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EFFECTS OF OESTROGENS AND PREGNANCY ON THE DISTRIBUTION OF SHEEP ERYTHROCYTES AND THE ANTIBODY RESPONSE IN MICE

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SUMMARY

Mice pre-treated with three oestrogenic preparations showed increased hepatic and reduced splenic uptake of ^{51}Cr-labelled sheep erythrocytes (SRBC). The antibody response to SRBC was also reduced. It was shown that, under certain conditions, the number of antibody-forming cells in the spleen parallels the amount of SRBC localizing in this organ and that both can be depressed or enhanced by appropriate pre-treatments with oestrogens or colloidal carbon. The effects of these agents are mediated through stimulation or 'blockade' of the phagocytic activity of liver macrophages.

Changes in localization of SRBC in pregnant mice were similar to those found after treatment with oestrogens. These changes were, however, rather small and the antibody response of pregnant animals was not affected.

INTRODUCTION

During pregnancy there is a spectacular increase of oestrogen production and excretion (compare Hytten & Leitch, 1971). Oestrogens administered to animals cause a marked stimulation of the phagocytic activity of the mononuclear phagocyte system (MPS) (for review see Vernon-Roberts, 1969; Šljivić & Warr, 1973a). A similar observation was made in patients undergoing therapy with oestrogens (Magarey & Baum, 1971). Under physiological conditions oestrogens are presumably responsible for increased MPS activity during pregnancy (Nicol *et al.*, 1964; Douglas & Grogan, 1970; Graham & Saba, 1973) and some stages of the oestrus cycle (Nicol *et al.*, 1964), as well as the higher activity in females as compared to males (Vernon-Roberts, 1969; Nothdurft & Flemming, 1971).

We have recently shown that administration of stilboestrol can alter the distribution of

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sheep erythrocytes (SRBC) by enhancing the phagocytic activity of hepatic macrophages (Warr & Šljivić, 1973) and have suggested that the concomitant reduced localization of this antigen in the spleen was responsible for the impaired antibody response seen in animals pretreated with this hormone (Warr & Šljivić, 1972; Šljivić & Warr, 1973b). The dose of stilboestrol used in these experiments was, however, far in excess of physiological or even therapeutic levels.

The present report considers the effects of pregnancy and pre-treatment with various doses of oestrogens on the distribution of, and the antibody response to, SRBC, and provides direct evidence that a depression of the antibody response can be caused by redistribution of this antigen *in vivo* resulting from MPS stimulation.

MATERIALS AND METHODS

Animals

Inbred, 2½–3½-month-old mice were used. Female CBA mice weighed 18–24 g, and male BALB/c mice 20–31 g. Pregnant mice were obtained by leaving males for a period of 24 hr with females in order to time the pregnancy and this was assessed further in some experiments by measuring the length of foetuses (Rafferty, 1970).

Oestrogens

Stilboestrol (diethylstilboestrol, Sigma Chemical Company, Norbiton Station Yard, Kingston-upon-Thames, Surrey) was dissolved at varying concentrations in arachis oil (Šljivić & Warr, 1974) and injected subcutaneously (s.c.) in 0·2 ml, at doses ranging from 10 to 1000 μg. Some control mice received arachis oil alone. Honvan (diethylstilboestrol diphosphate, Ward, Blenkinsop and Company, London) was injected intravenously (i.v.) at doses of 1, 5 and 10 mg in 0·2 ml saline (0·15 M NaCl). Animals receiving the two larger doses appeared to be heavily sedated after the injection, and the 10 mg dose was lethal to some mice. Oestradiol benzoate B.P. was injected s.c. at a dose of 1 mg in 0·5 ml ethyl oleate.

