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Ontogeny of secretory component in rat liver

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SUMMARY

The ontogeny of the expression of secretory component (SC), the receptor for transepithelial transport of polymeric immunoglobulins, in the rat liver was assessed by measuring the SC concentration in liver homogenates of rats killed from birth to 60 days of age, and by quantifying the daily SC secretion of *in vitro* cultured hepatocytes isolated from rats similarly killed from 2 days before birth to 60 days of age. With both methodologies, only very small amounts of SC were produced by the rat liver up to 20 days of age, whereafter expression of SC markedly increased. The development of SC production in the rat liver was co-ordinate with that previously reported in the rat small intestine, preceding the appearance of large numbers of intestinal IgA plasmacytes. These data further emphasize the importance of the liver contribution, via the bile, to the intestinal secretory IgA system of the rat.

INTRODUCTION

Secretory component (SC) is the soluble secreted form of the receptor for selective transport (Brandtzaeg, 1985) of polymeric (p) Igs (p-IgA and IgM) in milk, saliva, tears, intestinal and bronchial secretions and bile. This receptor is first synthesized as a larger transmembranous protein and is finally secreted, by enzymatic cleavage from its intracytoplasmic and membranous domains, into the smaller, soluble SC (Mostov, Friedlander & Blobel, 1984; Musil & Baenziger, 1987). In rabbits, mice and rats (Vaerman *et al.*, 1982; Delacroix *et al.*, 1983, 1984), the high IgA level in bile is due to hepatocytes which strongly express SC at their sinusoidal surface and actively transport p-IgA from plasma into hepatic bile. In humans, monkeys, dogs, sheep and guinea-pigs (Delacroix *et al.*, 1982, 1983, 1984), hepatocytes do not express SC; the smaller bile IgA level results mainly from SC-mediated transport in bile ducts and gall bladder (Nagura *et al.*, 1983; Vuitton *et al.*, 1985). About 90% of IgA in rat upper gut washings derives from bile (Lemaître-Coelho, Jackson & Vaerman, 1978), and high IgA antibody (Ab) titres occur in rat bile after intestinal immunizations (Hall *et al.*, 1979; Vaerman *et al.*, 1985).

Epithelial expression of SC during ontogeny should logically precede or be concomittant with the presence of IgA plasmacytes in mucosae. In humans, SC has been found as early as at 8 weeks of gestation, and regularly occurs at 13–17 weeks, long before any Ig-secreting cell, in bronchial, intestinal and kidney epithelia, but not in liver (Ogra *et al.*, 1972; Takemura & Eishi, 1985). In rats, SC was detected in urine at 35 days, 2–3 weeks, before secretory IgA (Hinton *et al.*, 1984). IgA plasmacytes occur in gut mucosa around 4 weeks (Nagura, Nakane & Brown, 1978; Sminia, Janse & Plesch, 1983). Only traces of SC were detected in rat enterocyte homogenates before Day 20, whereafter SC rapidly increased to reach adult levels at Day 40 (Buts *et al.*, 1984; Buts & Delacroix, 1985). Corticosterone and thyroxine reduced, but insulin increased the trace SC expression of rat enterocytes and/or hepatocytes at Days 10–20 (Buts, De Keyser & Dive, 1988). It is unknown whether membranous SC and soluble SC, the latter reflecting the activity of the cleaving enzyme(s), appear together during ontogeny.

Our study compares the *in vivo* or *in vitro* expression of rat SC in, respectively, liver homogenates or culture media of isolated hepatocytes from rats killed at various ages. Our data suggest that appreciable expression of rat hepatocyte SC, as for gut SC, starts at 20 days of age.

Abbreviations: Ab, antibody(ies); BSA, bovine serum albumin; FCS, fetal calf serum; PBS, phosphate-buffered saline; p-IgA, polymeric IgA; SC, secretory component.

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MATERIALS AND METHODS

Animals

Wistar rats (from the faculty animal house), housed in polystyrene cages in an air-conditioned room at $21 \pm 1°$ with a 12 hr light-dark cycle, were used throughout the *in vivo* studies for the liver homogenates. The day of birth was considered as Day 0. Sucklings remained with their mother from Day 0 (six rats per litter) and had free access to the nipples. The pups were separated from their mothers on Day 18 and weaned onto a chow pellet diet (No. 103, UAR, Villemoisson-sur-Orge, France). Six rats from the same litter were killed every other 5th day up to Day 40. Adult rats were from different litters.

