NOC was not concentration-dependent under the basal condition. However, when a submaximal vasoconstriction was elicited by Phe (10^{-7} M), when the PP had reached an average value of 97.9 ± 11.0 mm Hg (n = 24), NOC produced a concentration-dependent fall in PP, followed by a longer-lasting increase (fig.). NOC at the concentration of 10^{-8} M, which did not produce a direct vascular response by itself, effectively prevented the pressor response to periarterial stimulation and A II, but significantly increased the pressor effect of NA. The results are summarized in the table.

Discussion

The results of the present study indicate that NOC has a vasodilator action on resistance vessels of the isolated perfused kidney rabbit at the concentrations used. A similar long-lasting vasodilator action of NOC has also been observed in the rabbit basilar artery[4]. However, in isolated helically cut renal artery from the same species, NOC produces a long-lasting and slowly developing contractile response (unpublished observations). This difference may be due to differences in the action of the compound on large vessels and on resistance vessels. Lower concentrations of NOC, without producing a direct vascular response, inhibited the pressor response to electrical sympathetic stimulation in the isolated perfused rabbit kidney. A similar inhibitory effect of NOC was also observed on the pressor effect of exogenous A II. In contrast, NOC potentiated the pressor effect of NA. These findings indicate that the inhibitory effect of NOC against sympathetic stimulation is probably mediated by a presynaptic mechanism which causes an inhibition of NA release from adrenergic nerve endings. The mechanism of enhancement by NOC of the contractile effect of NA in perfused kidney may be due to the potentiation of the responsiveness of the postsynaptic alpha-adrenoceptors*, or to an inhibition of the re-uptake of NA. The present findings are consistent with those of several previously published observations showing inhibitory effects of various PGs on neurotransmitter release from adrenergic nerve endings[6, 7]. The effect of NOC in preventing gastric mucosal damage due to various noxious stimuli is well known[2, 3]. Prevention of the release of an adrenergic neurotransmitter from nerve endings has been shown to be one of the mechanisms of cytoprotection by PGs[8]. The findings presented here for the kidney support this hypothesis.

1 This work was supported by a grant from the Turkish Scientific and Technical Research Council (TAG-578). Nocloprost is the gift of Dr E. Schillinger, Head of Biochemical Pharmacology, Schering AG Research Laboratories, Berlin, FRG.

2 Zengil, H., Ercan, Z. S., Onuk, E., and Türker, R. K., Pros. Leuk. Ess. fatty Acids *40* (1990) 13.
3 Loge, O., and Radüchel, B., Kyoto Conference on Prostaglandins. Abstr. Nov. 25–28 Kyoto, Japan 1984.
4 Türker, R. K., Egemen, N., and Deda, H., Prostaglandins in Clinical Research: Cardiovascular System, p. 205. Eds K. Schrör and H. Sizinger. Alan R. Liss Inc., New York 1989.
5 Türker, R. K., Demirel, E., and Ercan, Z. S., Pros. Leuk. Ess. fatty Acids *31* (1988) 45.
6 Hedqvist, P., and Wennmalm, A., Acta physiol. scand. *83* (1971) 156.
7 Ercan, Z. S., Arch. int. Physiol. Biochem. *83* (1975) 799.
8 Schrör, K., Addicks, K., Darius, H., Ohlendorf, R., and Rosen, P., Thromb. Res. *21* (1981) 175.

0014-4754/91/010069-02$1.50 + 0.20/0

Changes in transglutaminase activity in carbon tetrachloride-damaged rat liver

H. Kohno, K. Kashimura, S. Katoh and Y. Ohkubo

Department of Radiopharmacy, Tohoku College of Pharmacy, Sendai, Miyagi 981 (Japan)
Received 26 February 1990; accepted 20 July 1990

Summary. A significant decrease in transglutaminase (TGase) activity was observed in the cytosol and nuclear fractions of carbon tetrachloride-damaged rat liver. The degree of decrease in TGase activity in the cytosol fraction was closely related to the serum transaminase level. Gel filtration studies revealed that TGase activity in 80 kDa fractions significantly decreased, but that in 160 kDa fractions slightly increased after carbon tetrachloride treatment.
Key words. Transglutaminase; ornithine decarboxylase; serum transaminase; liver damage; carbon tetrachloride; gel filtration.

