerant donor (Lew_T x BN; BN_T x Lew). In the second scheme, the cells were cultured alone or they were exposed to PWM as described above. In addition, they were mixed so that the cultures contained equal numbers of cells from (*a*) a tolerant or a nontolerant BN donor and a F_1(BN) hybrid (BN x F_1[BN]); (BN_T x F_1[BN]); (*b*) a tolerant or a nontolerant Lewis donor and a F_1(BN) hybrid (Lew x F_1[BN]); Lew_T x F_1(BN); and (*c*) a tolerant or a nontolerant Lewis or BN donor and a F_1(Buf) hybrid (BN x F_1[Buf]; BN_T x F_1[Buf]; etc.). Under these conditions, the F_1(BN) hybrid cells stimulated the transformation of the Lewis or BN cells without enlarging themselves (17). Hence, the reactions were unidirectional with

TABLE I

Summary of Graft Versus Host Reactions Runt Disease*

Group	Donor	Recipient	No. cells injected ($\times 10^6$)	No. survivors/ No. injected	AST‡
I	BN	Lewis	40–43	0/10	17.5
	BN_T	Lewis	40–45	10/10	—
II	BN	Long Evans	44	4/4	17.0
	BN_T	Long Evans	44	3/4§	17.0
III	Lewis	BN	36–44	0/10	15.0
	$Lewis_T$	BN	36–40	8/8	—
IV	Lewis	Long Evans	38	4/4	13.5
	$Lewis_T$	Long Evans	38	4/4	14.5

* T, transfused; i.e. $Lewis_T$, Lewis animals which were transfused with BN bone marrow cells within 24 hr of birth and were presumably tolerant to BN histocompatability antigens; BN, Brown Norway; and BN_T, Brown Norway presumably tolerant to Lewis because of Lewis bone marrow injections within 24 hr of birth.

‡ AST, average survival time.

§ The fourth animal showed definite runting symptomatology but did not succumb.

the parental cells reacting against the hybrid. In all cases, cultures were prepared in duplicate according to the method of Rieke and Schwarz (15). Beginning 70 hr after initiation, each culture was exposed to 4 μc/ml of thymidine-^{3}H (6.7 c/mM) for 12 hr. This was followed by washing the cells in BSS and dividing them into two aliquots. One group of cells was smeared for morphological evaluation (8) while the remaining cells were assayed for ^{3}H-activity by scintillation counting (16). The corrected counts per minute per total culture were defined by multiplying the observed readings by two and subtracting from the product the background count. On Text-figs. 1–3, the values from the nonmixed control and PWM-containing cultures are combined into single bars.

RESULTS

Induction of and Tests for Tolerance.—The results of the runt experiments are found in Table I and Figs. 1 and 2. As indicated in Table I, all Lewis animals which received cells from transfused BN donors survived while all recipients of

cells from noninjected donors succumbed with an AST of 17.5 days. The specificity of this inablity to produce runt disease was demonstrated by the destruction of Long Evans rats with cells donated by either a transfused BN or by a noninjected rat. Similar results were observed when cells from Lewis donors were injected into BN and LE recipients. 50% more cells were injected into the Lewis and BN recipients than were necessary to produce 100% fatal runting.

TABLE II

*Summary of the Normal Lymphocyte Transfer Reaction**

Donor	Recipient	No. cells injected ($\times 10^6$)	Diameter of reaction	Nature of reaction
			mm	
BN	F_1 (BN)	35	6–8	GVH
BN_T	F_1 (BN)	35	—	—
BN	F_1 (Buf)^R	35	6–8	GVH
BN_T	F_1 (Buf)^R	35	6–8	GVH
Lew	F_1 (BN)	35	8–10	GVH
Lew_T	F_1 (BN)	35	—	—
Lew	F_1 (Buf)	35	8–10	GVH
Lew_T	F_1 (Buf)	35	8–10	GVH

* Abbreviations: BN, Brown Norway; Lew, Lewis; T, animals transfused with bone marrow cells at birth and, hence, were presumably tolerant; Lew_T, Lewis rats presumably tolerant to BN cells; BN_T, BN animals presumably tolerant to Lewis cells; F_1 (BN), F_1 hybrid of Lewis and BN parental strains; F_1 (Buf), hybrid of Lewis and Buffalo parental strains; GVH, unidirectional response of grafted cells against the hosts tissues; and R, received 500 R total-body irradiation 24 hr before the injection of cells.

