EFFECT OF RECENT ANTIGEN PRIMING ON ADOPTIVE IMMUNE RESPONSES

I. SPECIFIC UNRESPONSIVENESS OF CELLS FROM LYMPHOID ORGANS OF MICE PRIMED WITH HETEROLOGOUS ERYTHROCYTES*

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Small lymphocytes from the thoracic duct lymph of rats (1) and mice (2) can initiate immune responses to antigens such as sheep erythrocytes and histoincompatible cells when transferred to appropriate hosts. Recently, it was demonstrated that for 1–2 days after the injection of such antigens in mice (3) or rats (4, 5), there was a specific deficiency in thoracic duct lymph of cells able to mount adoptive immune responses to these antigens. The results were interpreted in terms of antigen-induced selective recruitment of specific lymphocytes from the recirculating pool to appropriate lymphoid organs. As a corollary, it would be expected that at this time such organs, e.g. the spleen, would be enriched in cells specific for these antigens.

The present experiments were designed to test this corollary. They led to the unexpected and paradoxical observation that the reactivity of cells taken from lymphoid organs of mice given antigen 1–2 days previously was markedly and specifically depressed when tested in an adoptive transfer system under strict experimental conditions.

Materials and Methods

Animals.—In the experiments, the results of which are presented in Tables I–III and V and in Figs. 2–4 (performed at the Walter and Eliza Hall Institute), highly inbred CBA/H mice (obtained originally from Harwell, Didcot, Berkshire, England) were used. In the experiments, the results of which are presented in Tables IV and VI–IX and in Fig. 1 (performed at the Basel Institute for Immunology), a shortage of inbred strains necessitated the use of F1 crosses between CBA/J and C57BL/6 (the inbred strains being obtained originally from Jackson Laboratory, Bar Harbor, Maine). All mice were given Barastoc cubes and water ad libitum.

Cell Suspensions.—Thymus, spleen, bone marrow, mesenteric lymph nodes, and Peyer's

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patches were obtained from male and female mice aged 7-10 wk old. With the exception of bone marrow, cell suspensions were prepared by teasing the organs with fine forceps through an 80 mesh stainless steel sieve in cold Eisen's solution (6). Marrow cells were expressed from the femurs and tibiae by means of a syringe and needle using cold Eisen's solution. The marrow plugs were gently disrupted through a 26 gauge needle by aspiration. All cell suspensions were centrifuged once and resuspended in Eisen's solution except thymus cells, which were washed twice before resuspension. Counts of viable cells were performed using the trypan blue exclusion technique (7), the unstained cells being counted in a hemacytometer. The volume was then adjusted so that the cells could be injected in a volume of 0.2-0.5 ml.

Erythrocytes were obtained from the jugular veins of sheep (SRC) and horses (HRC) and stored in Alsever's solution. When required they were washed three times in saline and resuspended to an appropriate volume. In general, 10^9 erythrocytes were contained in 0.2 ml of a 25% suspension and 5 X 10^8 cells in 0.1 ml of a 25% suspension. The number of erythrocytes injected varied in different experiments and are shown in the tables.

**Injections.**—All suspensions of lymphoid cells and heterologous erythrocytes were injected intravenously via the lateral tail vein. Unless otherwise indicated, lymphoid cells were administered within 6 h of the recipients being irradiated.

**Irradiation.**—Mice were exposed to total body irradiation in a Perspex box. The dose given was 750 R to midpoint with maximum backscatter conditions and the machine operated under conditions of 250 kV, 15 mA, and a half-value layer of 1 mm of Cu. The focal skin distance was 50 cm and the absorbed dose rate 170 R per min. All irradiated mice received polymyxin B (100,000 IU/liter) and neomycin (10 mg/liter) in the drinking water.

**Thymectomy.**—Mice were thymectomized at 6 wk of age according to the technique outlined by Miller (8). Thymectomized mice were irradiated 3 wk after the operation.

**Plaque-Forming Cell (PFC) Assays.**—Spleen cell suspensions were prepared as mentioned above, washed once, and diluted to an appropriate volume in Eisen's solution so that 0.1 ml contained an estimated 100-1,000 PFC. Direct (19S) PFC were estimated by the method of Cunningham and Szenberg (9). Indirect (7S) PFC were determined by adding a rabbit anti-mouse immunoglobulin serum to the reactive mixture (10, 11). The number of 7S PFC was derived, by difference, from numbers obtained in assays performed in the presence or absence of this antiserum.

**Rosette-Forming Cell (RFC) Assays.**—Spleen cell suspensions and heterologous erythrocytes were mixed, centrifuged, and gently resuspended according to the method of Wilson (12). Only lymphocytes surrounded by a complete halo of erythrocytes were scored as rosettes. In order to estimate the number of RFC in a given suspension, spleen cells were screened using hemacytometers until 20-40 rosettes had been counted. This involved screening 5-15 X 10^4 spleen cells.

**Hemagglutinin assays** were performed by the method of Dietrich (13).

**Antisera.**—Anti-0-C3H serum was prepared by giving 10^7 CBA/J thymus cells once weekly to AKR mice as described by Reif and Allen (14). The mice were bled 7-10 days after the last of 9-10 injections. Serum from (CBA/J X C57BL/6)F1 mice was used as normal mouse serum (NMS). All sera were inactivated at 56°C for 30 min before use. Fresh guinea pig serum was used as a source of complement in experiments in which antisera were used to kill appropriate target cells.

**Statistical Analyses.**—Geometric mean and standard errors (SE) of the means were calculated from the log_{10} of the PFC counts. Arithmetic means and SE of the means were calculated from the reciprocal of the log_{10} values for the hemagglutinin titers. P values were deter-

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1 Abbreviations used in this paper: HRC, horse erythrocytes; NMS, normal mouse serum; NS, not significant; PFC, plaque-forming cells; 19S PFC, direct plaque-forming cells; 7S PFC, indirect (developed) plaque-forming cells; RFC, rosette-forming cells; SRC, sheep erythrocytes.
RESULTS

Specific Unresponsiveness of Lymphocytes Transferred 24 h after In Vivo Exposure to Heterologous Erythrocytes.—The adoptive response of spleen cells from normal mice given antigen 24 h before, i.e. "primed" for 24 h, was investigated as follows: normal mice were injected intravenously either with 10⁹ SRC, 10⁹ HRC, or left un.injected. 24 h later, 3 × 10⁷ viable spleen cells were given intravenously together with 5 × 10⁸ SRC and 5 × 10⁸ HRC to irradiated mice, and splenic PFC were measured at 7 days. Whereas spleen cells from unprimed mice (group 1, Table I) responded well to both SRC and HRC, cells from donors primed to SRC 1 day before transfer (group 2) responded well to HRC but poorly to SRC. Likewise, cells from donors primed to HRC 1 day previously responded well to SRC but not to HRC (group 3).

