

**RAPID ACTIVE TRANSPORT OF IMMUNOGLOBULIN A
FROM BLOOD TO BILE***

By EVA ORLANS, JANE PEPPARD, JOHN REYNOLDS, AND JOE HALL

(From the Chester Beatty Research Institute, Royal Marsden Hospital, Sutton, Surrey,
Great Britain)

The large amounts of IgA made by plasma cells in the gut and mesenteric nodes produce high levels of IgA in the intestinal and thoracic duct lymph which are difficult to reconcile with the low levels found in the blood of most species.

In rats, blood serum levels of 0.05–0.18 mg/ml have been reported while mesenteric or thoracic duct lymph with about one-third of the total immunoglobulin level of blood contain 0.6 mg/ml (1–5). Assuming a flow rate of 1–3 ml/h for thoracic duct lymph of rats and a blood plasma vol of 8 ml the amount of IgA which daily enters the blood from the thoracic duct is 20–50 times greater than the circulating plasma pool. The evident disappearance of this IgA from the blood suggests that it is either catabolized very fast or exported. The availability of IgA from rat plasmacytomas (6) now makes it possible to purify enough IgA to explore these possibilities.

Preliminary experiments had shown (a) that IgA disappeared from the blood very much faster than similarly labeled IgG₂; (b) that feces collected during the day of injection contained antigenically intact radiolabeled IgA; and (c) that in accord with Lemaitre-Coelho et al. (7) rat bile had IgA levels some 10 times higher, and IgG levels 30–50 times lower, than those of blood serum. As the liver of healthy mammals contains no cells considered capable of immunoglobulin synthesis, it seemed likely that the biliary IgA was derived from the blood by active transport.

Materials and Methods

Immunoglobulins and Antisera. IgA was isolated from the ascitic fluid of Lou/Wsl rats bearing the IR461 plasmacytoma. After salt precipitation the material was fractionated twice on a 2.6×100 cm column of Ultrogel Ac22. The IgA formed by IR461 is heterogeneous with regard to size, and, like normal rat IgA, mainly oligomeric (5). The fraction least contaminated with other proteins (Fig. 1) had a sedimentation rate of 13.2 S; it contained α -macroglobulin detectable in immunoelectrophoresis and estimated as 0.3 mg/ml. Other contaminants account for less than 5% of the total protein (5.4 mg/ml) so the fraction was about 90% IgA.

IgG₂ was made from serum of Lou/Wsl rats bearing the IR33 plasmacytoma which secretes IgG_{2a}. The material used in the clearance studies, eluted from DEAE cellulose with 0.01 M phosphate buffer pH 7.4, contained some IgG_{2b} but no IgG₁.

Antisera obtained by immunizing rabbits with purified myeloma IgA contained anti-idiotypic and other unwanted antibodies, most of which were absorbed out with a pseudoglobulin

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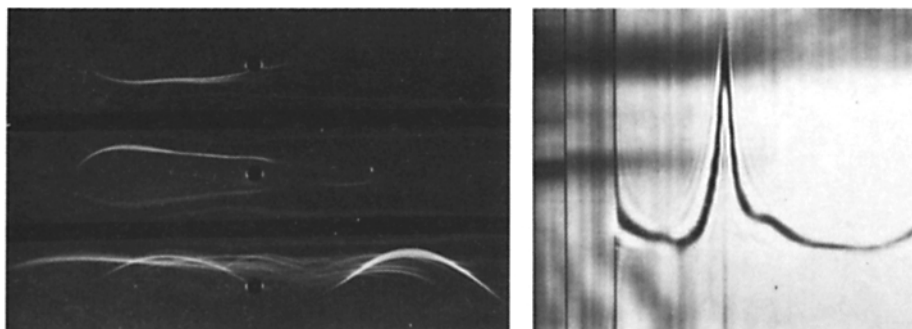


FIG. 1. IEP patterns formed by ascitic fluid in top and bottom wells and by the purified IgA fraction (5.4 mg/ml) (center well) with rabbit antisera to rat IgA (upper trough) and to rat serum (lower trough). Ultracentrifuge pattern of the IgA fraction after 16 min at 60,000 rev/min.

preparation of rat serum and with IgM. By injecting another rabbit with precipitates formed by the absorbed antiserum with polyclonal IgA from thoracic duct lymph an antiserum was obtained which, after absorption on an IgG-AH Sepharose column, was specific for α -chains.

Anti-IgG was made in rabbits against polyclonal IgG₂ and absorbed with F(ab')₂ coupled to AH-Sepharose when anti- γ chain specificity was required.

