Effects of streptozotocin on early rat embryos grown in culture

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27 June 1977

Summary. Addition of 1 mg/ml streptozotocin to serum in which 10-day rat embryos are cultured reduces their growth and viability. There is therefore a risk that administration of this drug to pregnant animals to induce diabetes could also have direct, deleterious effects on the embryos.

Although streptozotocin, the drug used to induce diabetes in laboratory animals[1,2], appears to act selectively on pancreatic β-cells and is eliminated from the body very rapidly[3], it is uncertain to what extent it may damage other tissues, or the embryos of a pregnant mammal. Reduced size and increased mortality have, however, been observed in fetuses of streptozotocin-diabetic female rats[4-6] as well as low incidences of brain, visceral and skeletal abnormalities[7]. In order to find out, therefore, whether these abnormalities could have been caused by the streptozotocin, rather than by the maternal diabetes, we have cultured embryos in serum containing concentrations of streptozotocin equivalent to those with which the rats were dosed to induce diabetes. The development of the embryos over a 24-h-period which includes major steps in organogenesis has been compared with that of controls cultured in normal serum.

Material and methods. 10-day Wistar rat embryos (day 0 = day of finding vaginal plug) were removed from the uterus and cultured in their membranes in watch-glasses as described by New[8]. For each embryo, 0.3 ml of maternal serum (obtained by heart puncture immediately before death) was used. 1 mg/ml of streptozotocin (Upjohn Ltd), freshly dissolved in ice-cold 0.01 M citrate buffer, pH 4.7, was added to the experimental serum, and an equivalent volume of citrate buffer alone to the controls. Cultures were gassed with 95% O_2/5% CO_2 and incubated at 37.5 °C for 24 h. After examining them alive, embryos were fixed in Bouin's fluid, dehydrated in alcohols and cleared in methyl benzoate. Cleared specimens were measured with an eyepiece micrometer, and histological studies were made on 8 μm sections stained with haematoxylin and eosin.

Results. a) Observations on whole embryos. 34 experimental and 34 control embryos were compared. 20 of the experimentals developed well (classed as 'very good' or 'good' in the table): i.e. the membranes had expanded to a large, transparent vesicle, the embryo had grown, differentiated and rotated normally[9] and its heart was beating rapidly, circulating red blood. In only 5 of these embryos ('very good' in the table) was blood circulating in the yolk sac, however, whereas 24 of the controls had a rapid yolk-sac circulation: these also had a more vigorous heart-beat than the 'good' embryos. By a χ^2-test, the numbers of 'very good' and 'good' controls are significantly higher than in the experimentals ($p < 0.01$). There was also only 1 retarded embryo and 3 deaths in the controls, as compared with 5 retarded and 9 dead experimental embryos. Embryonic size was assessed by measuring the widest diameter of the yolk sac vesicle, the trunk length and the tail length of each embryo. As the table shows, the mean values of all these dimensions were significantly higher in the controls than in the streptozotocin-treated embryos ($p < 0.01$, on a t-test).

b) Histological observations. Histogenesis of organs is not far advanced in 11-day rat embryos, so most attention was paid to the morphology of axial structures, nervous system, heart and liver. The table lists the main features observed. Neither the nervous system nor the heart appeared to be affected by the streptozotocin treatment (differences in incidence of abnormalities are not significant, between experimental and controls), but the liver appeared to be retarded. No liver was evident at all in 6 embryos, and it was barely detectable in 12, of the experimental group, whereas only 2 of the controls had a less than normally developed liver (figure a, b). This difference between experimental and control embryos is statistically significant ($p < 0.01$, on a χ^2-test).

Discussion and conclusions. The smaller size and the less frequent development of a yolk sac circulation in experimental embryos, as compared with controls, suggest that

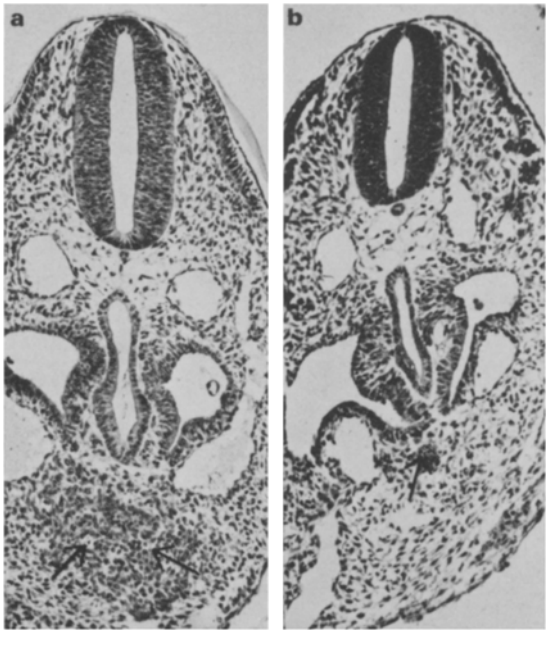

Fig. a. Transverse section of liver region in control embryo, ×45. Note fairly dense mass of liver cells (arrowed).