Transglutaminases (TGases, EC 2.3.2.13) are calcium-dependent enzymes that promote the formation of covalent linkages between the alkyl primary amine groups of 'amine donor' substrates and the gamma-carboxamide group of glutamine in some polypeptides that serve as 'amine acceptor' substrates. They are known to be widely distributed in various mammalian tissues both intracellularly and extracellularly[1–3]. Extracellular TGase has been shown to contribute to various biological events, including fibrin and seminal fluid clotting[1–3]. The biological function of intracellular TGase, however, has not been established, although the enzyme has been suggested to be involved in the processes of cell growth and differentiation[4, 5], and in various membrane-mediated

events, including receptor-mediated endocytosis[6,7], insulin release[8], cell-to-cell contact[9], and neurotransmitter release[10].

Mammalian liver tissue is very rich in TGase[11,12], but its physiological function there is almost unknown. It has recently been reported that liver TGase is distributed in the particulate fraction, which includes the plasma membrane, as well as in the cytosol fraction[13], and that the subcellular distribution of liver TGase fluctuates during tumor growth[14–16]. Moreover, Chang et al. showed that TGase from the particulate fraction of the malignant chondrocyte had a higher molecular weight than that from normal tissues[17].

The level of ornithine decarboxylase (ODC, EC 4.1.1.17) has been found to increase dramatically during rapid cell proliferation in response to various stimuli[18]. Reciprocal relationships between TGase and ODC activities have also been shown in lymphocytes[19], in retinoid-treated Chinese hamster ovary cells and in melanoma cells[20].

It has been reported that acute liver injury produced by various hepatotoxins including carbon tetrachloride is accompanied by increases in cellular calcium levels[21–23], ODC activity[24] and hepatic growth factor content[25] in liver. Considering these findings and the concomitant decrease in TGase activity during cell proliferation[4,5], we examined the activity and molecular weight changes in TGase during liver damage induced by carbon tetrachloride.

Materials and methods

Animals. Male Wistar rats weighing 180–250 g (SLC, Hamamatsu, Japan) were kept under conditions of a 12-h light-dark cycle (light: 09.00 h–21.00 h), 23 ± 1 °C temperature and 55 ± 5% humidity, and had free access to food (F2, Funabashi Farms, Funabashi, Japan) and tap water.

Chemicals. [1,4-^{14}C]putrescine dihydrochloride (^{14}C-PUT, 4.4 GBq/mmol, New England Nuclear, USA), DL-[5-^{14}C]ornithine (2.26 GBq/mmol, Amersham, England), N,N-dimethylcasein (Sigma, USA), putrescine dihydrochloride and L-ornithine hydrochloride (Nacalai Tesque, Japan) were used. All other reagents were of analytical grade.

Induction of liver damage. Rats were given a 20% suspension of carbon tetrachloride in olive oil (5 ml/kg) orally, after fasting for 16 h. Normal rats were used as controls, since no significant differences in serum alanine transaminase (ALAT) levels and liver TGase activity between normal and olive oil-treated groups were found.

Measurement of serum ALAT activity. Blood was drawn from the inferior vena cava under sodium pentobarbital anesthesia, and was centrifuged at 2000 × g for 10 min at 4 °C. Serum ALAT activity was measured using a commercially available kit (Eiken Chemicals, Japan) based on the method of Reitman and Frankel[26].

