could be obtained as readily when both the activatory and cytotoxic stages of the reaction were performed in medium containing foetal calf serum instead of human serum (Table II).

It was expected that lymphocytes activated by stimulants other than irradiated EB cells might be cytotoxic to EB cells in view of the reports that lymphocytes activated by a variety of stimulants are cytotoxic to Chang liver cells[7,8]. In practice, lymphocytes stimulated with phytohaemagglutinin or staphylococcal filtrate proved to be much less cytotoxic to EB cells than lymphocytes of the same donor stimulated with irradiated EB cells (Table II). The results may indicate that some specificity of the inducing agent is required. Lymphocytes activated with a high dose (10^6) of irradiated EB cells were more cytotoxic than lymphocytes activated with a low dose (10^5) even though the level of DNA synthesis measured was greater with the smaller dose. However, the specificity seems to be limited. Lymphocytes stimulated with either EB2 cells or Jiyoye cell line cells showed no differential killing effect towards the two cell types (Table III). We are currently investigating the question of specificity employing other cell lines[14].

Résumé. Les lymphocytes frais du sang humain, après culture avec des cellules lymphoïdes de lignées continues exposées aux rayons X, peuvent tuer ces cellules ou celles d'une autre lignée lymphoïde. Les lymphocytes humains stimulés par culture avec la phytohaemagglutinine n'ont pas cet effet.

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The Induction of Congenital Abnormalities in Mice by Means of Heterologous Anti-Mouse Placenta Serum[1]

The production of congenital malformations after the injection of heterologous anti-rat kidney sera into pregnant rats and mice was reported by different authors[2–6]. Similar results were obtained in rats using heterologous anti-rat placenta sera[7,8].

This paper is concerned with the effect of 2 different forms of heterologous anti-placenta sera in mice: against fresh and lyophilized placental tissue and the possible modification of this effect produced by absorption of the antisera with mice blood, always present in the antigenic tissues.

The interest of studying this teratogenic effect in mice is stressed by the observations about the presence of spontaneous congenital malformations in inbred animals[9].

Material and methods. Adult female Swiss albino mice weighing 25–30 g were mated overnight. Vaginal content was checked every morning and the day in which a plug was found was considered as day 0 of pregnancy. Animals were sacrificed on days 14, 16 or 18 and the dissected placentas were weighed. Aliquots of the 3 ages were pooled, homogeneized in an equal volume of saline in a cooled Virtis homogeneizer. A part of this homogenate was used as antigen without further treatment (fresh placenta). Another part was lyophilized and stored in a freezer at − 25 °C. When used, it was reconstituted with distilled water: 1 ml for each 100 mg of powder (lyophilized placenta).

15 New Zealand adults rabbits were used for the preparation of antisera. 6 of them were injected with fresh placenta, 6 with lyophilized placenta, 1 with mice serum, 1 with mice red cells and 1 with mouse whole blood. All rabbits were immunized with intradermic injections of 1 ml of antigen emulsified in equal volumes of Freund's complete adjuvant[10]. Booster injections of antigen alone were given usually at 15 day intervals and by different routes, during a period of 3–6 month. All sera obtained during and after immunization period were stored at − 25 °C.

Testing of antisera was done by immunodiffusion[11] and a passive hemagglutination test[12]. In both techniques, the placental antigen was the same suspension used for sensitization, except that it was centrifuged at 3500*g* for 90 min at 4 °C. The protein concentration in the supernatant was measured according to WADELL[13] and adjusted to the concentration of 10 mg/ml. Antibodies against mouse blood, present in the anti placenta sera were removed after inactivation at 56 °C for 30 min, incubated with heparinized mouse blood (0.2 ml/ml antisera) for 60 min at 37 °C in a water bath with constant agitation, and overnight at 4 °C. The mixture was centrifuged at 2500*g* for 30 min. The supernatant was separated, checked by immunodiffusion in presence of placental antigen and mouse serum and stored at − 25 °C.

5 groups of experiments were performed as shown in the Table. Age of pregnancies was established by the vaginal plug method as described above. Antisera were injected i.v. in the tail. A single injection of 1 ml/100 g body weight was given to each animal. Injected females were maintained in isolation with food and water ad libitum and sacrificed on the 18 th day of pregnancy by exsanguination. Uterine horns were exposed and implantation sites were registred. Fetuses were separated and inspected for external malformations.

