Experimental conditions. For $H_2$-production assay, the reactor was kept in a thermostat-controlled (30 °C) water bath illuminated from 2 sides with tungsten lamps at 5500 lux (fig. 2).

The chamber can be operated as a continuous flow reactor by feeding the substrate solution with a peristaltic pump at a predetermined rate. Nevertheless in the experiments reported here the reactor was filled with 600 ml of substrate solution (10 mM malate or industrial waste waters). The solution was replaced when the $H_2$-production rate declined owing to exhaustion of the substrate.

$H_2$-determination. $H_2$ in the gas phase was monitored by gas-chromatography. Aliquots (1 ml) were withdrawn from the atmosphere of the reactor and injected into a Fractovap (Carlo Erba s.p.a.) gas-chromatograph having a thermal conductivity detector and provided with a silica-gel (510/1100 mesh $cm^{-2}$) column (2 m × 6 mm). Nitrogen was used as the carrier gas at a flow rate of 60 ml $min^{-1}$; the column oven was set at 125 °C and the bridge current was 120 mA. The results were quantitated by relating the peak areas to a calibration curve.

*Results and discussion.* With the device described a good and lasting $H_2$-production both from malate and waste waters was obtained (tables 1 and 2). Indeed, the rate of $H_2$-evolution was remarkably stable over a 30-day-period (30–40 µl $H_2$ per mg of cells dry wt per h). These values are much higher than those reported by Bennett and Weetal[4] for agar trapped *Rhodospirillum rubrum* on malate (0.7 µl $H_2$ per mg of cells wet weight per h). Apart from the difference in the bacterial species used, the geometry of our agarized panel maximizes the contact between immobilized cells and substrate solutions so that the limitations due to diffusion of substrates and products are significantly reduced.

Under the experimental conditions employed, the final conversion of substrate into $H_2$ by *Rhodopseudomonas palustris* immobilized cells was 60%. High $H_2$-production and high COD (Chemical Oxygen Demand) removal were also obtained utilizing waste waters as substrates for the photo-dissimilative activity of the immobilized cells (table 2). A yield of 0.78 l $H_2$ from 1 l of sugar refinery wastes (initial COD = 1200 ppm) and 2.2 l $H_2$ from 1 l of straw paper mill effluent (initial COD = 5600 ppm) were achieved. The $H_2$-photoproductions thus obtained would allow an appreciable energy recovery; in fact the hydrogen produced from 1 l of straw paper mill effluent allows the recovery of 6.2 Kcal.

In summary, the reactor described in this paper is easy to build and to operate. Moreover, it attains high rates of $H_2$-production from various organic substrates. Hence it can be conveniently utilized for investigating the factors determining the efficiency of photosynthetic reactors employing immobilized cells in the recovery of hydrogen from waste waters.

1 J.E. Zajic, N. Kosaric and J.D. Brosseau, Adv. Microbiol. Physiol. *9*, 57 (1978).
2 H. Gest, J.G. Ormerod and K.S. Ormerod, Archs Biochem. Biophys. *97*, 21 (1962).
3 J.G. Ormerod, K.S. Ormerod and M. Gest, Archs Biochem. Biophys. *94*, 449 (1961).
4 M.A. Bennett and H.H. Weetal, J. Solid-Phase Biochem *1*, 137 (1976).

## Immunohistochemical localization of galactocerebroside in kidney, liver, and lung of golden hamster[1]

K. Sakakibara, M. Iwamori, T. Uchida and Y. Nagai

*Department of Pathobiochemical Cell Research, Institute of Medical Science, University of Tokyo, Shirokanedai, Minato-ku, Tokyo 108 (Japan), 10 November 1980*

*Summary.* Localization of galactocerebroside in kidney, liver, and lung of hamster was studied by the immunoperoxidase method using an affinity-purified specific antibody. Epithelial cells of the following anatomical sites were labelled with the antibody: distal tubuli, ascending limbs of Henle's loops, and collecting tubuli in kidney; periportal bile ducts and hepatic parenchyma in liver; bronchioli and alveoli in lung. The existence of galactocerebroside in these 3 organs was also confirmed by chemical analysis.