A similar inability of cells from transfused donors to produce graft versus host reactions in certain hybrids was seen when the NLT reaction was employed as the test for tolerance (Table II). Specifically, when lymph node cells from transfused BN donors were injected intradermally into F_1 (BN) hybrids, no reaction was discerned. In contrast, cells from noninjected donors induced reactions which measured 6–8 mm in diameter (Fig. 3). As with the runt experiments, the inability to produce NLT reactions was specific for the Lewis histocompatability antigens and was not due to an intrinsic defect in the lymph node cells. This was demonstrated by injecting cells from the transfused BN donors into F_1 (Buf) hybrids. Under these conditions, reactions measuring 6–8 mm in diameter were observed (Fig. 4). Identical 6–8 mm reactions were also observed in the F_1 (Buf) recipients with the cells from noninjected donors. In

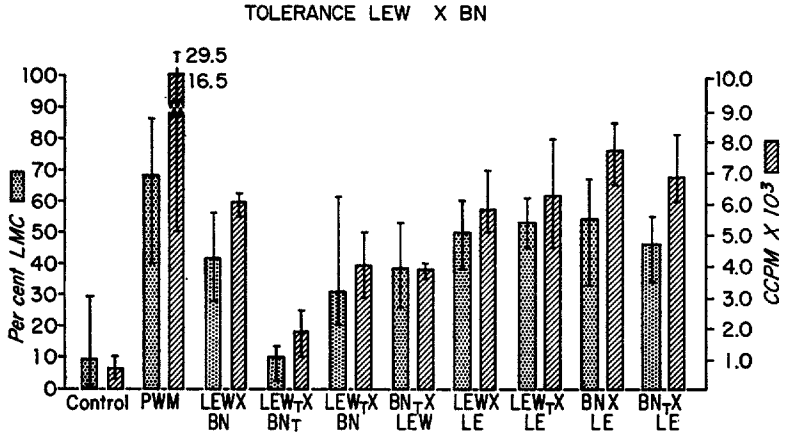
the case of the BN to F_1 (BN) hybrid NLT reaction, any reactions observed would have been unidirectional with parental cells transforming in response to the hybrid cells (17). This obtains since cells from the *noninjected* BN donors would have reacted to the Lewis histocompatability antigens of the hybrid. Reciprocally, the hybrids would not have reacted to the BN cells as the BN antigens were an intrinsic part of each hybrid. It follows that since the cells from the *transfused* BN donor were presumably tolerant to the Lewis antigens, no reaction would have occurred with cells from these tolerant donors.

In contrast to the BN $\rightarrow$ F_1 (BN) hybrid NLT reactions, when BN cells were injected into F_1 (Buf) hybrids, two reactions would have occurred. First, the BN cells from both the tolerant and nontolerant donors would have reacted against the Buffalo antigens and in the case of the nontolerant cells, would also have reacted to the Lewis antigens. Secondly, the F_1 hybrid would have recognized the BN antigens as foreign and would have responded to them. Therefore, the total reaction observed was the sum of a two-way or bidirectional response. To convert this bidirectional reaction to a unidirectional response on the part of the grafted cells, the F_1 (Buf) hybrids were given 500 R of total-body (^{60}Co) irradiation prior to injecting the BN cells. Because of the irradiation, the hosts were unable to respond to the foreign cells and the lesions observed reflected a reaction of the grafted cells against the tissues of the hosts.

The inability of cells from transfused donors to produce NLT reactions in F_1 (BN) hybrids was most probably not because an insufficient number of cells had been injected. This stems from the observation that the 35×10^6 cells routinely employed were 14 times the threshold dose of cells required to elicit an observable reaction with nontolerant BN cells. Although there was considerable variation, in general, the greater the number of cells injected, the larger the observed reaction. Similar NLT reactions to these observed with BN cells were detected when cells from transfused and noninjected Lewis donors were grafted into the hybrid strains. In these cases, the 8–10 mm reactions were unidirectional with parental cells reacting against the hybrid elements, but not vice versa. It appeared, therefore, that the results of the NLT reactions, together with those of the runt experiments, demonstrated that both the Lewis and BN animals were tolerant. Moreover, it was concluded that this tolerance was specific for the histocompatability antigens possessed by the bone marrow cells used to induce tolerance.

