taken into account that only an increase in the SCE rate of 100% is generally considered to be genetically important, since minor but statistically significant increases can be induced by changes in the culture conditions[14-16]. Furthermore, the SCE frequency did not increase when the concentrations of rat or human sera were raised from 10% to 100% (table). Since there were no dose-related effects of the sera on SCE frequency, micronuclei and structural chromosomal aberrations in exposed embryos, it may be assumed that the sera did not contain any genotoxic factors. Similarly, in a recent study no increase in SCE frequency, structural chromosomal abnormalities and micronuclei could be detected in 8-cell mouse embryos of the Balb C/6 × C3H strain during culture in media containing 10–30% human serum[12].

The present investigation proves that preimplantation mouse embryos can be cultured in the presence of media containing human and rat sera. There are several applications of such culture systems, e.g. the development of preimplantation mouse embryos in media containing human serum can be used to study the viability of different lots of human sera to be used in human in vitro fertilization programs[12]. Furthermore, in reproductive toxicology the culture of early mouse embryos in sera from exposed humans or rats holds promise for the detection of chemicals which are embryotoxic during the preimplantation period.

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Human serum as a culture medium for rat embryos

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Summary. A comparison was made between the development of post-implantation rat embryos in human serum and rat serum. Protein synthesis (growth) and somite number (differentiation) were retarded in human serum and there was an increased frequency of neural tube defects. Male and female human sera supported development equally well.
Key words. Embryo culture; human serum; neural tube defect.

Whole embryo culture techniques are now being used widely in the study of teratogenic mechanisms and are also being evaluated for possible applications in teratogen screening[1,2]. One approach is to use serum from an individual treated with the teratogens as the culture medium. This could combine any direct effects of an agent or metabolites of an agent on the embryo with effects resulting from changes in the serum produced by interactions between the agent and the maternal metabolism[3-5]. This procedure can also be used to study the effects on embryonic development of sera abnormal for reasons other than treatment with teratogenic agents (e.g. dietary deficiency[6]).

The potential value of this approach would be greatly increased if embryos could be grown in serum taken from humans dosed with a test substance. Unfortunately, human serum has not so far been shown to be satisfactory as a culture medium for rat embryos. In contrast, rat serum can support development in vitro comparable with that in vivo during the organogenetic phase[7].

Development in human serum can be improved by glucose supplementation[8]. However, when comparisons are made with other studies, using rat serum, it would appear that development in human serum is not as good as that in rat serum; there is

Table 1. Embryonic development in 90% human and 100% rat sera. The human sera were supplemented with glucose to 300 mg%. The rat sera were not supplemented

Group	No. of embryos	Heart-beat (at end of culture)	Yolk sac circulation (at end of culture)	Turning complete	Anterior neuropore closed	Optic vesicles	Otocysts	Two or more pharyngeal arches	Allantois fused with chorion	Limb buds	Somites $\bar{x} \pm SE$	Protein (μg) $\bar{x} \pm SE$
10 human sera	30	30 $p < 0.001$	30 $p < 0.001$	25 $p < 0.05$	14 $p < 0.001$	30	30	29	30	28	20 ± 1	139 ± 10
10 rat sera	30	18	18	18	28*	27	30	26	28	25	20 ± 1 ($n = 29$)	135 ± 13

*The somites in one of the two embryos with an open anterior neuropore were difficult to count accurately. Therefore, it is possible that the failure of the anterior neuropore to close may not have been abnormal for the stage of development.

growth retardation (reduced protein synthesis) and abnormal development. Other experiments where development in rat and human serum was compared have confirmed the occurrence in human serum of abnormalities[9,10] but not of growth retardation[10]. This uncertainty concerning development has adversely affected a more detailed examination of the occurrence of neural abnormalities in rat embryos grown in sera from human volunteers dosed with vitamin A (Steele, unpublished observations). A preliminary study[3] suggested that whole embryo culture could be used to detect teratogenic levels of vitamin A in human sera. However, in subsequent experiments there has been a high incidence of neural tube defects in predose control sera. Clearly, before data obtained from cultures using serum from treated humans can be adequately interpreted, further data on the development of rat embryos in normal human serum must be obtained.

This paper presents a more detailed analysis of rat embryonic development in human serum than has hitherto been published.

