Table 2. Effect of some nucleotides on poly-L-arginine-induced LDH release from PMNs. PMNs (3×10^6 per ml) were exposed to 100 nM poly-L-arginine and the indicated concentration of nucleotide for 20 min at 37 °C. Values given are the means of three experiments ± SD.

	% LDH release
–	55 ± 3
50 μM $GTP_\gamma S$	5 ± 1
50 μM GDPβS	40 ± 3
50 μM GTP	15 ± 2
50 μM ATP	43 ± 2

tions, and it has been suggested that different G-proteins might be involved [14–16].

There is a remarkable difference between the effect of $GTP_\gamma S$ on poly-L-arginine-induced LDH release, and on LDH release induced by poly-L-lysine. Whereas inhibition by pertussis toxin is about the same for both polycation, the inhibition by $GTP_\gamma S$ is much stronger for poly-L-arginine than for poly-L-lysine. It might therefore be that a G-protein has a modulating effect on the membrane-damaging effect of polycations. There remain some uncertainties, however, because the protective effect of pertussis toxin is modest, and because some other nucleotides, such as GTPβS and ATP also give a (relatively small) inhibition of poly-L-arginine-induced LDH release (table 2). GTP itself is furthermore a good inhibitor which could suggest that the guanine nucleotide-binding structure has no high GTPase activity. Shielding of positive charges on poly-L-arginine might affect its ability to interact with the PMN but it seems unlikely that inhibition by ATP and GDPβS is due to a charge effect because these nucleotides have no effect on poly-L-lysine-induced LDH release.

Though the molecular mechanism of guanine nucleotide inhibition of poly-L-arginine-induced membrane damage is far from clear, the results presented in this paper demonstrate that the damaging effect of the polycation on the plasma membrane is not direct. One or more guanine nucleotide-dependent structures are involved, which either induce membrane damage after activation by poly-L-arginine, or modify the membrane structure and thus enable poly-L-arginine to damage the membrane.

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Intact teratogenic immunoglobulins may reach the rat embryo

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Summary. It is known that heterologous antiserum against rat kidney homogenate may induce congenital malformations when injected into pregnant rats during the period of organogenesis. Teratogenic rabbit antibodies against a purified rat renal tubular glycoprotein were isolated, labelled with ^{125}I and injected into pregnant rats on the 10th day of gestation. Extracts of visceral yolk-sacs (VYS) and embryos were obtained 16 h later and chromatographed separately on a Sephacryl S-300 gel filtration column. The resultant chromatograms showed several radioactive peaks, one of which coincided with the eluate of intact rabbit immunoglobulins G (IgG). We interpret the result as an indication that some undigested intact teratogenic IgG were present in VYS and the embryo.
Key words. Teratogenesis; visceral yolk-sac; antibodies; embryos.

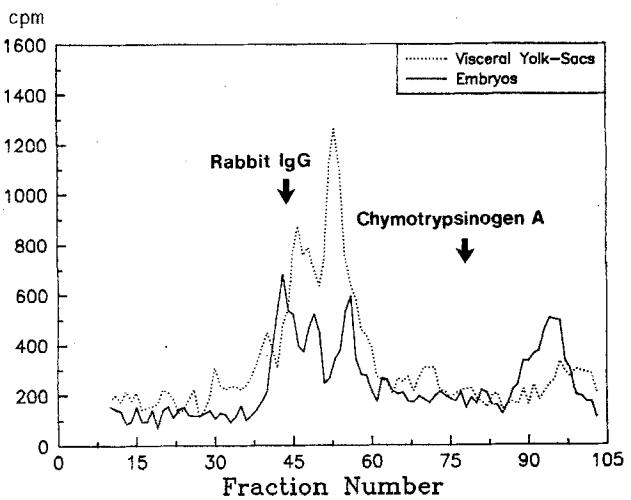
The discovery that rabbit antiserum against rat kidney homogenate induced abnormal embryonic development when injected into pregnant rats during the critical organogenetic period was reported more than a quarter of century ago [1]. Many investigators have since confirmed and extended these findings [2–8]. The biologic effects of the kidney antiserum appeared to be dependent on the dosage of antiserum administered but not on complement or other non-specific immunologic mediators [3]. Since many reported fluorescent antibody localization studies [4–7] demonstrated that the teratogenic antibodies localized in vivo within the visceral yolk-sac (VYS) endodermal cells of the developing embryo, it was hypothesized that the teratogenic antibodies might induce birth defects by causing VYS placental dysfunction. Both biochemical [9] and morphological [10] evidence has been presented to support such a hypothesis. However, it has not been determined if the injected teratogenic antibodies ever reach the developing embryo. Although immunofluorescent studies seemed to fail to demonstrate the presence of such antibodies in the embryo itself [7], it is possible that a small amount of antibodies might escape from detection by the immunofluorescent technique.