Galactocerebroside (GC), a biosynthetic precursor of sulfatide, is one of the major glycosphingolipids of the white matter of brain and known to be a useful cell surface marker for myelin sheath and oligodendroglial cells[2]. Chemical analyses have shown the presence of GC in kidney[3], intestine[3], spleen[4], and lung[5], but the precise histological location of this glycolipid in organs other than brain remains obscure. We previously reported an affinity chromatographic technique for preparing highly purified specific antibody against glycosphingolipids[6]. It is imperative to use such a purified antibody for unequivocal immunohistochemical identification of glycolipid antigens in cells and subcellular structures. The present paper describes the specific cellular distribution of GC in kidney, liver, and lung of hamster, determined by the immunoperoxidase method using this purified anti-GC antibody.

*Materials and methods.* Anti-GC antibody was raised in rabbits and purified by affinity chromatography as described before[6]. This anti-GC antibody was previously shown by the immunoperoxidase method to react selectively with the myelin sheath, oligodendroglial cells, and epithelial cells of choroid plexus in brain[7]. The antibody preparation used had a complement fixation titer of 1:200. Kidney, liver, and lung of 8-week-old male golden hamsters, which had been sacrificed under anesthesia with Nembutal (5 mg/animal), were immediately frozen at −70 °C and cut with a cryostat into 4-µm-thick sections. They were fixed in 100% acetone for 10 min at room temperature, dried in air, and then exposed to anti-GC rabbit antibody without further dilution for 30 min at 37 °C. After washing, the tissue sections were incubated for 30 min with goat antirabbit IgG labelled with horse raddish peroxidase (E-Y Laboratory, lot No. 1101B) diluted 1:30, followed by washing with PBS. They were immersed in a freshly prepared solution of 0.05% diaminobenzidine hydrochloride and 0.01% hydrogen peroxide in 0.05 M Tris buffer, pH 7.6 (Graham and Karnovsky's reagent) for 3–8 min to reveal peroxidase activity. After washing without

counter-staining, they were examined under a light microscope. The controls were as follows: 1. tissue sections treated with non-immunized rabbit serum, and then exposed to labelled secondary antibody, 2. tissue sections directly immersed in Graham and Karnovsky's reagent, 3. tissue sections exposed to antibody preabsorbed with GC-egg lecithin-cholesterol mixed micelles (1:4:10, by wt) according to the method reported previously[8], and then to labelled antibody.

For the chemical analysis, 10 g each of lung, liver and kidney were used. The tissues were homogenized in distilled water and lyophilized. Total lipids were extracted 5 times from the lyophilized powder with 9 volumes of chloroform-methanol mixture (2:1, 1:2, and 1:1). The extracts were combined and subjected to the determination of cholesterol[9] and cerebroside. GC was separated from glucocerebroside with a borate-impregnated thin-layer plate[10] and determined by gas-liquid chromatography[11] and high performance liquid chromatography[12].

*Results.* In kidney, a marked immunohistochemical reaction was found in the cytoplasm of the distal tubular epithelium of the cortex (fig. 1a). No staining reaction was found in either glomerulus or proximal convoluted tubular epithelium. The epithelial cells of ascending limbs of Henle's loops and collecting tubuli of the medullae, especially their plasma membrane, were also the sites of a positive reaction (fig. 1b). These locations of GC in kidney agreed with the immunofluorescence studies by Zalc et al. of sulfatide localization in rat kidney[13]. In liver, the cytoplasm of the epithelial cells of the periportal bile ducts was