Mixed Lymphocyte Reaction (MLR).—The results of the MLR experiments may be seen in Figs. 5 and 6 and Text-fig. 1–3. Since qualitatively identical results were seen with cells from either thymus, lymph node, or thoracic duct lymph, thymus cells were routinely employed. This allowed a simultaneous NLT reaction to be conducted with lymph node cells from each donor. In each case, except one described below, the NLT reaction correlated identically with the in vitro results. It is to be noted in Text-fig. 1 that when cells of the thymus

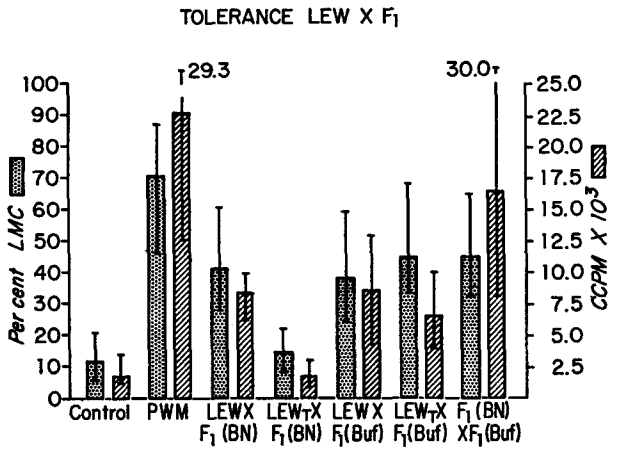
glands were cultured alone without stimulants, approximately 10% of the cells possessed nuclear diameters of greater than 7 μ. These cells incorporated slightly less than 1 $\times$ 10^3 cpm of ^3H activity. In contrast, when cells from two nontolerant donors were mixed together, a fourfold increase in the number of enlarged cells and a 3.5-fold increase in ^3H activity was observed (Text-fig. 1 and Fig. 5). However, when thymus cells from two tolerant donors were cultured together (Lew_T $\times$ BN_T), the MLR was depressed to approximately the levels of nonmixed controls (compare Figs. 5 and 6; Text-fig. 1). In addition,



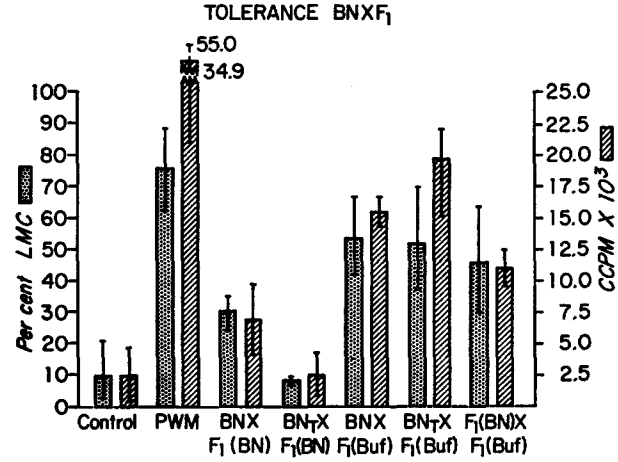
TEXT-FIG. 1. A graph demonstrating the results of MLR using as assays for transformation of thymus cells, the uptake of thymidine-^3H and the percentage of cells which after 3 days had nuclear diameters of greater than 7 μ (large and medium cells, LMC). The donors of the cells for the various types of cultures are indicated on the horizontal axis. The narrow bars represent the range of values observed. Lew, Lewis; BN, Brown Norway; T, tolerant; LE, Long Evans; ccpm, corrected counts per minute; and PWM, pokeweed mitogen.

transformation was observed to occur when one of the two donors was tolerant (Lew_T $\times$ BN; BN_T $\times$ Lew) and when either the tolerant or the nontolerant cells were mixed with cells from Long Evans donors (Lew $\times$ LE: Lew_T $\times$ LE; etc.). In the latter case, the Lewis or Brown Norway cells most probably responded to the Long Evans cells and vice versa. In the former case, the reaction was 25–35% less than that observed when both donors were nontolerant (Lew $\times$ BN).

Since the mixed lymphocyte reactions depicted in Text-fig. 1 were potentially bidirectional in nature, similar experiments were performed employing F_1 hybrid cells as the stimulating but nonresponding cell type (17). Results of these experiments may be seen in Text-fig. 2 and 3. In these experiments, when tolerant cells were exposed to cells from a hybrid rat possessing not only the donor's histocompatability antigens, but also those antigens to which the



TEXT-FIG. 2. A graph demonstrating the results observed when the MLR was used as an assay for tolerance of thymus cells. Culture conditions, parameters measured, and abbreviations are as in Text-fig. 1 with the following additions: F_1 (BN), F_1 (Lewis x BN) hybrid; F_1 (Buf), F_1 (Lewis x Buffalo) hybrid. In these cultures, the reactions represent a unidirectional response of the Lewis cells against the hybrid elements.



