not resume until 10-36 days later. Under similar test conditions a 1 mg daily dose of Provera, a compound with pronounced depot activity in domestic animals and man, induces an anestrous condition which persists for approximately 20 days following termination of treatment (unpublished data). These results suggest U-10293 may have significant depot activity in other test animals.

Induction of implantation with U-10293 was likewise consistent with an estrogenic effect. Blastocysts in Provera-treated intact rats remain free in the uterine cavity until a critical amount of exogenous estrogen is provided(2). Increasing estrogen doses beyond that required for nidation results in a loss of blastocysts from the uterine cavity. For example, Nutting and Meyer(3) reported 0.3 μg of estrone is a minimum dose supporting implantation in progesterone-treated castrate rats. Daily doses of 1 and 3 μg, but not 10 μg, were compatible with implantation. Their findings were comparable to results reported here with estrone in Provera-treated intact rats and are what would be anticipated with higher doses of U-10293.

Summary. 2,8-Dichloro-6,12-diphenyl-dibenzo[b,f][1,5] diazocine completely inhibited pregnancy when administered either orally or subcutaneously to female rats at doses as low as 0.5 mg daily for 7 days at time of mating. At higher doses, single treatments prior to completion of implantation were equally effective. Systemic or fetal toxicity did not occur at therapeutic doses. This compound also induced uterine growth, vaginal cornification, blastocyst implantation and inhibited gonadotropin activity. Each of these latter effects are indicative of estrogenic properties, and are probably involved with antifertility efficacy. This non-steroidal compound is a structurally unique addition to those agents with these biological activities.

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Hemolysin Formation in Newborn Mice of Different Strains.* (30638)

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The immune response of adult mice to sheep red blood cells (sRBC), measured by the Jerne technique(1), is known to be different in different strains(2). Thus Friedman found that adult AKR mice are the poorest responders among several strains tested(2). In contrast, we observed that newborn AKR mice give good early responses to sRBC as judged by numbers of hemolysin-forming cells (3). We therefore decided to extend our tests to 2 additional strains. This communication presents the results of these comparative studies during which we also tested strain differences in regard to the previously noted ability of oligodeoxyribonucleotides to stimulate immune responses(3,4).

Materials and methods. Pregnant female mice, one week before term, were obtained from the following sources: Ha/ICR from Millerton Research Farms, Millerton, N. Y.; C_{57}Bl/6 and AKR from Jackson Laboratory; Bar Harbor, Maine. The newborn animals of these mothers were immunized intraperitoneally between the third and tenth day of age, depending on the experiment, with 10^8

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washed sRBC. The litters were split, and half of the littermates were given oligodeoxyribonucleotides daily, the other half receiving biological saline, as described previously (3). The method of preparation of the oligodeoxyribonucleotides, by the enzymatic digestion of calf thymus DNA, also has been reported previously(4).

At different times after immunization, littermates were sacrificed and spleens, mesenteric and pancreatic lymph nodes were removed and placed into Eagle's Minimum Essential Medium containing 2 mM glutamine. Suspensions of single spleen cells were prepared by teasing the spleen and then passing the tissue suspension repeatedly through a syringe and needle. The lymph node pools were first washed twice in Eagle's medium and then homogenized gently in a Potter tissue homogenizer. Total cell counts were made in a haemocytometer. All cell suspensions were then plated in Eagle's medium containing 0.7% Special Agar-Noble (Difco) and 0.1 ml of a 1:6 suspension of sRBC. After one hour incubation at 37°C the plates were flooded with 1.5 ml of guinea pig complement (1:4 dilution of guinea pig serum), incubated again for one hour, and the number of plaques was counted. Circulating antibody was determined by standard hemolysin titrations on blood samples removed at time of sacrifice of the young mice.

Results and discussion. a) Onset of detectable responses. The earliest response to immunization with sRBC was detected both by the Jerne technique and by titration of circulating hemolysin in AKR mice immunized at five days of age (Table I and Fig. 1). No immune responses were detected in AKR animals immunized at 3 days of age.

In Ha/ICR mice, immunization at 5 days of age failed to elicit a detectable response to sRBC, whereas in animals immunized at 7 days of age an increase in the number of hemolysin-forming cells in the organs tested, and a rise in circulating hemolysin, was found (Table II and Fig. 2).

