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3. Many drugs affect the response to purine nucleotides, but also have other pharmacological actions: quinidine, imidazoline compounds, and caffeine. See 1972 and 1975 reviews by G. Burnstock for original references (*1*).
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8. Tissues were bathed for 1 hour in a modified Krebs-Henseleit solution (37°C), which was gassed with 95 percent O_2 and 5 percent CO_2 under 400 mg resting tension. All agonists were added stepwise in increasing concentration at 6-minute intervals. Agonists were present for 2 minutes, and the tissues were washed twice at 2-minute intervals before the next concentration was added.
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10. The spectra are identical to those seen in parallel experiments in which $ANAPP_3$ was dissolved in water and scanned before and after irradiation in quartz cuvettes with a UVS-11 Mineralight (253.4 nm; Ultra-Violet Products, Inc.). Irradiation, and not the tissues, caused the spectral changes. Bath temperature remained at 37°C during photoirradiation.
11. Since irradiation with the DVY visible light source had no effect on the tissues, control for light experiments were subsequently not irradiated. Interestingly, exposure of tissues in quartz cuvettes for 30 minutes with 253.4-nm ultraviolet light depressed the response to all concentrations of ATP, and $ANAPP_3$ after photoactivation had no effect under this condition.
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13. One reason for this discrepancy might be that the amount of activable $ANAPP_3$ available in the bath for binding to the tissue began to decline when irradiation was begun. The kinetics of the photoactivation was not studied. The binding of activated $ANAPP_3$ to the tissue would be expected to increase in a time-dependent fashion, in a manner analogous to the interaction of β-haloalkylamines to the α-adrenergic receptor. More effective blockade by $ANAPP_3$ in light experiments might be achieved in future studies by continuously replenishing with $ANAPP_3$ during irradiation.

14. We thank J. L. Mills, Texas Tech University, for mass spectral analysis of $ANAPP_3$ and G. Fromell for expert technical assistance. This work was supported, in part, by training grant T32-GM17039 from the National Institutes of Health.

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Evidence for a Vesicular Transport Mechanism in Hepatocytes for Biliary Secretion of Immunoglobulin A

Abstract. *Quantitative electron microscopic autoradiography and diaminobenzidine cytochemistry provide evidence for an uptake and vesicular transport mechanism for iodine-125-labeled immunoglobulin A from plasma to bile by hepatocytes in vivo. The data confirm the existence of a hepatobiliary pathway for secretion of immunoglobulin A into the intestine and are consistent with a vesicular transport mechanism for biliary proteins within liver parenchymal cells.*

Immunoglobulin A (IgA) is found in most mammalian secretions, including gut fluid (*1*, *2*). Intestinal IgA is dimeric and possesses the glycoprotein "receptor" referred to as secretory component (*3*, *4*). It has been proposed that this secretory immunoglobulin complex enters gut secretions by traversing small intestinal epithelial cells from the site of its synthesis by plasma cells in the lamina propria (*5*). Recently, secretory IgA was found in normal rat bile (*6–8*). Fisher *et al.* (*7*) showed that rat liver has an extraordinary capacity to remove polymeric IgA devoid of secretory component from portal blood and to secrete it into bile complexed to secretory component.

To investigate the morphological mechanisms involved in the transport of IgA into bile, dimeric IgA was prepared from rat myelomas (*7*) and labeled with ^{125}I to a specific activity of 0.1 μCi/μg. Purified labeled IgA (35 μCi) was injected either alone or with horseradish peroxidase (HRP) (10 mg per 100 g of body weight) directly into the portal veins of fasted male Sprague-Dawley rats weighing 250 to 300 g. Horseradish peroxidase provides a useful non-receptor-mediated marker for endocytically derived vesicles (*9*, *10*). One and 30 minutes after being injected, the livers were perfusion-fixed with glutaraldehyde and paraformaldehyde and then processed for light and electron microscopic autoradiography and diaminobenzidine cytochemistry to demonstrate HRP (*9*). To assess the secretion of IgA into bile, the common bile duct was cannulated with PE-10 tubing (Clay-Adams) and bile was collected at 10-minute intervals for 2 hours. Radioactivity from the labeled IgA was determined by counting equal portions of bile with a gamma counter. The HRP in the bile was assessed by observing the spectrophotometric change in absorbance due to oxidation of *o*-dianisidine at 460 nm (*11*). The density of silver grains revealed by autoradiography was determined by using two half-distances for all associations (*9*, *12*).

