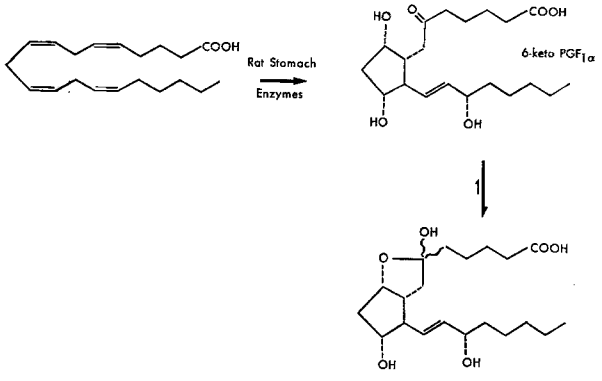
20 m*M* EDTA. The fundic portion (3.9 g) was dissected and homogenized in 20 volumes buffer (Polytron, top speed, 10 sec, 0°). A small portion of the homogenate (5 ml) was incubated with tracer arachidonic acid ($^3H_8$-20:4, 4×10^6 dpm, S.A. 93 Ci/mmole, New England Nuclear); the rest of the homogenate (73 ml) was incubated with unlabelled 20:4 (300 µg/g, Mann 99%). Incubation (20 min, 37°) was terminated by the addition of diethyl ether (2 vol), water (1 vol) and the mixture was rapidly acidified to pH 3 with *N* HCl. The ether phase was separated, washed to neutrality with water and evaporated in vacuo to complete dryness. The residue was transferred to a thin layer plate of silica gel G and after development in chloroform/methanol/acetic acid/water (90:9:1:0.65 v/v) the zone on the plate corresponding to PGE_2 was removed, washed with methanol, and the methanol eluate was taken to dryness. The residue, containing some PGE_2 formed during incubation, was dissolved in methanol (2 ml) and treated at 0° with sodium borohydride (15 mg). After stirring for 30 min at room temperature, the mixture was acidified to pH 3, and extracted with diethyl ether. The ether extract was washed to neutrality with water and taken to dryness. In other experiments PGE_2 was resolved from the mixture by conversion into PGB_2 after treatment with a solution of methanol: N KOH (1:1 v/v). After reaction the residue was purified again on thin layer plates of silica gel G (same developing solvent) and the zone on the plate corresponding to PGE_2 was again removed and eluted as described above. The eluate was taken to dryness and converted to the methyl ester derivative with a freshly prepared solution of ethereal diazomethane: methanol (9:1 v/v). Aliquots were converted to the methoxime and benzyloxime derivatives and analyzed by mass spectrometry after conversion of the hydroxyl groups to the corresponding trimethyl silyl ether (TMS) derivatives.



Structure proposed for novel metabolite of arachidonic acid formep by the rat stomach.

Results. Arachidonic acid is converted in good yield (> 60% of tracer 20:4) into a mixture of compounds with chromatographic properties similar to PGE_2. The major portion of compounds in the mixture are unreactive towards alkali and sodium borohydride, both of which convert PGE_2 into other products. Thus, PGE_2 in the mixture is easily removed through reaction with these reagents. That the residual compounds contained a keto group was determined by reaction of the methyl ester derivative with methoxylamine hydrochloride in pyridine. The resulting product was converted to the TMS derivative with Tri Sil Z (Pierce Chemical Co) and analyzed by combined gas chromatography-mass spectrometry. Only 1 major peak was observed (carbon value 25.2, 3% SE-30, Gas Chrom Q, T: 250°) with intense fragment ions at *m/e* 629 (M^+), 614 ($M-CH_3$), 598 ($M-OCH_3$), 558 ($M-C_5H_{11}$), 539 (M—TMSOH), 508 (M—(TMSOH + OCH_3)), 468 (M—(+C_5H_{11} + TMSOH)), 449 (M—(2×TMSOH)), 378 (M—(C_5H_{11} + (2×TMSOH)), 217 ($TMS\overset{+}{O}=C-CH=CH-OTMS$), 173 ($TMS\overset{+}{O}=CH-C_5H_{11}$) and 115 ($C_4H_8COOCH_3$, base peak). Further confirmation of the identity of the compound was obtained through analysis of the mass spectrum of the methyl ester benzyloxime-TMS derivative. This derivative showed 2 isomers on gas chromatography due to the bulky benzyloxime grouping (29.5, 29.9 carbon values) and showed characteristic shifts in the spectral fragmentation pattern when compared with the methoxime derivative. The base peak (1st isomer) was also found at *m/e* 115 corresponding to the C_1-C_5 fragment.