Results similar to those shown in Table I were obtained in more than 20 other experiments. In only two of these did the administration of one antigen to spleen cell donors 24 h before transfer fail to reduce the adoptive immune 7S PFC response to this antigen by more than 30-fold, as compared with the response obtained to this same antigen with spleen cells from donors primed to another antigen. The reduction in the 19S PFC response varied considerably in different experiments but it was usually more than 10-fold. Unresponsiveness was highly specific in that the response to the antigen to which the spleen cell donors had not been primed was usually not significantly lower than that

| TABLE I
| PFC in Spleens of Irradiated Mice Injected 7 Days before with SRC, HRC, and Spleen Cells from Mice Primed for 24 h with SRC or HRC

| Antigen given to donors of spleen cells 24 h before transfer | PFC per spleen at 7 days |
|-------------------------------------------------------------|-------------------------|
| Group no. | Anti-SRC | Anti-HRC |
|-----------|----------|----------|
| 19S | 7S | 19S | 7S |
| None | 1 | 1,150* | 62,580 | 1,920 | 15,030 |
| | | (3,920-2,860) | (73,850-53,040) | (2,450-1,500) | (17,290-13,070) |
| SRC | 2 | 280 | 1,020 | 1260 | 13,540 |
| | | (350-170) | (1,300-790) | (1,610-980) | (16,790-10,920) |
| HRC | 3 | 2,190 | 48,560 | 60 | 70 |
| | | (3,070-1,570) | (65,560-35,870) | (70-50) | (90-60) |

* 0.1 ml 25% suspension of erythrocytes (5 × 10⁷) given intravenously.
* 3 × 10⁷ viable cells injected intravenously.
* Spleen cells and antigen were given within 6 h of irradiating the recipients; results similar to those shown here were obtained when the recipients were irradiated 3 days before transfer.

**P values between groups—19S PFC to SRC: 1 cf. 2, <0.0005; 1 cf. 3, NS; 2 cf. 3, <0.005. 19S PFC to HRC: 1 cf. 2, <0.0005; 1 cf. 3, NS; 2 cf. 3, <0.005. 7S PFC to SRC: 1 cf. 2, <0.0005; 1 cf. 3, NS; 2 cf. 3, <0.005. 7S PFC to HRC: 1 cf. 2, NS; 1 cf. 3, <0.0005; 2 cf. 3, <0.0005.**

* Geometric mean, upper and lower limits of SE, five mice per group.
obtained by transferring unprimed spleen cells. Only in a few experiments was a two to threefold decrease observed as exemplified in Table II.

The specific unresponsiveness of lymphoid cells adoptively transferred from donors primed 1 day before was not restricted to spleen cells but was also observed with cells from mesenteric lymph nodes and Peyer’s patches (Table II). Here, as in the preceding experiments, the PFC responses were measured 7 days after cell transfer.

The hemagglutinin titer in serum of irradiated mice, obtained 7 days after cell transfer, were markedly and specifically reduced when the cell donors had received antigen 24 h before transfer (Table III). This excluded the possibility that the transferred spleen cells might have produced antibody in regions other than the spleen.

In the above experiments, specific unresponsiveness of cells exposed to antigen for 24 h was revealed upon adoptive transfer. Would such unresponsive-

### Table II

| Cells injected together with SRC and HRC | No. of mice per group | PFC per spleen at 7 days |
|-----------------------------------------|-----------------------|-------------------------|
|                                          |                       | Anti-SRC               | Anti-HRC               |
| 19S                                     |                       | 24,580                 | 181,150                |
| 2X 10^7 unprimed spleen                 | (28,030-21,570)       | (992,400-227,100)      | (29,490-21,180)        |
| 2X 10^7 unprimed lymph node              | 13,360                | 106,360                | 5,080                  |
|                                          | (126,470-49,380)      | (6,580-5,410)          | (57,590-54,200)        |
| 2X 10^7 unprimed                        | 3,190                 | 42,450                 | 2,690                  |
| Peyer’s patch                           | (5,970-3,510)         | (6,820-2,948)          | (2,779-1,929)          |
| 3X 10^7 SRC-primed spleen               | 1,890                 | 590                    | 7,130                  |
|                                          | (2,280-1,560)         | (1,128-29,480)         | (4,880-10,290)         |
| 2X 10^7 SRC-primed lymph node            | 3,300                 | 240                    | 6,750                  |
|                                          | (570-290)             | (1,190-50)             | (8,170-5,580)          |
| 3X 10^7 HRC-primed spleen               | 12,610                | 105,310                | 1,170                  |
|                                          | (15,380-10,340)       | (130,580-84,930)       | (650-250)              |
| 2X 10^7 HRC-primed lymph node            | 12,090                | 100,830                | 530                    |
|                                          | (16,650-10,410)       | (115,930-87,680)       | (570-200)              |
| 10^6 HRC-primed                         | 1,450                 | 16,940                 | 90                     |
| Peyer’s patch                           | (1,695-1,210)         | (20,330-14,120)        | (100-60)               |

