tail flukes safely and quickly relaxed and calmed the animal, thereby greatly facilitating intubation.

The percentage of halothane to be used for induction and maintenance was determined by trial and error. On the basis of 13 successful experiments with the porpoise (three *Lagenorhynchus obliquidens*, ten *Tursiops truncatus*) we arrived at the following generalizations for the animals intubated while awake. Administration of 1.5 percent halothane resulted in a half-hour induction period before the loss of "tube bucking" and the proper depression of reflexes; administration of 2.5 percent gave a smooth induction in 15 minutes. The induction period for 3.5 percent halothane ranged from 5 to 15 minutes, and 0.75 to 1.0 percent halothane was sufficient to maintain surgical anesthesia. Occasionally an animal might require administration of 1.5 percent halothane for 2 to 5 minutes for proper maintenance during major surgery. When theopental was given before intubation, no more than 2.0 percent halothane was necessary for initial induction. Swimming movements of the free tail flukes were found to be the most reliable indication of depth of anesthesia. When these movements disappeared the subject was sufficiently anesthetized for surgery to begin. During induction, the swimming movements disappeared just after the loss of strong corneal and eyelid reflexes. The proper degree of anesthesia was maintained with the lowest concentration of halothane necessary to inhibit movement of the tail fluke. The lid and corneal reflexes were the next most dependable criteria for assessing the depth of anesthesia. All other reflexes (except anal reflex) were not prominent during periods of surgical anesthesia.

Animals were allowed to recover on 60 percent ambient air and 40 percent oxygen. In 10 to 15 minutes all reflexes except the blowhole reflex returned, spontaneous movement of the jaw and flipper occurred, and the animal's eyes followed a finger moved near them. Depending on the duration of anesthesia, the blowhole reflex returned approximately 15 to 45 minutes after the start of the recovery period. At this time extubation could be safely performed.

The swimming reflex cannot be easily observed if the porpoise is restricted with rigid wooden retainers like those used by Nagel, Morgane, and McFarland (*5*). The animal can be effectively restrained with sea belts, which do not impair the movement of the tail fluke, over the anterior and thoracic regions (Fig. 1).

Measures of gases and the *p*H of blood samples taken from the tail-fluke artery during anesthetization correlated well with corresponding adjustments of pulmonary ventilation and changes in systemic measuremens of expired CO_2. Because the central artery of the tail fluke is surrounded by a venus plexus, venous and arterial blood might occasionally intermingle during puncture of the artery or veins. However, during a recent surgical procedure for studies of cochlear potential, blood specimens were simultaneously collected from the external carotid artery and the central artery of the tail fluke, and the *p*H, P_{O_2}, and P_{CO_2} of the respective samples were found to be identical.

We have used halothane during abdominal surgery. Two ovariohysterectomies were performed on adult *Tursiops truncatus*, and an orchidectomy was performed on another adult. In each case, recovery from anesthesia was rapid and uneventful. Ten months after the surgery, the two females are completely healed, in apparent good health, and being trained for behavioral studies. The male, however, died 6 days after surgery. A postmortem examination indicated that death was caused by an infection that was aggravated by the surgery.

We have also used halothane anesthesia in the porpoise for a laprotomy, the removal of a cyst, and the repair of a corneal ulcer. Each of these animals recovered completely.

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Antigenic Competition: Cellular or Humoral

Abstract. *The injection of one antigen into mice inhibited the response to a second when 1 to 10 days separated the two injections. When the same type of inhibition was attempted in γ-irradiated mice reconstituted with normal spleen cells, the inhibition was greater in mice receiving 50 million spleen cells than in those receiving 10 million. The results are interpreted as favoring a humoral mechanism of inhibition.*

The injection of one antigen into an animal may partially suppress that animal's immune response to another antigen given a few days later. This phenomenon known as antigenic competition is well established. Adler (*1*) has reviewed in detail the various factors which affect its demonstration.

Antigenic competition has been explained either as a competition by the antigens for a limited number of multipotential precursor cells or as competition for a limited amount of some humoral factor. The experiments we report provide evidence that supports the humoral rather than the cellular hypothesis. However, a variation in the humoral hypothesis is advanced, namely that a humoral factor is produced during the response to the first antigen, and this factor acts as a feedback repressor of the response to the second antigen.

Adult LAF_1/J (C57L/J♀ X A/He J♂) mice from R. B. Jackson Memorial Laboratories, Bar Harbor, Maine, were used as donors and re-

cipients. All mice were of the same sex (M) and approximately the same age (17 to 19 weeks); they were fed Purina chow and tap water.

Recipient mice were irradiated from a C-10,000 Co^{60} unit (*2*) at a distance of 80 cm and approximately 80 roentgens per minute to give a total calculated dose of 1000 r. During exposure, mice were kept in a glass tray covered with a perforated plastic sheet.

Pools of singly dispersed spleen cell suspensions were made from three to ten donors. Spleens were minced and passed through a stainless steel screen (*3*). Cells were suspended in medium 199 and appropriate amounts containing 10×10^6 or 50×10^6 cells were injected intravenously into recipients 16 hours after γ-irradiation. In some experiments both groups of 10×10^6 and 50×10^6 transferred donor spleen cells were from the same pool.

