shown) to be unaffected by PGE_1 as well. The possibility that PGE_1 might affect the activity of acetyl-CoA carboxylase in vivo cannot, however, be completely excluded since: 1. the effect of PGE_1 on the activity of this enzyme might disappear in the course of the in vitro technical procedure of the enzymatic measurement; 2. under the optimal experimental conditions described in the literature for the in vitro measurement of the activity of this enzyme, a possible effect of PGE_1 might be masked.

The increase in 1-^{14}C acetate incorporation into fatty acids of perfused livers might be due to an effect of PGE_1 on the fatty acid synthesis, the esterification, or possibly on both. Its effect upon esterification could, in fact, result in a shift of fatty acids toward esterification which would upset the equilibrium of the fatty acid synthesis itself.

γ-Glutamyl Transpeptidase in Human Nephroblastoma Grown in Nude Mice*

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Summary. A cell line from human nephroblastoma was employed to induce a solid, transplantable tumor in *nu/nu* mice. GGTP was produced by these tumor cells, and its presence in the serum of mice accurately reflected the presence of tumor.

Human nephroblastoma (Wilm's tumor), a renal malignancy found generally in young children, is associated with a variety of distinctive measurable features. Nephroblastoma patients have abnormal mucinous material in their circulation, urine and tumor tissues[2–4]; cytotoxic reactions between their lymphocytes and tumor cells occur[5]; and unusual antigens can be isolated from tumor tissue[6,7]. The significance of these assorted findings could be more quickly appreciated if a suitable in vivo model were available for their systematic evaluation. Such a model may be found in the use of congenitally athymic (*nu/nu*) mice as a host for heterotransplantation of human tumors, a system offering unparalleled experimental access to a number of problems requiring controlled studies of in situ tumor growth[8–10].

The aim of our study was to follow the growth of a transplantable tumor derived from an established human nephroblastoma cell line and to study the fate of the γ-glutamyl transpeptidase-activity (GGTP, E.C.2.3.2.1.) in this tumor when grown both in vitro and in vivo.

Material and methods. A human nephroblastoma cell line (CCL31, TuWi) maintained in monolayer culture was

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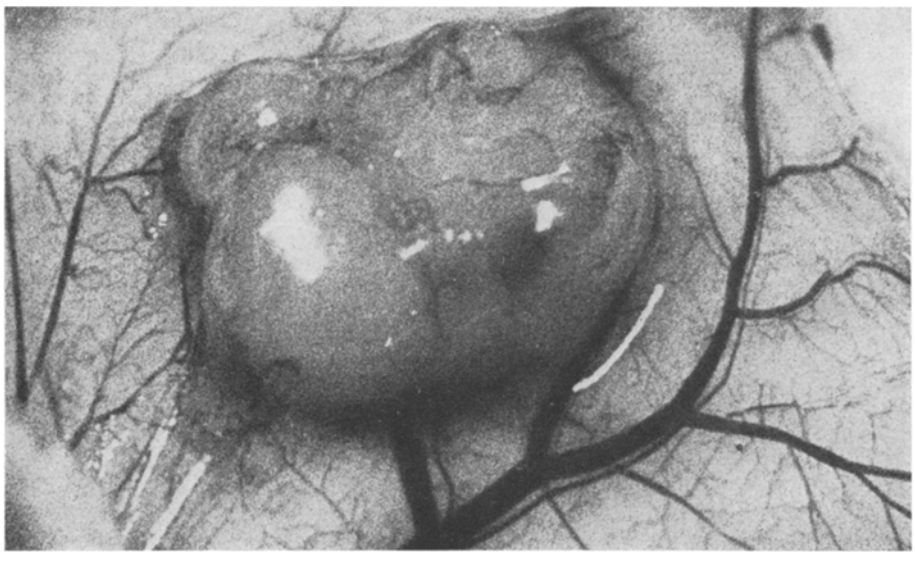

Fig. 1. Solid tumor 4 weeks after subcutaneous inoculation of 5×10^6 trypsinized TuWi cells grown in vitro, showing encapsulation and extensive peripheral blood supply. Tumor diameter approximately 0.7 cm.