Materials and methods. Explantation, culture and assessment of embryos. Rat embryos of the CFHB strain were explanted in cold Hanks saline during the afternoon of the 10th day of gestation (1st day = day of sperm detection in vaginal smear). They were cultured at 37.5 °C in serum in a glass bottle rotated at 30 or 60 rev/min[1]. The medium was equilibrated with a gas mixture containing 5%, 20% or 40% O_2 (see later), 5% CO_2 and the remainder nitrogen. 48 h after the start of culture the presence or absence of a heartbeat and yolk sac circulation was noted. The embryos were then dissected from their membranes and the following assessed: 'turning' (adoption of the fetal position); the presence or absence of optic vesicles, otocysts, pharyngeal arches and anterior limb buds; closure of the neural tube; fusion of allantois and chorion; and somite number. Finally, the embryos were dissolved in N/2 NaOH and their protein content determined spectrophotometrically[11].

Blood donation and serum preparation. Human volunteers were bled circa 09.00 h after overnight fasting (at least 9 h). They did not consume alcohol the previous evening and the two smokers (one male and one female) did not smoke for at least 1 h before being bled. None were receiving medication.

Male and female rats of the CFHB strain weighing about 200 g were allowed food and water ad libitum. They were anesthetized with ether and bled from the aorta.

Immediately-centrifuged sera were prepared using the method described by Steele and New[12] except for human sera in A (see below) which were prepared according to procedures described by Chatot et al.[8]. Samples from individuals were kept separate. Following the addition of penicillin (60 µg/ml) plus streptomycin (100 µg/ml) and heat-inactivation at 56 °C they were stored at −20 °C until used for culture.

A) Comparison of development in human and rat sera.

The serum from each of five male and five female human volunteers was divided into two equal aliquots. Before culture the sera were assayed for glucose[13] and supplemented with glucose to a final concentration of 300 mg%. The ability of the first aliquot of human serum to support embryonic development was compared with that of rat serum not supplemented with glucose, and the second aliquot with that of rat serum supplemented with glucose to 300 mg%.

The culture procedure matched the method described by Chatot et al.[8]. Bottles containing three embryos/2 ml medium were rotated at 30 rev/min. The medium was equilibrated with a gas mixture containing 5% O_2 for the first 24 h, 20% O_2 for the next 18 h and 40% O_2 for the remainder of the culture period.

B) Comparison of development in female and male human sera.

The glucose concentration of the sera was adjusted to 300 mg%. Bottles containing one embryo/1 ml serum were rotated at 60 rev/min. The proportion of O_2 in the gas phase was increased from 5% to 20% after 22 h, and kept at 20% until termination of the culture.

Statistical comparisons were made using Student's t-test (protein content and somite number) and χ^2 (all other parameters).

Results. With one exception (see table 1), all embryos with an open anterior neuropore had 14 or more somites. Since the anterior neuropore normally closes at 14 somites[14] the open anterior neuropores should be considered as abnormalities and not merely developmental delay.

A) Comparison of development in human vs rat sera.

There was little difference in protein content (growth) and somite number (differentiation) in human serum supplemented with additional glucose compared with unsupplemented rat serum (table 1) but closure of the anterior neuropore occurred less frequently in human serum ($p < 0.001$). There were fewer embryos with a heartbeat and yolk sac circualtion after 48 h culture in unsupplemented rat serum compared with human serum

Table 2. Embryonic development in 90% human and 100% rat sera. All sera were supplemented with glucose to 300 mg%

Group	No. of embryos	Heart-beat (at end of culture)	Yolk sac circu-lation (at end of culture)	Turning complete	Anterior neuropore closed	Optic vesicles	Otocysts	Two or more pharyngeal arches	Allantois fused with chorion	Limb buds	Somites $\bar{x} \pm SE$	Protein (µg) $\bar{x} \pm SE$
10 human sera*	30	30 (100%)	30 (100%)	23 (77%)	18 (60%) $p < 0.01$	29 (97%)	30 (100%)	30 (100%)	30 (100%)	28 (93%)	19 ± 0 $p < 0.0005$	125 ± 8 $p < 0.0005$
8 rat sera	24	24 (100%)	24 (100%)	19 (79%)	23 (96%)	24 (100%)	24 (100%)	24 (100%)	24 (100%)	20 (83%)	22 ± 1	174 ± 8

* Same donors as table 1.