One minute after injection of the proteins, electron microscopic autoradiography showed grains associated primarily with the sinusoidal plasma membranes of parenchymal cells (Fig. 1). Thirty minutes after the injection, grains were observed over hepatic parenchymal cell cytoplasm but were concentrated over bile canalicular regions (Fig. 2). Quantitative electron microscopic autoradiography showed grains associated with sinusoidal hepatocyte plasma membranes and forming endocytic vesicles (58 percent), and with endocytic vesicles approximately 1000 Å in diameter within the cell (7 percent), as determined by their content of HRP 1 minute after the injection (Table 1). Grains were associated, to a lesser extent, with other subcellular organelles in the hepatocytes (Table 1). After 30 minutes, the number of grains near hepatocyte plasma membranes declined to 10 percent, whereas those associated with HRP-containing endocytic vesicles 1000 Å in diameter, now located in the interior of the cell, increased to 26 percent (Table 1). These grains and the associated vesicles were abundant around bile canaliculi, and vesicles were occasionally observed fusing with the bile canalicular membrane. Grains associated with other organelles were also observed (Table 1). In the ab-

Table 1. Association of silver grains with organelles in the hepatocytes. Approximately 300 grains were counted at each time.

Organelle	Distribution of grains (%)		Organelle volume per unit volume of hepatocyte (%)
	1 minute after protein injection	30 minutes after protein injection	
Plasma membrane	58	10	
Endocytic vesicles	7	26	1
Smooth endoplasmic reticulum	14	35	8
Rough endoplasmic reticulum	6	8	6
Mitochondria	5	5	19
Nucleus	3	3	8
Golgi-rich area	1	1	3
Lysosomes	1	3	< 1

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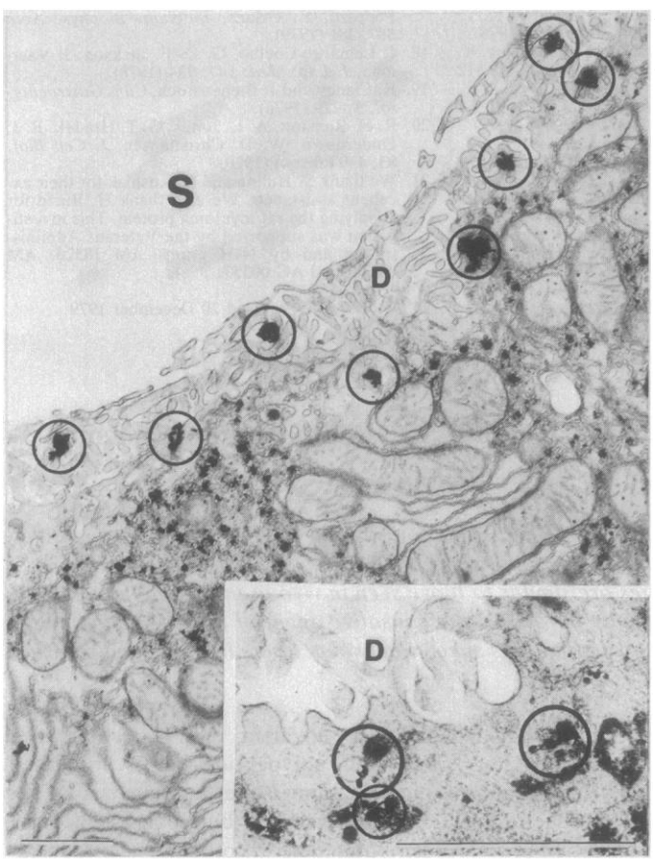