Labeling with ^{125}I and Estimation of Radioactivity. Purified IgA and IgG were lightly labeled by the chloramine T method (8) to an activity of $0.02 \mu\text{Ci}/\mu\text{g}$. Radioactivity was assayed in a Packard Auto Gamma Scintillation Spectrometer S260 (Packard Instrument Co., Inc., Downers Grove, Ill.). Free ^{125}I was estimated after precipitation of protein with 10% trichloroacetic acid.

Animals: Surgical and Experimental Procedures. Ligation of the common bile duct close to its junction with the duodenum, or insertion of cannulae (nylon, external diameter 0.94 mm, Portex Ltd., Hythe, U. K.) were done in adult, male Wistar rats weighing 300 g under pentobarbitone anesthesia (Sagatal, May and Baker, Ltd., Dagenham, U. K.) at 30 mg/kg bodyweight. The rats were placed in restraining cages and the next day they, and some unoperated rats, each received an i.v. injection containing 2×10^7 cpm of labeled Ig in 0.4 ml.

Blood samples of about 0.1 g were taken from the tail into weighed tubes at the times shown in Fig. 2. Bile from the cannulated rats was collected quantitatively into containers that were changed whenever a blood sample was taken. The flow of bile was about 0.4 ml/h and its protein content 3–5 mg/ml.

Autoradiographs were obtained by exposing washed and dried immunoelectrophoretic or Ouchterlony plates to X-ray film for 24 h.

Sedimentation studies were done by Mr. Neville Buttress, Agricultural Research Council Institute of Animal Physiology, Babraham, with a Spinco model E Analytical Ultracentrifuge.

Results

During the 3 h after the injection of ^{125}I -IgA, the radioactivity in the blood of both control and cannulated rats fell by at least 90%, while in rats with ligated bile ducts the fall was 53% (Fig. 2). The bile collected during this period contained a quarter of the injected dose of ^{125}I -IgA (Table I). At the peak of biliary excretion, 1–2 h after injection, the radioactivity/ml of bile was 12 times that of serum; if expressed in cpm/mg of protein the ratio approaches 200.

Free ^{125}I accounted at most for 10% of the counts in serum and 18% of those in bile. Fig. 3 shows the presence of antigenically intact labeled IgA in bile.

In one experiment similar to those described above, radiolabeled 7 S monomer prepared by reduction and alkylation from the 13.2 S material was injected into two cannulated and two control rats. The disappearance of the 7 S IgA from the

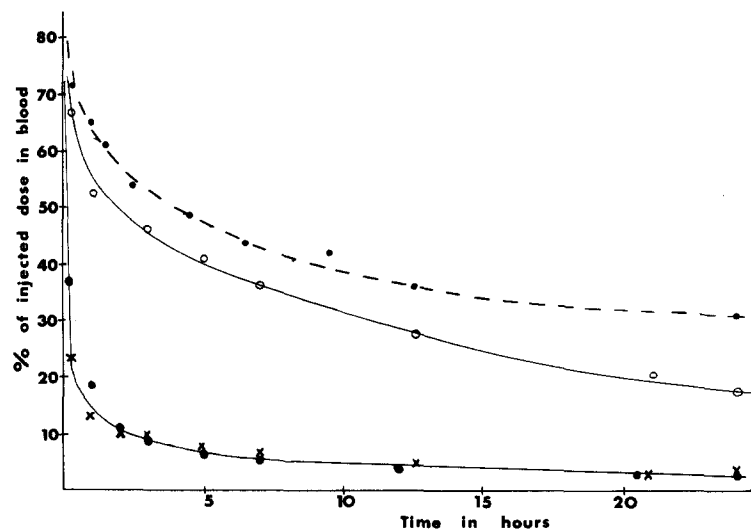


FIG. 2. The clearance of ^{125}I -IgA from the blood of normal rats (\times — \times) and rats with ligated (\circ — \circ) or with cannulated (\bullet — \bullet) bile ducts. Similar data for IgG₂ in normal rats is shown for comparison (\bullet — \bullet). The activity of the blood samples is plotted as the percentage of the injected dose in cpm/grams blood.

Table I
Radioactivity in Serum and Bile after i.v. Injection of ^{125}I -IgA

Time after injection	Blood serum	Bile	Bile
h	cpm/ml	cpm/ml	cpm/sample
0.25	8.2×10^5	4.2×10^5	1.5×10^6
1	4.5×10^5	5.4×10^5	2.9×10^6
2	3.3×10^5	1.6×10^6	1.0×10^6
3	2.8×10^5	0.4×10^6	0.4×10^6
5	2.2×10^5		
Total = 5.8×10^6			

Measurements made during the 5 h after i.v. injection of 2×10^7 cpm of ^{125}I -IgA. The values for serum are from samples taken at the beginning and end of each period of bile collection, and are means from assays on seven rats, three of which had been cannulated.

blood was indeed slower (17 h for 90% clearance)—but still much faster than for IgG—and, again, labeled IgA appeared rapidly in the bile.