TEXT-FIG. 3. A graph depicting the results of experiments identical to those in Text-fig. 2 except that thymus cells from BN and not Lewis donors are employed.

donors were tolerant, no greater reactions occurred than were seen in nonstimulated control cultures. This may be seen by comparing the $Lew_T \times F_1$ (BN) and $BN_T \times F_1$ (BN) with the equivalent nontolerant mixtures. That this suppression was specific for the tolerance-inducing histocompatability antigens was demonstrated by exposing cells tolerant to Lewis or to BN antigens to

lymphoid elements from an unrelated F_1 (Buf) hybrid ($Lew_T \times F_1$ [Buf]; $BN_T \times F_1$ [Buf]). As may be seen in Text-fig. 2 and 3, the transformation observed in these cultures compared favorably with similar cultures of nontolerant cells. In the case of mixtures involving cells from Lewis donors, the reactions were unidirectional with the Lewis cells reacting against the Buffalo component of the F_1 hybrids. However, when cells from a Brown Norway donor were mixed with cells from an F_1 (Buf), the reaction was bidirectional with BN cells reacting against the Buffalo antigens and the Lewis $\times$ Buffalo cells reacting against the BN antigens. To convert this reaction to a one-way response, the cells of the F_1 hybrid were irradiated with 1000 R from a ^{60}Co source before culture was initiated. Under these conditions, the irradiated cells stimulated transformation of the BN cells but were incapable of enlarging themselves (18). It was concluded from these studies that the MLR was suppressed by the neonatal induction of tolerance. Moreover, this suppression was specific for the histocompatability antigens of the cells used to induce the tolerance. As such, the MLR correlated with the NLT reactions which were performed simultaneously and with the runting experiments which were conducted separately.

In three instances, tolerance induced by the method described above did not depress the MLR to control levels. In these cases, two of which involved mixtures of tolerant cells and one of which involved a mixture of tolerant Lewis and F_1 hybrid cells, the per cent LMC and thymidine-^3H uptake was 92–358% higher than nonmixed controls and 18–40% less than the nontolerant mixtures. In one of the three instances, the NLT reaction was slightly, if at all, present while NLT reactions were not performed in the other two. These findings were interpreted as indicating that at least one of the donors to the mixtures of tolerant cells and the single parental donor of the F_1 hybrid cultures were partially, if at all, tolerant. It may also be suggested from these observations that the MLR is a more sensitive measure of tolerance than is the NLT reaction.

DISCUSSION

While the classical skin graft has not been utilized in this study as a test for tolerance, previous investigators have applied this test to Lewis and BN rats after administering bone marrow cells during the neonatal period (11). Inducing tolerance by methods identical to those employed in the present study, these investigators found 100% of the injected animals to be tolerant to the histocompatability antigens of the injected cells as measured by skin graft survival. When this observation is coupled with the runt and NLT results of the present study, it is reasonable to conclude that the injected animals used for the mixed lymphocyte reactions were tolerant. Moreover, the fact that the cells from tolerant animals were able to produce runting and NLT reactions in unrelated or partially related strains of rats demonstrates the specificity of this tolerance. Since the donors of the cells for the suppressed MLR were tolerant as tested by

NLT reactions and runting, it was concluded that the MLR reflected the tolerant status of the animals tested.

If a test for tolerance is to be maximally useful, it must be simple, rapid, reproducible, sensitive, quantitable, and should require little, if any, manipulation of the "tolerant" animal. Certain characteristics of the MLR suggest that it fulfills the majority of these requirements. Included are (*a*) the in vitro nature of the reaction, (*b*) the small number of cells which are required for moderately reproducible results in 3 days, (*c*) a semiquantitable end point of thymidine-^{3}H uptake, (*d*) the sensitivity of the end point as compared with the relatively sensitive NLT reaction and the insensitive runt or skin graft assays, and (*e*) the minor manipulation required to obtain blood from the animal to be tested. In relation to the latter characteristic, since the thoracic duct lymph cells demonstrated the suppression of transformation, it may be assumed that blood lymphocytes would also manifest this phenomenon. Hence, the cells for the test may be obtained from the blood of the test animal without risking an alteration in his immune state with skin grafts or NLT reactions. It seems reasonable to suggest, therefore, that the MLR may be used as an in vitro test for tolerance.

