course of hypertension from peripheral resistance measurements. If the curve relating cardiac output to peripheral resistance is known, then the resistance value for a given cardiac output is meaningful and the difference in resistance produced by hypertension or, for example, by norepinephrine is seen clearly. These findings have clinical application since patients with mild hypertension have been reported as having an increased cardiac output with a resistance within the normal range (3). The seemingly normal levels of resistance may, in fact, be elevated at that cardiac output.

Summary. Peripheral resistance has been found to fall exponentially as cardiac output increases in dogs. A similar curve with a higher peripheral resistance at all levels of cardiac output is observed in hypertensive animals. The measurement, therefore, of peripheral resistance alone, or its changes, to indicate the state of the peripheral vascular bed is of little value unless level of blood flow is held constant or the relationship between blood flow and peripheral resistance have already been determined.

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The Effect of RES Blockade on Cellular Antibody Formation to Sheep Erythrocytes* (32996)

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Processing of particulate antigens by cells of the reticuloendothelial system (RES) is considered to be an important step in the "afferent limb" of antibody formation (1–4). Circulating and fixed macrophages have been shown to "digest" particulate antigens such as foreign erythrocytes or bacteria following their inoculation into experimental animals (4,5). Results by a number of investigators have suggested that, as a consequence of such phagocytosis, a product of the macrophage, possibly an informational nucleic acid product or a processed antigenic determinant, probably associated with RNA, is released and may act directly in stimulating antibody formation by lymphoid cells (6–11).

Blockade of the RES is regarded as a classic method of profoundly influencing the immune capability of animals toward a variety of antigens (2–4). In a number of such experiments, treatment of experimental animals with agents such as carbon, colloidal iron, or oil emulsion interfered with production of specific serum antibody, or development of protective immunity against challenge infection with a microorganism. Some investigators have reported an inhibitory effect of RES activity by blockading agents, others have observed no effect, and yet others have observed stimulation of RES activity by blockading agents (12–18). Many of these diverging observations have been related to the dependence of dosage and timing for either stimulation or depression of RES activities by large particulate matter and to the variations in phagocytic activities of individual animals.

In general, the role of phagocytic cells as related to antibody formation has been studied only on the humoral antibody level. In the present study, experiments were performed concerning the effect of RES blockading agents on the cellular immune response of

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mice to sheep red blood cells (S-RBC). The number of lymphoid cells forming detectable antibody to S-RBC was determined in either normal or immunized mice injected with a sufficient quantity of colloidal suspension of carbon to blockade the RES system and suppress phagocytosis.

Methods and Materials. Experimental animals. Random bred mice of the NIH albino A strain, bred and maintained as a closed colony for a period of at least 7 years by a local dealer in the Philadelphia area, were used for these experiments. At the time of testing most of the animals were 6–8 weeks of age. They were kept in groups of six in plastic cages and fed water and Purina mouse pellets *ad libitum*. For individual experiments, mice of the same age and sex were used. For RES blockade, a colloidal suspension of carbon (Pelikan) manufactured by Gunther Wagner, Hannover, Germany was used. The Pelikan carbon, designated C11/1431a, contained 10% carbon, 4.3% fish glue, and 1% phenol in water. The size of the carbon particles ranged between 200 to 300 Å in diameter. A dosage of 10 mg of carbon was injected in 0.1- to 0.5-ml volumes into experimental mice intraperitoneally (i.p.). In some initial experiments, Higgins carbon (India ink) was used similarly.

For immunization, mice were injected intraperitoneally with S-RBC which had been washed three times with physiological saline solution (PSS) in the cold by serial centrifugation. The dosage was adjusted so that each animal received 4.0×10^8 S-RBCs in 0.5 ml of PSS.

The number of hemolysin forming cells appearing in the spleens of experimental and control mice was determined by the hemolytic plaque assay in agar gel, essentially as described previously (19,20). In brief, mice were sacrificed at various times following carbon injection and/or immunization and spleen cell suspensions prepared by "teasing" with fine needles and forceps. Cell suspensions were washed three times by serial centrifugation in the cold with Hanks balanced salt solution, and used for the plaque assay. In general, a suspension containing 4.0×10^5 to 2.0×10^7 viable nucleated cells in 0.1 ml of Hanks was added to 2 ml of warm (45–48°C) 0.7% Noble agar, containing 1.0 mg of DEAE dextran and 0.1 ml of a 10% suspension of target S-RBC. This mixture was rapidly layered on the surface of a base layer of previously prepared 1.4% Noble agar, in a 100-mm diameter sterile plastic Petri dish. When the upper layer had solidified, the plates were incubated at 37°C for 1 hour, and then treated with 5–10 ml of a 1:15 dilution of guinea pig serum (containing 4–5 units of complement activity). Following incubation for an additional 30 min at 37°C, the plates were examined for the presence or absence of localized zones of hemolysis (plaques), indicating hemolysin forming cells. The plates were stained with H_2O_2-benzidine solution as described previously. The number of detectable antibody forming cells (pfc) per million cells, or per whole spleen, was calculated. In general, two to three different concentrations of spleen cells were tested in duplicate or triplicate for each animal. In all experiments, equal numbers of control, untreated mice and blockaded animals were tested at the same time.