Tissue preparation. Each rat was anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and was systemically perfused with cold physiological saline from the heart. The liver was immediately removed, minced and homogenized with 4 volumes of ice-cold buffer (0.25 M sucrose, 3 mM Tris, 1 mM EDTA, pH 7.4) by 10 strokes of a loose Dounce homogenizer. A subcellular fractionation of the liver was performed by the method of Barnes et al.[15] with some modifications. The homogenate was centrifuged at 600 × g for 10 min at 4 °C. The precipitate (nuclear fraction) was washed once with the same volume of the homogenization buffer and recentrifuged at 600 × g for 10 min at 4 °C. The supernatant fractions obtained from the two centrifugations were mixed and centrifuged at 105,000 × g for 60 min at 4 °C. The precipitate (cytoplasmic particulate fraction) was washed once with the same volume of the homogenization buffer and recentrifuged at 105,000 × g for 60 min at 4 °C. The supernatant (cytosol fraction) was used for each assay. The nuclear and cytoplasmic particulate fractions were suspended in a Potter-Elvehjem homogenizer with 4 volumes of homogenization buffer containing 0.2% Triton X-100.

Gel filtration chromatography. Each fraction was adjusted to 10 mg/ml protein with elution buffer (50 mM Tris-HCl, containing 1 mM EDTA and 0.5 mM dithiothreitol, pH 7.4). A 3-ml volume of the solution was applied to a Bio-Gel A 0.5 m column (2.6 cm × 100 cm), and eluted with the elution buffer at a speed of 0.5 ml/min, and each 5-ml fraction was collected.

Measurement of TGase activity. TGase activity was assayed by the incorporation of ^{14}C-PUT into N,N'-dimethylcasein using the filter paper technique described by Lorand et al.[27] with minor modifications. The final assay medium contained 50 mM Tris-HCl (pH 7.4), 10 mM dithiothreitol, 5 mM $CaCl_2$, 0.5 mg of N,N'-dimethylcasein, and 1 mM PUT including 0.925 kBq of ^{14}C-PUT. The reaction was initiated by the addition of the sample solution (20 µl), and was incubated at 37 °C in a total volume of 50 µl. The enzyme reaction was terminated by spotting 40 µl of the reaction mixture onto a Whatman 3MM filter paper which was immersed in 10% trichloroacetic acid (TCA) solution and fixed on to a multi-vacuum filter unit. The filter paper was washed twice, each time with 1 ml of 10% TCA. The assays were corrected against Ca^{2+}-free blanks. The filters were transferred to scintillation counting vials and 3 ml of scintillation counting fluid (Clearsol I, Nacalai Tesque, Japan) was added. The vials were vigorously shaken using a vial mixer and counted by liquid scintillation counter (LS 7800, Beckman) after overnight extraction in the dark. The enzyme activity was expressed as nmol PUT incorporated into dimethylcasein · min^{-1} · mg protein^{-1}.

Measurement of ODC activity. ODC activity was measured by the method of Djurhuus[28] with minor modification. The final assay medium contained 50 mM Tris-

HCl (pH 7.4), 0.1 mM EDTA, 5 mM dithiothreitol, 0.2 mM pyridoxal phosphate, and 5 mM L-ornithine including 18.5 kBq of DL-[5-^{14}C]ornithine. The reaction was initiated by the addition of the sample solution (30 µl), and was incubated for 60 min at 37 °C in a total volume of 50 µl. The enzyme reaction was terminated by spotting 20 µl of the reaction mixtures onto a Whatman P-81 cation exchange paper fixed on a multi-vacuum filter unit. The paper was washed twice with 4 ml of 0.1 M ammonium solution. The assays were corrected against sample-free blanks. The radioactivity of the papers was counted as described above. The enzyme activity was expressed as pmol PUT formation · min^{-1} · mg protein^{-1}.

Protein assay. The protein content was determined according to the method of Bradford[29], using bovine serum albumin as a standard.

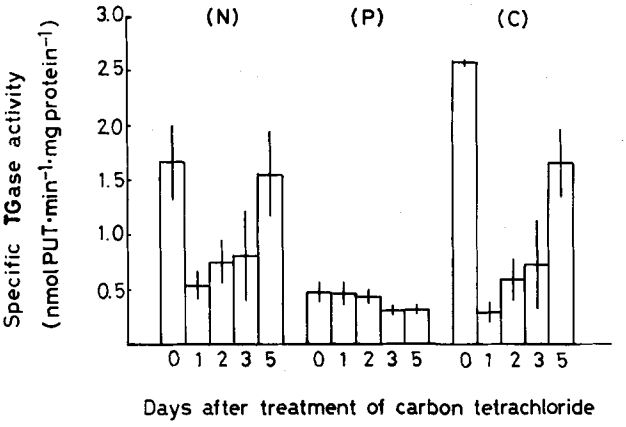

Figure 1. Changes in specific transglutaminase (TGase) activity in the nuclear (N), cytoplasmic particulate (P) and cytosol (C) fractions of liver after carbon tetrachloride treatment. The day-0 values indicate those of normal rat liver. Each column is the mean ± SE of 4–9 experiments.