In spite of considerable investigation, the exact nature of the MLR remains speculative. The depression of this reaction by the neonatal induction of tolerance strongly suggests an immune basis for the transformation. Further support for this concept may be found in studies with more classical antigens (19, 20). In these cases, the antigens were unable to induce the transformation of lymphoid cells or the production of antibody when cells from an animal exposed to the antigen immediatelyafter birth were subsequently reexposed to the same antigen in vitro. In addition, the MLR has been shown to be enhanced during and after rejection of a skin graft when cells from the donor and recipient of the skin were cultured together (5, 6). It has also been suggested that the inability of mixed cultures to survive beyond 8–9 days represented an in vitro homograft reaction with cellular destruction (21). However, a later report claimed prolonged survival when the cell mixtures were transferred to homologous layers of fibroblasts (22). In spite of this latter report, it appears that the majority of the evidence supports the concept that the MLR is an immune phenomenon governed by the histocompatability differences of the reacting cells.

In the mixtures of tolerant cells where the MLR was only partially suppressed, the question may be raised as to whether one or both of the tolerant donors were only partially tolerant. While no direct evidence is available, the following two observations suggest that, of the two donors, the Lewis was only partially tolerant to BN cells. First, Lewis animals are known to be more mature immunologically at birth than BN rats. This is evidenced by the larger number of cells required to induce tolerance and runt disease in these animals as compared with the BN and the shorter time interval after birth during which

successful tolerance-inducing injections may be made (11).[4] Secondly, of the four littermates of the BN donors employed in the two exceptional cases, none evidenced a lack of tolerance when their cells were analyzed by the one-way reactions involving F_1 hybrids. In contrast, the two Lewis rats which contributed cells to the partially tolerant mixtures were littermates.

SUMMARY

Tolerance, as defined by an inability to produce runt disease and the failure to elicit a normal lymphocyte transfer reaction, was induced in Lewis and Brown Norway rats by the neonatal injection of bone marrow from the opposite strain. When thymus, thoracic duct lymph, or lymph node cells from such tolerant Lewis and Brown Norway rats were cultured together, or were individually cultured with similar cells from an F_1 hybrid of these strains, transformation was suppressed to the levels observed in nonmixed control cultures. In contrast, mixtures of cells involving nontolerant donors demonstrated significant transformation as measured by per cent enlarged cells and thymidine-^{3}H uptake. The specificity of the tolerance was confirmed by the presence of transformed cells in mixtures involving cells from a tolerant donor and an unrelated or a partially related third strain of rats. From these results, it is suggested that the mixed lymphocyte reaction may be used as a simple test for tolerance and that it most likely represents an in vitro immune response.

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Addendum.—Since the preparation of this paper, a report has appeared in which a depression of the MLR was noted when the donor animals were tolerant as judged by skin graft survival (Wilson, D. B., W. K. Silvers, and P. C. Nowell. 1967. Quantitative studies on the mixed lymphocyte interaction in rats. II. Relationship of the proliferative response to the immunologic status of the donors. *J. Exptl. Med.* **126**:655). As such, the present study confirms and extends these findings.

BIBLIOGRAPHY

1. Bain, B., M. R. Vas, and L. Lowenstein. 1964. The development of large immature mononuclear cells in mixed leucocyte cultures. *Blood.* **23**:108.
2. Bach, F., and K. Hirschhorn. 1964. Lymphocyte interaction: a potential histocompatability test *in vitro*. *Science.* **143**:813.
3. Johnson, G. J., and P. S. Russell. 1967. Normal lymphocyte transfer and lymphocyte stimulation as practical histocompatability tests. *Brit. Med. J.* **2**:202.
4. Elves, M. W., and M. C. G. Israëls. 1965. Lymphocyte transformation in cultures of mixed leucocytes: a possible test of histocompatability. *Lancet.* **I**:1184.
5. Oppenheim, J. J., J. Wang, and E. Frei. 1965. The effect of skin homograft rejection on recipient and donor mixed leukocyte cultures. *J. Exptl. Med.* **122**:651.

[4] Schwarz, M. R. 1967. Unpublished data.