Erythrocytes from sheep (S-RBC), goat, horse (H-RBC), ox, and pig preserved in Alsevers solution were used (*4*). Red cells were washed once with physiological saline, twice with medium 199, and suspended in medium 199. Dose of immunization was 0.2 ml of 20 percent red cell suspension. In most experiments the antigen was injected intraperitoneally. The only exception was the group of mice reported in Table 2 in which the S-RBC were mixed with the spleen cells and injected intravenously.

Spleens were assayed for hemolytic plaque-forming cells (PFC) by the method of Jerne and Nordin (*5*). A 0.1-ml portion of the spleen cell suspension and 0.15 ml of 20 percent freshly washed S-RBC were mixed with 2 ml of a 0.7 percent purified Bacto-Agar made up in Earle's solution and kept soft in a water bath at 45°C. This mixture was immediately poured into a petri dish (15 by 100 mm) containing a base layer of solidified 1.4 percent Bacto-Agar in Earle's solution. The overlayed mixture of spleen cells, red cells, and soft agar was allowed to solidify at room temperature for 5 to 10 minutes. The plates were then incubated for 2 hours at 37°C under 5 percent CO_2 and adequate moisture. After this first incubation, 2 ml of guinea pig serum, diluted in medium 199 (1:3), was added. Plates were again incubated for 1 hour, and after the second incubation they were ready for counting. Generally two plates were made for each spleen cell suspension. Plaques were counted with a 3M-brand overhead projector (*6*) and a glass screen. This procedure permitted rapid scoring of plaques on a glass screen, with the use of a wax pencil and cell counter.

Table 1. The number of antibody-forming cells per spleen, as tested against red cells from five species.

Red cell injected	Number of antibody-forming cells against				
	Sheep	Goat	Ox	Pig	Horse
None	104	16	0	56	24
Sheep	*73,000*	34,000	14,000	200	180
Goat	88,000	*340,000*	29,000	240	48
Ox	34,000	35,000	*370,000*	1,600	100
Pig	2,000	2,400	11,000	*25,000*	140
Horse	340	430	2,000	1,200	*21,000*

Six mice were used in an experiment to test the cross-reactions between the red cells of five species. Five mice were injected with 0.2 ml of a 20 percent suspension of five different red cells: sheep, goat, ox, horse, and pig. The sixth mouse served as control. Four days later, the mice were killed, and cell suspensions were made from the spleens. The number of PFC for each of the five red cells was determined on each spleen cell suspension (Table 1). In each spleen cell suspension, the largest number of PFC was against the red cells injected into that mouse. However, the cell suspension from the mouse injected with sheep cells contained almost half as many goat PFC as sheep PFC. Paradoxically, the cell suspension from the mouse injected with goat cells contained more sheep PFC than the cell suspension from the mouse injected with sheep cells. Horse and sheep cells cross-reacted approximately 1 percent in both directions and were selected for the following two types of experiments.

The first experiment was designed to determine the effect of H-RBC on the response of intact mice to S-RBC. This experiment consisted of 76 mice, of which 25 were used as controls and the remaining 51 were divided into eight experimental groups consisting of 5 to 12 mice each. The first antigen (H-RBC) was given to all experimental groups on day 0, and the second antigen (S-RBC) was given on days 0, 1, 2, 3, 4, 5, 7, or 10. The control group received only S-RBC. All mice were killed 4 days after the S-RBC injection, and the number of PFC for S-RBC per 10^6 spleen cells was determined. The mean and standard error for each group were calculated. The results are plotted as functions of time between first and second antigen (Fig. 1). Compared to the control group, a significant drop in the number of PFC to the second antigen (S-RBC) was observed when the second antigen was given 2 to 7 days after the first antigen. The maximum drop was more than tenfold when 4 days separated the two antigens.

The second type of experiment was designed to determine the effect of H-RBC on the response of irradiated and reconstituted mice to S-RBC. Two different experiments involved a total of 83 irradiated recipients. These two experiments differ in the source of donor cells used to reconstitute the irradiated mice and in the route and time intervals at which S-RBC antigen were injected.

For the first experiment, spleen cells from unimmunized donors and from donors injected with H-RBC 2 days before transfer were mixed with S-RBC antigen and injected intravenously into Co^{60}-irradiated recipients. All recipients were killed 6 days after the S-RBC in-

Table 2. Response to S-RBC of Co^{60}-irradiated recipients after the injection of spleen cells from unimmunized donors and from donors injected with H-RBC 2 days before transfer. Five irradiated recipients given S-RBC on day 0, but not given spleen cells averaged eight PFC per spleen.

Source of transferred cells	10×10^6 Cells transferred		50×10^6 Cells transferred	
	No. mice	Response (PFC)*	No. mice	Response (PFC)*
Unimmunized donors	6	100 ± 17	8	252 ± 34
Donors given H-RBC	6	136 ± 49	10	66 ± 5

* Mean PFC for S-RBC per million cells transferred in spleen of recipient 6 days after injection of S-RBC $\pm$ S.E.