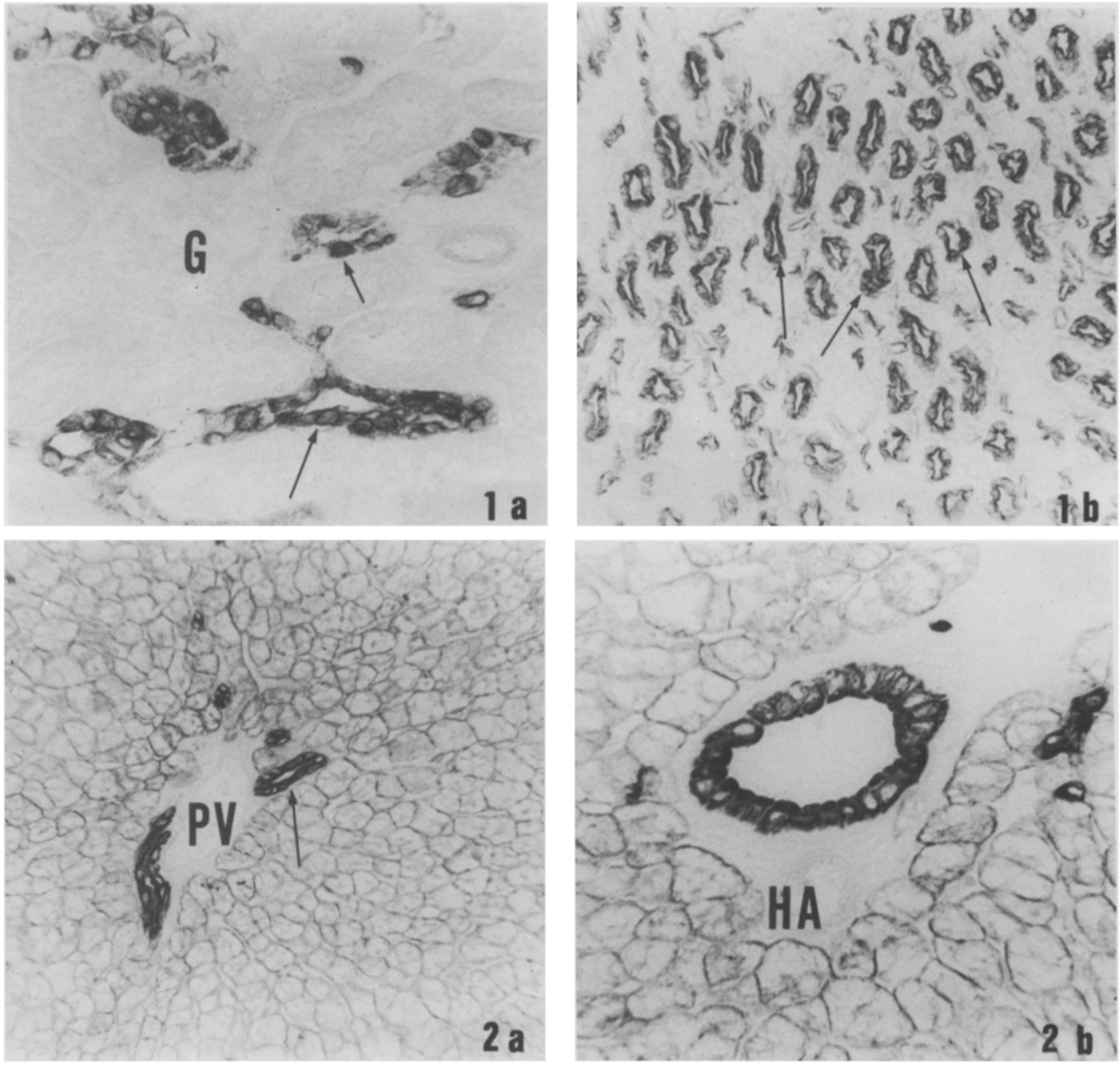


Figure 1. *a* Anti-GC antibody reacts selectively with epithelial cells of distal (arrow) and collecting tubuli (arrow) of the renal cortex. G, glomerulus. × 600. *b* In the lower medulla of kidney, cells of the ascending limbs of Henle's loops are stained positively (arrows). × 600.