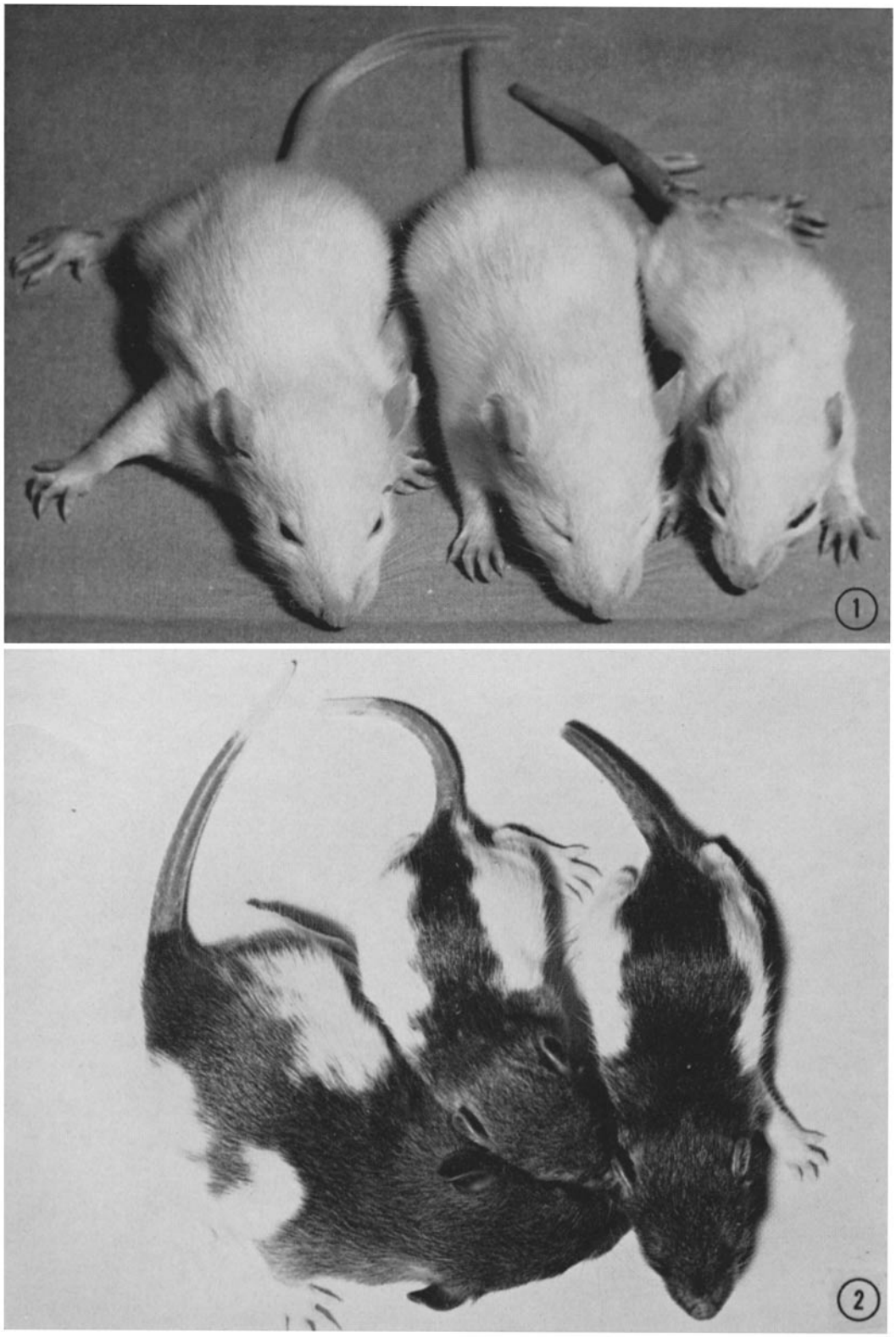
6. Moynihan, P. C., J. F. Jackson, and J. D. Hardy. 1965. Lymphocyte transformation as an *in vitro* histocompatability test. *Lancet.* **I**:453.
7. Harris, R., C. A. Clark, A. L. Jones, P. M. Sheppard, D. Lehane, M. McCarthy, S. D. Lawler, and H. S. Shatwell. 1966. Further studies on the normal lymphocyte transfer test in man. *Brit. Med. J.* **1**:509.
8. Schwarz, M. R. 1966. Blastogenesis in cultures containing genetically-disssimilar thymic cells. *J. Immunol.* **10**:281.
9. Billingham, R. E., and W. K. Silvers. 1959. Inbred animals and tissue transplantation immunity. *Plastic Reconstruc. Surg.* **23**:399.
10. Billingham, R. E., B. A. Hodge, and W. K. Silvers. 1962. An estimate of histocompatability loci in the rat. *Proc. Nat. Acad. Sci. U.S.* **48**:138.
11. Billingham, R. E., V. Defendi, W. K. Silvers, and D. Steinmuller. 1962. Quantitative studies on the induction of tolerance of skin homografts and on runt disease in neonatal rats. *J. Natl. Cancer Inst.* **28**:365.
12. Schwarz, M. R. 1967. Transformation of rat small lymphocytes with allogeneic lymphoid cells. *Am. J. Anat.* **121**:559.
13. Ford, W. L. 1967. A local graft-versus-host reaction following intradermal injection of lymphocytes in the rat. *Brit. J. Exptl. Pathol.* **48**:355.