These findings are in agreement with the structure proposed in the Figure. The isolated compound appears to exist mainly in the cyclic form, a lactol, based on the following evidence:1. lack of reaction with sodium borohydride in methanol; 2. lack of reaction with *n*-butylboronic acid in dimethoxy-propane except after prior reaction with methoxylamine hydrochloride in pyridine (C. PACE-ASCIAK, to be published). In this case an MO-NBB derivative is formed. 3. Positive reaction with methoxylamine hydrochloride and benzhydroxylamine hydrochloride in pyridine. Further studies are in progress to determine the mechanism of formation of this compound.

Effect of PGE_1 on Lipogenesis in Perfused Rat Liver

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29 September 1975.

Summary. In perfused livers of 24 hour-fasted rats, PGE_1 (prostaglandin E_1) infused continuously into the perfusate, was found to cause a 45% increase in the incorporation of 1-^{14}C acetate into liver fatty acids. PGE_1 was found to have no effect, however, on the activity of the key lipogenic enzymes.

Although several studies of the effect of PGE_1 on lipogenesis in rat adipose tissue have appeared[1–4], only one has been published concerning this effect in rat liver[5].

In a study on glycerol esterification[6], it was shown that PGE_1 caused a significant increase in the esterification of 1-^{14}C glycerol into hepatic glycerides of perfused liver obtained from rats fasted for 48 h. It might therefore be expected that PGE_1 would cause an increase in fatty acid synthesis in perfused rat liver. This was in fact observed by CALANDRA and MONTAGUTI[5] in liver slices, using low concentration of PGE_1.

In the present study, perfused rat liver was used to determine the effect of PGE_1 on the ^{14}C acetate incor-

Table I. Incorporation of 1-^{14}C acetate into liver fatty acids

	Total radioactivity (μCi in total fatty acids of the liver)	Specific radioactivity (μCi/mg of fatty acids)	Weight of fatty acids in total liver (mg)
Livers + PGE_1 (I)	$2.17 \cdot 10^{-2} \pm 0.19 \cdot 10^{-2}$ $n = 5$	$0.86 \cdot 10^{-4} \pm 0.08 \cdot 10^{-4}$ $n = 5$	256 ± 14 $n = 5$
Controls (II)	$1.48 \cdot 10^{-2} \pm 0.08 \cdot 10^{-2}$ $n = 5$	$0.59 \cdot 10^{-4} \pm 0.04 \cdot 10^{-4}$ $n = 5$	253 ± 22 $n = 5$
I/II	1.47	1.46	0.99
p	$0.01 < p < 0.02$	$0.025 < p < 0.05$	n.s.

Values are means ± SEM. PGE_1 was infused at a concentration of 0.5 μg/min during 5 min and 40 sec. The incorporation time of 1-^{14}C acetate was 30 sec.

Table II. Effect of PGE_1 on activities of acetyl-CoA carboxylase and fatty acid synthetase

	Acetyl-CoA carboxylase in supernatant	Purified acetyl-CoA carboxylase	Purified fatty acid synthetase
Assays + PGE_1	$2.36 \cdot 10^{-3} \pm 0.107 \cdot 10^{-3}$ $n = 5$	$4.10 \cdot 10^{-2}$	0.353
Controls	$2.38 \cdot 10^{-3} \pm 0.110 \cdot 10^{-3}$ $n = 5$	$3.56 \cdot 10^{-2}$	0.329

PGE_1 was added at a concentration of 0.1 μg/ml of assay. Activity of acetyl-CoA carboxylase is expressed as: μmoles $NaH^{14}CO_3$ incorporated/min/mg protein. Acetyl-CoA carboxylase was purified until the DEAE-column step. Activity of fatty acid synthetase is expressed as: μmoles NADPH oxidized/min/mg protein. Fatty acid synthetase was entirely purified. Assays with purified enzymes were performed in a simple experiment.

poration into fatty acids. The effect of PGE_1 on activity of acetyl-CoA carboxylase and of fatty acid synthetase were also determined.