Results. Treatment of mice with a suspension of Pelikan carbon either within 15 min or 24 hours prior to immunization with a standard inoculum of S-RBC resulted in a markedly altered cellular immune response. As shown in Table I, normal mice responded to immunization with the appearance of relatively large numbers of antibody plaque forming cells in their spleens. There was an average of 40,000–80,000 pfc per spleen on the fourth day after immunization. Mice injected with carbon 24 hours prior to immunization had much fewer pfc. For example, in six separate experiments the average number of pfc in the spleens of carbon treated mice was approximately 3500 pfc. In general, the pfc response was less than 10% of that observed in normal immunized controls. Some carbon treated mice had no detectable pfc response and, on rare occasion, some animals had a 50–70% depression.

There was no shift in the day of peak pfc response in mice treated with carbon, as compared to controls (Fig. 1) There was a marked depression in the number of plaque

TABLE I. Effect of Time Interval Between Administration of Carbon Suspension and Immunization with S-RBC on Peak PFC Response.

Time interval between immunization and carbon injection[a]		Peak PFC response[b]	
		Per 10^6 WBC	Per spleen
No carbon		235.6	48,600
Same day		143.5	31,550
Days before RBC	1	28.6	6580
	2	10.5	8649
	3	14.8	7385
Days after RBC	1	242.6	58,500
	2	230.1	43,460

[a] All mice immunized i.p. with 4×10^8 S-RBC; 10.0 mg of Pelikan carbon administered on day indicated.

[b] Average of at least 4–6 mice per group 4 days after immunization.

forming cells appearing in the spleens of carbon treated mice throughout the time period during which control mice were producing large numbers of plaque forming cells in their spleens. For example, normal mice had background counts of 100–200 pfc prior to immunization and about twice as many 24 hours later. There were several thousand pfc 2 days after immunization and often 10,000–20,000 pfc per spleen by the third day after immunization. The peak response occurred on day 4, followed by a rapid drop in pfc for about a week to 10 days thereafter.

Mice treated with carbon 24 hours prior to immunization had a lowered pfc response

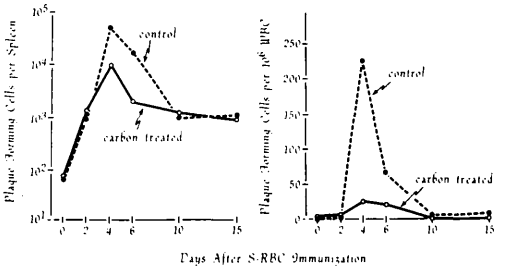

FIG. 1. The effect of administration of Pelikan carbon to mice on appearance of antibody pfc following immunization with S-RBC (4×10^8/mouse i.p.). Carbon suspension (10 mg) injected i.p. into test mice 24 hours prior to immunization. Each point represents mean plaque count of at least 3–4 mice.

throughout this time period (Fig. 1). The first detectable plaque forming cells appeared in the spleen by the second day after immunization. By days 3 and 4 there was usually less than 10% of the number of pfc as detected in spleens of control mice. There was no increase in the number of hemolysin forming cells during the next 10 days. In general, detectable pfc in the spleens of normal mice decreased to a range of about 400 or 500 by day 15 after immunization. There were usually about 200–300 pfc in the spleens of carbon treated mice at this time. Mice receiving Pelikan carbon but no S-RBC had no detectable change in pfc background count as compared to other normal control mice. There was no marked difference in the suppressive activity of Pelikan carbon as compared to Higgins carbon, a less purified commercial India ink product. However, bacterial endotoxins, or other similar products, may be present in carbon suspensions such as Higgins India ink, but probably not in Pelikan, since there was some stimulating effect on background pfc when Higgins carbon was administered without antigen. Mice injected with Pelikan, and no S-RBC, had no demonstrable change in the number of background pfc's over an observation period of one to ten days. However, mice injected with 10 mg of commercial India ink had a 30–80% increase in background pfc by the fourth day after administration. Injection of carbon, either the Pelikan or Higgins preparation, induced a moderate increase in spleen weight over a period of about 5 days. The spleen weight of normal, nonimmunized mice averaged 150–200 mg. Immunization with S-RBC generally increased the spleen weight to 250–300 mg within 4–6 days. Injection of carbon alone resulted in similar increases in spleen weight. Mice injected with carbon and RBC generally had spleen weights of 350–400 mg within 4 days.