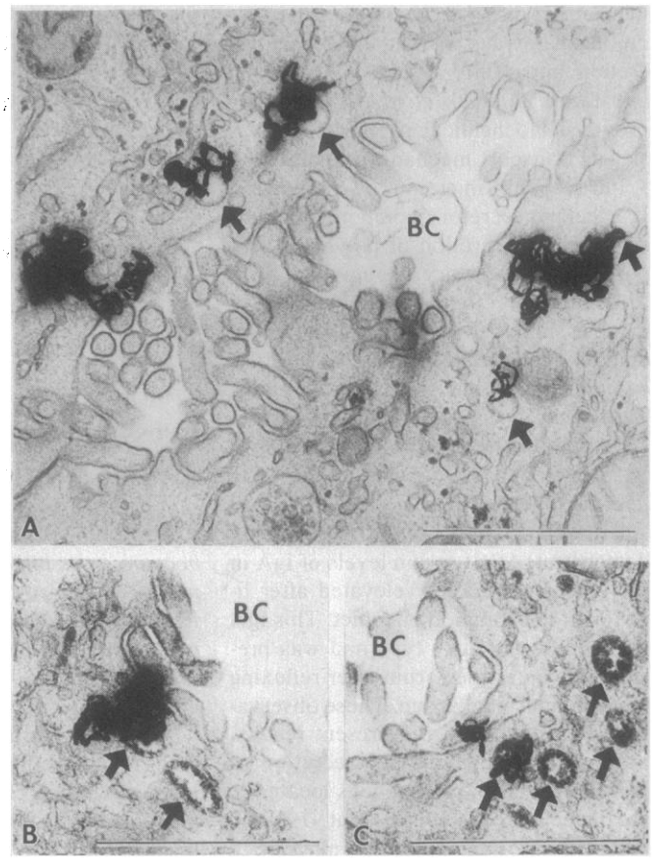

Fig. 1 (left). Electron microscopic autoradiographs showing the localization of silver grains (circled) along hepatocyte plasma membranes 1 minute after the intraportal injection of ^{125}I-labeled IgA alone or (inset) combined with horseradish peroxidase-diaminobenzidine cytochemistry. Silver grains are associated with endocytically derived HRP-containing vesicles immediately subjacent to the hepatocyte plasma membrane (inset). *D*, space of Disse; and *S*, sinusoidal space. Scale bar, 1 μm. Fig. 2 (right). Electron microscopic autoradiographs showing association of silver grains with endocytic vesicles (arrows) in the vicinity of biliary canaliculi 30 minutes after injection of ^{125}I-labeled IgA and HRP. Vesicles devoid of HRP from animals injected with ^{125}I-labeled IgA alone (A) and those containing dense diaminobenzidine reaction product due to the presence of HRP injected simultaneously with ^{125}I-labeled IgA (B and C) are readily observed in the pericanalicular cytoplasmic region. Approximately 70 percent of the grains in 1 μm of a biliary canalicular membrane are associated with an endocytic vesicle. *BC*, bile canaliculus. Scale bar, 1 μm.

sence of HRP, the distribution of the grains was the same, except that endocytic transport vesicles lacked diaminobenzidine reaction product. Comparison of associated grains with organelle volume densities (*9*) demonstrates the high degree of correlation of intracellular IgA with endocytic vesicles.

When the grains were studied in a 1-μm region of cytoplasm surrounding the bile canaliculus [the pericanalicular cytoplasm (*13*)], approximately 70 percent of them were found associated with HRP-containing vesicles 30 minutes after the injection, whereas none was present in this region 1 minute after the injection. A 70 percent association value (grains with HRP-containing vesicles) approaches the theoretical maximum association of 75 percent at two half-distances, implying that all grains in this region might have been associated with HRP-containing vesicles.