Results

Since no significant difference in serum ALAT and TGase activity between normal and olive oil-treated rats (table 1) was observed, normal rats were used as control. Serum ALAT activity increased steeply after the treatment with carbon tetrachloride (table 2). The maximum value was observed on day 1, after which there was gradual recovery nearly to the control levels on day 5. The TGase activity in each fraction is shown in figure 1. In control liver, the activity was highest in the cytosol fraction and lowest in the cytoplasmic particulate fraction. The activity in the cytosol and nuclear fractions decreased drastically after the carbon tetrachloride treatment. The lowest activity was observed on day 1, and subsequently increased in both fractions. No significant changes in the level in cytoplasmic particulate fraction were seen.

Since the degree of liver damage varied considerably among carbon tetrachloride-treated rats, the relationship between serum ALAT activity and TGase activity of the cytosol fraction was examined. A significant correlation ($r = -0.86153$, $p < 0.01$) was observed between these values on the 2nd day after treatment (fig. 2).

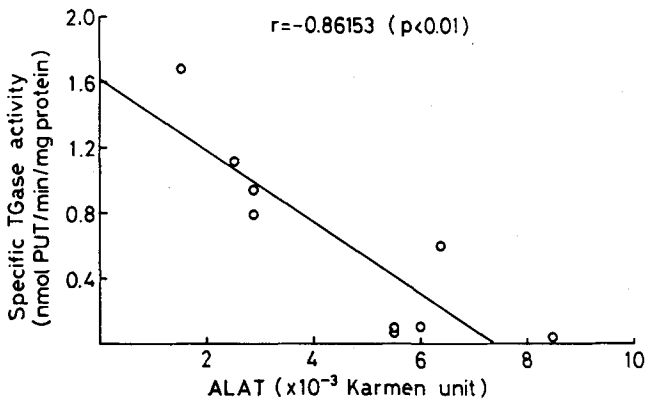

Figure 2. Correlation between the specific transglutaminase (TGase) activity in the cytosol fraction and the serum alanine transaminase (ALAT) activity on the 2nd day after carbon tetrachloride treatment.

The elution patterns of TGase activity in the nuclear, cytoplasmic particulate and cytosol fractions of normal and damaged livers on the 2nd day after treatment are shown in figure 3. The peak activity in the nuclear fraction of normal rat liver appeared at the fraction number

Table 1. Serum alanine transaminase (ALAT) and specific transglutaminase (TGase) activity in the nuclear (N), cytoplasmic particulate (P) and cytosol (C) fractions of the liver from normal and olive oil-treated rats

	ALAT (Karmen units)	Specific TGase activity (nmol PUT · min^{-1} · mg protein^{-1}) N	P	C
Normal rats	23.8 ± 2.9	1.665 ± 0.335	0.481 ± 0.086	2.598 ± 0.031
Olive oil-treated rats	27.0 ± 6.9	1.653 ± 0.549	0.429 ± 0.067	2.554 ± 0.083

Data from olive oil-treated rats were the values on the 2nd day after the treatment. Each value is the mean ± SE of 4 experiments.

Table 2. Changes in serum alanine transaminase activity after carbon tetrachloride treatment

Days	0	1	2	3	5
Karmen units	23.8 ± 2.9	6287.5 ± 883.3	4643.3 ± 757.7	821.3 ± 216.5	36.3 ± 6.4

Each value is the mean ± SE of 4–9 experiments.