Organ uptake

SRBC in Alsever's solution (Tissue Culture Services, Slough, Bucks.) were washed and labelled with ^{51}Cr (sodium chromate B.P., Radiochemical Centre, Amersham, Bucks.) as described before (Warr & Šljivić, 1973). They were then suspended in sterile saline and a known number injected i.v. in 0·2 ml. Organ distribution was measured as before (Warr & Šljivić, 1973) and the degree of uptake is given as a percentage of the dose injected. Uptake was measured both 15 min and 24 hr after injection. While early organ uptake (15 min) was considered to give an indication of relative phagocytic activities (Warr & Šljivić, 1973), 24-hr uptake was measured in some experiments to assess the final organ distribution when all SRBC had been cleared from the blood. It should be noted that while the 15 min uptake in the spleen is representative of that seen at 24 hr, the major proportion of injected SRBC has localized in the liver of both control and stimulated mice by 24 hr, unless a dose of SRBC (greater than those used in these studies), sufficient to saturate the phagocytic capacity of the liver, is given (Warr & Šljivić, 1973).

Antibody response

Mice were injected i.v. with a known number of SRBC in 0·2 ml saline and the number of

direct (IgM) haemolytic plaque-forming cells (PFC) was determined as described elsewhere (Šljivić & Warr, 1973b).

Carbon blockade

In order to block the hepatic uptake of SRBC (Souhami, 1972) colloidal carbon (C11/1431a, Günther Wagner, Pelikan Werke, Hanover) was injected i.v. 6 hr before SRBC. CBA mice received 50 μl and BALB/c mice 75 μl of the original suspension in 0·3 ml of 1% gelatin solution.

Statistics

Results are given as means ± standard errors. Significance of differences between experimental and control groups was analysed by the Student–Welch's *t*-test (Cochran & Cox, 1957).

RESULTS

Effects of oestrogens on organ distribution of SRBC and the antibody response

The organ uptake of ^{51}Cr-labelled SRBC in mice pretreated with stilboestrol, oestradiol benzoate and Honvan is shown in Table 1. All three oestrogens caused a marked increase

TABLE 1. Effect of oestrogens on organ distribution of SRBC

Treatment	Liver		Spleen		Lungs (percentage uptake)
	Weight (g/100 g of body weight)	Percentage uptake	Weight (mg)	Percentage uptake	
None	4·88 ± 0·05	44·5 ± 4·1	123 ± 15	9·9 ± 0·8	4·0 ± 0·4
Arachis oil	4·50 ± 0·12*	50·8 ± 3·3	125 ± 12	11·1 ± 1·5	3·1 ± 0·6
Stilboestrol (1 mg)	5·19 ± 0·06	91·7 ± 1·1*	115 ± 4	2·0 ± 0·4*	0·2 ± 0·02*
Oestradiol benzoate (1 mg)	4·98 ± 0·08	88·7 ± 3·8*	127 ± 14	3·0 ± 0·8*	0·2 ± 0·04*
Honvan (1 mg)	4·84 ± 0·19	72·1 ± 0·6*	112 ± 12	6·8 ± 0·6*	2·2 ± 0·3*
Honvan (5 mg)	5·24 ± 0·01*	91·0 ± 0·7*	143 ± 8	3·3 ± 0·6*	0·4 ± 0·05*
Honvan (10 mg)	6·80 ± 0·40*	92·1 ± 1·5*	157 ± 6	1·1 ± 0·05*	0·4 ± 0·1*

Three BALB/c mice in each group were injected with 8×10^8 ^{51}Cr-labelled SRBC 3 days after administration of oestrogens and the uptake was measured 15 min later.

* Significantly different from untreated control ($P < 0·05$ to $P < 0·001$).

in hepatic uptake, as measured at 15 min after injection, and a reduction in the splenic and lung uptake. This effect is similar to that described for stilboestrol alone (Warr & Šljivić, 1973).

The effect of oestrogens on the primary antibody response to SRBC, measured at the peak of the response, is shown in Table 2. The numbers of PFC were significantly reduced in mice pre-treated with any of the hormones tested.

The relationship between the hormone dose on one hand, and the organ uptake and antibody response on the other, was studied in two parallel experiments. Stilboestrol was

TABLE 2. Effect of oestrogens on the primary response to SRBC

Treatment	Direct PFC/spleen		Direct PFC/10^6 spleen cells		Number of mice
	Mean $\log_{10}$	Geometric mean	Mean $\log_{10}$	Geometric mean	
None	4·78±0·10	59,730	2·43±0·09	268	5
Stilboestrol (1 mg)	4·02±0·10*	10,390	1·75±0·12*	56	3
Oestradiol benzoate (1 mg)	3·81±0·14*	6473	1·71±0·11*	52	3
Honvan (5 mg)	3·66±0·11*	4581	1·45±0·14*	28	3

BALB/c mice were immunized with 10^7 SRBC 3 days after treatment (2 days after Honvan) and the PFC response was measured 4 days later.