*P values between groups—19S PFC to SRC: 1 cf. 4, <0.0005; 1 cf. 7, <0.005; 4 cf. 7, <0.005; 2 cf. 8, NS; 5 cf. 7, <0.005; 3 cf. 9, <0.005; 4 cf. 9, <0.005; 1 cf. 7, <0.005; 4 cf. 7, <0.005; 2 cf. 8, NS; 3 cf. 9, <0.005; 3 cf. 9, <0.005; 3 cf. 9, <0.005; 4 cf. 9, <0.005; 1 cf. 7, <0.005; 4 cf. 7, <0.005; 2 cf. 8, NS; 3 cf. 9, <0.005; 3 cf. 9, <0.005; 4 cf. 9, <0.005; 1 cf. 7, <0.005; 4 cf. 7, <0.005; 2 cf. 8, NS; 3 cf. 9, <0.005; 3 cf. 9, <0.005; 4 cf. 9, <0.005; 1 cf. 7, <0.005; 4 cf. 7, <0.005; 2 cf. 8, NS; 3 cf. 9, <0.005; 3 cf. 9, <0.005; 4 cf. 9, <0.005. 7S PFC to SRC: 1 cf. 4, <0.005; 4 cf. 7, <0.005; 1 cf. 7, <0.005; 2 cf. 8, NS; 3 cf. 9, <0.005; 3 cf. 9, <0.005; 4 cf. 9, <0.005; 1 cf. 7, <0.005; 4 cf. 7, <0.005; 2 cf. 8, NS; 3 cf. 9, <0.005; 3 cf. 9, <0.005; 4 cf. 9, <0.005. 7S PFC to HRC: 1 cf. 4, <0.005; 4 cf. 7, <0.005; 1 cf. 7, <0.005; 2 cf. 8, NS; 3 cf. 9, <0.005; 3 cf. 9, <0.005; 4 cf. 9, <0.005. 10^6 PFC to SRC: 1 cf. 4, <0.005; 4 cf. 7, <0.005; 1 cf. 7, <0.005; 2 cf. 8, NS; 3 cf. 9, <0.005; 3 cf. 9, <0.005; 4 cf. 9, <0.005; 1 cf. 7, <0.005; 4 cf. 7, <0.005; 2 cf. 8, NS; 3 cf. 9, <0.005; 3 cf. 9, <0.005; 4 cf. 9, <0.005. 10^6 PFC to HRC: 1 cf. 4, <0.005; 4 cf. 7, <0.005; 1 cf. 7, <0.005; 2 cf. 8, NS; 3 cf. 9, <0.005; 3 cf. 9, <0.005; 4 cf. 9, <0.005. 10^6 PFC to SRC: 1 cf. 4, <0.005; 4 cf. 7, <0.005; 1 cf. 7, <0.005; 2 cf. 8, NS; 3 cf. 9, <0.005; 3 cf. 9, <0.005; 4 cf. 9, <0.005. 10^6 PFC to HRC: 1 cf. 4, <0.005; 4 cf. 7, <0.005; 1 cf. 7, <0.005; 2 cf. 8, NS; 3 cf. 9, <0.005; 3 cf. 9, <0.005; 4 cf. 9, <0.005. 10^6 PFC to SRC: 1 cf. 4, <0.005; 4 cf. 7, <0.005; 1 cf. 7, <0.005; 2 cf. 8, NS; 3 cf. 9, <0.005; 3 cf. 9, <0.005; 4 cf. 9, <0.005. 10^6 PFC to HRC: 1 cf. 4, <0.005; 4 cf. 7, <0.005; 1 cf. 7, <0.005; 2 cf. 8, NS; 3 cf. 9, <0.005; 3 cf. 9, <0.005; 4 cf. 9, <0.005.

1. Number of viable cells given intravenously.
2. 0.2 ml of 25% suspension of erythrocytes given intravenously.
3. 0.1 ml of 25% suspension of erythrocytes (5X 10^7) given intravenously.
4. Geometric mean, upper and lower limits of SE.
ness have occurred had the cells not been transferred? To determine this, normal mice received two doses of antigen 24 h apart; either $10^8$ SRC or $10^9$ HRC were given intravenously, and 1 day later both groups of mice and a third group that had not been given erythrocytes were injected intravenously with $5 \times 10^8$ SRC and $5 \times 10^9$ HRC. PFC in the spleens of these mice were measured 7 days later. As seen in Table IV, injecting two doses of the same antigen 24 h apart had no effect on the $19S$ and $7S$ PFC response to that antigen but produced a three to fivefold decrease in the response to the other antigen, probably as a result of antigenic competition (15).

Specific unresponsiveness after adoptive transfer was also demonstrable with cells from mice primed to antigen 1 mo previously. However this occurred only if these mice received the same antigen 24 h before their spleen cells.

| TABLE III |
|-----------------------------|
| Hemagglutinin Titers of Serum Obtained from Irradiated Mice 7 Days after Injection of SRC, HRC, and Spleen Cells from Mice Primed with SRC or HRC 24 h Previously |

| Antigen given to donors of spleen cells 24 h before transfer | 1/log₂ Hemagglutinin titers | Anti-SRC | P value | Anti-HRC | P value |
|-------------------------------------------------------------|-----------------------------|----------|---------|----------|---------|
| SRC                                                         | 1.1±0.1                     | <0.0005  | 5.8±0.3 | <0.0005  |
| HRC                                                         | 7.0±0.4                     |          |         |          |

* 0.1 ml of 25% suspension of erythrocytes ($5 \times 10^8$) given intravenously.
† $3 \times 10^9$ viable cells given intravenously.
§ 0.2 ml of 25% suspension of erythrocytes ($10^9$) given intravenously.
|| Arithmetic mean ± SE, six mice per group.

| TABLE IV |
|-----------------------------|
| PFC in Spleens of Normal Mice Primed with Either SRC or HRC and Challenged 24 h Later with Both SRC and HRC |

| Antigen injected at Day 0* | Day 11 Group | PFC per spleen at day 8 Anti-SRC | Anti-HRC |
|----------------------------|--------------|---------------------------------|---------|
|                            |              | 19S | 7S | 19S | 7S |
| None                       | SRC + HRC    | 19S | 7S | 19S | 7S |
| SRC + HRC                 | 2            | 5,720-5,800 (70,260-41,560) | 4,730   | 38,190 |
| HRC + SRC                 | 3            | 1,550 (14,330-14,150) | 1,930   | 9,310 |

P values between groups—$19S$ PFC to SRC: 1 cf. 2, NS; 1 cf. 3, <0.01; 2 cf. 3, NS. $7S$ PFC to SRC: 1 cf. 2, NS; 1 cf. 3, <0.005; 2 cf. 3, <0.005. $19S$ PFC to HRC: 1 cf. 2, <0.005; 1 cf. 3, NS; 2 cf. 3, NS. $7S$ PFC to HRC: 1 cf. 2, <0.005; 1 cf. 3, NS; 2 cf. 3, <0.005.

* Antigen given as 0.2 ml of 25% suspension of erythrocytes ($10^9$) intravenously.
† Antigen given as 0.1 ml of 25% suspension of erythrocytes ($5 \times 10^8$) intravenously.
§ Geometric mean, upper and lower limits of SE, six mice per group.
were transferred. The results of such an experiment are shown in Table V. Groups of normal mice were injected intravenously with SRC or HRC. 1 mo later half the mice in each group were given the same antigen. 24 h later, \(10^7\) spleen cells from mice of each of the four groups were transferred to irradiated recipients together with both SRC and HRC and numbers of PFC measured at 7 days. Challenge of the cells with the priming antigen 24 h before transfer produced a five to sixfold reduction in both the 19S and 7S response to this antigen, except in the case of the 19S response to HRC where a 30-fold decrease occurred.

Certain parameters were common to the experiments described above: (a) a dose of \(10^6\) erythrocytes was used to prime the cell donors; (b) cells were transferred 24 h after priming; (c) irradiated recipients of cells were challenged with antigen immediately upon transfer; (d) the PFC responses were measured 7 days after cell transfer. The effects of varying each of these four parameters individually will now be considered.