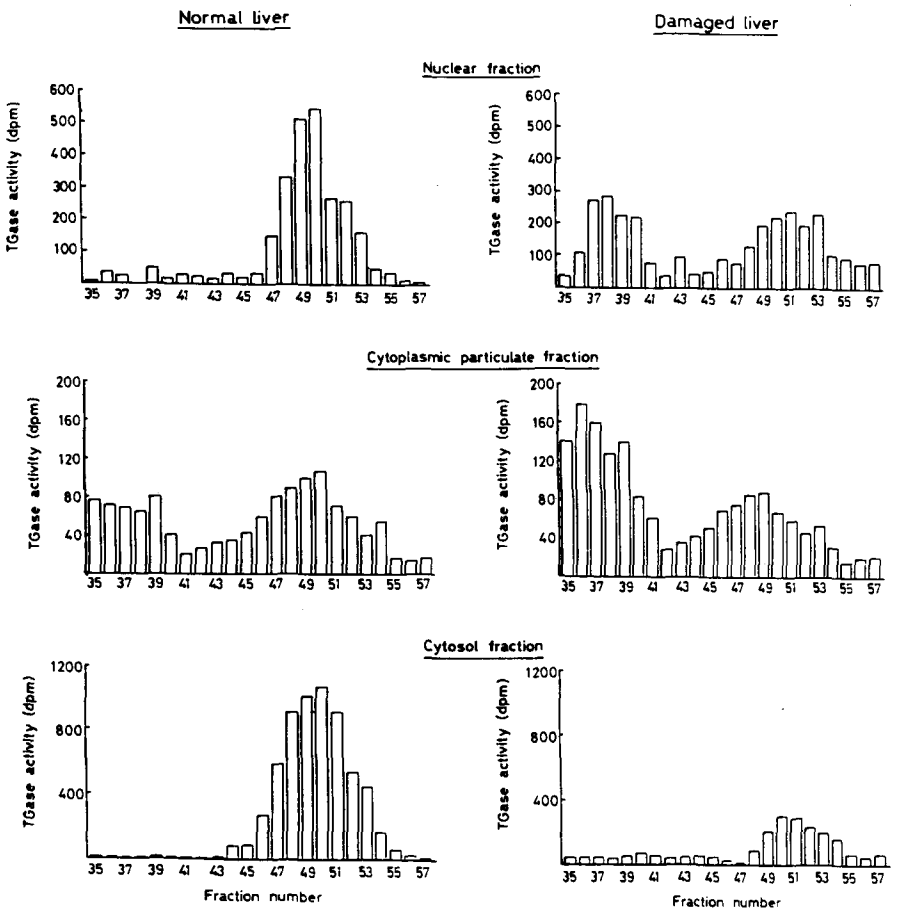

Figure 3. Elution of transglutaminase (TGase) activity in the nuclear (top), cytoplasmic particulate (middle) and cytosol (bottom) fractions of normal (left) and damaged (right) liver on the 2nd day after carbon tetrachloride treatment. Incubation time: 2 h for the nuclear and cytoplasmic particulate fractions, 1 h for the cytosol fraction.

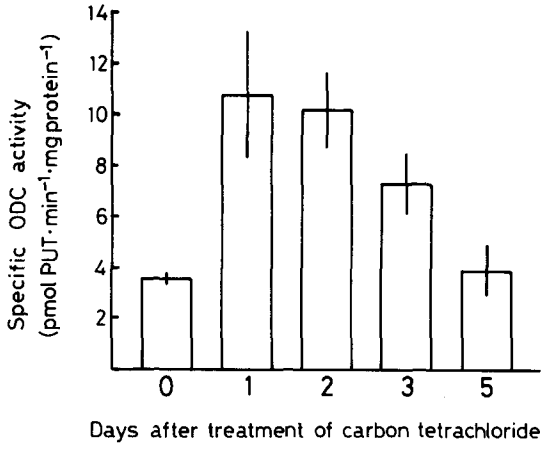

Figure 4. Changes in specific ornithine decarboxylase (ODC) activity in the cytosol fraction of liver after carbon tetrachloride treatment. The day-0 values indicate those of normal rat liver. Each column is the mean ± SE of 4–9 experiments.

