fluorescence covering the bile canaliculi is observed, resulting from an ongoing secretion during this period[16]. These observations are in accordance with the view that excretion of IgA follows a transcellular and not a paracellular pathway.

Studying the secretion of horseradish peroxidase Kacich et al.[23] have obtained different results, suggesting that only microtubules but not microfilaments are involved. Whether this difference is due to possible alternative routes for the secretion of IgA and horseradish peroxidase or could be explained in another way is currently being investigated.

Each of the observed effects of these drugs on IgA transport seemed to be specifically mediated by their influence on elements of the cytoskeleton, as has also been found for the effect of vinblastine on canalicular morphology[18]. A general toxic deterioration of cell metabolism caused by these agents could be ruled out by measuring metabolic functions such as urea synthesis[18] and by performing viability tests (e.g. staining with trypan blue or leakage of lactate dehydrogenase) which all revealed a normal behavior of the drug-exposed hepatocytes.

The findings reported here suggest that both microtubules and microfilaments are involved in the transcellular vesicular transport of IgA and that primary cultures of hepatocytes may provide a suitable model for further defining the particular role of these cytoskeletal elements in this kind of secretion.

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2 Allison, A.C., in: Locomotion of Tissue Cells. CIBA Foundation Symposium vol. 14, p. 109. Ed. M. Abercrombie. Associated Scientific Publishers, New York 1973.
3 Bardele, C.F., Cytobiologie *7* (1973) 442.
4 LeMarchand, Y., Patzelt, C., Assimacopoulos-Jeannet, F., Loten, E.G., and Jeanrenaud, B., J. clin. Invest. *53* (1974) 1512.
5 Redman, C.M., Banerjee, D., Howell, K., and Palade, G.E., J. Cell Biol. *66* (1975) 42.
6 Feldmann, G., Maurice, M., Sapin, C., and Benhamou, J.P., J. Cell Biol. *67* (1975) 237.
7 Stein, O., Sanger, L., and Stein, Y., J. Cell Biol. *62* (1974) 90.
8 Gregory, D.H., Vlahcevic, Z.R., Prugh, M.F., and Swell, L., Gastroenterology *74* (1978) 93.
9 Dubin, M., Maurice, M., Feldmann, G., and Erlinger, S., Gastroenterology *79* (1980) 646.
10 Reichen, J., Berman, M.D., and Berk, P.D., Biochim. biophys. Acta *643* (1981) 126.
11 Mullock, B.M., Jones, R.S., Peppard, J., and Hinton, R.H., FEBS Lett. *120* (1980) 278.
12 Godfrey, P.P., Lembra, L., and Coleman, R., Biochem. J. *208* (1982) 153.
13 Jung, W., Gebhardt, R., and Mecke, D., Eur. J. Cell Biol. *27* (1982) 230.
14 Gebhardt, R., Jung, W., and Robenek, H., Eur. J. Cell Biol. *29* (1982) 68.
15 Gebhardt, R., and Jung, W., J. Cell Sci. *56* (1982) 233.
16 Gebhardt, R., Gastroenterology *84* (1983) 1462.
17 Gebhardt, R., and Mecke, D., Exp. Cell Res. *124* (1979) 349.
18 Gebhardt, R., Exp. Cell Res. *144* (1983) 218.
19 Orlans, E., Peppard, J., Fry, J.F., Hinton, R.H., and Mullock, B.M., J. exp. Med. *50* (1979) 1577.
20 Socken, D.J., Jeejeebhoy, K.N., Bazin, H., and Underdown, B.J., J. exp. Med. *50* (1979) 1538.
21 Wilson, L., Bamburg, J.R., Mizel, S.D., Grisham, L.M., and Creswell, K.M., Fedn Proc. *33* (1974) 158.
22 Phillips, M.J., Oda, M., Mak, E., Fisher, M.M., and Jeejeebhoy, K.N., Gastroenterology *69* (1975) 48.
23 Kacich, R.L., Renston, R.H., and Jones, A.L., Gastroenterology *80* (1981) 1360.

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Rabbit immunization to xenogeneic red blood cells following anterior eye chamber inoculation

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Summary. Inoculation of human red blood cells (HRBC) into the anterior chamber of the eye (AC) of rabbits effectively stimulated systemic antibody production. Intraocular (i.o.) inoculation was observed to be more effective a route of immunization than i.v. or i.m. inoculation of antigen. These results contradict the accepted belief that the AC is an immunologically privileged site in the body.