**Dose of priming antigen:** Only high doses of the priming antigen rendered lymphoid cells unresponsive upon adoptive transfer. Thus intravenous injections of from \(10^4\) to \(10^6\) SRC to donors 24 h before cell transfer produced no decrease in either the 19S (Fig. 1 a) or 7S (Fig. 1 b) response to SRC. A marked decrease was observed with higher doses of antigen and with \(10^6-10^9\) SRC

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**TABLE V**

| PFC in Spleens of Irradiated Mice Injected with Spleen Cells* from Donors Primed with SRC or HRC 1 mo Previously. Effect of Injecting Donors with a Second Dose of Antigen 24 h Before Adoptive Transfer |
|---|---|---|---|
| **Antigen given to spleen cell donors before adoptive transfer** | **Antigen injected together with transferred spleen cells** | **PFC per spleen at 7 days** |
| | Group no. | | Anti-SRC | Anti-HRC |
| | | | 19S | 7S | 19S | 7S |
| SRC | none | SRC + HRC | 1 | 11,810 | 119,330 | 1,350 | 10,070 |
| | | | | (19,050-11,130) | (120,490-112,580) | (1,650-1,100) | (16,010-6,330) |
| SRC | SRC | SRC + HRC | 2 | 2,000 | 21,980 | 1,050 | 5,430 |
| | | | | (1,780-2,630) | (21,950-29,150) | (1,220-990) | (6,760-4,730) |
| HRC | none | SRC + HRC | 3 | 2,910 | 24,860 | 21,390 | 65,990 |
| | | | | (3,320-2,560) | (29,500-29,150) | (23,960-19,100) | (75,890-57,180) |
| HRC | HRC | SRC + HRC | 4 | 6,500 | 25,520 | 11,540 | 660 |
| | | | | (5,580-3,630) | (42,660-25,750) | (43,640-6,410) | (1,060-410) |

*P values between groups—19S PFC to SRC: 1 ef. 2, <0.0005; 3 cf. 4, NS. 7S PFC to SRC: 1 ef. 2, <0.0005; 3 cf. 4, NS. 19S PFC to HRC: 1 ef. 2, NS; 3 cf. 4, <0.0005. 7S PFC to HRC: 1 ef. 2, NS; 3 cf. 4, <0.0005.
* \(10^9\) viable cells given intravenously.
1 0.1 ml of 25% suspension of erythrocytes (5 × 10^9) given intravenously.
2 0.2 ml of 25% suspension of erythrocytes (10^9) given intravenously.
3 Geometric mean, upper and lower limits of SE, six mice per group.
the response to SRC was virtually abolished. Such doses of SRC produced no reduction in the responses to HRC.

Interval between priming and adoptive transfer: Normal mice were injected with SRC or HRC and then killed immediately or 1, 2, 3, or 5 days later. Spleen cells from each group were injected into irradiated mice, together with SRC and HRC, and PFC responses were measured 7 days after transfer. Responses obtained with spleen cells transferred immediately after the donors had received one of the two antigens were similar to those occurring in recipients of cells from donors not given this antigen (Fig. 2). By contrast, and as already shown in experiments described above, a delay of 1 day between antigen priming and cell transfer resulted in a marked and specific reduction of both 19S and 7S PFC responses to the antigen used for priming. This reduction was much less when the cells were transferred 2 days after antigen priming and was not detectable at 3 or 5 days postpriming. A reduction was observed after 2 days’ priming in the 19S PFC response of the transferred cells to the antigen not used for the priming. The 7S PFC responses here were not reduced.

Interval between adoptive transfer and antigen challenge: Spleen cells from normal mice injected with SRC or HRC 24 h previously were transferred to irradiated mice that were then challenged with both SRC and HRC either immediately or 1, 5, 9, or 16 days later. PFC responses in mice of each group were determined 7 days after antigen challenge. In order to exclude the possi-
specific unresponsiveness in recently primed cells

Fig. 2. Numbers of 19S PFC to SRC (group a) and to HRC (group b) and of 7S PFC to SRC (group c) and to HRC (group d) in spleens of irradiated mice 7 days after intravenous injection of $5 \times 10^8$ SRC and $5 \times 10^8$ HRC given together with $3 \times 10^7$ viable spleen cells from mice injected intravenously at various times before transfer with $10^9$ SRC (●) or $10^9$ HRC (○). Geometric means of the results obtained from six mice are given, and the vertical bars represent the upper and lower limits of SE. P values between groups—(a): day 1, <0.0005; day 2, <0.0005; day 3, <0.0005; (b): day 1, <0.0005; day 2, NS; day 3, NS; (c): day 1, <0.0005; day 2, <0.0005; day 3, NS; (d): day 1, <0.0005; day 2, <0.0005; day 3, NS.

Bility that hemopoietic stem cells present in the spleen cell suspensions might differentiate to T cells in the irradiated host and thus obscure the response of the transferred spleen cells per se, all recipients were thymectomized 3 wk before irradiation. A marked specific reduction in PFC responses was observed when the challenge antigen was given together with the transferred spleen cells or 1 day later (Fig. 3). This applied to both the 19S and 7S PFC responses except in the case of the 19S PFC response to HRC (Fig. 3 b) where a delay of 1 day in the administration of the challenge antigen was associated with a response similar to that obtained with cells from donors not primed to HRC. Striking differences were observed when challenge with antigen was delayed.
Fig. 3. Numbers of 19S PFC to SRC (group a) and to HRC (group b) and of 7S PFC to SRC (group c) and to HRC (group d) in spleens of irradiated mice 7 days after intravenous injection of 5 x 10^8 SRC and 5 x 10^8 HRC given either together with—or 1, 5, 9, or 16 days after—an inoculum of 3 x 10^7 viable spleen cells from donor mice primed 24 h previously with 10^8 SRC (●) or 10^8 HRC (O) intravenously. Geometric means of the results obtained from six mice are given, and vertical bars represent upper and lower limits of SE. P values between groups—(a): day 1, <0.0005; day 5, NS; day 9, <0.025; (b): day 1, <0.05; day 1, NS; day 5, <0.05; day 9, <0.01; (c): day 1, <0.0005; day 5, <0.01; day 9, <0.0005; day 5, <0.0005; day 9, <0.01.

for 5 days. Thus, in the case of the 7S PFC response to SRC, the numbers of PFC given by SRC-primed spleen cells significantly exceeded those given by HRC-primed spleen cells (Fig. 3 c). Similar changes were noted with both the 19S and 7S PFC responses to HRC (Fig. 3 b and d), although the 19S PFC response to SRC was similar whether or not the spleen cells were taken from donors primed to this antigen (Fig. 3 a). It is clear from Fig. 3 that increasing the time interval between cell transfer and antigen challenge from 1
to 5 days was associated not only with an increase in the response of the primed cells to the priming antigen but also with a concomitant decrease in the response to this antigen by cells primed to the other antigen. Essentially similar changes were observed when antigen challenge was delayed for 9 or 16 days.