$C_{57}Bl$ mice immunized at 5 days of age did not show a detectable response either in spleen and lymph nodes or in the circulation. Immunization at 10 days of age§ did

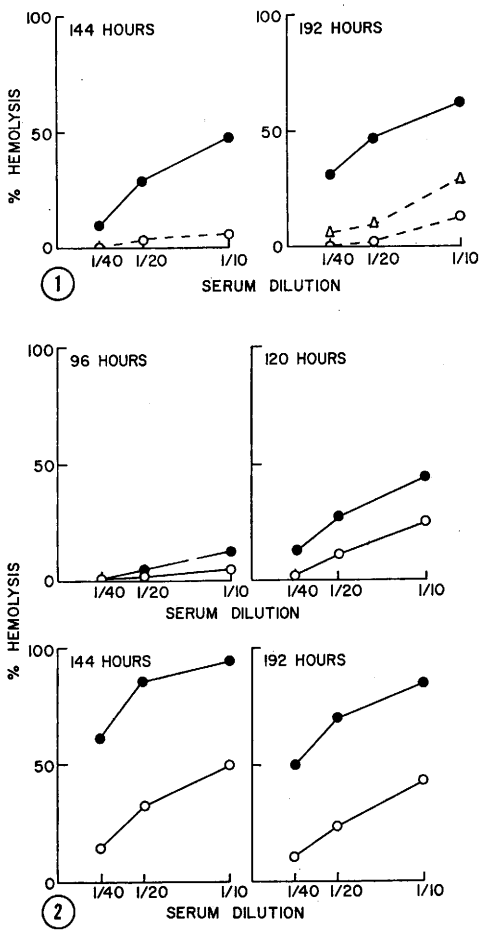

FIG. 1. Hemolysin titers in AKR mice immunized either at 5 (○---○) or 7 (△---△) days of age, and in $C_{57}Bl$ mice immunized at 10 days of age (●——●). Times indicated on the graph are hours after immunization.

FIG. 2. Hemolysin titers in Ha/ICR mice immunized at 7 days of age. Antigen administered with saline (○——○); antigen administered with oligodeoxyribonucleotides (●——●). Times indicated on the graph are hours after immunization.

bring about a poor but detectable response in the spleen and circulation, and a very poor response in the lymph nodes (Table III and Fig. 1).

More detailed data with newborn AKR mice(3) revealed that the *order of onset of detectable responses,* once competence was reached in animals immunized at 5 days of age, was spleen→pancreatic lymph nodes→

§ Seven-day immunizations were not studied on account of the poor responses obtained when animals were immunized at 10 days of age.

mesenteric lymph nodes. The data with Ha/ICR and $C_{57}Bl$ mice appear to follow a somewhat similar pattern. In Ha/ICR mice, which responded after immunization at 7 days of age, only spleen and pancreatic lymph nodes showed a significant number of hemolysin-forming cells during the period of observation. In $C_{57}Bl$ mice, which are late in maturing immunologically to this antigen (*i.e.*, responses are not obtained after immunization at 5 days of age, but are obtained at 10 days of age) only the spleen responded and the lymph nodes tested gave virtually no response.

b) Magnitude of responses. The response in terms of numbers of hemolysin-forming *spleen cells* showed a considerable individual variation in all strains tested (Tables I to III). Despite such variation, the range of responses in individual spleens of animals of the three strains is similar when one compares responses not in terms of chronological age but in terms of time after onset of detectable competence. Thus the differences among strains are in time of maturation rather than in magnitude of response once maturation has been attained.

On first inspection of the data the situation would appear to be different as far as lymph node responses are concerned. AKR mice show a relatively high number of plaque-forming cells in both the pancreatic and mesenteric lymph nodes, whereas the corresponding figures are markedly lower for the Ha/ICR and $C_{57}Bl$ strains. However, it must be kept in mind that prior tests with AKR mice had revealed a much later maturation of lymph node responses compared to spleen responses(3). Therefore, it is possible that in Ha/ICR and $C_{57}Bl$ mice, where, as indicated by the spleen responses, onset of responsiveness to sRBC is later than in AKR

TABLE I. Hemolysin Production in AKR Mice Immunized at 5 Days of Age with 10^8 Sheep Red Blood Cells.

Time of assay (hr after immunization)	No. of hemolysin-producing cells/animal in: Spleen		Pool of mesenteric lymph nodes		Pool of pancreatic lymph nodes	
	Antigen administered with:					
	DNA digest	Saline	DNA digest	Saline	DNA digest	Saline
72	13	17	0	0	0	0
	7	17				
	8	3				
	142	46				
96	0	52	0	.25	31	0
	2	1				
	3	6				
	2	148				
	44					
120	50	20	2	0	112	86
	0	80				
	10	10				
	10	730				
	750	50				
	600					
144	140	20	108	2	1912	821
	140	80				
	60	60				
	0					
192	130	230	167	4	62	217
	20	207				
	40	35				
	86					
216	4	56	693	360	81	12
	141	59				
	14	26				
	210	1				
Background	0		0		0	

mice, the maturation of lymph node responses may be delayed beyond the period here employed for immunization.