For *in vitro* culture of hepatocytes, Sprague–Dawley rats (IFFA-Credo, Les Oncins, France) were killed at Days −2, 0, +1, +5, +12, +20, +25, +27, +35 and +60.

Rat liver homogenates

After killing the rats by decapitation, the liver was dissected and a specimen of 30 mg of wet tissue was weighed from each animal for all age groups. The specimen was homogenized with a Potter (tight pestle) homogenizer for 30 seconds at 2600 r.p.m. at 4° in 3 ml of 0·1 M Tris-HCl buffer, pH 7·4 (representing a 1/100 dilution), and then sonicated for 30 seconds at 21 watts at 4° in a B12-Sonifier (Branson Sonic Power, Danbury, CT). A 1·0-ml aliquot of homogenate was taken for total protein measurement (Lowry *et al.*, 1951) with bovine serum albumin (BSA) as standard. Two other aliquots of homogenate were taken for SC assay. Both SC and total protein were assayed immediately after homogenization.

Hepatocyte cultures

From the fetal period to 5-day-old neonatal rats, hepatocytes were isolated according to Guguen-Guillouzo *et al.* (1980). Briefly, the livers were excised, pooled, minced with scissors, and washed three times with HEPES buffer, pH 7·6. The tissue dissociating solution was the same HEPES buffer with 0·025% collagenase (Boehringer-Mannheim, Meylan, France) and 5 mM $CaCl_2$. Liver pieces were stirred gently at 37° in 30 ml of dissociating medium for 10 min; two further stirrings, each lasting 10 min, were carried out in new dissociating medium. For 12–60-day-old rats, hepatocytes were isolated by cannulating the portal vein and pefusing the liver with the same collagenase solution, as described earlier (Guguen-Guillouzo *et al.*, 1975). In both cases, cells were collected in Leibovitz medium containing 1·0 mg of BSA and 5 μg of bovine insulin per ml. Cell suspensions were filtered on gauze and allowed to sediment for 20 min in order to eliminate debris, blood and sinusoidal cells. The cells were then washed three times by centrifugation at 50 *g* and tested for viability. Hepatocytes were then suspended in a mixture (75/25) of Eagle's minimum essential medium and medium 199 with Hanks' salts, containing 10% fetal calf serum (FCS) and, per ml: kanamycin (50 μg), streptomycin (50 μg), penicillin (7·5 IU), bovine insulin (5 μg), BSA (1·0 mg) and $NaHCO_3$ (2·2 mg), and plated in 25 cm^2 Nunclon flasks. The medium was changed 3–4 hr later and then every day with the same medium supplemented with 10^{-7} M dexamethasone. The number of hepatocytes put into culture was estimated by cell protein content of culture dishes. The cultures were washed with buffer, and hepatocytes were selectively separated from fibroblasts after incubation in a phosphate-buffered Ca-free collagenase solution (0·05%, pH 7·6) for 10 min at 37°. Hepatocytes were then potterized, sonicated, and their protein content was measured using the Bio-Rad reagent (Bradford, 1976), with BSA as standard. Inter- and intra-assay variations did not exceed 10%. Culture media were collected daily and stored at −20°.

Immunoradiometric assay of rat SC

All hepatocyte culture media were lyophilized and reconstituted with water before this assay. The procedure has been described earlier (Buts *et al.*, 1988). Rabbit or goat antisera against rat-free SC (Acosta-Altamirano *et al.*, 1980) reacted well with both membranous and soluble SC. Polystyrene beads (6 mm diameter) were coated with the IgG fraction (20 mg/ml in 0·05 M glycine-NaOH buffer, pH 9·5), obtained by either protein A–Sepharose CL-4B (Pharmacia, Uppsala, Sweden) chromatography for rabbit, or by DEAE–cellulose chromatography for goat Ab. After three washes with 0·01 M phosphate-buffered saline, pH 7·2 (PBS), beads were incubated for 4 hr at 37° with 200 μl of liver homogenate or hepatocyte culture medium, undiluted or diluted 1/100 in either 10% goat serum in PBS for liver homogenates, or in 10% FCS in PBS for culture media. After three more washes (= a total of 15 ml of deionized water), incubation was continued for 18 hr at 22° with 250 μl of iodine-labelled (McConahey & Dixon, 1966) affinity-purified goat or rabbit anti-rat SC Ab (1000 c.p.m./μl). This was followed again by three washes, and the radioactivity bound to beads was then counted. All individual liver homogenates were assayed in duplicate. For *in vitro* culture media, three or six separate cultures of hepatocytes for each age were performed, and each medium was assayed in duplicate. The SC standard was either purified free SC (Acosta-Altamirano *et al.*, 1980) or a pool of diluted sera from bile duct-ligated rats standardized with free SC. The standards were made in 10% FCS in PBS. A standard curve was run with each series of assays. Results were expressed as ng of SC per mg of liver protein for homogenates, or as ng of SC per μg of cell protein per 24 hr for culture media.