Methods and results. Except for experiments with purified enzymes, livers of adult female white rats fasted for 24 h were used for the experiments. This fasting period was chosen in order to amplify the possible differences between the control livers and those treated with PGE_1, and to approach, as closely as possible, the experimental conditions under which PGE_1 was observed to cause an increase in glycerol esterification into liver glycerides[6].

First set of experiments: Livers were perfused by the isolated in situ technique described by HEMS et al.[7]. PGE_1, at a concentration of 0.5 μg/min was introduced as previously described[6] by continuous infusion until the end of the experiment. PGE_1 was omitted in the control experiments. 5 min after the PGE_1 infusion was begun, 1-^{14}C acetate (10 μCi = 0.17 μmoles) was introduced at approximately the same point as had been the PGE_1, by means of an injection which lasted 10 sec. 30 sec after this injection, the perfusion was stopped and the livers were removed, weighed and homogenized. The total radioactivity of the liver tissue was measured by counting an aliquot of the homogenate which had been dissolved into soluene. The rest of the homogenate was saponified and the fatty acids extracted and counted.

PGE_1 was found to cause an increase in the incorporation of 1-^{14}C acetate into fatty acids as shown by the increase in both total and specific radioactivity (Table I). The magnitude of the increase was approximately 45%. The maximum discrepancy in the radioactivity of the liver tissue was found to be about 14%.

Second set of experiments: The enzymatic activity of acetyl-CoA carboxylase in the supernatant of the centrifuged homogenate was assayed according to the following method[8]. Added, in vitro, to half of the assays, PGE_1, at the concentration used, was found to have no effect on this activity (Table II). The activities of purified acetyl-CoA carboxylase[9] and fatty acid synthetase[10,11] were also unaffected by PGE_1, at the concentration used (Table II).

Discussion. Particle-free supernatants were used in the assays of enzymatic activities in an attempt to determine whether or not PGE_1 had a direct effect on enzyme activity in preparations containing no cellular membrane. Not surprisingly, it was found to have no effect at all.

PGE_1 was then infused continuously into the perfusate for 10 and for 45 min in order to determine whether it could affect acetyl-CoA carboxylase activity under these experimental conditions. This was found (results not

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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

* Dedicated to Prof. Dr. G. TÖNDURY, Zürich, on the occasion of his 70th birthday.

[1] We are grateful to Professor W. SCHMID for preparation of karyograms, Dr. S. KUMAR for providing the TuWi cell line, Dr. S. IVANIC for performing the electrophoresis, Mrs. M. ACKLIN for technical assistance and Miss R. LEEMANN for preparation of this manuscript.

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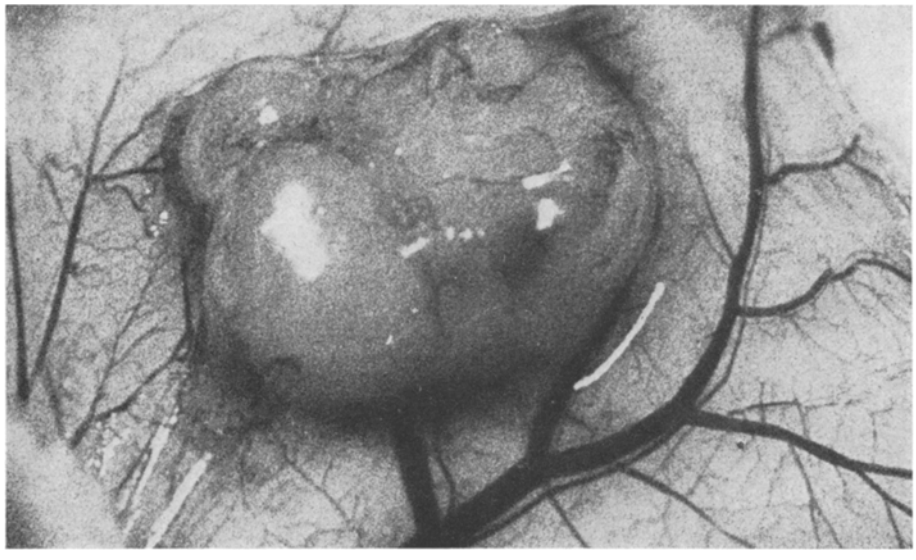

Fig. 1. Solid tumor 4 weeks after subcutaneous inoculation of 5×10^6 trypsinized TuWi cells grown in vitro, showing encapsulation and extensive peripheral blood supply. Tumor diameter approximately 0.7 cm.