The time of administration of the carbon seemed important since administration on the same day as immunization had less effect than injection 1–3 days previously (Table I). Mice treated with carbon 2 or 3 days prior to immunization generally had a somewhat lower pfc response than mice treated 24 hours

prior to immunization. This was noticeable only when pfc was calculated per 10^6 WBC, and not per spleen (Table I). Injection of Pelikan carbon 1 or 2 days after immunization had little or no suppressive effect on the subsequent peak plaque response on day 4.

Discussion. The results of these experiments indicate that treatment of mice with colloidal carbon suspensions prior to immunization with S-RBC may markedly effect the expected cellular antibody response. There was no difference in the day of appearance of peak numbers of pfc in carbon treated mice as compared to controls. However, there was an 85–95% suppression in the number of pfc on the day of peak response in carbon treated mice as compared to controls. Such suppression was most marked when the number of pfc was calculated per million cells. This difference seemed to be due to the effect of carbon on spleen weight and total number of nucleated cells. Animals injected with carbon alone, without red cells, had an increase in spleen weight and total number of spleen cells within a few days. Injection of both S-RBC and carbon resulted in an even greater increase in total weight and cell number.

The role of the RES in immunity has been investigated in numerous studies by means of blockade experiments, either alone or combined with other experimental methods such as splenectomy, chemical suppression, or X-irradiation. Many of these studies have indicated that use of colloidal substances such as colloidal carbon may markedly interfere with phagocytic activity (2–4). Stern *et al.* (21) studied the effect of polyvinyl pyrrolidine as a RES blockading agent on antibody response. They observed a decrease in both circulating hemolysin and hemagglutinin titers in such treated animals. Similarly, Derby and Rogers observed that RES blockade with thorium dioxide decreased bacterial clearance from blood (22). In contrast, Fisher (23) and Jenkin *et al.* (24) found that pretreatment by thorium dioxide enhanced circulating antibody titers such as serum opsonin or hemagglutinins. In other studies, we have found that during the depression of the pfc response in mice treated with charcoal there is also a significant delay in clearance of radio-labeled sheep red blood cells or charcoal suspensions from the circulation of the mice[2].

The effect of RES blockading agents on the number of antibody forming cells have not been reported to date. The previously observed increases or decreases in circulating antibody could reflect either a change in the total number of antibody forming cells, or a change in the amount of antibody secreted by individual cells. Use of the localized hemolytic plaque assay, first described by Jerne *et al.* (19) and by Ingraham and Bussard (25) as methods applicable to the screening of large numbers of antibody forming cells *in vitro*, has permitted analysis of the effect of administration of carbon suspensions, at a dose and route capable of suppressing RES activity, on the immune response of mice to S-RBC. Data obtained in prior experiments indicated that such treatment suppressed the appearance of serum agglutinins and hemolysins to sheep red cells in mice (26–28). The results of this study, showing that immunodepression is associated with a marked inhibition in appearance of individual antibody forming cells in spleens of carbon treated mice, suggest that RES blockade may interfere with vital steps in the "afferent limb" of antibody formation.

The inability of charcoal to prevent the primary antibody plaque response once antigen has been injected suggests that the suppressive effects were not due to a toxicity or lethal effects on antibody forming cells per se. In addition, the observation that administration of carbon within 1 or a few days prior to challenge immunization resulted in marked suppression suggests that the observed immunodepression may be related directly to a prior commitment of macrophages to carbon, preventing their functioning as specific antigen "processors" for the S-RBC, a step possibly needed for the immune response.

Other studies in progress concerning the distribution of antibody forming cells in various organs of carbon blockaded animals immunized with S-RBC indicate that the immunosuppression, both on the serum as well as cellular level, is nearly complete and that

[2] Melnick, H., Newlin, C., Sabet, T., and Friedman, H., in preparation.

there are no significant pfc's in other lymphoid organs which may have taken over the antibody producing function of the spleen. Other experiments in progress concerning cell transfer of macrophage-rich peritoneal exudates from normal or immune donors into blockaded animals, and transfer of similar cells from carbon-treated donors to normal recipients, may provide additional information as to the cellular basis of the immunosuppressive effect of RES blockade on antibody forming cells.

Summary. Reticuloendothelial cell blockade has been used to study the possible role of phagocytic cells in the primary immune response of mice to S-RBC. Administration of colloidal carbon (10 mg per mouse) resulted in a marked suppression in appearance of normal numbers of antibody plaque forming cells to S-RBC in the spleens of treated mice. There was a suppression in both the total number of pfc in the spleen and the amount of pfc per million viable nucleated cells. However, the day of peak antibody response was the same for carbon treated mice as for controls. Administration of carbon 24–48 hours prior to injection of S-RBC resulted in the greatest suppression, as compared to treatment on the same day as immunization. Injection of carbon 1–2 days following immunization had little effect on subsequent appearance of plaque forming cells.

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