Immunoassay of rat serum albumin in culture media

Rat albumin was assayed in the culture media because it was a good parameter of the viability and functional state of the hepatocytes in culture. Laser immunonephelometry was used as described elsewhere (Ritchie, 1975), using rabbit anti-rat albumin as Ab and purified rat serum albumin as standard, both from Cappel Laboratories, Cochranville, PA. Standards and culture media were mixed in an appropriate dilution of antiserum in saline containing 4% polyethylene glycol. Light scatter was measured after incubation for 1 hr at room temperature. The validity of the rat albumin assay was confirmed by the lack of detectable cross-reactivity with BSA and by the linear reference curve obtained for rat albumin in medium, over a 1–35 μg/ml range. The sensitivity limit was about 1·0 μg/ml. Inter- and intra-assay variations did not exceed 10%.

RESULTS

Liver homogenates

The levels of SC measured in liver homogenates from rats killed at different ages are illustrated in Fig. 1. Only traces of SC were detected up to the age of 20 days. Thereafter, the SC concentration increased rapidly to reach a plateau at 40 days of age. There was no further increase in SC content in liver homogenates from 40 to 60 days of age.

In vitro hepatocyte cultures

The daily excretion of SC and albumin in hepatocytes cultured *in vitro* for 4 days was first measured for cells isolated from rats 20 and 27 days old. These ages were chosen because the preceding *in vivo* results (Fig. 1) indicated that liver expression of SC remained very low until 20 days of age, but rose markedly at 25–30 days, despite the virtual absence of IgA-secreting cells at this age (Nagura *et al.*, 1978; Sminia *et al.*, 1983). For these

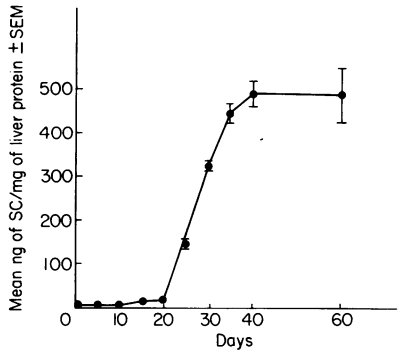

Figure 1. SC content of liver homogenates prepared from rats killed at various ages. Each point represents the mean ± SEM of six rats. When absent, the SEM was smaller than the point.

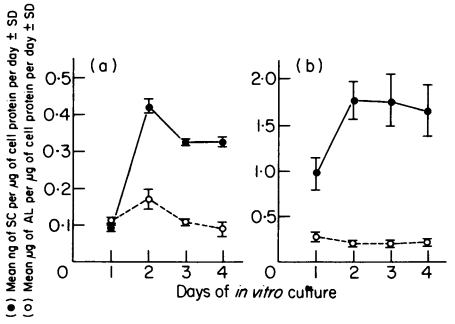

Figure 2. Daily *in vitro* synthesis of SC (●) and albumin (AL) (○) by cultured isolated hepatocytes from rats aged 20 (a) or 27 days (b). Each point represents the mean ± SD of three cultures.

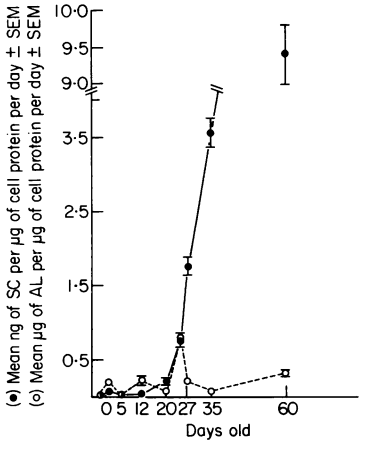

Figure 3. Daily *in vitro* synthesis of SC (●) and albumin (AL) during the third day of culture of isolated hepatocytes from rats killed from −2 to +60 days of age. Each point represents the mean ± SEM of three to six cultures.

experiments, secretion per day was standardized by dividing the amount of SC or albumin secreted in the medium by the number of µg of cell protein in the culture dish after each day. The results are illustrated in Fig. 2. For SC, the amount secreted during the first day *in vitro* was significantly smaller than the amount secreted during the next 3 days, and this held for hepatocytes isolated from both rats aged 20 and 27 days. For albumin, this increase was not observed and the daily secretion of this protein was roughly constant during the 4 successive days of culture. These data prompted us to measure the daily *in vitro* secretion of SC during the third day of culture of hepatocytes of various ages.