Iodine-125-labeled IgA was detected in bile within minutes of its intraportal injection (Fig. 3). Peak secretion of IgA occurred after 50 to 60 minutes, and declined to negligible levels after 2 hours. The presence of HRP caused a slight reduction in the biliary secretion of IgA. The HRP, like the labeled IgA, appeared in bile within minutes and peaked 50 to 60 minutes after the injection (Fig. 3). The extraction of ^{125}I-labeled IgA from portal blood and the secretion of this immunoglobulin into bile by the liver were very efficient: 2 hours after being injected, about 90 percent of the IgA and 70 percent of the IgA combined with HRP had been so processed.

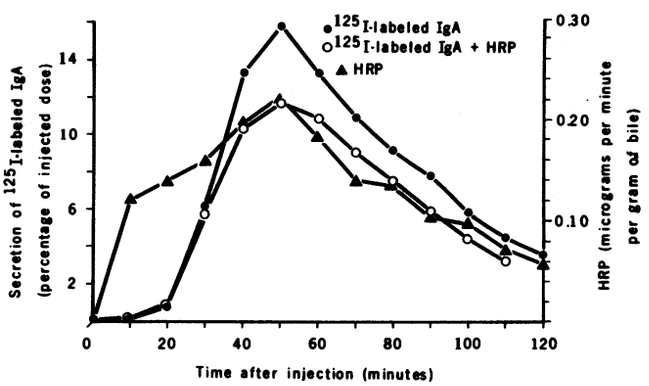

Fig. 3. Biliary secretion of ^{125}I-labeled IgA, ^{125}I-labeled IgA + HRP, and HRP alone. All three proteins appear in the bile within 10 minutes and reach peak levels of secretion within 50 to 60 minutes of the injection.

These results extend the light microscopic autoradiographic findings of Birbeck *et al.* (*14*) and are consistent with studies from our laboratory demonstrating, in the rat hepatocyte, a unique vesicular transport and secretory mechanism for proteins destined for biliary secretion (*9*, *15*, *16*). This protein secretion

mechanism differs from other protein-secreting pathways in that the biliary secretion apparently bypasses the Golgi apparatus. Mullock *et al.* (*17*) recently provided biochemical support for a vesicular transport mechanism for IgA.

Intravenous injection of polymeric IgA lacking secretory component resulted in biliary secretion of polymeric IgA possessing secretory component (*7*). This indicates that hepatocytes, in a manner similar to that of other mucosal epithelial cells, may synthesize secretory component for use as an IgA receptor. Thus secretory component may be responsible for the cytoplasmic transfer of IgA across hepatocytes and its secretion into the biliary space.

Lemaitre-Coelho *et al.* (*18*) recently demonstrated that serum levels of IgA in rats become markedly elevated after ligation of the common bile duct. This IgA was of the secretory type and was presumed to enter the serum after refluxing into the sinusoidal space. These observations, together with the present results, indicate that hepatobiliary secretion of IgA derived from plasma cells located in the lamina propria of the gut (*19*) and secreted into portal blood may be a major pathway by which secretory immunoglobulin reaches the intestinal lumen (*20*).