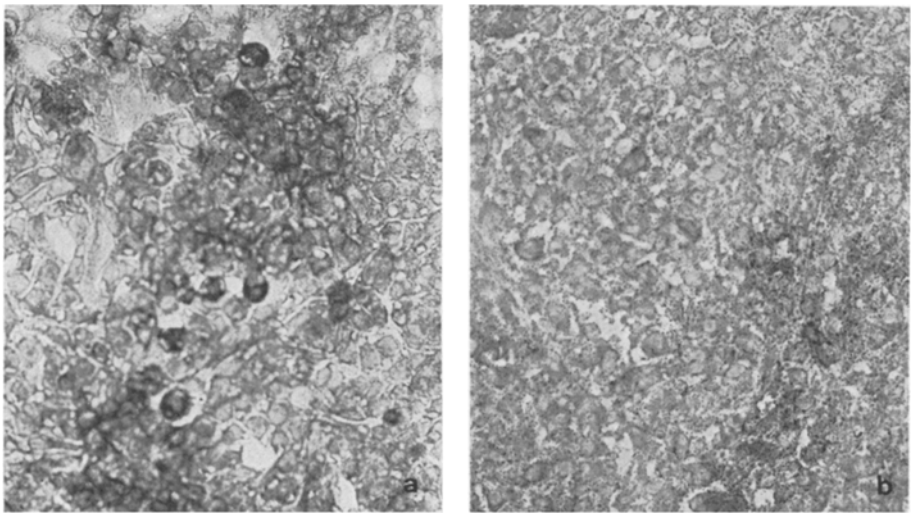

Fig. 2. Distribution of γ-glutamyltranspeptidase as reaction product a) in monolayer culture of human nephroblastoma cell line (CCL31, TuWi) and b) in solid tumor derived from this cell line grown in *nu/nu* mice.

adapted to growth as a solid tumor in vivo by subcutaneous inoculation of trypsinized, washed cells into male mice carrying the *nu/nu* homozygous genotype on a mixed BALB/c and A2G genetic background[11]. 5 serial in vivo passages have been performed to date by transferring 5–10 mm³ of minced tumor tissue, freshly obtained from a previous mouse passage, to a new host by subcutaneous trocar. Per gram tumor tissue to be examined, 2 ml phosphate buffered saline containing 0.02% tetrasodium ethylenediamine were added; the tissue was homogenized in a Potter-Elvehjem apparatus and shaken at room temperature for 1 h. This homogenate was centrifuged 30 min at 6000 × *g*, the supernatant was saved and the pellet was resuspended to the original homogenate volume. GGTP was determined for both of these fractions and for the whole homogenate[12]. The enzyme-activity was also determined in the sera of tumor-bearing mice at different times before and after surgical removal of the tumor. The GGTP isoenzymes were revealed by the cellogel strip electrophoresis technique described by PATEL et al.[13]. The histochemical localization was examined by the method of RUTENBERG et al.[14] using the substrate N-(γ-L-glutamyl)-4-methoxy-2-naphthylamide (Cyclo Chemical Corp., Los Angeles, Calif., USA).

Extraction of GGTP from solid tumor

	GGTP activity[a]	Activity[b] (%)
Tumor homogenate	1100/–	100
Supernatant fraction	720/740	65
Sediment[c]	500/600	50

[a] International units (IU) per liter[12]. Values are given for duplicate determinations from tumor pools of 3 hosts. [b] Based on comparison with whole homogenate. [c] Residual supernatant activity in the unwashed, suspended pellet material probably accounts for the apparent excess recovery of GGTP activity.

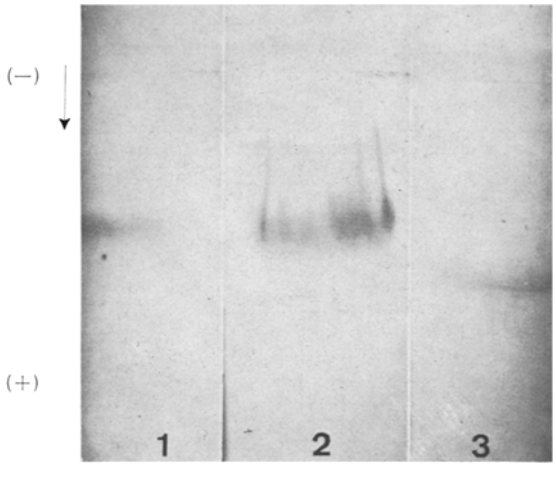

Fig. 3. Electrophoretic separation of γ-glutamyltranspeptidase in tissue extracts of 1. nephroblastoma tumor grown in *nu/nu* mice, 2. fetal human kidney and 3. fetal human liver.

Results and discussion. 5 out of 7 mice receiving the initial inoculum developed tumors by approximately 3 weeks. The remaining 2 failed to grow tumor even after 10 weeks. The resulting subcutaneous tumor (Figure 1) showed multiple encapsulated lobes with varying degrees of central necrosis, and a high degree of peripheral vascularization. Macroscopically, no invasiveness was apparent. This was confirmed by histological examination, which also failed to demonstrate metastasis to the liver, spleen, kidney or draining lymph nodes of tumor-bearing mice. The solid tumor showed a rather uniform picture of undifferentiated cells with frequent mitoses. Elements of connective tissue were found at the periphery of tumor lobes. Karyograms of the original culture and of cultures derived from explants of the solid tumor confirmed that the tumor growing in mice represented the original cell line. Both demonstrated a clearly human chromosome morphology, and a high frequency of cells with chromosome numbers between 60–65, characteristic of the TuWi cell line[15]. Thus the tumor was easily transplantable, and retrievable as an in vitro culture. Surgical removal of tumors was possible without subsequent tumor relapse. Histochemical detection of GGTP-activity revealed remarkably dense deposits of enzyme reaction product on