14. Schwarz, M. R. 1967. Transformation of rat small lymphocytes with pokeweed mitogen (PWM). *Anat. Record.* In press.
15. Rieke, W. O., and M. R. Schwarz. 1964. The culture and karyotype of rat lymphocytes stimulated with phytohemagglutinin. *Anat. Record.* **150**:383.
16. Caffrey, R. W., N. B. Everett, and W. O. Rieke. 1966. Radioautographic studies of reticular and blast cells in the hemopoietic tissues of the rat. *Anat. Record.* **155**:41.
17. Silvers, W. K., D. B. Wilson, and J. Palm. 1967. Mixed leukocyte reactions and histocompatability in rats. *Science.* **155**:703.
18. Kasakura, S., and L. Lowenstein. 1967. Irradiated and preserved leucocytes in mixed leucocyte cultures. *Proc. Soc. Exptl. Biol. Med.* **125**:355.
19. Dutton, R. W. 1964. The effect of antigen on the proliferation of spleen cell suspensions from tolerant rabbits. *J. Immunol.* **93**:814.
20. Friedman, H. 1966. Absence of agglutinin formation in spleen cell cultures from immunologically tolerant mice. *J. Immunol.* **96**:289.
21. Maclaurin, B. P. 1965. Homograft interaction in the test tube. *Lancet.* **II**:816.
22. Stanfield, A. B., J. B. Cubberley, J. L. Parsons, and C. A. L. Stephens, Jr. 1967. *In vitro* studies of reacted human lymphocytes in perfusion chambers. *Ann. Allergy.* 25:359.