50, corresponding to a molecular weight of 80 kDa. In the damaged liver, the TGase activity of this peak fraction was found to have decreased significantly, while another peak fraction, corresponding to a high molecular weight of 160 kDa, increased (fig. 3, top). In the cytoplasmic particulate fraction of control liver, two distinct peaks were observed at almost the same positions as in the nuclear fraction of the damaged liver. There were minor changes in the elution profile and an increase in the high molecular weight fraction (fig. 3, middle). In the cytosol fraction of control liver, only one single peak of TGase activity, corresponding to 80 kDa, was observed. A significant decrease of TGase activity in this peak was observed in the cytosol fraction of damaged liver (fig. 3, bottom). ODC activity of the cytosol fraction significantly increased following the carbon tetrachloride treatment (fig. 4). The activity reached a maximum, 360% of the control value, one day after the treatment and subsequently decreased.

Discussion

The subcellular distribution pattern of TGase activity in normal rat liver was very similar to that reported by Barnes et al.[15]. TGase activity was significantly reduced in both the nuclear and the cytosol fractions following treatment of the rats with carbon tetrachloride. The results from gel filtration studies suggest that a significant reduction of TGase activity is due to the loss of TGase with a molecular weight corresponding to 80 kDa.

In tumor tissues, TGase activity also decreased in the soluble fraction, but it increased in the cytoplasmic particulate fraction[14–16]. In the cytoplasmic particulate fraction of carbon tetrachloride treated liver, TGase activity in a higher molecular weight fraction corresponding to 160 kDa also increased, but TGase activity in a lower molecular weight fraction corresponding to 80 kDa inversely decreased. Specific TGase activity in the cytoplasmic particulate fraction, however, did not totally change following the carbon tetrachloride treatment. This was very different from the results observed in the cytoplasmic particulate fraction of tumor tissues.

Chang et al. reported that TGase from the particulate fraction of the malignant chondrocyte had a higher molecular weight, 100 kDa, than that from normal tissues, 80 kDa[17]. We observed that TGase activity in the higher molecular weight fraction corresponding to 160 kDa increased, both in the nuclear and cytoplasmic particulate fractions, following the carbon tetrachloride treatment, but we did not find TGases of molecular weight 100 kDa following the carbon tetrachloride treatment.

There is a possibility that high-molecular-weight TGase in the nuclear and cytoplasmic particulate fraction is a dimer of 80 kDa-TGase through a γ-glutamyl ε-lysine bridge, since TGase itself has been suggested to be a substrate for its own enzymatic activity[30,31], and the intracellular Ca^{2+} level of rat liver has generally been found to increase after carbon tetrachloride treatment[21–23].

It is interesting that the existence of high-molecular-weight TGase was also observed in the normal liver, but only in the cytoplasmic particulate fraction. This result suggests that TGase functions physiologically at those particular sites in cells which are readily exposed to a high concentration of Ca^{2+} responding to various stimuli. An absence of the high molecular weight form of TGase in the nuclear and cytosol fractions of normal rat liver suggests that intracellular Ca^{2+} does not reach the micromolar concentration required for the activation of TGase[32] in the physiological state without any stimulation.

The mechanism of the significant decrease in cytosol TGase activity following carbon tetrachloride treatment is almost unknown. Recently, Kawashima et al. found a drastic decrease in TGase activity in the brains of Alzheimer's patients[33]. They postulated that TGase was exhausted after it had functioned, or was inactivated by self-crosslinking when intracellular Ca^{2+} increased. A significant decrease in the cytosol TGase activity after carbon tetrachloride treatment may be due to a mechanism similar to that suggested in Alzheimer's disease.

A close relationship between the elevation of serum ALAT levels and the reduction of TGase activity in the liver cytosol fraction was observed on the 2nd day after the carbon tetrachloride treatment. The cell proliferation activity seems to be more extensive on the 2nd day than on the 1st day after treatment with carbon tetrachloride, since the most significant increase in ^{3}H-thymidine incorporation into DNA was observed at 2 days after the treatment[34]. Moreover, hepatocyte growth factor has very recently been shown to increase markedly in rat liver after the injection of carbon tetrachloride[25]. Considering the reciprocal relationship between ODC and TGase activity, significant reduction of TGase activity in the damaged liver, or changes in its subcellular distribution, may be related to the subsequent cell proliferation process. It is not clear whether the changes in TGase activity are only a result of cell injury or are necessary for cell proliferation. The same question can also be asked about the process of carcinogenesis. Studies on this point are in progress.