*Interval between antigen challenge and PFC assays:* In order to determine whether responses occurred at times other than 7 days after cell transfer and antigen challenge, splenic PFC were measured in irradiated mice at 5, 7, 8, and 11 days after transfer of SRC-primed or HRC-primed spleen cells given together with both SRC and HRC. Whereas a reduction in both 19S and 7S PFC responses to the priming antigen was evident at 5 and 7 days, it was less apparent at 8 days, and only very slight at 11 days (Fig. 4). In the case of the 19S PFC responses, these changes can largely be accounted for by a decline in the PFC responses of the respective control groups after 7 days. With the 7S responses, however, a clear increase was observed between 7 and 11 days and associated with only a slight decrease in the responses of the controls.

Specific Unresponsiveness in Both T and B Lymphocytes.—Since the PFC response of mice to sheep erythrocytes requires collaboration between thymus-derived (T) and non-thymus-derived (B) cells (16, 17), it was of interest to determine whether the specific unresponsiveness of recently primed cells observed on adoptive transfer was due to a defect occurring in either T cells, B cells, or in both types of cells. This was investigated by determining whether the unresponsiveness could be reversed by adding either normal T or normal B cells. The experimental design was the same as that used to obtain the results shown in Table I. Thus spleen cells primed to SRC or HRC 24 h before were transferred either with SRC and HRC alone or together with 10^6 thymus cells or 2 × 10^7 bone marrow cells; thymus and bone marrow were used as sources of T and B cells, respectively (17). As shown in Table VI, the addition of bone marrow to SRC- or HRC-primed spleen cells did not overcome the unresponsiveness of the cells to the priming antigen (groups 3 and 6); this applied to both the 19S and 7S PFC response. Thymus cells, by contrast, did produce partial reconstitution of the response (groups 4 and 7), particularly the 19S response. That thymus and bone marrow were suitable as sources of T and B cells in these experiments was shown by their capacity to respond to the antigens when injected together (group 10) but not when injected separately (groups 8 and 9).

The failure of thymus cells to effect full reconstitution of the response of the antigen-primed spleen cells might have been due to one of the following: (a) suboptimal numbers of competent T cells in the suspensions used; (b) existence of suppressor T cells (18); (c) specific unresponsiveness affecting the B cell population of the primed spleen.

In order to investigate these possibilities, spleen cells from normal mice or from mice primed 24 h before with SRC or HRC were incubated in vitro with anti-θ-serum and complement (see footnote to Table VII) so as to eliminate
Fig. 4. Numbers of 19S PFC to SRC (group a) and to HRC (group b) and of 7S PFC to SRC (group c) and to HRC (group d) in spleens of irradiated mice at various times after intravenous injection of $5 \times 10^8$ SRC and $5 \times 10^5$ HRC given together with $3 \times 10^7$ viable spleen cells from donor mice primed 24 h previously with $10^9$ SRC (●) or $10^9$ HRC (○) intravenously. Geometric means of the results obtained from six mice are given, and vertical bars represent upper and lower limits of SE. P values between groups—(a): day 7, <0.0005; day 8, NS; day 11, NS; (b): day 7, <0.0005; day 8, NS; day 11, NS; (c): day 7, <0.0005; day 8, <0.0005; day 11, <0.025; (d): day 7, <0.0005; day 8, <0.0005; day 11, <0.01.

T cells. The effect of adding $10^8$ normal thymus cells to such spleen cell suspensions was then investigated by adoptive transfer. PFC responses in irradiated recipients were measured at 7 days and compared with responses given in recipients of primed spleen cells treated in vitro with NMS and complement before transfer. The results are shown in Table VII. It is clear that anti-θ-
### Table VI

| Cells injected together with SRC§ and HRC§ | PFC per spleen at 7 days | Anti-SRC | Anti-HRC |
|------------------------------------------|--------------------------|----------|----------|
|                                          |                          | 19S      | 7S       | 19S      | 7S       |
| Unprimed spleen                          |                          | 1        |          | 2        |          |
|                                          | (14,470)                 | 159,560  | 9,940    | 133,570  |
| SRC-primed spleen                        |                          | 2        |          | 3        |          |
|                                          | (780-410)                | (197,850)| (128,680)| (225,830)| (131,590)|
| SRC-primed spleen +                      |                          | 3        |          | 4        |          |
| 2 × 10⁷ bone marrow                      |                          | (200-90) | (190-10) | (11,000-1,010) | (166,500-109,000) |
| SRC-primed spleen + 10⁶ thymus           |                          | 4        |          | 5        |          |
|                                          | (1,840-2,950)            | (10,000-6,400) | (16,620-21,420) | (451,090-230,870) |
| HRC-primed spleen                        |                          | 5        |          | 6        |          |
|                                          | (27,780-11,170)          | (370,310-222,330) | (1,470-1,010) | (2,590-130) |
| HRC-primed spleen + 10⁶ thymus           |                          | 6        |          | 7        |          |
| 2 × 10⁷ bone marrow                      | (29,320-16,690)          | (135,160-87,970) | (660-330) | (1,170-70) |
| HRC-primed spleen + 10⁶ thymus           |                          | 7        |          | 8        |          |
| 2 × 10⁷ bone marrow +                    | (35,830-23,710)          | (338,330-275,750) | (14,860-5,100) | (7,370-4,100) |
|                                          | (10-1)                   | (10-1)   | (10-1)   | (10-1)   |
| 10⁸ thymus                               |                          | 9        |          | 10       |          |
|                                          | (20-1)                   | (10-1)   | (10-1)   | (10-1)   |
|                                          | (110-1)                  | (160-1)  | (20-1)   | (20-1)   |
| 2 × 10⁷ bone marrow + 10⁶ thymus         |                          | 10       |          | 11       |          |
|                                          | (1,380)                  | 3,520    | 1,110    | 6,190    |
|                                          | (1,930-980)              | (6,620-4,600) | (1,500-820) | (8,630-1,750) |