* Significantly different from untreated controls ($P<0·02$ to $P<0·005$).

administered, in doses ranging from 50 to 1000 μg, to groups of mice and 3 days later these were either immunized or used to determine the hepatic and splenic uptake of ^{51}Cr-labelled SRBC. The results of these two experiments are shown together in Fig. 1. The minimum dose

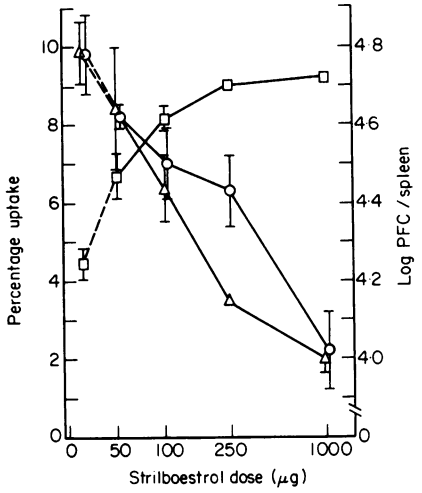

FIG. 1. Effect of varying doses (log scale) of stilboestrol on the organ uptake of ^{51}Cr-labelled SRBC and the primary antibody response to SRBC. The hormone was administered 3 days before SRBC. The uptake was measured 15 min and the PFC response 4 days after SRBC injection. Figures for hepatic uptake were divided by 10 to allow plotting on the same scale. Each point is a mean for three to five animals. (□) Liver. (○) PFC. (△) Spleen.

of stilboestrol (not shown here), which gave a noticeable effect in terms of organ uptake, was 10 μg. As the dose of stilboestrol increased, the uptake of SRBC by the liver also increased, but the splenic uptake and numbers of PFC in the spleen progressively declined.

In order to provide more formal evidence that oestrogens can depress the antibody response to SRBC by diverting the antigen from the spleen to the liver, and not by interfering with antibody-producing cells, the following experiments were designed. They were based, in

part, on the observation made by Souhami (1972) that prior administration of colloidal carbon reduced the hepatic and increases the splenic uptake of SRBC. Four groups of mice in each of the two parallel experiments received one of the following treatments: (a) SRBC only; (b) 1 mg of stilboestrol 3 days before SRBC; (c) colloidal carbon 6 hr before SRBC; and (d) stilboestrol 3 days and colloidal carbon 6 hr before SRBC. In one experiment mice were injected with ^{51}Cr-labelled SRBC for uptake studies 24 hr later, and in the other with 10^7 SRBC for the PFC response 4 days later. The results of these two experiments are shown in Fig. 2, and can be summarized as follows. Stilboestrol alone caused a slight increase in hepatic uptake (this was not marked since the uptake was measured at 24 hr