It is well established that allografts transplanted to the AC survive longer than grafts transplanted orthotopically or heterotopically to other body sites[2-5]. Unfortunately, the concept arose that the AC is an immunologically privileged site in an absolute sense, i.e. that foreign grafts transplanted there stimulate no immunity and are not rejected. Although the AC lacks lymphatic drainage[6,7], it does not lack vascular drainage, and grafts to the AC are not permanent. The following experiments were performed in an effort to clarify our understanding of the immunologically privileged status of the AC. The results of these experiments demonstrate that inoculation of HRBC into the AC of the rabbits eye vigorously stimulates the hosts immune system and results in the production of high titers of systemic antibody.

Materials and methods. Human blood (type - 0) in Alsever's solution was washed 5 times in Earle's balanced salt solution (EBSS) to remove serum components and buffy coat lymphocytes. HRBC were collected and diluted 1:3 in EBSS and used to immunize New Zealand White rabbits (2.0-2.5 kg each). All immunizations consisted of 0.25 ml of diluted HRBC. The immunization protocol is shown in table 1. I.m. inoculations were to the right flank, i.v. inoculations to the right marginal ear vein, and i.o. (intraocular) inoculations to the right AC. Rabbits immunized by the i.o. route were heavily sedated with sodium pentobarbital (Nembutal, Abbot Laboratories) and the periorbital nerves blocked by s.c. administration of lidocaine HCl (Xylocaine, Astra Laboratories). After sedation and anesthesia the eye was immobilized and a 27-ga needle was inserted through the sclera at the lateral limbus and directly into the AC. AC fluid (0.25 ml) was removed and replaced with an equal volume of HRBC. Loss of AC fluid and trauma to the eye was minimal.

Table 1. Immunization protocol and results of immunization of rabbits with HRBC

Rabbit	Group	Titer[a] of serum day 0	Phase 1 Route of primary immunization day 1	Route of secondary immunization day 34	Titer of serum day 39	Phase 2 Route of tertiary immunization day 68	Titer of serum day 73
101	i.o.	0	i.o.	i.o.	32,000	i.v.	1,000,000
104	i.o.	0	i.o.	i.o.	32,000	i.v.	250,000
105	i.o.	0	i.o.	i.o.	32,000	i.v.	3,000
208	i.v.	0	i.v.	i.v.	8,000	i.v.	32,000
209	i.v.	0	i.v.	i.v.	16,000	i.v.	64,000
210	i.v.	0	i.v.	i.v.	32,000	i.v.	16,000
302	i.m.	0	i.m.	i.m.	4,000	i.v.	64,000
306	i.m.	0	i.m.	i.m.	1,000	i.v.	1,000
307	i.m.	0	i.m.	i.m.	4,000	ND	ND
411	Control	0	ND	ND	0	i.v.	8
412	Control	0	ND	ND	0	i.v.	64

[a] Titer is recorded as the reciprocal of the highest serum dilution which agglutinated HRBC. All antibody analyses were performed in duplicate or triplicate and yielded virtually results. HRBC, Human red blood cells; i.o., intraocular; ND, not done.

Blood was collected from rabbits by intracardiac puncture before primary immunization, at 5 and 7 days after secondary immunization and at 5 days following tertiary immunization. Serum was separated from clotted blood and stored at −20 °C until assayed. A sensitive microagglutination assay modified from Thompson et al.[8] was developed to measure antibody to HRBC. HRBC in Alsever's solution were washed 5 times in normal saline containing 0.1% bovine serum albumin (SS) and diluted to 2×10^8 cells/ml in SS. Rabbit serum for analysis was diluted 2-fold in SS. HRBC (0.05 ml) were mixed with serum dilutions (0.05 ml) and incubated for 15 min at room temperature. HRBC incubated with SS alone or with serum collected from rabbits before immunization served as negative controls. Cells were gently mixed while incubating. The serum-cell mixture was then drawn into a 1.1-mm diameter microhematrocrit tube and one end sealed with clay. The tubes were centrifuged for 1 min and then placed at a 45° angle, cell pellet up, for 15 min. If agglutination occurred the cells remained pelleted at the upper end of the hematocrit tube. If agglutination did not occur the cells 'rolled' down the tube and were observed as a 'streamer' of cells. Titer was recorded as the reciprocal of the final dilution of serum which agglutinated HRBC. All analyses were performed in duplicate or triplicate.