Table 3. Embryonic development in 90% male and 90% female human sera. All sera were supplemented with glucose to 300 mg%

Group	No. of embryos	Heart-beat (at end of culture)	Yolk sac circu-lation (at end of culture)	Turning complete	Anterior neuropore closed	Optic vesicles	Otocysts	Two or more pharyngeal arches	Allantois fused with chorion	Limb buds	Somites $\bar{x} \pm SE$	Protein (µg) $\bar{x} \pm SE$
Female sera (4 donors)	40	40	39	35	19	39	40	40	39	40	21 ± 0	270 ± 9
Male sera (4 donors)	40	40	39	35	25	40	40	40	39	40	21 ± 0	289 ± 8

($p < 0.001$). Complete turning occurred less frequently in unsupplemented rat serum ($p < 0.05$).

Supplementation of the rat serum with glucose greatly improved embryonic development (table 2). There were significant differences ($p < 0.0005$) in protein content and somite number compared with embryos grown in supplemented human serum. As before, the difference in frequency of closure of the anterior neuropore was statistically significant ($p < 0.01$), there being more embryos with an open anterior neuropore in human serum.

B) Comparison of development in female vs male human sera. There were no statistically significant differences between data obtained from embryos cultured in female serum and those in male serum (table 3). The protein content of the embryos was approximately double that of embryos cultured in human serum described in A. Closure of the anterior neuropore again occurred in only about 50% of the embryos.

Discussion. The comparison of development in rat and human sera (section A) showed that human serum did not support development as well as rat serum when both had the same glucose concentration. Growth and differentiation were retarded in human serum and there was an increased frequency of neural tube defects. Only when the rat serum was not supplemented with glucose, and used at the relatively low volume of 0.6 ml/embryo, did development in human serum compare favorably in terms of growth and differentiation. This is in accord with the observations made by Ellington[15] on the effects of rat starvation and the subsequent reduction in serum glucose concentration on the ability of sera to support normal development. Even so, the use of rat serum, though unsupplemented, still resulted in a lower incidence of neural tube defects compared with supplemented human sera.

The present observations on growth are not in accord with those of Gupta and Beck[10] who found that there was no significant difference in protein content between embryos grown in human serum compared with those in rat serum. However, the overall conclusion, that rat embryos cannot be reliably cultured in human serum, is the same. Potential solutions to this problem include dilution of human serum with rat serum[9] or a synthetic culture medium.

The growth (protein content) of embryos cultured in human serum was greater in section B than in section A. The most likely explanation for this is the increase in the amount of serum from 0.6 ml/embryo to the more widely accepted 1 ml/embryo. The results obtained in section B showed that male and female human sera supported development equally well, confirming the preliminary observations of Chatot et al.[8] with human sera and New[16] with rat sera. This indicates that male human volunteers could possibly be used to screen for teratogens thereby avoiding ethical problems which would arise from the use of female human volunteers of reproductive age.

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Lysozyme activity in female genital tissues of normal and genetically lysozyme-deficient rabbits[1]

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Summary. The internal genitalia of female normal rabbits and mutant lysozyme-deficient rabbits, which lack genetically the leukocytic isozyme of lysozyme, were assayed for lysozyme activity. The ovaries, uteri, and vaginas of the lysozyme-deficient rabbits had less than 20% of the lysozyme activity of normals. The oviducts, and in particular the caudal portions of the oviducts, had lysozyme activities up to 71% of the levels in normals. These observations suggest that the lysozymes of oviduct and leukocytes of rabbits are under the control of different genes.

Key words. Rabbit; lysozyme-deficient; genitalia; lysozyme activity; isozymes.

Lysozyme (EC 3.2.1.17) hydrolyzes the β-1,4 glucosidic linkages of N-acetylmuramic acid and N-acetylglucosamine and is a cationic, small, and ubiquitous enzyme[2]. The primary identification of lysozyme is based upon its lysis of the cell walls of the bacterium *Micrococcus luteus (lysodeikticus)*. Bacterial cell walls are composed of alternating N-acetylmuramic acid and N-acetylglucosamine moieties, the substrate of lysozyme[3]. These observations suggest that the role of lysozyme is antibacterial. In spite of extensive investigations, however, the role of lysozyme in mammals has not been established.

Lysozyme from chicken egg white has been extensively studied and has been well characterized. The majority of studies on lysozyme have utilized this source of the enzyme and hypotheses on the role of lysozyme in mammals have often been based on studies with chicken egg white lysozyme. Lysozyme occurs in high concentration in human leukocytes and it has been suggested that it functions as an antibacterial agent in leukocytes. Some studies have utilized human leukocytic lysozyme isolated from the urine of human patients with leukemia[4].

Recent studies with several species indicate that lysozyme exists