Figure 3 shows the daily amounts of SC and albumin secreted *in vitro* during the third day of culture of hepatocytes isolated from rats killed at various ages from 2 days before birth up to 60 days after. Again, when hepatocytes of rats aged from −2 to +20 days were put into culture, they daily secreted only small amounts of SC. But after 20 days, the secretion of SC increased markedly, and continued *in vitro* to raise up to the secretion of hepatocytes of 60-day-old rats. The daily secretion of albumin, despite a larger amount (µg instead of ng for SC) and a possible transient rise at Day 25, did not display a large increase at Days 27, 35 and 60.

DISCUSSION

The cleavage of membranous SC into soluble SC is a constitutive enzymatic process, localized at the canalicular membrane of rat hepatocytes (Musil & Baenziger, 1988), and thought (but not formally proven) to be synchronous in ontogeny with the production of membranous SC, because cleavage does occur even if there is no p-IgA ligand to transport (Mullock, Jones & Hinton, 1980). Nothing is known about the ontogeny of the cleaving enzyme(s). Homogenization of rat hepatocytes may also lead to this cleavage (Musil & Baenziger, 1987). Nevertheless, we cannot state if our liver homogenates only contained cleaved, soluble SC, or a mixture of soluble and membranous SC in unknown proportion. This proportion could affect the validity of our absolute SC levels, as membranous SC could be under-estimated by the sandwich type of radioimmunoassay used. We do not think, however, that the proportion of soluble and membranous SC could change dramatically in our homogenates prepared in a very standardized manner. Even if this is incorrect, preliminary (unpublished) data from our group showed that the addition of 1% of Triton X-100, in order to solubilize membranous SC, did not yield higher SC levels in liver homogenates made from rats aged 5, 25 and 40 days, suggesting that they contained almost only soluble SC. Finally, these possible limitations would only apply to the homogenates, and not to the culture media which only contain cleaved soluble SC.

The parallelism between the timing of SC expression of rat enterocytes and hepatocytes is strongly outlined by our data from both liver homogenates and *in vitro* hepatocyte cultures. Very little SC was produced before 20 days of age; thereafter, SC production markedly increased. Whereas in liver homogenates the SC content was roughly equal at 40 and 60 days of age, hepatocytes from rats aged 60 days produced *in vitro* about twice as much SC as hepatocytes from rats aged 40 days (Fig. 3). This indicates that SC synthesis could be regulated *in vivo* (homogenates) by some unknown mechanism which would not work *in vitro* (culture). If one compares SC content in crypt enterocytes (Buts & Delacroix, 1985) at 20 and 40 days of age (60 versus 580 ng/mg of protein, respectively) with the SC content of liver homogenates at the same ages (20 versus 490 ng/mg of protein, respectively), at 40 days of age, both types of cells have roughly the same SC content (0·5 µg of SC/mg of cell protein)

but, at 20 days, crypt enterocytes display a three times higher SC content than do hepatocytes. It is possible that this difference reflects a stronger IgA-transport function in the gut at that age: more IgA would reach the intestinal lumen at 20 days of age via the intestinal wall than via the bile. Also, at 20 days, the vast majority of the IgA plasmacytes are mainly found in the gut mucosa.

In vitro, hepatocytes showed a marked increase in SC, but not albumin secretion from Day 1 to Day 2 of culture, both for hepatocytes taken from rats aged 20 and 27 days. The reason for this difference is not known. Albumin secretion could be regulated by BSA in the culture medium, or the change from *in vivo* conditions to *in vitro* culture medium could have more influence on SC than on albumin secretion.