In phase 1 of the experimental protocol, animals received 2 identical inoculations of HRBC separated by a 34-day rest period. Animals were bled 5 and 7 days following secondary immunization. Only results of 5-day serum are shown in table 1, as 5- and 7-day serum had virtually identical titers. In phase 2 of the experimental protocol all rabbits were inoculated with HRBC by the i.v. route and bled 5 days following immunization (table 1). The results are summarized in table 2.

Results and discussion. In animals inoculated via the i.o. route, the entire AC of the eye became clouded with the HRBC suspension. The cells sedimented within 4–6 h. and could be observed as a pellet of cells at the inferior aspect of the iris angle in the AC. The pellet decreased in size over a 3-day period following inoculation and was gone by 4 days. No hemolysis was ever observed in the AC. Results of serum titers indicate that inoculation of HRBC to the AC vigorously stimulates systemic antibody production (table 1). It appears the i.o. route is at least as effective a route of immunization as the i.v. route (table 2).

Table 2. Average titers for each experimental group of rabbits immunized with HRBC

Experimental phase	Experimental group	Average titer[a]
1	i.o.	15.0 ± 0
	i.v.	14.1 ± 1.0
	i.m.	11.3 ± 1.1
	Control	0
2	i.o.	17.0 ± 3.6
	i.v.	15.0 ± 1.0
	i.m.	13.0 ± 4.3
	Control	4.5 ± 2.1

[a] Titer is recorded as the serum dilution to the $\log_2$ which agglutinated HRBC. Results are average titers for each experimental group ± SD. Abbreviations: See legend for table 1.

The immunization method described is an accurate and sensitive immunizing protocol, but it requires heavy sedation and anesthesia to preclude immediate or prolonged pain to the experimental animal. Therefore, I would not suggest the i.o. route be adopted as a standard route of immunization as it is too tedious and technically demanding for routine use.

Other investigators have recently analyzed the immunologic nature of the AC and all reports contradict the concept of its privileged status. A variety of grafts have been used to demonstrate the development of both humoral and cell mediated immunity following transplantation to the AC[9–12]. Kaplan and Streilein explained i.o. immunization (in the absence of lymphatic drainage) by demonstrating the importance of the spleen in the development of immunity[9,11]. They also reported that rats inoculated i.o. with F_1 hybrid lymphocytes developed both humoral and cellular immunity. They suggested that motile lymphocytes actively migrated to the spleen where they stimulated immunity[10]. They could not demonstrate hemagglutinating antibody following transplantation of trypsinized epithelial cells or whole skin grafts to the AC. They also suggested antibody production was enhanced by retention of lymphocytes in the AC which acted as a depot for retention of antigen[11]. My results substantiate the suggestion that cells inoculated to the AC are retained, and this may explain the high levels of

antibody that are produced following i.o. immunization. In these experiments, HRBC inoculated into the AC never hemolyzed, but were slowly absorbed over a period of 3 days. Note that the AC is in virtually direct contact with the venous system. The anterior ciliary vein of the eye is in direct contact with the canal of Schlemm which is separated from the spaces of Fontana and the AC by a single, very porous layer of endothelial cells. HRBC were apparently able to readily escape the AC and enter the venous circulation. Absorption must be passive as HRBC are not motile, as are lymphocytes. Active egress from the AC, as suggested by Kaplan and Streilein is not necessary. Kaplan and Streilein used trypsinized epithelial cells and whole skin grafts as AC implants, but were unable to measure hemagglutinating antibody using a relatively insensitive microtiter method of analysis. In my methods, HRBC were used as immunogens and as the test cell for antibody titration, and a highly sensitive microagglutination assay (100 times more sensitive than microtiter agglutination) was used. It is well established the i.v. route is superior for humoral immunization as compared to the i.m. route. This is supported by these results. Although the experiments were not designed as quantitative studies of antibody production, it appears that the i.o. route is at least as effective, and possibly more effective than the i.v. route for sensitization and stimulation of systemic antibody production. Such results are surprising, but may be due to an antigen depot effect.

Conclusion. The interpretation of these results are clear. Inoculation of HRBC into the AC vigorously stimulates systemic antibody production. This interpretation is not compatible with the theory that the AC of the eye is an immunologically privileged site.