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Cross-Linking of Lens Crystallins in a Photodynamic System: A Process Mediated by Singlet Oxygen

Abstract. *In a dye-sensitized photooxidation system, lens crystallin polypeptides become cross-linked, and a blue fluorescence that is associated with the proteins is produced. These changes are similar to those seen in vivo in the aging human lens. Evidence implicating singlet oxygen as the causative agent of the effects in vitro is presented, and the possibility that this species may play a role in aging and cataractogenesis in vivo is discussed.*

A number of changes occur to human lens crystallins during aging and cataractogenesis. Such changes include protein aggregation (*1*), increased production of insoluble protein (*2*, *3*), oxidation of sulfhydryl groups (*4*), production of non-disulfide covalent cross-links between crystallin polypeptides (*5*, *6*), increased pigmentation in the lens nucleus (*5*, *7*), and production of blue fluorescence not attributable to tryptophan (*8*). Considerable effort has been made to implicate

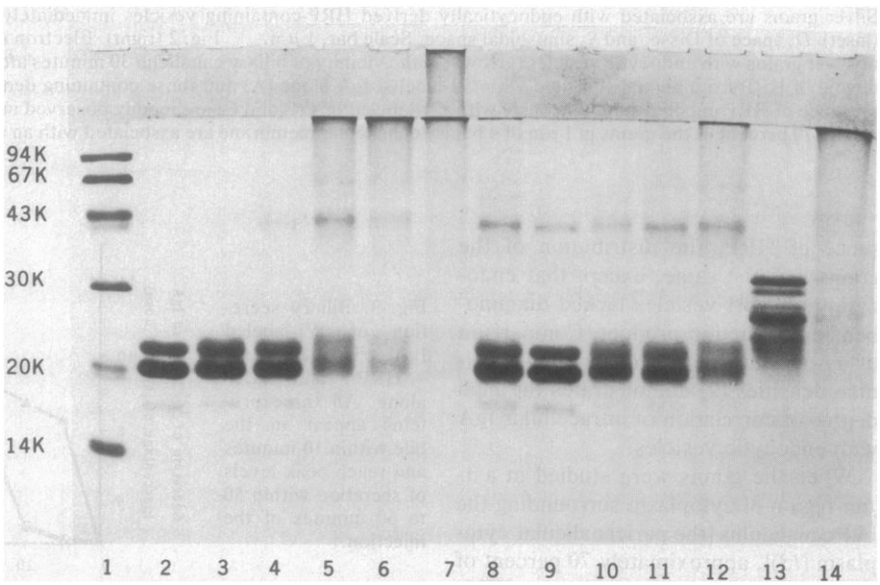

Fig. 1. Polypeptide patterns for bovine lens crystallin samples on a sodium dodecyl sulfate (SDS) polyacrylamide slab gel (4.5 percent stacking gel; 15 percent running gel). All samples were heated for 2 minutes at 100°C in 1 percent SDS and 1 percent 2-mercaptoethanol before being placed on the gel. Sample concentrations were 2.4 mg/ml in phosphate-buffered saline, *p*H 7.5; if present, the methylene blue concentration was $10^{-4}M$. (Lane 1) Standard mixture (Pharmacia) containing phosphorylase b, bovine serum albumin, ovalbumin, carbonic anhydrase, soybean trypsin inhibitor, α-lactalbumin. (Lane 2) α-Crystallin normal control. (Lane 3) α-Crystallin dark control. (Lane 4) Deaerated α-crystallin, irradiated 1 hour with methylene blue. (Lane 5) α-Crystallin, irradiated 1 hour with methylene blue. (Lane 6) α-Crystallin, irradiated 2 hours with methylene blue. (Lane 7) α-Crystallin, irradiated 6 hours with methylene blue. (Lane 8) α-Crystallin, irradiated 1 hour with methylene blue and 2 m*M* cyclohexadiene. (Lane 9) α-Crystallin, irradiated 1 hour with methylene blue and 15 m*M* sodium azide. (Lane 10) α-Crystallin, irradiated 1 hour with methylene blue and 2 m*M* glutathione. (Lane 11) α-Crystallin, irradiated 0.5 hour with methylene blue. (Lane 12) α-Crystallin, irradiated 0.5 hour with methylene blue in buffer prepared in D_2O. (Lane 13) Bovine β_H-crystallin, normal control. (Lane 14) Bovine β_H-crystallin irradiated 1.5 hours with methylene blue.

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