Other changes in SC expression due to age or hormones have been described in rats. The six-fold decrease in transport of rat polymeric IgA from plasma to bile observed in Fischer rats with increasing age from 3 to 12 months (Schmucker *et al.*, 1985) was shown to be due in part to a simultaneous decline in expression of SC by hepatocytes (Daniels, Schmucker & Jones, 1985). There was, however, no concomittant age-dependent decrease of SC expression by rat enterocytes (Daniels *et al.*, 1988). Positive hormonal influences on adult rat SC expression include androgen regulation of SC secretion in tears (Sullivan, Hann & Vaerman, 1988), oestrogen regulation of SC in uterine fluid (Sullivan, Underdown & Wira, 1984), and cortisone-enhanced excretion of SC into canalicular bile by hepatocytes (Wira & Colby, 1985). Whether the latter also applies to neonatal rat hepatocytes *in vivo* warrants further studies.

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REFERENCES

Acosta-Altamirano G., Barranco-Acosta C., Van Roost E. & Vaerman J.P. (1980) Isolation and characterization of secretory IgA (sIgA) and free secretory component (FSC) from rat bile. *Mol. Immunol.* **17,** 1525.

Bradford M.M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. *Anal. Biochem.* **72,** 248.

Brandtzaeg P. (1985) Role of J chain and secretory component in receptor-mediated glandular and hepatic transport of immunoglobulins in man. *Scand. J. Immunol.* **22,** 111.

Brown P.J. (1979) The development of immunoglobulin-containing cell populations in intestine, spleen and mesenteric lymphnode of the young rat. *Veter. Immunol. Immunopathol.* **1,** 15.

Buts J.P., De Keyser N. & Dive C. (1988) Intestinal development in the suckling rat: effect of insulin on the maturation of villus and crypt cell functions. *Eur. J. clin. Invest.* **18,** 391.

Buts J.P. & Delacroix D.L. (1985) Ontogenic changes in secretory component expression by villous and crypt cells of rat small intestine. *Immunology*, **54,** 181.

Buts J.P., Delacroix D.L., De Keyser N., Paquet S., Horsmans Y., Boelens M., Van Craynest M.P. & De Meyer R. (1984) Role of dietary iron in maturation of rat small intestine at weaning. *Am. J. Physiol.* **246,** G725.

Daniels C.K., Schmucker D.L., Bazin H. & Jones A.L. (1988) Immunoglobulin A receptor of rat small intestinal enterocytes is unaffected by aging. *Gastroenterology*, **94,** 1432.

Daniels C.K., Schmucker D.L. & Jones A.L. (1985) Age-dependent loss of dimeric immunoglobulin A receptors in the liver of the Fischer 344 rat. *J. Immunol.* **134,** 3855.

Delacroix D.L., Furtado-Barreira G., De Hemptinne B., Goudswaard J., Dive C. & Vaerman J.P. (1983) The liver in the IgA secretory immune system. Dogs, but not rats and rabbits, are suitable models for human studies. *Hepatology*, **3,** 980.

Delacroix D.L., Furtado-Barreira G., Rahier J., Dive C. & Vaerman J.P. (1984) Immunohistochemical localization of secretory component in the liver of guinea pigs and dogs, versus rats, rabbits and mice. *Scand. J. Immunol.* **19,** 425.

Delacroix D.L., Hodgson H.J.F., McPherson A., Dive C. & Vaerman J.P. (1982) Selective transport of polymeric immunoglobulin A in bile. Quantitative relationships of monomeric and polymeric immunoglobulin A, immunoglobulin M, and other proteins in serum, bile, and saliva. *J. clin. Invest.* **70,** 230.

Guguen-Guillouzo C., Boisnard M., Le Cam A. & Bourel M. (1975) Etude ultra-structurale de monocouches d'hépatocytes de rat cultivés en présence d'hémisuccinate d'hydrocortisone. *Biol. Gastroentérol.* **8,** 223.

Guguen-Guillouzo C., Tichonicky L., Szajnert M.F. & Kruh M. (1980) Changes in some chromatin and cytoplasmic enzymes of perinatal rat hepatocytes in culture. *In Vitro* **16,** 1.

Hall J., Orlans E., Reynolds J., Dean C., Peppard J., Gyure L. & Hobbs S. (1979) Occurrence of specific antibodies of the IgA class in the bile of rats. *Int. Arch. Allergy Appl. Immunol.* **59,** 75.

Hinton R.H., Lau D.Y.H., Sanderson C. & Mullock B.M. (1984) IgA in the bile, saliva and urine of rats. *Protides Biol. Fluids*, **32,** 81.