Figure 2. *a* Epithelial cells of the intrahepatic bile ducts (arrow) are strongly labelled with anti-GC antibody, while hepatocytes, especially their plasma membranes, also show specific reaction. PV, portal vein, × 600. *b* A higher magnification of periportal area of the same liver section as shown in fig. 2a. HA, hepatic artery. × 1080.

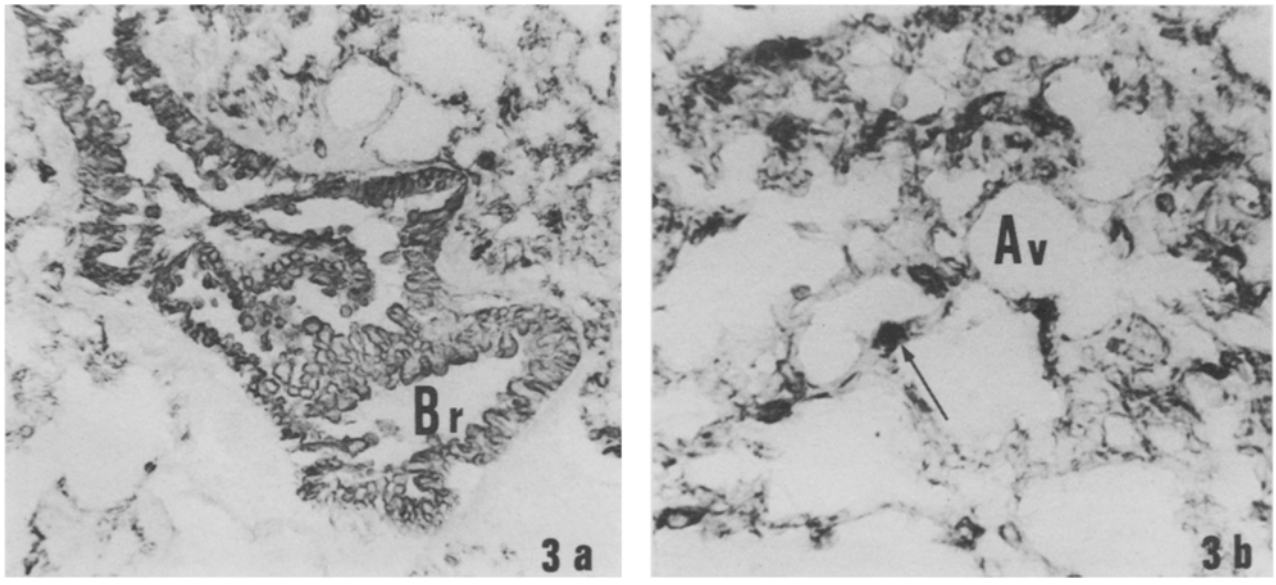


Figure 3. *a* Plasma membranes of bronchiolar epithelium are the sites strongly reacted with anti-GC antibody in the lung. Br, bronchiolus. × 600. *b* A certain type of alveolar cell, presumably type II alveolar cells (arrow) shows prominent staining with anti-GC antibody, while in other cell types a weak but seemingly definite staining is observed. Av, alveolus. × 1080.

the most intensely labelled with anti-GC antibody (fig. 2a). Parenchymal hepatocytes, especially their plasma membranes, were also stained but with less intensity (fig. 2b). In lung, bronchiolar epithelium (fig. 3a) and type II alveolar cells showed marked labelling, whereas a weak reaction was found in the other epithelial components (fig. 3b). There was no staining of the cells of mesenchymal origin in any of these 3 organs. The controls always gave negative results.