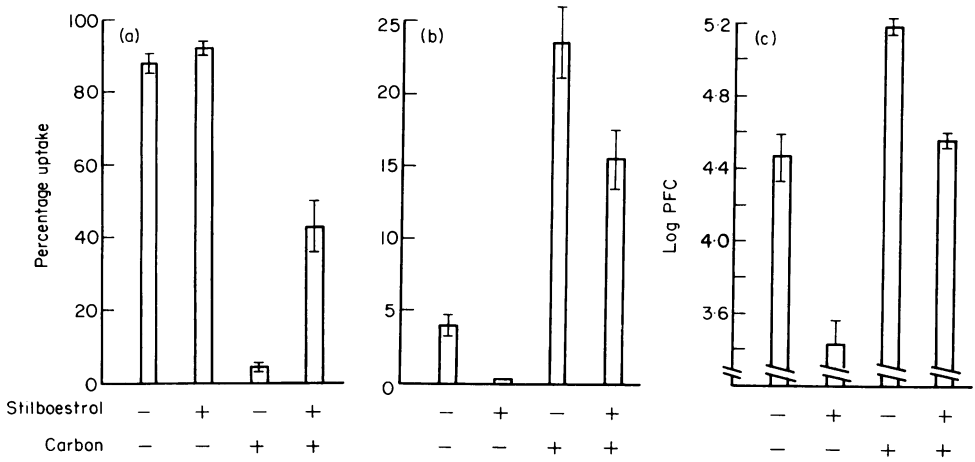

FIG. 2. Effect of stilboestrol and colloidal carbon on organ localization of ^{51}Cr-labelled SRBC and the primary PFC response to SRBC. See text for details of treatment. Values are means for four to five mice in each group. (a) Liver. (b) Spleen. (c) PFC/spleen.

by which time most of the injected SRBC had been taken up by the liver in control animals) and a distinct drop in splenic uptake and PFC numbers. Colloidal carbon alone had an effect opposite to that of stilboestrol, i.e. reduced the hepatic uptake and increased the splenic uptake and the PFC response. Finally, administration of stilboestrol followed by colloidal carbon had an effect which was intermediate to those obtained by each agent given separately. The dose of colloidal carbon used was presumably insufficient to block hepatic phagocytosis in animals stimulated by stilboestrol as effectively as it did in control mice.

Uptake of SRBC and antibody response in pregnant mice

In view of the increased production of oestrogens during pregnancy and suggestions that they are responsible for the greater phagocytic activity of the MPS, as mentioned in the Introduction, two experiments were carried out in order to determine whether effects obtained by administration of oestrogens could be detected under physiological conditions during pregnancy. In the first experiment the hepatic and splenic uptake of ^{51}Cr-labelled SRBC was measured in control mice and in mice 18–20 days pregnant. The hepatic uptake in pregnant mice was significantly increased and the splenic uptake slightly, but significantly, reduced (Table 3).

TABLE 3. Organ uptake of SRBC in pregnant mice

Group	Liver		Spleen	
	Weight (g/100 g of body weight)	Percentage uptake*	Weight (mg)	Percentage uptake*
Non-pregnant	5·11 ± 0·11	40·0 ± 3·8	76 ± 8	6·66 ± 0·59
Pregnant†	6·08 ± 0·42	54·5 ± 1·8‡	88 ± 2	4·04 ± 0·59‡

* Percentage of the dose given 15 min after injection of 5×10^8 ^{51}Cr-labelled SRBC. There were four CBA mice in each group.

† Eighteen to 20 days pregnant at the time of the test.

‡ Significantly different from controls ($P < 0·02$).

In the other experiment 18–20 day pregnant females and their non-pregnant controls were immunized with either a low (5×10^6) or a high (10^9) dose of SRBC, and the number of antibody-forming cells was measured 4 days later. As shown in Table 4, no difference could be detected between pregnant and non-pregnant mice in terms of their PFC response.

TABLE 4. Primary antibody response to SRBC in control and pregnant CBA mice

Group	Dose of SRBC	Direct PFC/spleen*		Spleen weight (mg)	Spleen cell count ($\times 10^{-6}$)	Number of mice
		Mean $\log_{10}$	Geometric mean			
Non-pregnant	5×10^6	4·82 ± 0·03	66,180	98 ± 6	179 ± 22	5
Pregnant†	5×10^6	4·75 ± 0·11	55,590	129 ± 9	262 ± 24	5
Non-pregnant	10^9	4·90 ± 0·07	78,940	132 ± 11	287 ± 29	5
Pregnant†	10^9	4·91 ± 0·05	81,510	133 ± 2	198 ± 28	4

* Determined 4 days after immunization.

† Eighteen to 20 days pregnant at the time of SRBC injection.

DISCUSSION

It was shown earlier that stilboestrol administered to mice causes stimulation of the MPS which results in an altered distribution of intravenously injected particulate materials, with a characteristic reduction in splenic localization (Warr & Šljivić, 1973). In addition, it was shown that stilboestrol had an immunosuppressive effect during the primary response to suboptimal and optimal doses of SRBC and it was suggested, on the basis of experiments in which the dose of SRBC was varied, that the magnitude of the antibody response in the spleen is determined by the amount of antigen reaching this organ (Šljivić & Warr, 1973b). The present experiments extend these findings and provide more direct evidence concerning the mechanism of depression of the antibody response which is the result of oestrogen administration.