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Malformations and resorptions caused by anti-mouse placenta serum

Type of antiserum	Injection (day)	No. of litters	No. of fetuses	Resorptions[a]	Malformed
Rabbit anti-fresh mouse placenta	7	7	78	6 (7.7%)	6 (7.7%)
	8	18	179	42 (23.4%)	37 (20.8%)
	9	6	51	15 (29.4%)	0
	11	6	60	4 (6.6%)	0
Rabbit anti-fresh mouse placenta absorbed with mouse blood	7	4	43	10 (23.3%)	0
	8	5	48	12 (25%)	2 (4.2%)
Rabbit anti-lyophilized mouse placenta[b]	8	14	122	57 (46.7%)	1 (0.8%)
Rabbit anti-lyophilized mouse placenta absorbed with mouse blood	8	5	50	15 (30.0%)	0
Controls (injected with normal rabbit serum)	8	6	57	4 (7.0%)	0
Controls	–	7	76	5 (6.6%)	0

[a] Included macerated fetuses. [b] Pooled results of group injected with four different batches of serum.

Results. 1 anti-fresh placenta serum and 4 anti-lyophilized placenta sera were selected by higher titers (1/4096 to 1/8192) in the passive hemagglutination tests. As shown in the Table, the first series of experiments indicated that the injection of anti-fresh placenta sera was teratogenic, producing a high incidence of resorptions and malformations. Injections made on the seventh, eighth, and ninth day were effective, while those on the eleventh day did not produce embryonic damage. Most of the malformed fetuses showed varying degrees of exencephaly and anencephaly (Figure). In only 2 cases, the defect in the nervous system was accompained by other malformations: a total medial facial cleft (central animal in the Figure) and an umbilical hernia. About half of the fetuses included in the Table as malformed were alived when extracted; the rest had died recently and not detectable degree of maceration was present. Macerated fetuses, even if malformed, were included as resorptions.

In the other series of experiments pregnant mice were injected with anti-lyophilized placenta serum. Injections were made on the eight day because this was the period in which the maximum number of malformations was obtained previously. It was found that this serum was specially toxic both for mother and fetus: a large number of pregnant mice injected with this serum died within the first hour with symptoms of reverse anaphylaxis, even using as little as 0.3 ml/100 g body wt. The Table includes only survivors. In these cases a low proportion of malformations but a very high incidence of resorptions were observed. The absorption of both types of antisera with mouse blood decreased the total amount of fetal damage. Pregnant mice injected with normal rabbit serum did not contain malformed fetuses and the proportion of resorptions in them was similar to that observed in uninjected controls.

Mouse fetuses, 18 days of age, showing different degrees of exencephaly. Mothers were treated with Rabbit anti-placenta serum.

Mice injected with anti-mouse serum, whole blood and red cells did not tolerate the injection and most of them died with symptoms of anaphylaxis. Those injected with anti-mouse serum sera which survived showed an increase number of resorptions: 19.5% (not included in the Table).

Discussion. Our results in mice agree with those of SLOTNICK and BRENT[7] and BRENT[8] in rats. In their experience, however, the external malformations had a much wider distribution. This discrepancy may be due to species differences. This explanation is supported by the fact that MERCIER-PAROT et al.[5], injecting heterologous anti-kidney serum into pregnant mice, obtained malformations which, as in our experiments, were mainly restricted to the nervous system.

The difference observed in teratogenic activity between fresh and lyophilized anti-placenta sera, could be merely due to differences of intensity in the attack (the fetuses dead before they developed malformations).

TAN and KAPLAN[14] observed cross-reactivity between a β-globulin of mouse serum and the kidney glomerular basement membrane. Furthermore, by means of immunofluorescent techniques, we observed[15] the localization of heterologous anti-placental sera in the latter structure. Our findings that absorption with whole blood decrease the teratogenic action of antisera is thus probably due to the elimination of antibodies against this common antigen and we have actually observed reduction of titer against placental antigen. Our data suggest also the possible teratogenic or letal effect of antiblood sera.

Résumé. Des souris en gestation ont été injectée avec deux types hétérologues de sérum anti-placenta: l'un préparé avec du tissu frais et l'autre avec du tissu lyophilisé. Une haute proportion de réabsorptions et de malformations a été enrégistrée, spécialement depuis l'injection au 8ème jour de la grossesse. Pratiquement dans tous les cas les malformations ont été localisées dans l'extrémité céphalique du SNC, avec différents degrés d'exencéphalie et d'anencéphalie. L'absorption des antisérums avec du sang de souris a réduit le titre antiplacentaire et aussi l'action tératogène.

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