Strain differences also became apparent in the magnitude of *circulating hemolysin* levels. The differences for this parameter, however, are not in line with the differences detected by organ assays. At the lowest feasible serum dilution tested (1:10), AKR animals never yielded 50% hemolysis (Fig. 1). Ha/

TABLE II. Hemolysin Production in Ha/ICR Mice Immunized at 7 Days of Age with 10^8 Sheep Red Blood Cells.

Time of assay (hr after immunization)	No. of hemolysin-producing cells/animal in Spleen		Pool of mesenteric lymph nodes		Pool of pancreatic lymph nodes	
	Antigen administered with:					
	DNA digest	Saline	DNA digest	Saline	DNA digest	Saline
96	50	4	0	.4	1.2	0
	6	0				
	10	4				
	8	4				
	134	0				
120	25	90	.3	0	304	.4
	0	30				
	725	0				
	120	150				
	80	0				
	10					
144	110	45	5	0	133	20
	115	90				
	215	185				
	370	20				
	240	870				
	120					
192	480	65	0	10	30	0
	140	40				
	305	15				
	5	255				
	150	65				
Background	1		0		3	

TABLE III. Hemolysin Production in $C_{57}Bl/6$ Mice Immunized at 10 Days of Age with 10^8 Sheep Red Blood Cells.

Time of assay (hr after immunization)	No. of hemolysin-producing cells/animal in Spleen		Pool of mesenteric lymph nodes		Pool of pancreatic lymph nodes	
	Antigen administered with:					
	DNA digest	Saline	DNA digest	Saline	DNA digest	Saline
96	8	2	0	0	0	1
	22	580				
	162					
	146					
144	35	15	5	0	6	1
	25	5				
	70	0				
	50	15				
192	40	10	0	5	0	5
	4	39				
	386	34				
	70					
Background	0		0		0	

ICR mice, immunized at 7 days of age, gave a maximum titer of 50% hemolysis at a 1:10 dilution at 144 hours after immunization (Fig. 2). C_{57}Bl animals, immunized at 10 days, gave a maximum titer of 50% hemolysis at a 1:15 dilution at 192 hours after immunization (Fig. 1). It is likely that the differences between titers of circulating antibody and numbers of antibody-forming cells in spleen and lymph nodes reflect differences in sites of antibody formation and/or differences in capacity of antibody formation by committed individual cells. The present data for newborn animals clearly show that hemolysin titers do not necessarily reflect the number of hemolysin-forming cells in the organs assayed, whereas according to Friedman's data(2) there is such a correlation in adult animals. In line with this it is noteworthy that the strain differences in responsiveness observed in adult mice(2) follow the same trend in newborn only when circulating hemolysins are taken into consideration and not when comparisons are made on the basis of tissue responses.

c) Stimulatory effects of oligodeoxyribonucleotides. The concurrent administration of antigen and oligodeoxyribonucleotides causes a significant increase in the early number of hemolysin-forming spleen cells in adult AKR mice(4). Comparable effects are difficult to discern in the newborn in the present study. This difficulty is the result of the great individual variation in spleen responses mentioned above, which interferes with the calculation of any significant averages.

An oligodeoxyribonucleotide effect is discernible in the responses of pancreatic and mesenteric lymph nodes of AKR mice and in the pancreatic lymph nodes of Ha/ICR animals. In contrast, the mesenteric lymph nodes of Ha/ICR and all of the lymph nodes tested in C_{57}Bl showed a lack of stimulation by oligodeoxyribonucleotides. This lack of stimulation may be associated with the very poor basic response to antigen which is typical of these organs in young animals of these strains.

The only strain in which the administration of oligodeoxyribonucleotides caused an elevated hemolysin titer was Ha/ICR. In these mice, differences in circulating antibody were observed 120 through 192 hours after immunization, as shown in Fig. 2.

In general, administration of oligodeoxyribonucleotides with the antigen at an age at which a particular strain normally failed to respond did not elicit any detectable responses.

Summary. Newborn mice of AKR, Ha/ICR and C_{57}Bl strains start to respond to immunization with sheep red blood cells at different ages (as measured by assays on circulating hemolysins and numbers of antibody-forming cells in spleen and lymph nodes). Once competence to respond has been attained, the magnitude of the response, in terms of numbers of antibody-forming spleen cells, is similar for all strains tested. However, there is no correlation between numbers of hemolysin-forming cells in the organs tested and circulating hemolysins. Only the latter parallel the strain differences in responsiveness observed by others in adult animals of these strains. When antigen is administered in conjunction with oligodeoxyribonucleotides, the early immune responses are enhanced, but the time of competence to respond to the antigenic stimulus is not advanced.

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