The results from the immunohistochemical study were confirmed by chemical analysis as shown in the table. The 3 tissues, lung, kidney and liver, were found to contain GC in various concentrations. Liver contained a small but definite amount of GC, which is not in accord with the result reported on rat liver by Svennerholm who showed the absence of GC by thin-layer chromatography[4].

*Discussion.* The results show the unique cellular distribution of GC in kidney, liver and lung of hamster. Immunohistochemical data using an affinity chromatography purified antibody, and biochemical analyses indicate that the distal nephron of the kidney, the bronchiolar epithelium of the lung, and the bile ducts of the liver are the major carrier of the GC, whereas all other cells either contain no GC, or contain it only to a marginal extent. It is of interest to note that our antibody decorates not only the plasma membrane, but also the cytoplasm. Marcus and Ianus[14] found by immunofluorescence studies that globoside, GalNAc($\beta$, 1→3)Gal($\alpha$, 1→4)Gal($\beta$, 1→4)Glc($\beta$, 1→1)ceramide, was present in both plasma membrane and cytoplasm of epithelial cells of proximal convoluted tubuli of human kidney. They suggested an association of globoside with mitochondria. We would rather suggest some close association of this and other glycolipids with the cytoskeleton, based on another immunocytochemical study on GC using cultured epithelial cell lines, JTC-12, HeLa, and MDCK[15]. The solution to this problem will be provided by precise immunoelectronmicroscopic study of the subcellular localization of glycosphingolipids.

Despite the extensive studies of Karlsson et al.[16,17] the true physiological functions of sulfatide remain unclear. The present study, however, revealed the common location of GC in glandular epithelium which is generally known to perform secretion. We suggest, therefore, that this glycolipid as well as sulfatide may be involved in some processes of secretion performed by these particular types of epithelial cells.

Monoglycosylceramides and cholesterol content of hamster lung, liver and kidney

| | Cholesterol (μmoles/g dry wt) | Galactocerebroside (nmoles/g dry wt) | Glucocerebroside |
|---|---|---|---|
| Lung | 29.6 | 6.8 | 10.1 |
| Liver | 8.7 | 0.2 | 6.2 |
| Kidney | 35.8 | 6.3 | 16.7 |

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2 W.T. Norton and L.A. Autilio, J. Neurochem. *13*, 212 (1966).
3 K.A. Karlsson, in: Structure of Biological Membranes, p. 245. Ed. S. Abrahamsson and I. Pascher. Plenum Press, New York 1977.
4 E. Svennerholm and L. Svennerholm, Nature *198*, 688 (1963).
5 R. Narasimhan and R.K. Murray, Biochem. J. *179*, 199 (1979).
6 T. Uchida and Y. Nagai, J. Biochem. *87*, 1829 (1980).
7 T. Uchida, K. Takahashi, H. Yamaguchi and Y. Nagai, Jap. J. exp. Med. *51*, 21 (1981).
8 Y. Nagai and T. Ohsawa, Jap. J. exp. Med. *44*, 451 (1974).
9 M. Iwamori and H.W. Moser, Clin. Chem. *21*, 725 (1975).
10 E.L. Kean, J. Lipid Res. *7*, 449 (1966).
11 C.C. Sweeley and B. Walker, Analyt. Chem. *36*, 1461 (1964).
12 M.D. Ullman and R. McCluer, J. Lipid Res. *19*, 910 (1978).
13 B. Zalc, J.J. Welwig, M.S. Ghandour and L. Sarlieve, FEBS Lett. *92*, 92 (1978).
14 D.M. Marcus and R. Janis, J. Immun. *104*, 1530 (1970).
15 K. Sakakibara, T. Momoi, T. Uchida and Y. Nagai, Nature, Lond. submitted.
16 K.A. Karlsson, B.E. Samuelsson and G.O. Steen, Biochim. biophys. Acta *176*, 429 (1969).
17 K.A. Karlsson, B.E. Samuelsson and G.O. Steen, Biochim. biophys. Acta *316*, 317 (1973).