*P* values between groups—19S PFC to SRC: 1 cf. 2, <0.0005; 2 cf. 3, <0.01; 2 cf. 4, <0.0005; 4 cf. 5, <0.01. 7S PFC to SRC: 1 cf. 2, <0.0005; 2 cf. 3, NS; 2 cf. 4, <0.05; 2 cf. 5, <0.0005; 4 cf. 5, <0.01. 19S PFC to HRC: 1 cf. 5, <0.02; 5 cf. 6, <0.02; 2 cf. 7, <0.005; 7 cf. 7, NS; 2 cf. 5, <0.01. 7S PFC to HRC: 1 cf. 5, <0.0005; 5 cf. 6, NS; 5 cf. 7, <0.005; 1 cf. 7, <0.0005; 2 cf. 5, <0.005.

| 10⁸ thymus | 110-30 |
| 2 × 10⁷ bone marrow | 1,380 |

† ‡ PFC treatment of normal spleen cells caused a marked reduction in both 19S and 7S PFC responses to SRC and HRC after transfer and that this reduction was largely prevented by supplementing with thymus cells. Likewise, the effect of pretreatment with anti-β-serum in abolishing the response of antigen-primed spleen cells to the antigen not used for priming was not evident when thymus cells were also given. These results imply that the thymus cell suspensions used contained sufficient numbers of competent T cells to allow normal adoptive responses. When thymus cells were added to antigen-primed spleen cells pretreated with anti-β-serum, PFC responses to the priming antigen, however, were only slightly, though still significantly, above those given by NMS-incubated primed cells.

The results obtained above thus suggested that defects in both T and B cells contributed to the failure of recently primed spleen cells to respond on adoptive transfer to the priming antigen. There was no evidence that T cells in the transferred spleens were suppressing the response. Hence complete restoration should occur by supplementing the spleen cells with a mixture containing both T and...
TABLE VII

PFC in Spleens of Irradiated Mice Injected with Anti-0-Serum-Treated Spleen Cells from Unprimed Mice or from Mice Primed 24 h Previously with SRC* or HRC*. Partial Restoration of the Immune Response by Injection of Normal Thymus Cells

| Cells injected together with SRC* and HRC* | Serum used for incubation of spleen cells in vitro in presence of complement | PFC per spleen at 7 days | Group no. |
|------------------------------------------|--------------------------------------------------------------------------------|-------------------------|----------|
|                                          |                                                                              | Anti-SRC    | Anti-HRC |
| Unprimed spleen NMS 1                    | 8,620f                                                                           | 107,440      | 3,060    | 90,220 |
|                                          | (10,610-7,010)                                                                   | (136,440-84,600)     | (3,350-7,780) | (101,050-80,560) |
| Unprimed spleen Anti-0 2                 | 230                                                                              | 2,140        | 10       | 200    |
|                                          | (600-170)                                                                        | (3,240-1,540)       | (30-4)   | (370-190) |
| Unprimed spleen Anti-0 3                 | 2,180                                                                            | 41,740       | 2,760    | 50,520 |
|                                          | (3,590-2,810)                                                                    | (56,370-30,030)    | (3,420-2,230) | (59,100-43,180) |
| SRC-primed spleen NMS 4                  | 150                                                                              | 200          | 1,516    | 34,710 |
|                                          | (250-90)                                                                         | (380-50)       | (1,110-1,370) | (47,890-25,130) |
| SRC-primed spleen Anti-0 5               | 40                                                                               | 20           | 20       | 240    |
|                                          | (50-30)                                                                          | (30-7)        | (50-5)   | (330-160) |
| SRC-primed spleenAnti-0 6                | 1,430                                                                            | 3,990        | 1,920    | 41,420 |
|                                          | (1,739-1,150)                                                                    | (5,166-5,310)     | (2,570-1,430) | (45,560-37,650) |
| SRC-primed spleen + 10^8 thymus          |                                                                                 | Anti-SRC     | Anti-HRC |
| HRC-primed spleen NMS 7                  | 2,530                                                                            | 51,620       | 180      | 30     |
|                                          | (3,110-2,040)                                                                    | (57,820-46,090)   | (210-130) | (110-10) |
| HRC-primed spleen Anti-0 8               | 100                                                                              | 1,230        | 20       | 9      |
|                                          | (160-60)                                                                         | (2,169-530)      | (10-10)  | (30-2) |
| HRC-primed spleen Anti-0 9               | 5,260                                                                            | 74,710       | 650      | 1,580  |
|                                          | (6,860-3,500)                                                                    | (100,860-55,140) | (1,100-380) | (1,950-1,190) |
| 10^8 thymus                              | 10                                                                               | 6            | 10       | 210    |
|                                          | (20-2)                                                                           | (50-3)        | (40-3)   | (270-160) |

P values between groups—19S PFC to SRC: 1 cf. 2, <0.0005; 1 cf. 3, <0.005; 2 cf. 3, <0.005; 4 cf. 5, <0.02; 4 cf. 6, <0.0005; 7 cf. A, <0.0005; 8 cf. 9, <0.005; 7 cf. NS. 75 PFC to SRC: 1 cf. 2, <0.0005; 1 cf. 3, <0.02; 2 cf. 3, <0.005; 4 cf. 5, <0.05; 7 cf. 8, <0.005; 8 cf. 9, <0.005; 7 cf. NS. 19S FFC to HRC: 1 cf. 2, <0.005; 1 cf. 3, NS; 2 cf. 3, <0.0005; 4 cf. 5, <0.005; 4 cf. 6, NS; 5 cf. 6, <0.005; 7 cf. 8, <0.02; 7 cf. 9, <0.005. 75 FFC to HRC: 1 cf. 2, <0.0005; 1 cf. 3, <0.005; 2 cf. 3, <0.005; 4 cf. 5, <0.0005; 4 cf. 6, NS; 5 cf. 6, <0.0005; 7 cf. 8, NS; 7 cf. 9, <0.005. 0.2 ml of 23% suspension of erythrocytes (10^8) given intravenously.

1 0.1 ml of 23% suspension of erythrocytes (5 X 10^8) given intravenously.

2 Spleen cells were incubated at 5 X 10^7 cells per ml with 1:4 dilution of serum at 37°C for 20 min, washed once, and then incubated for 20 min at 37°C in complement. 2 X 10^7 cells were then transferred intravenously with antigen to irradiated mice. Viability of transferred cells: NMS-treated = 80-90%; anti-0-treated = 45-60%.

3 Geometric mean, upper and lower limit of SE, five mice per group.

B cells. The experimental results given in Table VIII substantiate this prediction in that addition of normal spleen cells from unprimed mice to spleen cells from mice primed 1 day before allowed good adoptive immune responses to the antigen used for priming.