The levels of ^{125}I -IgG₂ in the blood, also plotted in Fig. 2, agree with previous reports (9).

Discussion

Biliary excretion does not account completely for the rapid decline of labeled IgA in the blood as some fall occurred in the blood of rats with ligated bile ducts. Equilibration with the extravascular protein pool, secretion through other mucous membranes and genuine catabolism, must be involved.

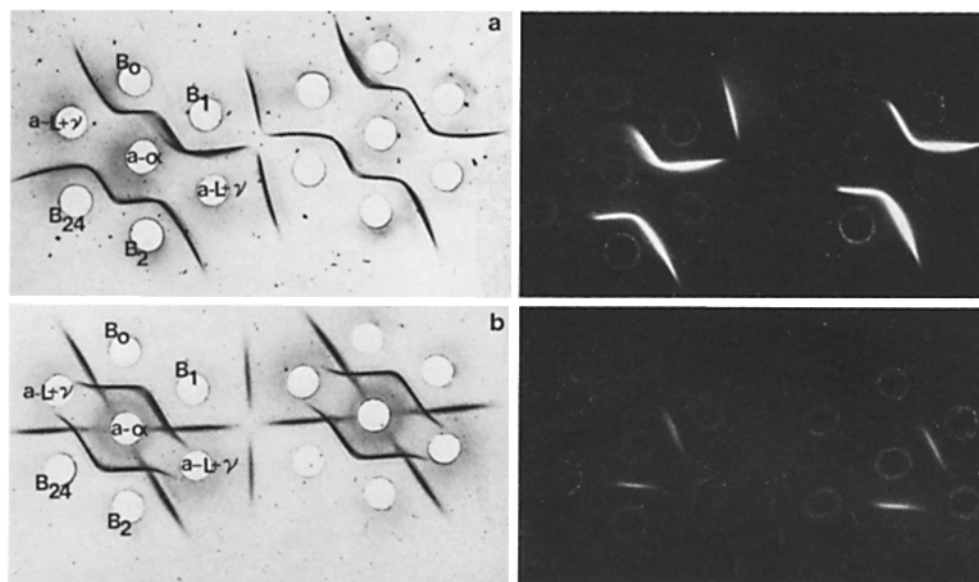


FIG. 3. Ouchterlony plates stained for protein (left) and corresponding autoradiographs on right, showing the reactions of eight different bile samples (a) with rabbit antiserum to IgA (a - α) and to IgG (a - L + γ). The bile samples from two rats were collected before injection (B₀); $\frac{1}{2}$ -1 h (B₁); 1-2 h (B₂); and 24-27 h (B₂₄) after injection. In (b) the same samples were mixed with a 1:30 dilution of serum from a rat with an IgA tumour. The continuous line formed by the bile samples in (a) with anti- α and anti-L + γ sera shows that bile IgA has both α - and light chains. Both patterns (a) and (b) show that the IgA output is fairly constant, with labeled IgA present in B₁ and B₂. In (b) unlike (a) lines due to γ -chains can be seen, showing that the IgG content of this bile is much less than that of serum diluted 1:30.

However, the rapid transport of a substantial proportion of intravenously injected IgA into the bile and thence to the gut accounts for the discrepancy between IgA levels in blood and thoracic duct lymph in rats, and could well explain the increased levels of both IgA (10) and of anti-coliform antibodies (11) in patients with hepatobiliary diseases. It shows also how the IgA antibodies which are formed locally in response to gut antigens and discharged into the intestinal lymph instead of directly into the mucous secretions may yet gain access to the lumen of the gut.

Summary

Immunoglobulins were isolated from the serum or ascitic fluid of Lou/Wsl rats bearing plasmacytomas and labeled with ^{125}I . When labeled IgA was injected i.v. it disappeared from the blood serum much more rapidly than IgG₂ so that after 3 h less than 10% remained. This rapid disappearance of the injected IgA was not seen in rats with ligated bile ducts. In rats with cannulated bile ducts, the labeled IgA appeared rapidly in the bile so that 25% of the injected dose was recovered in 3 h; at the peak of this biliary excretion the specific radioactivity of the bile (cpm/milligram protein) was about 200 times greater than that of the blood serum.

Thus much of the IgA which finds its way into the blood is rapidly and

actively transported across the liver so that it enters the gut lumen via the biliary tract.

Rats of the Lou/Wsl strain were kindly provided by Dr. H. Bazin; the IR461 tumour by Professor J. L. Gowans, and some ascitic fluid by Dr. B. Ogilvie.

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