A high molecular weight glycoprotein (gp340) has been isolated from rat renal proximal tubules and antibodies against

this glycoprotein were demonstrated to be potent teratogens[11]. Using ^{125}I-labelled anti-gp340 antibodies, we examined the presence of radiolabelled antibodies in the embryo following the administration of radiolabelled antibodies to the mother. This communication presents our findings which suggest that intact antibodies against gp340 might have reached the embryo during the period of organogenesis. Thus, it is possible that teratogenesis may also be a result of direct action of antibodies on the embryo itself.

Methods. Random-bred Wistar rats of both sexes were placed overnight in the same cage to mate. Female rats were considered to be on their first day of pregnancy when sperms were found in vaginal smears in the morning.

Teratogenic rabbit anti-gp340 IgG were purified by ammonium sulfate precipitation at 33% saturation, pH 7.8, followed by DE 52 cellulose ion-exchange chromatography as described previously[11]. The IgG were radiolabelled with ^{125}I by the chloramine-T method[12]. ^{125}I carrier-free sodium iodide, free of thiosulfate, was purchased from Amersham Corporation (Arlington Heights, IL). One mg of IgG dissolved in 0.5 ml of 0.05 M phosphate buffer, pH 7, was radiolabelled with 500 µCi of ^{125}I having specific activity of 13–17 mCi/µg. After the removal of the free ^{125}I by gel filtration through Sephadex G 25 and dialysis, the labelled IgG molecules at a dose of $2.4-2.8 \times 10^7$ cpm were injected i.p. into 18 pregnant rats in the afternoon on the 10th day of pregnancy. Individual embryos and VYS were carefully dissected out 16 h later under a dissecting microscope and immediately stored in a −60 °C freezer. A total of 25 VYS and 109 embryos were collected. The pooled embryos and VYS were separately homogenized in 2 ml of a detergent-buffer solution containing 0.025 M Tris-HCl, 0.15 M NaCl, 0.1% SDS, pH 7.2. After centrifugation at 105,000 × g for 1 h, the clear supernatant extracts of both VYS and embryos were recovered and separately chromatographed in a gel filtration column of Sephacryl S-300 (2.5 × 61 cm) equilibrated with the same detergent-buffer. The gel column was calibrated with normal rabbit IgG and chymotrypsinogen A dissolved in the same detergent-buffer system.



Radioactivity profiles of Sephacryl S-300 gel filtration chromatography of supernatant extracts of 25 visceral yolk-sacs and 109 embryos. The mothers were injected with ^{125}I-labelled teratogenic IgG. The gel column was calibrated with rabbit IgG (150,000 daltons) and chymotrypsinogen A (25,000 daltons). The arrows indicate the respective eluates of these two molecular standards.

Results. Counts of radioactivity of the effluents of Sephacryl S-300 column are shown in the figure. Four peaks with radioactivities above the background were observed for supernatant extract of the embryo homogenate; two major peaks were observed for supernatant extract of VYS homogenate. The first radioactive peak of each chromatogram appeared to coincide with the eluates which contained normal rabbit IgG (mol. wt 150,000). The other retarded peaks appeared to contain the digested radioactive fragments of anti-gp340 IgG, one of the peaks from the embryo extract appeared to contain IgG fragments less than 25 kdaltons.

Discussion. The results of our present investigation seem to suggest that both rat VYS and embryos contained some intact rabbit anti-gp340 IgG. The finding of heterologous IgG in the rat embryo is not at all unexpected since it is known that heterologous IgG can be transported across the maternofetal barrier in the direction of the mother to the fetus in both rodents and rabbits[13–16]. The results seemed to indicate that there were more IgG in the VYS than in the embryo at the 11th day of gestation. Since it is known that the VYS has an embryotrophic function and contains numerous lysosomal hydrolases, it is likely that most of the anti-gp340 IgG might have been digested intracellularly by the VYS endoderm. However, it is possible that some of the IgG escaped from the digestive process and might have reached the embryo. Furthermore, the intact ^{125}I-labelled IgG in the VYS and embryo could not be newly synthesized proteins since cells cannot utilized the digestion product ^{125}iodotyrosine for synthetic purpose[17]. The finding that intact IgG localized in the VYS tends to confirm our previous observation from in vivo immunofluorescent studies[7,11]. However, the presence of intact IgG within the embryo is a new significant finding and has obvious important implication on the possible mechanism of teratogenesis.

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