Table 3. Response to S-RBC of Co^{60}-irradiated recipients receiving spleen cells and H-RBC and S-RBC at various intervals after cell transfer. Five irradiated recipients given S-RBC on day 4 but not given spleen cells averaged eight PFC per spleen.

Group	10×10^6 Cells transferred		50×10^6 Cells transferred	
	Mice (No.)	Response (PFC)*	Mice (No.)	Response (PFC)*
I S-RBC Day 0	8	66 ± 14	7	218 ± 25
II S-RBC Day 4	7	181 ± 49	8	102 ± 17
III H-RBC Day 0, S-RBC Day 4	11	58 ± 7	12	12 ± 5

* Mean PFC for S-RBC per million cells transferred in spleen of recipient 6 days after injection of S-RBC $\pm$ S.E.

jection, and the number of PFC for S-RBC in each spleen per million transferred cells was determined. The mean and standard error for each group were calculated (Table 2). Only when 50×10^6 spleen cells were transferred, a highly significant inhibition of nearly fourfold was found in the experimental group.

The second experiment with irradiated recipients consisted of one experimental and two control groups. In all these groups, spleen cells were transferred intravenously, but antigens were injected intraperitoneally. The experimental group received spleen cells and H-RBC on day 0 and S-RBC on day 4. One of the control groups received spleen cells and S-RBC on day 0, and the other control group received spleen cells on day 0 and S-RBC 4 days later. Spleens of recipients in all groups were assayed for S-RBC 6 days after injection of S-RBC, and PFC per million cells transferred and the mean and standard error were calculated (Table 3).

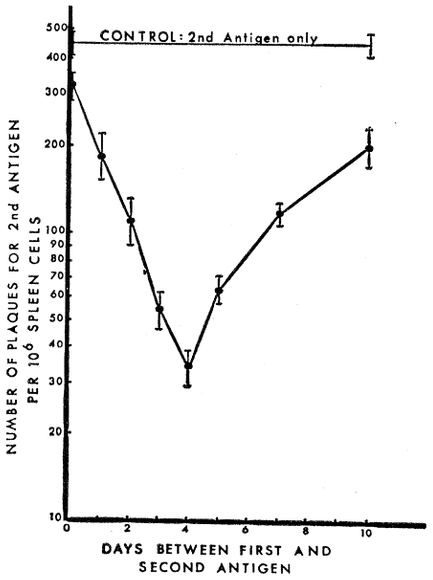

Fig. 1. The response of mice to sheep red blood cells (S-RBC) at various time intervals after the injection of horse red blood cells (H-RBC). Vertical bars indicate standard error.

The prior injection of H-RBC produced a highly significant inhibition (eightfold) in the response to S-RBC only in the mice receiving 50 million spleen cells. There was a threefold inhibition in the mice receiving 10 million spleen cells, but this was not statistically significant (at $P > .05$). The experiments reported in Table 2 also indicate that the response of irradiated mice may depend on two other factors: the number of spleen cells injected and the time of injection.

For these experiments, sheep and horse erythrocytes were selected because in mice they cross-reacted less in both directions than any of the other combinations tested. Despite the low cross-reaction (approximately one percent) the injection of one antigen, H-RBC, inhibited the response to the second given 1 to 10 days later. The maximum inhibition was approximately 13-fold when 4 days separated the two antigens. Other experiments indicated that the inhibition of the second response was equally good when the order of the injection was reversed.

Experiments were designed to determine whether the observed inhibition represented a competition for a precursor cell or for some humoral factor. Under conditions of spleen-cell transfer to irradiated recipients, it might be expected that competition would increase for a limited supply of precursor cells if fewer cells were injected. On the other hand, competition for a humoral factor would increase with injection of more donor cells. The results of two different experiments clearly show a greater competition with 50 million than with 10 million cells injected. These experiments thus favor a humoral hypothesis and are consistent with the cell-selection theory of antibody formation (*7*) and with the demonstration that individual cells make only one kind of antibody (*8*).

The finding that maximum inhibition of the second antigen occurs when 4 days separate the two antigens makes it seem unlikely that the phenomenon known as antigenic competition is in fact competition for anything. In that antibody responses to erythrocytes in normal mice reach a peak in 4 to 5 days and rapidly decline, it would be expected that maximum competition would occur when the two responses coincided. However, under these conditions (simultaneous injection) no significant inhibition of either response occurs. For this reason, it is suggested that the inhibition of the response to the second antigen may result from a humoral feedback inhibition by the response to the first antigen. As such, this type of inhibition might be part of a general control mechanism that prevents overgrowth of lymphoid tissue. Or it might be related to the humoral inhibitor of the antibody response found in spleen, lung, and thymus by Thompson (*9*).

The possibility was considered that the humoral factor might be a cross-reacting nonhemolytic antibody. This cannot be ruled out. However, this explanation seems unlikely because of the rapid decline in the inhibition after reaching a peak at 4 days. The amount of circulating antibody declines much more slowly after reaching a peak between days 4 and 6.

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