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monolayer cultures of TuWi cell line, and in sections and imprints of the solid tumor derived from this line. All cultured tumor cells showed a high degree of enzyme activity, with extensive deposits of reaction product occurring in cells with mitosis and in areas of dense cell growth in late cultures (Figure 2a). The solid tumor provided a massive amount of uniform tissue rich in GGTP (Figure 2b) and it was therefore easy to extract from the tumor homogenate sufficient amounts, approximately 65% of total enzyme activity, into a supernatant fraction (Table).

The proposed role of this widely distributed, membrane-associated enzyme as a translocase in amino acid transport[16] and its accumulation in various tissue, notably the kidneys, which are actively involved in this process, undescore its possible significance in nephroblastoma. Fetal and adult kidneys show high concentrations of GGTP in the brush borders of proximal convoluted tubules[14]. The presence of this enzyme in several renal tumors, as reported by ALBERT et al.[17], and shortly thereafter also described in Wilm's tumor (nephroblastoma)[18], has been advanced as evidence for their origin in cells of the proximal tubules.

As GGTP has been shown to express tissue-specific isoenzyme forms[19], and to be present in the serum elevated levels during certain stages of some malignant disorders[20–22], we also made attempts in these two directions. Repeated examinations using cellogel strip electrophoresis technique showed that enzyme extracted from the nephroblastoma had a mobility comparable to that GGTP derived from normal fetal and adult human kidney, but slower than that of the enzyme from fetal liver (Figure 3). As a control, kidney and pancreas tissue extracts from the host of the tumor (the tumorbearing mouse) were used. Here again the tumor tissue extract showed mobility identical with that of the kidney and different from that of the pancreatic enzyme. Adult mouse liver did not show any GGTP activity[14, 23].

Preliminary examinations of GGTP activity in the serum of nephroblastoma-bearing mice showed activity 4 days before surgical removal of the 3-week-old tumor and on the day of removal an average of 15 IU/l. 3 and 7 days after tumor resection either no enzyme activity or traces only could be found in the serum. Since the serum level of GGTP in tumor-bearing mice was quite remarkably elevated over that found in control mice before the tumor implantation, and initial experiments showed that these values returned to normal following surgical resection, this suggested that serum GGTP originated in the tumor. While the tumor is encapsulated and not invasive, its extensive peripheral vascularization may permit ready access of tumor-derived substances into the circulation. It remains unclear whether the enzyme is actively secreted or is released upon necrotic destruction of tumor tissue.

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Sulfur Dioxide as a Sulfur Source in Duckweeds (*Lemna minor* L.)[1]

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Summary. Isotope competition experiments with *Lemna minor* L. indicate that SO_2-sulfur enters the sulfur amino acids of the proteins and the sulfoquinovose of the sulfolipids following oxidation to SO_4^{2-} and subsequent reduction.

Plants take up a great amount of sulfur dioxide, an atmospheric pollutant which is mainly produced by burning of fossil fuels[3]. Thus, plants can play a significant role in reduction of the concentration of a toxic gas; at the same time they can satisfy at least part of their sulfur requirements, unless the concentration of SO_2 is so high that they are damaged or killed[4]. In plants, the absorbed SO_2 occurs predominantly in the form of SO_3^{2-}[5]. In vitro studies with plant extracts have demonstrated that SO_3^{2-} can either be oxidized to SO_4^{2-}[5,6] or reduced to H_2S[7–9]. If, in vivo, the absorbed SO_2 is oxidized, the sulfur is metabolized as sulfate, the normal sulfur source of plants[10]. If the SO_2 is reduced, the sulfide could be used directly for formation of cysteine[11] in a reaction which is quantitatively the most important step for sulfur incorporation into organic compounds[10]. Another important reaction, in which SO_3^{2-} could be used without prior reduction or oxidation, is the formation of 6-sulfoquinovose found in the sulfolipids of plants[12].

The results reported here show to what extent the systems for reduction and oxidation of SO_3^{2-}, which have been demonstrated in vitro, may be significant in vivo in the duckweed *Lemna minor* L. and how far SO_2 is used directly for the formation of sulfolipids.

In the Figure results of an isotope competition experiment are presented in which duckweed was cultivated in an atmosphere with 0.3 ppm SO_2 on a nutrient solution containing $^{35}SO_4^{2-}$. The specific activities of the sulfur in the sulfate, the proteins, and the sulfolipids, reached

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