when they encounter neuronal processes and synapses, they change their course.

In summary, tip cells of the vascular cord may grow several tentacles and thereby contribute to the formation of the vascular network. At this developmental stage of the rat cerebral cortex, the widened intercellular spaces facilitate the vascularization, as recently reported by Caley[3] and Bär[4].

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Immunopotentiators and the protection they give against carbon tetrachloride hepatotoxicity

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Summary. Immunopotentiators such as BCG, levamisole, PS-K and OK-432 prevent carbon tetrachloride (CCl_4) hepatotoxicity, and in spite of exposure to CCl_4 the liver tissue levels of thiobarbituric acid (TBA) reactive substances were not increased in rats pretreated with such immunopotentiators.

It has been suggested that the liver injury caused by carbon tetrachloride (CCl_4) is due to lipid peroxidation in liver microsomes[1]. On the other hand, many immunopotentiators such as BCG, the anthelmintic compound levamisole, a protein polysaccharide from myceria Coriolus vesicolor PS-K[2] and a streptococcal preparation OK-432[3] are now in clinical use in Japan. Levamisole was shown to be an antioxidant in rat liver microsomes in vitro, and also inhibited lipid peroxidation induced by X-irradiation[4]. The present study was undertaken in order to determine the preventive effect of such immunopotentiators against liver damage induced by CCl_4, and its inhibitory effect on lipid peroxidation due to CCl_4. These immunopotentiators were compared with the well known antioxidant α-tocopheryl acetate (vitamin E) for their ability to prevent CCl_4 hepatotoxicity.

Materials and methods. Wistar strain female rats, weighing 250 mg, were treated with BCG; 2.5, 10.0, 25.0 mg/kg, i.p.: levamisole; 10.0, 25.0, 50.0 mg/kg, p.o.: OK-432; 2.5, 3.8, 5.0 mg/kg, i.p.: PS-K; 250, 500, 1000 mg/kg, perorally, and α-tocopheryl acetate; 20.0, 100.0, 200.0 mg/kg, i.p. for 7 successive days. At the end of the treatment period, CCl_4 was injected once in a dose of 0.5 ml/kg, i.p. 24 h after injection of CCl_4, all rats were killed. The liver was perfused with 0.9% NaCl via the portal vein before homogenization. After washing with 0.9% NaCl, tissue homogenates were prepared at a ratio of 1.0 g of wet tissue to 9.0 ml of 1.15% KCl, using a Teflon Potter-Elvehjem homogenizer. Lipid peroxide levels in the liver homogenates were determined by the thiobarbituric acid (TBA) method according to Ohkawa et al.[5]. Glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) were measured according to Reitman and Frankel[6].

Results. Serum GOT and GPT of rats treated with immunopotentiators or vitamin E were not elevated after the administration of CCl_4. The protective effect of immunopotentiators against CCl_4 hepatotoxicity was dose-dependent (table). Histological examination also indicated that,

Effects of immunopotentiators or vitamin E on serum transaminases and liver TBA reacting materials in the rats treated with CCl_4

Pretreatment dosage (mg/kg)	Treatment (0.5 ml/kg)	GOT (Karmen U)	GPT (Karmen U)	TBA reactants[b] (nmoles/100 mg wet wt)
BCG (i.p.)	+ CCl_4			
2.5		968.2 ± 425.1[a,c]	137.3 ± 74.2[c]	54.9 ± 12.5[c]
10.0		561.5 ± 219.7[c]	73.0 ± 40.5[c]	36.0 ± 6.2[c]
25.0		338.2 ± 104.3[c]	34.5 ± 6.2[c]	35.3 ± 5.7[c]
Levamisole (p.o.)	+ CCl_4			
10.0		3137.2 ± 1134.6	984.9 ± 310.6	95.0 ± 33.5[c]
25.0		1051.2 ± 437.4[c]	299.2 ± 104.5[c]	56.9 ± 27.7[c]
50.0		897.9 ± 467.8[c]	165.7 ± 93.4[c]	47.6 ± 8.5[c]
OK-432 (i.p.)	+ CCl_4			
2.5		1779.0 ± 723.9	363.0 ± 129.1[c]	151.6 ± 51.8
3.8		1279.6 ± 511.5[c]	235.5 ± 78.2[c]	92.5 ± 41.5[c]
5.0		522.0 ± 118.1[c]	49.8 ± 16.7[c]	31.5 ± 4.2[c]
PS-K (p.o.)	+ CCl_4			
250.0		1860.3 ± 697.3	749.2 ± 299.6	84.8 ± 29.5[c]
500.0		911.8 ± 379.2[c]	360.8 ± 84.6[c]	67.3 ± 29.3[c]
1000.0		291.7 ± 82.9[c]	58.0 ± 30.4[c]	38.1 ± 9.8[c]
α-Tocopheryl acetate (i.p.)	+ CCl_4			
20.0		1141.7 ± 301.6[c]	204.9 ± 50.5[c]	37.2 ± 5.2[c]
100.0		628.2 ± 292.1[c]	144.0 ± 67.3[c]	36.6 ± 4.4[c]
200.0		196.4 ± 35.4[c]	19.0 ± 5.1[c]	33.0 ± 6.4[c]
Saline (control) (i.p.)	+ CCl_4	2986.5 ± 816.1	925.6 ± 258.5	177.6 ± 36.7
Saline (i.p.)		203.1 ± 41.3[c]	22.4 ± 6.3[c]	34.1 ± 6.3[c]

[a] Data represent mean ± SD of 10 rats in each group. [b] Measured as nmoles of malondialdehyde per 100 mg wet wt. [c] $p < 0.001$ for difference from controls by Student's t-test.

as was the case with the transaminase value, the extent of liver damage was less in rats treated with immunopotentiators or vitamin E than in controls treated with saline. The liver tissue levels of TBA reacting substances were not elevated in rats treated with immunopotentiators or vitamin E even after injection of CCl_4, which fact suggested that lipid peroxidation in liver tissue by CCl_4 might be inhibited. The effect of immunopotentiators against CCl_4-induced lipid peroxidation was dose-dependent (table).