Capacity to Bind Antigen.—It might be suggested that the specific unresponsiveness of adoptively transferred spleen cells from 1-day-primed mice resulted from a specific inability of the cells to recognize antigen after transfer. For example, contact with antigen before transfer could have led to a temporary loss of antigen-specific receptors. It was thus decided to determine the number of cells in spleens of unprimed mice or of mice primed 24 h before to SRC that...
TABLE VIII

PFC in Spleens of Irradiated Mice Injected with Spleen Cells from Mice Primed 24 h Previously with SRC* or HRC.* Effect of Injection of Normal Spleen Cells

| Cells injected together with SRC\ and HRC\ | PFC per spleen at 7 days |
|------------------------------------------|-------------------------|
|                                          | Anti-SRC    | Anti-HRC    |
|                                          | 19S  | 7S  | 19S  | 7S  |
| Unprimed spleen 1                       | 5,425| 100,370| 6,960| 61,730|
|                                          | (7,540-3,900) | (107,400-93,810) | (8,630-6,610) | (82,720-46,070) |
| SRC-primed spleen 2                     | 90   | 190  | 2,100| 27,620|
|                                          | (110-70) | (330-140) | (2,560-2,080) | (28,820-22,500) |
| SRC-primed spleen 3                     | 8,160| 177,370| 24,620| 281,160|
| + unprimed spleen                       | (10,300-6,460) | (235,750-153,900) | (28,330-21,400) | (334,200-238,770) |
| HRC-primed spleen 4                     | 6,000| 97,810| 410  | 9   |
|                                          | (7,740-4,650) | (108,570-88,120) | (480-320) | (40-2) |
| HRC-primed spleen 5                     | 9,810| 115,150| 7,250| 56,030|
| + unprimed spleen                       | (11,200-6,330) | (132,120-100,130) | (8,700-6,600) | (67,800-46,300) |

P values between groups—19S PFC to SRC: 1 cf. 2, <0.005; 1 cf. 3, NS; 1 cf. 4, NS; 2 cf. 3, <0.005. 7S PFC to SRC: 1 cf. 2, <0.005; 1 cf. 3, <0.005; 1 cf. 4, NS; 2 cf. 3, <0.005. 19S PFC to HRC: 1 cf. 4, <0.0005; 1 cf. 5, NS; 2 cf. 5, <0.001; 4 cf. 5, <0.0005. 7S PFC to HRC: 1 cf. 4, <0.0005; 1 cf. 5, NS; 1 cf. 2, <0.01; 4 cf. 5, <0.0005.

* 0.2 ml of 3% suspension of erythrocytes (10⁹) given intravenously.
† 3 × 10⁷ viable cells of each spleen cell suspension given intravenously.
‡ 0.1 ml of 3% suspension of erythrocytes (5 × 10⁸) given intravenously.
§ Geometric mean, upper and lower limits of SE, six mice per group.

could form rosettes to SRC. In five separate experiments, the number of RFC to SRC found in spleens of SRC-primed mice were reduced to 28–48% of those found in spleens of unprimed mice (Table IX). In the one experiment in which numbers of cells forming rosettes to HRC were determined, more rosettes were found in SRC-primed spleens than in unprimed spleens.

**DISCUSSION**

Spleen, mesenteric lymph node, and Peyer’s patch cells from mice recently primed with heterologous erythrocytes were markedly and specifically unresponsive to the priming antigen, but only on adoptive transfer. Unresponsiveness was reversible and the transferred cells recovered their reactivity after about 5 days. There are several reports that primary immunization with antigen, given in doses normally considered immunogenic rather than tolerogenic, can suppress the immune response to the priming antigen provided that antigen challenge was given soon afterwards:

Tao (19) observed that fragments of lymph nodes from normal rabbits or rabbits primed 3 days before with φX 174 responded well to this antigen when cultured in vitro. Fragments from donors primed 1–2 days previously, however, produced only small amounts of antibody in culture. No explanation was given for this finding.

Katz, Paul, and Benacerraf (20) reported that in guinea pigs, the effect of carrier preimmunization in promoting secondary antihapten antibody responses to heterologous hapten-carrier conjugates was prevented by supple-
TABLE IX
Numbers of Rosette-Forming Cells in Spleens of Mice Injected with SRC* 24 h Previously

| No. of experiment | Antigen given to donors of spleen cells | RFC per 10^8 cells† to SRC | RFC per 10^8 cells† to HRC |
|------------------|----------------------------------------|---------------------------|---------------------------|
| 1                | SRC                                    | 212                       | —                         |
|                  | SRC                                    | 90 (41%)§                 | —                         |
| 2                | SRC                                    | 449                       | —                         |
|                  | SRC                                    | 172 (38%)                 | —                         |
| 3                | SRC                                    | 556                       | —                         |
|                  | SRC                                    | 157 (28%)                 | —                         |
| 4                | SRC                                    | 331                       | —                         |
|                  | SRC                                    | 156 (47%)§                | —                         |
| 5                | SRC                                    | 443                       | 467                       |
|                  | SRC                                    | 213 (48%)§                | 627 (134%)§               |

* 0.2 ml of 25% suspension of erythrocytes (10^9) given intravenously.
† Numbers of RFC were determined on suspensions pooled from the spleens of two mice. Total cell numbers in spleens of mice injected with SRC were 3-15% higher than in controls not given antigen.
§ Compared with donors not given antigen.

mental immunization with the carrier 1 day before administering the hapten conjugated to this carrier. This effect appeared nonspecific in that it was also observed when an unrelated carrier was used for supplemental immunization. Cells were not transferred adoptively in these studies, and the authors interpreted their observation in terms of a suppressive influence exerted by T cells.

Armstrong and Kraft (21) separated mouse spleen cells on the basis of size by velocity sedimentation and tested immune responses to polymerized flagellin. They found that the capacity of cells to form antibody in vitro or in vivo in the presence of antigen was restricted to cells with a slow sedimentation rate (small cells) when taken from unprimed donors but mostly to fast moving cells (blasts) when taken from mice primed to the antigen 48 h before. The competence of cells from mice primed for 24 h was likewise restricted to the large cell fraction when tested in vitro. Paradoxically, however, none of the cell fractions from 24-h primed mice produced antibody in vivo. This was considered to reflect "the manner of antigen contact in the irradiated host."

The phenomenon reported in this paper is probably analogous to that observed by Armstrong and Kraft (21), although in their study, it was not established whether the unresponsiveness of cells transferred in vivo was specific since the response to other antigens was not investigated. Neither was it shown whether the transferred cells regained their reactivity. On the other
hand, the effects described in the present studies are unlikely to be related to those described by Katz et al. (20) since, in our studies, unresponsiveness was specific, demonstrable only after adoptive transfer and not the result of a suppressive influence exerted by T cells (18). The relationship of our findings to those described by Tao (19) is not clear.