In accordance with the findings of a number of authors that oestrogens stimulate the phagocytic activity of the MPS (compare Vernon-Roberts, 1969; Šljivić and Warr, 1973a)

all three compounds used in this study caused a marked increase of the 15 min hepatic uptake of ^{51}Cr-labelled SRBC. At the same time the splenic uptake was reduced, a feature not found when other methods of stimulating the MPS are used (Warr & Šljivić, in preparation). It has been shown elsewhere (Warr & Šljivić, 1973) that this depression of splenic localization is not caused by some deficiency in the normal function of this organ, but is the result of a competition with the liver, the phagocytic activity of which is greatly enhanced. Stimulation of hepatic phagocytosis appears to be caused, at least in part, by an increase in macrophage numbers (Warr & Šljivić, 1973) which, in turn, seems to be brought about by migration and proliferation of cells of extra-hepatic origin (Warr & Šljivić, 1974). However, the intimate mechanisms by which oestrogens initiate all these processes are not understood.

In addition to changes in localization of ^{51}Cr-labelled SRBC, all three oestrogenic compounds used in the present study caused a depression of the primary antibody response to a given dose of SRBC. Two experiments contribute to the understanding of this depression. In the first, graded doses of stilboestrol, ranging from 50 to 1000 μg, caused a progressive decline of the splenic uptake and numbers of PFC in the spleen. This parallelism in the change of the two indices may be taken to suggest that the number of antibody-producing cells is related to the amount of antigen taken up by the spleen. The alternative possibility, that oestrogens may have a direct deleterious effect on immunologically competent cells, while at the same time causing redistribution of antigen, is very unlikely in the light of the experiments involving 'blockade' of the phagocytic activity of the MPS. Administration of colloidal carbon 6 hr prior to SRBC reduced the hepatic uptake and increased both the uptake and PFC numbers in the spleen, thus confirming the finding of Souhami (1972). In mice pre-treated with stilboestrol the 'blockade' induced by colloidal carbon was less effective than in untreated animals, but was nevertheless sufficient to greatly enhance the splenic uptake and the PFC response as compared to animals which received stilboestrol only. The present experiments thus confirm in a direct manner the earlier suggestion (Šljivić & Warr, 1973b) that the depression of the antibody response after treatment with stilboestrol is caused by reduced availability of antigen, resulting from its redistribution, and is not mediated by an effect of the hormone on the function of antibody-producing cells.

Greater phagocytic activity of the MPS during pregnancy has been demonstrated on several occasions (Nicol *et al.*, 1964; Wexler & Kantor, 1966; Douglas & Grogan, 1970; Graham & Saba, 1973) and this has been attributed either to increased oestrogen production (Nicol *et al.*, 1964) or elevated opsonin levels (Graham & Saba, 1973). These findings have been confirmed in the present experiments in terms of enhanced hepatic uptake of ^{51}Cr-labelled SRBC. In addition, the splenic uptake was reduced, an observation also made by Graham & Saba (1973) using a different test system. The pattern of changes of organ uptake is, therefore, similar to that found in animals treated with oestrogens. It should be pointed out, however, that changes in organ distribution during pregnancy are comparatively small and would correspond, approximately, to those induced by a single dose of 10–50 μg of stilboestrol.

Antibody responses during pregnancy have been studied by several authors in a variety of species (Merritt & Galton, 1969; Woodrow, Elson & Donohoe, 1971; Fabris, 1973; Outteridge & Dufty, 1973) and no depression, as compared to non-pregnant animals, has been found. The results of the present experiments are in agreement with these reports.

Although the antibody response was assayed under conditions which were optimal for the demonstration of the depression after administration of oestrogens, no difference was found between pregnant and non-pregnant mice. The fact that rather small changes in organ localization of SRBC are found during pregnancy, and that large doses of oestrogens are required for the depression of the antibody response, lead to the condlusion that physiological variations of oestrogen levels are insufficient to affect antibody formation.

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