1 Folk, J. E., and Finlayson, J. S., Adv. Prot. Chem. *31* (1977) 1.
2 Folk, J. E., A. Rev. Biochem. *49* (1980) 517.
3 Lorand, L., and Conrad, S. M., Molec. cell. Biochem. *58* (1984) 9.
4 Birckbichler, P. J., and Patterson, M. K. Jr, Ann. N.Y. Acad. Sci. *312* (1978) 354.
5 Birckbichler, P. J., and Patterson, M. K. Jr, Prog. clin. biol. Res. *41* (1980) 845.
6 Maxfield, F. R., Willingham, M. C., Davies, P. J. A., and Pastan, I., Nature *277* (1979) 661.
7 Davies, P. J. A., Davies, D. R., Levitzki, A., Maxfield, F. R., Milhaud, P., Willingham, M. C., and Pastan, I. H., Nature *283* (1980) 162.
8 Bungay, P. J., Owen, R. A., Coutts, I. C., and Griffin, M., Biochem. J. *235* (1986) 269.
9 Slife, C. W., Dorsett, M. D., and Tillotson, M. L., J. biol. Chem. *261* (1986) 3451.
10 Pastuszko, A., Wilson, D. F., and Erecinska, M., J. Neurochem. *46* (1986) 499.
11 Chung, S. I., Ann. N.Y. Acad. Sci. *202* (1972) 240.
12 Juprelle-Soret, M., Wattiaux-De Coninck, S., and Wattiaux, R., Biochem. J. *250* (1988) 421.
13 Slife, C. W., Dorsett, M. D., Bouquett, G. T., Register, A., Taylor, E., and Conroy, S., Archs Biochem. Biophys. *241* (1985) 329.
14 Birckbichler, P. J., Orr, G. R., and Patterson, M. K. Jr, Cancer Res. *36* (1976) 2911.
15 Barnes, R. N., Bungay, P. J., Elliott, B. M., Walton, P. L., and Griffin, M., Carcinogenesis *6* (1985) 459.
16 Hand, D., Elliott, B. M., and Griffin, M., Biochim. biophys. Acta *970* (1988) 137.
17 Chang, S. K., and Chung, S. I., J. biol. Chem. *261* (1986) 8112.
18 Morris, D. R., and Fillingame, H., A. Rev. Biochem. *43* (1974) 303.
19 Vanella, A., Campisi, A., Guglielmo, P., Cacciola, E. Jr, Cunsolo, F., Geremia, E., Tiriolo, P., Pappalardo, P., and Crisafi, G., Acta haemat. *76* (1986) 33.
20 Scott, K. F. F., Meyskens, F. L. Jr, and Russell, D. H., Proc. natl Acad. Sci. USA *79* (1982) 4093.
21 Moore, L., Davenport, G. R., and Landon, E. J., J. biol. Chem. *251* (1976) 1197.
22 Farber, J. L., Life Sci. *29* (1981) 1289.
23 Farber, J. L., Lab. Invest. *47* (1982) 114.
24 Holtta, E., Sinervirta, R., and Janne, J., Biochem. biophys. Res. Commun. *54* (1973) 350.

25 Asami, O., Ihara, T., and Nakamura, T., J. biol. Chem. (1990) in press.
26 Reitman, S., and Frankel, S., Am. J. clin. Path. *28* (1957) 56.
27 Lorand, L., Campbell-Wilkes, L. K., and Cooperstein, L., Analyt. Biochem. *50* (1972) 623.
28 Djurhuus, R., Analyt. Biochem. *113* (1981) 352.
29 Bradford, M. M., Analyt. Biochem. *72* (1976) 248.
30 Birckbichler, P. J., Orr, G. R., Carter, H. A., and Patterson, M. K. Jr, Biochem. biophys. Res. Commun. *78* (1977) 1.
31 Kannagi, R., Teshigawara, K., Noro, N., and Masuda, T., Biochem. biophys. Res. Commun. *105* (1982) 164.
32 Hand, D., Bungay, P. J., Elliott, B. M., and Griffin, M., Biosci. Rep. *5* (1985) 1079.
33 Kawashima, S., Ihara, Y., and Inomata, M., Biomed. Res. *10* (1989) 17.
34 Goyette, M., Petropoulos, C. J., Shank, P. R., and Fausto, N., Science *219* (1983) 510.

