may be important in the injury of both large and small vessels, *e.g.*, in immune reaction and large vessel disease such as atherosclerosis.

Summary. Incubation of platelets with antigen-antibody complexes, thrombin, collagen, latex particles, ADP, AMP or aggregated human serum albumin is associated with aggregation of platelets and release of permeability factors into the ambient fluid. The permeability response was most intense with the platelet-antigen-antibody complex and platelet-thrombin mixtures. It is postulated that the increased permeability is due to release of histamine, serotonin and lysosomal enzymes from the aggregated platelets.

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Influence of Oligodeoxyribonucleotides on the Immune Response of Newborn AKR Mice.* (30357)

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Adult AKR mice immunized with sheep red blood cells and treated with oligodeoxyribonucleotides display, 48 hours after antigen administration, a significantly greater number of hemolysin-producing cells in the spleen than animals that received antigen only(1,2,3). These differences become less pronounced as time between immunization and assay increases(1) and they are not detectable in the early responses of mesenteric and pancreatic lymph nodes(2). This paper reports additional results of similar studies carried out in newborn AKR mice.

Methods. Five- or seven-day-old AKR littermate mice were injected intraperitoneally with 10^8 sheep red cells in the presence or absence of oligodeoxyribonucleotides (= oligonucleotides), which were prepared by enzymatic digestion of calf thymus DNA as described previously(4). Treatment with oligonucleotides, or injection of corresponding amounts of biological saline into controls, was started with 0.05 ml on the day of immunization, was continued on the next 2 days, and increased to 0.1 ml on the fourth day after immunization. All injections were intraperitoneal and were continued daily until sacrifice. Animals were sacrificed at various times after immunization. Their blood was collected for determination of circulating antibody, and cells from individual spleens, as well as from pools of mesenteric and pancreatic lymph nodes, respectively, were as-

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TABLE I. Average Number of Hemolysin-Forming Cells in Spleens of AKR Mice Immunized with 10^8 Sheep Red Blood Cells at 5 Days of Age.

Spleen donor given:	Avg No. of hemolysin-forming cells (± S.E.) after 48 hr	72 hr	96 hr	120 hr	144 hr	192 hr	216 hr
Biological saline	35 ± 24	20 ± 9	51 ± 34	180 ± 140	53 ± 18	157 ± 62	35 ± 14
Oligodeoxyribonucleotides	2 ± 2	43 ± 33	10 ± 8	236 ± 143	85 ± 34	69 ± 25	92 ± 50

sayed according to Jerne's technique(5). Total numbers of cells were estimated by direct counts in a haemocytometer. The data to be presented are based on averages obtained with 3-6 littermates per determination, and the results are typical of those obtained in at least triplicate experiments.

Results and discussion. The response in spleen cell populations of animals immunized at 5 or 7 days of age showed a similar pattern in both oligonucleotide-treated and untreated (saline-injected) animals (Table I). As indicated by the standard errors of the averages in Table I, a considerable individual variation in the response of spleens occurred at all assay times. Oligonucleotide treatment did not cause a significant increase in total cell numbers in any of the organs assayed and, therefore, all data are presented as absolute numbers of hemolysin-forming cells. In the spleen, and also in the lymph nodes that will be discussed next, the background number of hemolysin-forming cells at the time of immunization was zero.

Fig. 1 shows the response of the pancreatic and mesenteric lymph nodes of animals immunized at 5 days of age. In contrast to the lack of stimulatory effects of oligonucleotides on the number of hemolysin-producing *spleen* cells, an obvious early enhancement of the number of hemolysin-forming cells could be observed in the *lymph nodes* of oligonucleotide-treated animals. More specifically, the pancreatic lymph nodes of the oligonucleotide-treated animals showed a striking increase in numbers of antibody-producing cells 24 hours prior to any detectable increases in the untreated controls. However, 120 hours after immunization similar levels of response were attained in both groups. An even greater difference in onset of the immune response could be noted in the mesenteric lymph nodes, where the first marked increase in number of hemolysin-producing cells was detected 144 hours after immunization in the oligonucleotide-treated mice, and only 216 hours after immunization in the controls that received antigen plus saline. Similar stimulatory effects were observed in animals immunized at 7 days of age.