Discussion. It has been accepted that the liver injury caused by CCl_4 is due to lipid peroxidation in liver microsomes[1]. In the present study, experimental liver injury induced by CCl_4 could be inhibited by immunopotentiators such as BCG, levamisole, OK-432 and PS-K, and in spite of exposure to CCl_4 the liver tissue levels of TBA reacting substances were not increased in rats pretreated with such immunopotentiators. Levamisole has been shown to inhibit lipid peroxidation induced by X-irradiation[4]. This study suggests the possibility that such immunopotentiators might exhibit a protective action against lipid peroxidation damage induced by CCl_4. Lipid peroxidation mediated by free-radicals is believed to be one of the important causes of cell membrane destruction and cell damage, for the cell membrane contains much lipid, especially unsaturated fatty acids[7]. The possibility that immunopotentiators might inhibit the CCl_4-induced lipid peroxidation damage deserves special attention from the standpoint of protection against, and treatment of, various kinds of damage arising from lipid peroxidation.

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Immunogenic potency of the zona pellucida

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Summary. New Zealand white rabbits were immunized with low doses of pig zona pellucida material with the aim of reducing nonspecific antibodies in the antiserum. The antibody levels were assayed by the standard precipitation and immunofluorescence methods. The titers produced were comparable with those obtained using large amounts of zona material.

Injection of zona pellucida material into laboratory animals provokes antibody-formation against this membrane[1,2]. Treatment of the zona with antiserum containing antibody-activity against zona-antigens produces a precipitation layer on the surface of the zona pellucida (fig. 1). This precipitation line blocks zona digestion by trypsin[3]. The inhibition of fertilization by anti-zona serum has been reported in the mouse and hamster[4,5]. These anti-zona sera probably interfere with the so-called sperm-attachment sites, or sperm receptors, on the zona pellucida[6], and can be used as immunological vaccines for contraception[7]. Sacco[8] and Dietl et al.[9] demonstrated a crossreactivity between the porcine and the human zona pellucida. As pig zona material is easily obtainable it is therefore an ideal model for immunological studies of the human zona pellucida and can help us to analyse further the immunological properties of this extraordinary membrane.

The intention of the present study was to determine whether a low dose of 100–400 zonae pellucidae (equivalent to 330 ng to 1.32 µg protein) is enough to produce a detectable antibody-titer in a rabbit following immunization.

Fresh pig ovaries were obtained from a slaughterhouse and were kept frozen at −20 °C.

Follicular fluid was gained by aspiration with a fine glass pipette and the follicular eggs were collected from the pooled fluid using a stereomicroscope. Eggs were washed several times in PBS and then suspended in sodium citrate. In order to obtain isolated zonae the cumulus-free eggs were drawn into and expelled from a micropipette with an internal bore slightly less than the diameter of the oocyte plus its zona. The egg is destroyed during this procedure. Isolated zonae were collected and washed twice in PBS.

6 groups of New Zealand rabbits, each consisting of 3 females, 5–6 months old, were immunized as follows:

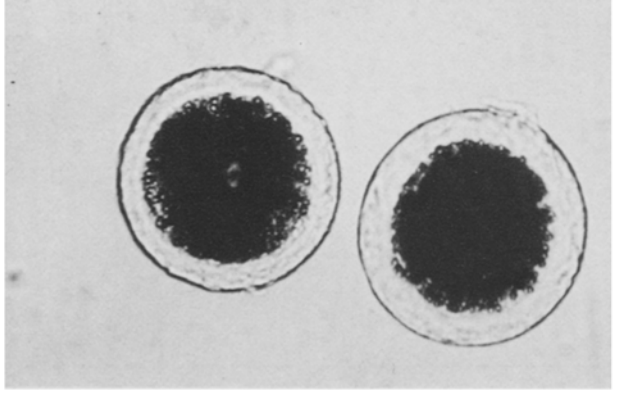

Figure 1. Precipitation layers on pig egg cells.

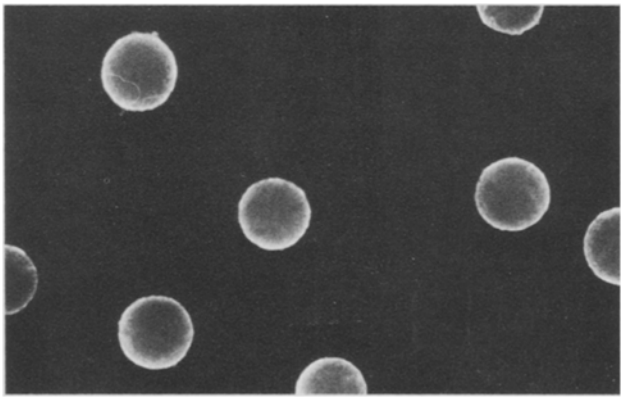

Figure 2. Positive immunofluorescence of pig zonae pellucidae.