Fig. b. Transverse section of liver region in experimental embryo, ×45. Note very small liver rudiment (arrowed).

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	External features (34 experimental and 34 control embryos)							Histological observations (26 experimentals and 26 controls)				
	Very good	Good	Retarded	Dead	Vesicle diameter	Body length	Tail length	CNS abnormal	Heart abnormal	Liver normal	small	absent
Experimentals	5	15	5	9	2.97 ± 0.067	1.97 ± 0.079	1.75 ± 0.077	12	5	8	12	6
Controls	24	6	1	3	3.44 ± 0.079	2.49 ± 0.070	2.32 ± 0.088	9	4	24	1	1

Vesicle, body and tail measurements are in mm, ± SE; other figures represent numbers of embryos in each category.

streptozotocin has deleterious effects on the growth and viability of embryos at this stage. The apparently delayed development of the liver may be linked with the failures in yolk sac circulation, since the liver develops from the proximal anterior wall of the yolk sac and is also a site of haemopoiesis. None of these effects, however, parallels the abnormalities that have so far been observed in embryos of streptozotocin-diabetic rats (i.e. malformations of the nervous system and heart, skeletal deficiencies and exomphalos[7]). So it seems likely that these latter abnormalities were attributable to the maternal diabetes rather than to any direct effects of streptozotocin. Moreover, since the female rats were injected with streptozotocin either before mating or on day 0, and it is eliminated from the body within 4–6 h[3], only early cleavage stages of embryos could be at much risk of exposure to the drug. The present work shows, however, that administration of streptozotocin to rats at post-implantation stages of pregnancy could have severe effects on the further development of the embryos.

Modification of hepatotoxic effects of aflatoxin B_1 in rabbits by immunization[1]

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Summary. Reduction of acute toxic effect of aflatoxin B_1 was achieved by immunizing the rabbits with small amounts of bovine serum albumin-aflatoxin B_1 conjugate. Rabbits after immunization showed lower mortality, near normal serum isocitric dehydrogenase activity, no abnormality in livers when challenged with a single dose of aflatoxin B_1. The results suggest that immunization might be used prophylactically against aflatoxicosis.

Aflatoxin B_1 (afla B_1) is one of the most potent environmental carcinogens and hepatotoxins produced by *Aspergillus parasiticus* and *A. flavus*. Because of the potential hazard of this toxin to human and animal health, the chemistry and the biochemical and pathological effects of afla B_1 have been studied extensively in the last decade[4,5]. Unlike most bacterial toxins, afla B_1 and other mycotoxins are small molecular weight fungal metabolites with diverse chemical structures. While these toxins are devoid of any antigenicity, an afla B_1-1-(0-carboxymethyl)-oxime can be prepared through derivation[6,7]. The new derivative has a carboxyl group which is readily coupled to a protein for immunization. Using this approach, investigators in this and other laboratories[7,8] have produced antibody in rabbits showing high affinity to afla B_1 after the animals were immunized with either bovine serum albumin (BSA)-alfa B_1 conjugate or with polylysine-afla B_1 conjugate. The antibody was also found to be useful in the radioimmunoassay for afla B_1. The present study was carried out in order to find whether or not immunization might be used prophylactically against aflatoxicosis.

Material and methods. Since rabbits are among the most sensitive animals with regard to afla B_1 toxicity, this species was selected for study. Albino female rabbits weighing 3.5 kg were divided into 6 groups of 3–7 rabbits each. 3 groups of rabbits were immunized with BSA-afla B_1 conjugate (210 µg per rabbit) which contained 13 moles of afla B_1/mole of BSA, according to the method previously described[8]. Rabbits in the other 3 groups were raised under the same conditions but were not immunized. Antibody titers of the immunized rabbits were determined by a binding method[9] every week starting from the 4th week after immunization. 6 weeks after immunization, all the animals were challenged with a single dose of pure alfa B_1 by i.p. injection. Mortality and serum isocitric dehydrogenase activity (ICDH, Sigma method[10]) were monitored. Limited histological examinations on

1 Supported by the College of Agricultural and Life Sciences, the University of Wisconsin, by Public Health Service research grant number CA 15064 from the National Cancer Institute, and by contribution from food industries to the Food Research Institute of University of Wisconsin.

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3 The authors wish to thank Prof. R. D. Hinsdill for his comments during the preparation of the manuscript.

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