approximately 9.3% of the methadone was metabolized in 24 h to at least 2 compounds. These results suggest that HTC cells, even though transformed, have retained the enzymes required to metabolize opiates.

The results obtained with the lymphoma cells are shown in figure 2. As with the HTC cells the chromatograms indicate that a substantial amount of the radioactivity does not have the same R_f as methadone. When the amount of metabolism was estimated as described above, it was found that 26% of the total radioactivity migrated in the acidic solvent system after a 29-h incubation.

To examine further the radioactive material produced by the lymphoma cells, a quantity of the medium extract was chromatographed with the basic solvent system. The chromatogram (similar to figure 2H) was bisected at R_f 0.4, the material from each piece eluted with CM, and rechromatographed using the neutral solvent system. The material which migrated in the basic solvent system with an R_f of less than 0.4 was labeled Peak A and the material with an R_f greater than 0.4 was labeled Peak B. The results obtained after chromatography with the neutral solvent system are shown in figure 3. Peaks A and B were both found to be composed of at least 2 radioactitve substances. Unaltered methadone was found in Peak B and had an R_f in the neutral solvent system of 0.1. The results in figure 3 therefore indicate that lymphoma cells produce at least 3 radioactive compounds from [1-^{3}H] methadone.

Both HTC and lymphoma cells appear to produce 2-ethyl-5-methyl-3,3-diphenyl-1 pyrroline as shown by chromatographic mobility[18]. However, the metabolites could not be definitely identified due to the minute quantities produced. After metabolism, the radioactivity is probably still associated with the bulk of the methadone molecule because tritium present in a methyl group does not readily exchange and because most metabolites still possess the number one methyl group[3–10, 21, 22].

It was not the objective of this study to examine the rate of methadone metabolism by cultured cells, and the estimates of the extent of metabolism given above can only be considered first approximations. It is likely that these figures are too low as some metabolites may not migrate in the acidic solvent system. However, the rates obtained (9.3% per day by HTC cells and 22% per day by lymphoma cells) are similar to the 20–30% per day estimated in vivo with non tolerant rats and men[4, 23]. Cultured cells may therefore serve as useful models for studying the metabolism of opiates by specific cell types.

15 W. D. Noteboom and G. C. Mueller, Molec. Pharmac. *5*, 38 (1969).
16 H. M. North and D. W. Martin, Proc. West. Pharmac. Soc. *15*, 87 (1972).
17 S. H. Madin and N. B. Darby, Proc. Soc. exp. Biol. Med. *98*, 574 (1958).
18 A. L. Misra, N. L. Vadlamani and S. J. Mule, J. Chromat. *67*, 379 (1972).
19 K. Randerath, Thin Layer Chromatography, p. 177. Academic Press, New York 1968.
20 G. W. Carter and K. Van Dyke, Clin. Chem. *17*, 576 (1971).
21 W. J. George and T. R. Tephly, Molec. Pharmac. *4*, 502 (1968).
22 A. Pohland, H. E. Boaz and H. R. Sullivan, J. med. Chem. *14*, 194 (1971).
23 R. C. Baselt and L. J. Casarett, Clin. Pharmac. Ther. *13*, 64 (1972).

Suramin stimulates renal growth in the rat[1]

L. Selmeci, K. Szepesházi, E. Pósch and L. Hársing[2]

Institute of Pathophysiology and 1st Institute of Pathology, Semmelweis University Medical School, Budapest (Hungary), 11 July 1977

Summary. Suramin given to rats in a single high dose (20 mg/100 g i.v.) stimulated renal growth. Maximum changes are taking place after 6–9 days of treatment.

A single high dose of folic acid has been shown to induce a marked increase in kidney weight, DNA- and RNA-synthesis, followed by an intensive mitosis rate throughout the kidney[3–5], This phenomenon has been called 'chemically-induced' hypertrophy and used as a model for studying induced cell proliferation. Here we present evidence of induced renal growth in the rat by a single i.v. dose of suramin (sodium salt of 8-[3-benzamido-4-methylbenzamido]-naphtalene-1, 3, 5,-trisulphonic acid, mol.wt 1492). Suramin is a trypanocidal drug used in the human therapy and prophylaxis of sleeping sickness. Following its i.v. administration, a high concentration is achieved in the plasma. This falls fairly rapidly for a few h, then more slowly for a few days, after which a low concentration is maintained for as long as 3 months. The persistence of suramin in the circulation is due to its firm binding to plasma proteins. The compound apparently does not enter cells readily, since none is present in erythrocytes, and tissue concentrations are uniformly lower than those in the plasma. It is of particular interest that, in experimental animals, the kidneys have been found to contain considerably more suramin than other organs[6].