Several possibilities can be invoked to explain the results reported here:

(a) In preparing single cell suspensions for adoptive transfer, certain cells, viz those “transformed” as a result of antigen activation (21), might be abnormally sensitive to the trauma associated with making cell suspensions; clonal expansion of a few cells surviving such trauma might account for the gradual return of reactivity. According to this explanation, it would have to be assumed that cells should be much more sensitive to trauma when exposed to antigen in vivo for 1 day than for 3 or more days (Fig. 2). Furthermore, one would expect that single-cell suspensions from 1 day-primed mice should be unresponsive in vitro, whereas tissue fragments, where cell damage should be minimal, ought to respond. In the experiments of Armstrong and Kraft (21), however, cell suspensions were responsive in vitro, whereas in the experiments of Tao (19) tissue fragments were not. It would appear therefore that our findings are unlikely to be explained on the basis of a heightened susceptibility of blast cells to the trauma of preparing cell suspensions.

(b) A large proportion of blast cells from rats (22) or mice (23), injected with antigen 3-5 days before, migrated to the intestines rather than to the spleen after intravenous injection. Such cells might not therefore reach sites conducive to their differentiation into antibody-forming cells. The work of Armstrong and Kraft (21), mentioned above, does not support this possibility. Thus, although after 24 h priming, their large cell fraction responded well to the priming antigen when cultured in vitro but not when transferred in vivo, after 48 h priming similar fractions of large cells produced high responses not only in vitro but also in vivo.

(c) Lymphocytes bear surface “receptors” sensitive to trypsin and neuraminidase (24, 25), which control their pattern of migration. As previously mentioned, it has been suggested that lymphocyte migration is altered soon after contact with antigen (3). Possibly this results from subtle changes in the cell surface membrane that induces cells to become localized in areas conducive to the production of antibody, e.g., the primary follicles and germinal centers. Once the cells reach these areas, however, membrane changes might become sufficiently extensive to prevent them from reentering such areas if removed and replaced in the bloodstream. Spleen cell suspensions from mice recently injected with antigen would thus be unable to mount an adoptive immune response in vivo until the surface membrane structures of the activated cells had reverted to normal. Detailed knowledge of the migration of recently activated lymphocytes and their subsequent fate must be obtained before this possibility can be further evaluated.
(d) The unresponsiveness observed in the present system might be due to transient induction of high zone tolerance. It could be argued, for example, that, unlike small lymphocytes, cells recently transformed in response to antigen are easily tolerized, but that antigen clearance mechanisms in the intact animal normally protect such cells from exposure to antigen in tolerogenic form or concentration. Such a protective mechanism, however, might no longer operate during the preparation of single-cell suspensions shortly after priming with high doses of antigen. The activated cells in these suspensions might then be freely exposed to antigen capable of tolerizing. It should be noted that it is necessary to invoke a greater sensitivity to tolerance induction of activated rather than nonactivated cells, since, in the case of heterologous erythrocytes, the high adoptive responses shown by unprimed spleen cells were not suppressed by supplemental injection of 1 day-primed spleen cells (Table VIII). A heightened susceptibility of blast cells to tolerance induction would account for the findings of Haskill and Marbrook (26) that cells transformed in response to SRC in culture differentiated to PFC in vitro only if antigen was not added. To test this possibility, we investigated whether washing 1 day-primed spleen cells (to remove excess antigen) before transfer and withholding antigen challenge of the cells would overcome their unresponsiveness. This was observed but only in one of four experiments and then only with the 19S PFC response (unpublished data of J. S. and J. F. A. P. M.). Nevertheless, other results have indicated that 1 day-primed spleen cells, washed and cultured for 4 h without antigen, partly regained their capacity to respond to the priming antigen 7 days after transfer in vivo with the antigen. This was not the case, however, when the cells were cultured together with the priming antigen before transfer. Further experiments along these lines are in progress.

(e) The reduced antigen-binding capacity of 1 day-primed spleen cells (Table IX), also reported for cells exposed to antigen in vitro for 18 h (26), might imply that activated cells express fewer surface receptors. This might prevent the cells from being restimulated on further exposure to antigen. The finding of Diener and Paetkau (27) that the capacity of lymphocytes to bind polymerized flagellin was considerably increased after exposure to antigen for 6 h in vitro makes it unlikely that antigen activation reduces the density of surface receptors. Possibly the reduction in numbers of RFC in the present study was the result of receptor blockade by soluble antigen released from macrophages.

The mechanism by which short-term exposure to antigen in vivo renders lymphocytes specifically unresponsive on transfer is clearly not understood. Since unresponsiveness involved both T and B cells and both 19S and 7S responses, the defect is clearly a basic one. It may perhaps be of general importance since recent studies have shown that alloantigens may induce a similar phenomenon. Thus spleen cells from mice exposed to alloantigens for 1 day in vivo failed to proliferate or evoke classical graft-vs.-host reactions
upon reexposure to these antigens (unpublished results of J. S. and J. F. A. P. M.).

Our findings challenge the concept that lymphocytes are selectively recruited from the recirculating pool to organs such as the spleen and lymph nodes after antigen injection (3-5). Thus, it could be argued that cells are not withdrawn from the circulation but merely become transiently unresponsive as a result of a process similar to that occurring in spleen as described in the present studies. Investigation of this possibility will be the subject of the next paper in this series.

**SUMMARY**

When spleen, mesenteric lymph node, or Peyer's patch cells from mice primed 24 h before with either sheep erythrocytes (SRC) or horse erythrocytes (HRC) were transferred together with both SRC and HRC to irradiated mice, antibody responses measured 7 days later were very low to the priming antigen but high to the other antigen. This was demonstrated either by measuring numbers of antibody-forming cells in spleen or levels of hemagglutinins in serum. Specific unresponsiveness of the transferred cells was evident in both the 19S and 7S responses. It was observed only when strict experimental conditions were followed: (a) the cell donors had to be primed with not less than $10^9$ erythrocytes given intravenously; (b) the cells had to be transferred between 1 and 2 days after antigen priming; (c) antibody responses in the recipients were measured within 7 days of cell transfer, i.e., partial recovery was evident by 11 days; (d) the transferred cells had to be challenged in the recipients within 1 day after cell transfer: when challenge was delayed for 5 days or longer, responsiveness returned.

The failure of cells from recently primed donors to respond to the priming antigen on adoptive transfer could be overcome by supplementing with normal spleen cells, but not with thymus alone or bone marrow alone. This implied that unresponsiveness occurred at the levels of both T and B lymphocytes, and was not due to a suppressive influence exerted by T cells. Further work is in progress to determine the mechanism of this transient state of specific unresponsiveness.

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