In regard to the onset of a detectable im-

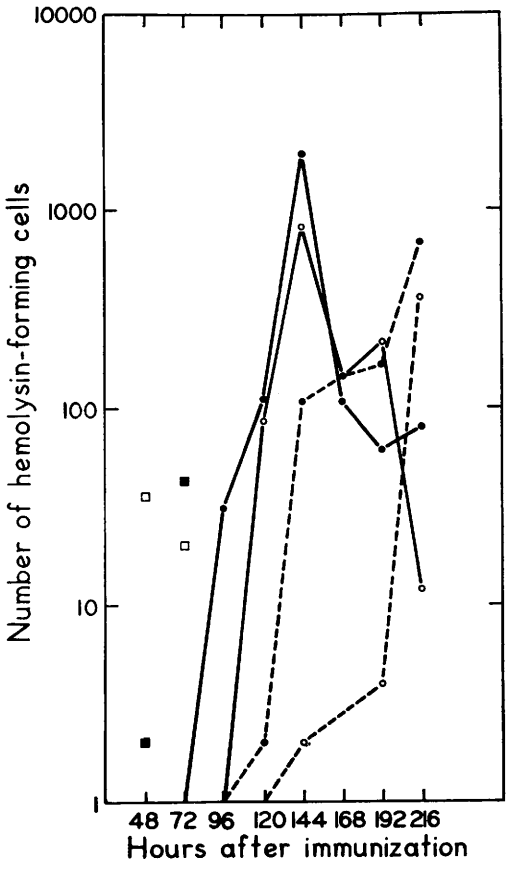

FIG. 1. Number of hemolysin-forming cells in pancreatic (——) and mesenteric (------) lymph nodes of mice which received either antigen plus DNA digest (●—●, ●----●) or saline (○—○, ○----○). The squares indicate early responses in the spleen of immunized animals given DNA digest (■) or saline (□).

mune response in the 3 organs assayed, it was observed that the sequence of appearance of hemolysin-producing cells followed a constant pattern, independent of treatment with oligonucleotides. As can be seen in Fig. 1, the first organ to respond to the antigenic stimulus was the spleen, followed by the pancreatic lymph nodes, and finally by the mesenteric lymph nodes. Although oligonucleotide treatment of immunized mice shortens the period required for initial detectable responses in the lymph nodes, but not in the spleen, the sequential pattern of response remained unchanged. Contrary to the findings in adult animals reported by Friedman(6), the peak number of antibody-producing cells is not reached simultaneously in these organs when newborn mice are assayed. Tests with adult AKR mice, employing the Jerne technique, have shown that in such animals mesenteric and pancreatic lymph nodes respond simultaneously to sheep red blood cells, and this response occurs 24 hours after the first detectable splenic response(2). As shown here, in newborn animals the first detectable hemolysin response of lymph node cells does not occur until at least 48 hours following a response of spleen cells. Such observations suggest that the lymph nodes of newborn animals may be less mature immunologically than their spleens in terms of their ability to respond to antigenic stimulation. This conclusion would agree with that of Harris *et al* (7) who observed that the lymph node cells of the rabbit developed immunologic competence later than spleen cells.

Although the Jerne technique revealed a large number of hemolysin-producing cells in the organs of the newborn mice assayed in this study, the circulating hemolysin levels of these mice remained low at the time periods tested (48 to 216 hours after immunization). In fact, the lowest feasible serum dilution tested (1:10) never yielded 50% hemolysis. Oligonucleotide treatment had no effect on these low titers of circulating antibody.

In comparison to data collected with adult AKR mice(2), the response of the lymph nodes of newborn animals to the stimulatory effects of oligonucleotides is strikingly greater. One possible reason for this difference may be the likely presence, or easy release of stimulatory oligonucleotides in adults as a consequence of immunological and other injuries. Whatever the cause, the present data indicate that newborn mice are well suited for the further exploration of stimulated immune responses.

Summary. Oligodeoxyribonucleotides, administered to newborn AKR mice in conjunction with sheep red blood cells, influence the time of appearance of hemolysin-forming cells in pancreatic and mesenteric lymph nodes. Thus, in comparison with animals that received the antigen only, 5- and 7-day-old animals injected with antigen *and* oligodeoxyribonucleotides, subsequently showed hemolysin-forming lymph node cells one to 3 days earlier. Such differences were not detected in assays on spleen cell populations and circulating hemolysins. The sequence of first appearance of antibody-producing cells in the organs assayed was spleen→pancreatic lymph nodes→mesenteric lymph nodes, and this sequence was not affected by administration of oligodeoxyribonucleotides.

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