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2 Green, H.S.N., Can. Res. *9* (1949) 728.
3 Kaplan, H.J., and Stevens, T.R., Transplantn *19* (1975) 302.
4 Vesella, R.L., Raju, S., and Grogan, J.B., Fedn Proc. *33* (1974) 783.
5 Woodruff, M.F.A., and Woodruff, H.G., Phil. Trans. R. Soc. *234* (1950) 559.
6 Kaplan, H.J., Stevens, T.R., and Streilein, J.W., J. Immun. *115* (1975) 800.
7 Medawar, P.B., Br.J. exp. Path. *29* (1948) 58.
8 Thompson, J.S., Severson, C.D., Lavender, A.R., Foreland, M., and Russe, H.P., Transplantation *6* (1968) 728.
9 Kaplan, H.J., and Streilein, J.W., Trans. Proc. *9* (1977) 1193.
10 Kaplan, H.J., and Streilein, J.W., J. Immun. *118* (1977) 809.
11 Kaplan, H.J., and Streilein, J.W., J. Immun. *120* (1978) 689.
12 Vesella, R.L., Grogan, J.B., Raju, S., and Cockrell, J.O., J. Sug. Res. *22* (1977) 1.

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Increase of lymphocytic H-Y antigen in female 21-hydroxylase deficiency

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Summary. H-Y antigen was found to be increased in lymphocytes from 10 female 21-hydroxylase deficiencies, suggesting a correlation between the degree of virilization of these patients and their H-Y+ lymphocytes proportions. Furthermore, these findings demonstrate the ability of a 46,XX female subject to produce, in some circumstances, an excess of H-Y antigen.

H-Y antigen is a minor histocompatibility antigen which is detectable on male cells, at least in species where the male is the heterogametic sex[1-3]. Although its role in testicular organization seems to be very important[4], it has not clearly been demonstrated how H-Y antigen can act on gonadal organogenesis. Erickson suggested that androgens could easily play an important role in the expression of H-Y antigen[5]. Therefore we studied lymphocytic H-Y antigen in adrenal hyperplasia due to 21-hydroxylase deficiency. This deficiency results in decreased cortisol synthesis and secondary increased production of adrenocorticotrophic hormone (ACTH). In its turn, excess ACTH secretion leads to overproduction of androgens and consequent virilisation[6-8].

Materials and methods. The study was performed on 16 21-hydroxylase deficient patients. 11 had congenital adrenal hyperplasia with increased hydroxyprogesterone and testosterone levels (group 1, 2, 3 and 3'): 5 boys (3 with pubic hair and 2 with salt wasting crises), 3 little girls (1 with pubic hair and 2 with salt wasting crises) and 3 adults 46,XX with female pseudohermaphroditism[9]; 5 female cases (group 4) were late forms of adrenal hyperplasia (hirsutism in early adulthood associated with increased hydroxyprogesterone, testosterone and delta-4-androstenedione levels). H-Y control values were obtained from 159 healthy volunteers (89 male and 70 female). H-Y antigen was studied in human peripheral blood lymphocytes by indirect immunofluorescence[10]. Anti H-Y serum was raised in (C_{57}Bl/6 × DBA/2) F_1 female mice by i.p. inoculations of spleen cells from inbred males. Pooled sera were absorbed with human female blood buffy-coat cells before testing. The anti-mouse immunoglobulin was a fluorescein isothiocyanate (FITC)-conjugated $F(ab')_2$ fragment goat anti-mouse IgG (Cappel) absorbed with pooled human buffy coat cells. Since a close correlation between the male B lymphocyte counts and the male H-Y+ cell counts had been shown, and not between female counts[11], Ig+ cells (B lymphocytes) counts were used as control for each patient. B lymphocytes were identified by direct immunofluorescence with FITC-conjugated $F(ab')_2$ fragment goat antiserum to human immunoglobulins (polyvalent) (Meloy).

Results and discussion. A correlation was found between the proportion of H-Y+ cells from the little boys (group 1), from the little girls (group 2), from 2 pseudohermaphrodites (group 3) and that from the male controls (table). Moreover the proportions of H-Y+ cells were significantly greater in the little girls (group 2) and in 2 female pseudohermaphrodites (group 3) than in the female controls