0014-4754/91/010070-06$1.50 + 0.20/0

Colloidal gold as a permanent marker of cells

B. H. J. Juurlink[a] and R. M. Devon

Departments of Anatomy[a] and Oral Biology, University of Saskatchewan, Saskatoon (Saskatchewan, Canada S7N OWO)
Received 28 February 1990; accepted 15 May 1990

Summary. We have demonstrated that colloidal gold-labelled serum proteins are taken up by a number of cells in cultures established from the postnatal rodent neopallium. The colloidal gold enters and remains within secondary lysosomes over extended periods of time and, as well, persists after the subculture of these cells. The cell types that readily take up the label in our culture system are type-1 astrocytes, glial precursor cells and macrophages, whereas, only a small number of oligodendrocytes take up the label. The use of serum proteins to introduce colloidal gold into cells therefore seems to be a convenient and easy way to permanently mark cells.
Key words. Astrocytes; colloidal gold; culture; label; lysosomes.

In certain experimental situations it is often desirable to be able to recognize a particular cell or its progeny and then follow its differentiation and development to its final location in the body. One way of doing this is to take advantage of naturally occurring differences between intracellular components such as difference in nuclear size and then follow the fate of these 'labelled' cells in chimeric embryos. This technique has been performed for several decades [1-3]. A recent twist to the use of stable tracers in the study of the development of the nervous system is to generate one's own label by introducing retroviruses into single cells and detecting such cells cytochemically [4]; the major drawback to this latter technique is that one has very little control over which particular cell will take up the virus.

Other labelling techniques have also been employed to tag cells to follow their developmental fate. More commonly, these cells are marked with labels that are diluted as the cells proliferate. The first of such labels used were low signal dyes such as Nile blue sulfate that were taken up by the cells of interest [5]. More recently enzymes or enzyme-linked conjugates which then can be detected cytochemically [6] have been used, as have chemicals with stronger signals such as a variety of fluorescent dyes [7]. One setback in using such markers is that they can be degraded and thus have a limited life within cells. More permanent markers are obtained by incorporating tritiated thymidine [8] or bromodeoxyuridine [9] into cells during the S phase of the cell cycle. The marked cells are identified through the use of autoradiography and immunocytochemistry respectively. The use of these labels, however, requires that the cell population of interest be a proliferative one at the time of labelling.

Other marking methods exist that do not require the cells of interest to be proliferative but only requires that they be capable of phagocytosis. An example of this method is the uptake of polystyrene microspheres into the lysosomes of cultured astrocytes as demonstrated by Emmet and co-workers [10].

We have recently demonstrated that astrocytes in culture readily phagocytose colloidal gold-labelled serum proteins [11] and that the gold ultimately is sequestered within lysosomes. Therefore, it seemed to us that such an accumulation of gold within cells would also serve as a marker of such cells. The objective of this study was firstly to determine whether such a colloidal gold-labelled cell is 'permanently' marked and secondly to determine whether other cell types could be labelled in this fashion.

Materials and methods

Culture preparation and treatment. Cultures consisting mainly of astrocytes were prepared from newborn Swiss mouse neopallium [12] whereas cultures consisting mainly of oligodendrocytes and oligodendrocyte precursor cells were prepared from two-week-old Swiss mouse neopallium [13]. The growth medium consisted of a modified Eagle's Minimum Essential Medium containing 5% (v/v) horse serum [12]. After two weeks of culture the cells were fed with a medium containing colloidal gold-labelled horse serum proteins for a period of four hours. The