Lemaitre-Coelho I., Jackson G.D.F. & Vaerman J.P. (1978) Relevance of biliary IgA antibodies in rat intestinal immunity. *Scand. J. Immunol.* **8,** 459.

Lowry O.H., Rosebrough N.J., Farr A.L. & Randall R.J. (1951) Protein measurement with the Folin phenol reagent. *J. biol. Chem.* **193,** 265.

McConahey P.J. & Dixon F.J. (1966) A method of trace iodination of proteins for immunologic studies. *Int. Arch. Allergy Appl. Immunol.* **29,** 185.

Mostov K.E., Friedlander M. & Blobel G. (1984) The receptor for transepithelial transport of IgA and IgM contains multiple immunoglobulin-like domains. *Nature* (*Lond.*), **308,** 37.

Mullock B.M., Jones R.S. & Hinton R.H. (1980) Movement of endocytic shuttle vesicles from the sinusoidal to the bile canalicular face of hepatocytes does not depend on occupation of receptor sites. *FEBS Lett.* **113,** 201.

Musil L.S. & Baenziger J.U. (1987) Cleavage of membrane secretory component to soluble secretory component occurs on the cell surface of rat hepatocyte monolayers. *J. Cell. Biol.* **104,** 1725.

Musil L.S. & Baenziger J.U. (1988) Proteolytic processing of rat liver membrane secretory component. Cleavage activity is localized to the bile canalicular membranes. *J. Biol. Chem.* **263,** 15799.

Nagura H., Nakane P.K. & Brown W.R. (1978) Breast milk IgA binds to jejunal epithelium in suckling rats. *J. Immunol.* **120,** 1333.

Nagura H., Tsutsumi Y., Hasegawa H., Watanabe K., Nakane P.K. & Brown W.R. (1983) IgA plasma cells in biliary mucosa: a likely source of locally synthetized IgA in human hepatic bile. *Clin. exp. Immunol.* **54,** 671.

Ogra S.S., Ogra P.L., Lippes J. & Tomasi T.B. (1972) Immunohistologic localization of immunoglobulins, secretory component, and lactoferrin in the developing human fetus. *Proc. Soc. exp. Biol. Med.* **139,** 570.

Ritchie R.F. (1975) Automated immunoprecipitation analysis of serum proteins. In: *The Plama Proteins: Structure, Function and Genetic Control* (ed. F.W. Putnam), Vol. II, p. 375. Academic Press, New York.

SCHMUCKER D.L., GILBERT R., JONES A.L., HRADEK G. & BAZIN H. (1985) Effects of aging on the hepatobiliary transport of dimeric immunoglobulin A in the male Fischer rat. *Gastroenterology*, **88**, 436.

SMINIA T., JANSE E.M. & PLESCH B.E.C. (1983) Ontogeny of Peyer's patches of the rat. *Anat. Rec.* **207**, 309.

SULLIVAN D.A., HANN L.E. & VAERMAN J.P. (1988) Selectivity, specificity and kinetics of the androgen regulation of the ocular secretory immune system. *Immunol. Invest.* **17**, 183.

SULLIVAN D.A., UNDERDOWN B.J. & WIRA C.R. (1983) Steroid hormone regulation of free secretory component in the rat uterus. *Immunology*, **49**, 379.

TAKEMURA T. & EISHI Y. (1985) Distribution of secretory component and immunoglobulins in the developing lung. *Am. Rev. Resp. Dis.* **131**, 125.

VAERMAN J.P., DE RYCK-LANGENDRIES A., RITS M. & DELACROIX D.L. (1985) Neutralization of cholera toxin by rat biliary secretory IgA antibodies. *Immunology*, **54**, 601.

VAERMAN J.P., LEMAITRE-COELHO I., LIMET J. & DELACROIX D.L. (1982) Hepatic transfer of polymeric IgA from plasma to bile in rats and other mammals: a survey. In: *Advances in Mucosal Immunity* (eds W. Strober, L.A. Hanson and K.W. Sell), pp. 233–250. Raven Press, New York.

VUITTON D.A., SEILLES E., CLAUDE P., SAVA P. & DELACROIX D.L. (1985) Gall bladder: the predominant source of bile IgA in man? *Clin. exp. Immunol.* **62**, 185.

WIRA C.R. & COLBY E.M. (1985) Regulation of secretory component by glucocorticoids in primary cultures of rat hepatocytes. *J. Immunol.* **134**, 1744.