EXPLANATION OF PLATES

PLATE 97

FIG. 1. Three Lewis littermates 17 days after birth. The animal on the left is the noninjected littermate control. The animal in the center received, within 24 hr of birth, an intravenous injection of 40 million lymph node cells obtained from a BN donor. This donor had been injected with Lewis bone marrow cells during the neonatal period. The animal on the right received, within 24 hr of birth, an intravenous injection of 40 million lymph node cells obtained from a normal, noninjected Lewis donor. The animal on the right demonstrated the classical signs of runt disease while the recipient in the center showed no variation from the noninjected control.

FIG. 2. Three Long Evans littermates, 17 days postpartum. The animal on the left is the noninjected control. The animal in the center, within 24 hr of birth, was injected intravenously with 44×10^6 lymph node cells from a BN donor. This BN donor had received an injection of Lewis bone marrow cells within 24 hr of its birth and presumably, was tolerant. The animal on the right received, within 24 hr of birth, an intravenous injection of 44 million lymph node cells from the normal noninjected Lewis donor. Both animals which received cells evidenced runting, thus demonstrating the specificity of the tolerants depicted in Fig. 1.



(Schwarz: Tolerance and the mixed lymphocyte reaction)

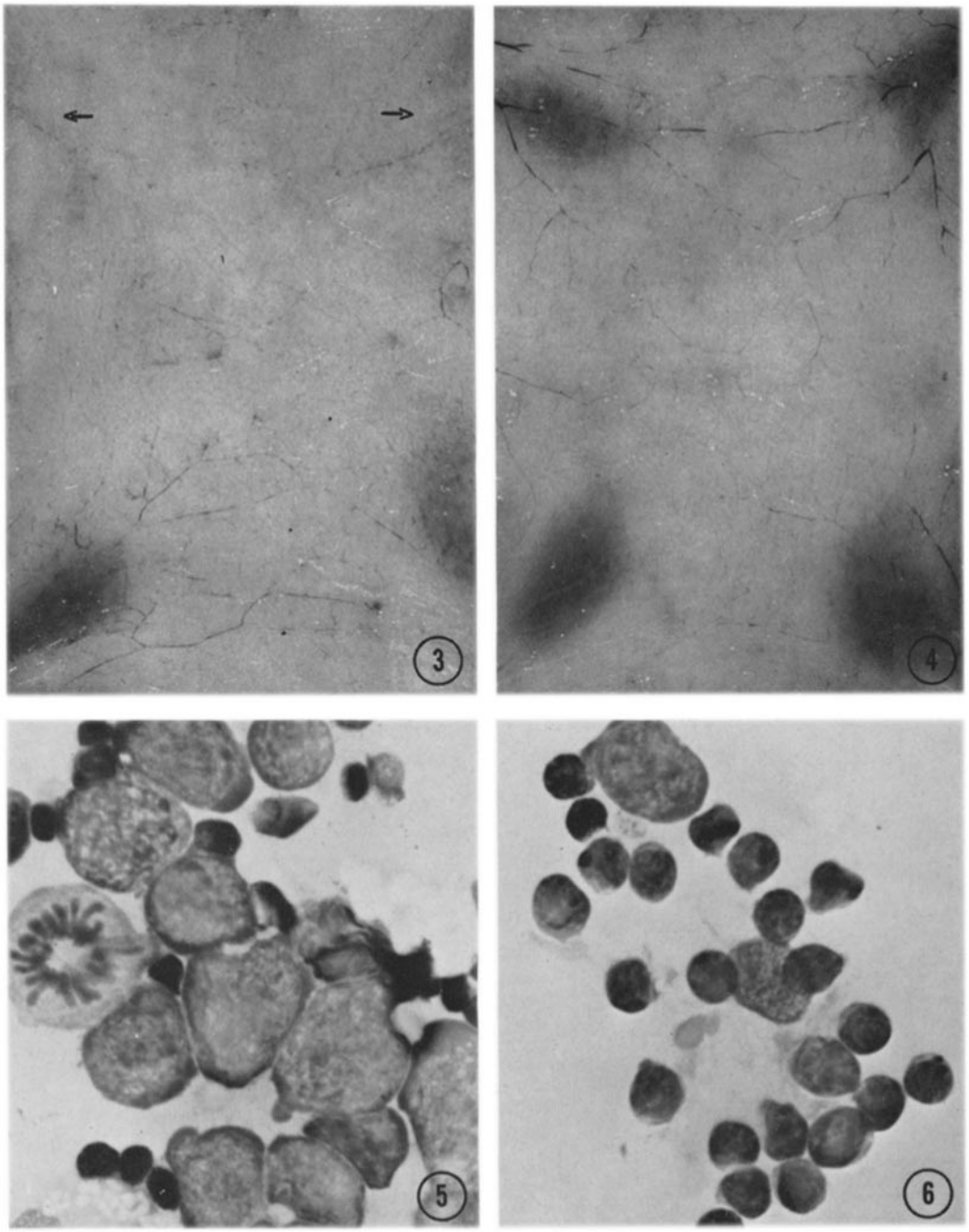
PLATE 98

FIG. 3. The skin from a F_1 (Lew x BN) hybrid which had been injected intradermally with 35×10^6 lymph node cells from a normal BN donor (lower right and lower left) and a BN donor tolerant to Lewis cells (upper right and upper left, at the arrows). Note the reaction produced by the nontolerant cells and the absence of reactions (arrows) at the site where the tolerant cells were injected. Tolerance was defined as an inability to produce runt disease (see Figs. 1 and 2). × 2.5.

FIG. 4. The skin from a F_1 (Lew x Buf) hybrid which had received 500 R total-body irradiation from a ^{60}Co source. 24 hr after irradiation, 36×10^6 lymph node cells were injected intradermally. The cells were obtained from a normal BN donor (lower right and left) and from a BN donor tolerant to Lewis histocompatability antigens (upper right and left). Tolerance was defined as in Fig. 3. Note that the cells from both donors initiated reactions which demonstrated that the tolerance was specific for the histocompatability antigens of the cells used to induce the tolerance. × 2.5.

FIG. 5. A high-power view of the cells observed in mixed cultures of thymus cells donated in equal numbers by a nontolerant Lewis and a nontolerant BN rat. Note the numerous transformed cells and the mitotic figure. × 900.

FIG. 6. Identical conditions to those described in Fig. 5 except that the cells were donated by a Lewis rat tolerant to BN cells and a BN animal tolerant to Lewis cells (see Figs. 1–4). Note the single enlarged cell and the large numbers of small lymphocytes. The cells are clustered about a phagocytic reticular cell, the cytoplasm of which is faintly discernible at the lower left. × 900.



(Schwarz: Tolerance and the mixed lymphocyte reaction)