Materials and methods. Suramin (Bayer 205) was dissolved in physiological saline in a concentration of 10% (w/v) and injected i.v. in a single dose (20 mg/100 g) to male albino rats of inbred strain (CFY) weighing 130–160 g. At various days after treatment, rats were anesthetized with ether at the same period of the day (between 9 and 10 a.m.), their kidneys removed, stripped of their capsules, blotted on filter paper weighed and immediately processed for chemical determinations (left kidney) or put in an oven at 105 °C (right kidney) for 48 h and then

1 Suramin (Bayer 205) was generously provided by Bayer (Leverkusen, Federal Republic of Germany).
2 The authors thank Miss J. Telegdy and Mrs I. Harmos for technical assistance and Mrs M. Herczeg for the preparation of the sections.
3 D. M. Taylor, G. Threlfall and A. T. Buck, Nature *212*, 472 (1966).
4 G. Threlfall, D. M. Taylor and A. T. Buck, Am. J. Path. *50*, 1 (1967).
5 R. Baserga, D. Thatcher and D. Marzi, Lab. Invest. *19*, 92 (1968).
6 J. M. Rollo, in: The Pharmacological Basis of Therapeutics, p. 1081. Ed. L. S. Goodman and A. Gilman. Macmillan, New York 1975.

Table 1. Changes in rat kidney weights following a single i.v. dose (20 mg/100 g) of suramin

Days after treatment	Wet kidney weight (mg/100 g b.wt) Control	Suramin	Dry kidney weight (mg/100 g b.wt) Control	Suramin	Percentage dry weight Control	Suramin
1	404.2 ± 29.9 (8)	428.4 ± 51.0 (8)	93.0 ± 6.7	99.6 ± 7.1	23.0 ± 0.94	23.4 ± 2.2
2	–	427.7 ± 23.6 (8)	–	97.7 ± 5.1	–	22.9 ± 0.54
3	424.4 ± 21.2 (8)	466.7 ± 34.7* (8)	101.5 ± 4.6	107.4 ± 7.7	23.9 ± 0.64	23.0 ± 0.50
6	408.0 ± 25.4 (8)	542.1 ± 66.6** (8)	94.4 ± 6.7	112.1 ± 7.1**	23.1 ± 0.62	20.8 ± 1.35
9	386.2 ± 31.0 (8)	544.1 ± 45.6** (7)	92.4 ± 9.2	117.0 ± 3.8**	24.0 ± 0.51	21.5 ± 0.92

*Significantly different from control $p < 0.02$; **$p < 0.001$. Student's t-test was used for calculating statistical significance. In parentheses No. of rats. Values represent means ± SD

Table 2. Number of mitoses in rat kidney after a single i.v. dose (20 mg/100 g) of suramin

Days after treatment	Cortex Number of mitotic figures per 10^4 cells Control	Suramin	Medulla Number of mitotic figures per 10^4 cells Control	Suramin
3	4.5 ± 3.2 (6)	3.3 ± 3.5 (6)	1.8 ± 1.3	4.5 ± 3.1
6	4.5 ± 3.7 (6)	14.3 ± 6.1* (6)	2.2 ± 2.4	6.0 ± 4.5
9	6.8 ± 4.5 (6)	19.5 ± 7.5** (4)	6.0 ± 2.5	5.7 ± 4.3
12	5.3 ± 3.4 (5)	3.3 ± 3.0 (3)	5.0 ± 1.2	6.3 ± 4.9

*Statistically different from controls $p < 0.01$; **$p < 0.001$. Student's t-test was used for calculating statistical significance. In parentheses No. of rats. Values represent means ± SD.

reweighed. Data are compared with those of saline-treated controls killed on the same day, except on the 2nd day when no control group was killed and the values of suramin-treated rats were compared with controls killed on the 1st day.

For histological study the kidney was cut into halves lengthwise across the papilla and fixed in formalin (10% w/v) neutralized with calcium carbonate, embedded in paraffin, sectioned at a thickness of 2 μm. Sections were stained either with hematoxilyn and eosin or PAS. Histological slides were carefully examined by light microscopy, and mitotic figures counted both in cortex and medulla. Renal RNA[7] and DNA[8] contents were determined after 7 days of treatment.

Results. Suramin given in a single i.v. dose considerably increased both wet and dry kidney weights. Highest values were reached on 6 and 9 days. The percentage dry weight values remained fairly constant (table 1). At 6 and 9 days of treatment, mitotic figure exhibited a sharp increase in the cortex but not in the medulla, and by 12 days it declined to normal (table 2). At 7 days, both renal RNA and DNA contents (mg per kidney/100 g b.wt) were significantly elevated compared to values of saline-treated control. $RNA_{control}$: 1.780 ± 0.234, $RNA_{suramin}$: 2.866 ± 0.497 ($p < 0.001$), $DNA_{control}$: 1.609 ± 0.183, $DNA_{suramin}$ 2.461 ± 0.203 ($p < 0.001$).

Discussion. Suramin has been demonstrated to inhibit some enzymes such as urease, hexokinase, succinic oxydase[9], lysozyme[10], acid phosphatase, beta-glucuronidase[11], lysosomal proteolytic activity of rat liver[12], the complement systems[13], C_1 esterase, thrombin, plasma kallikrein[14] and membrane ATPase[15].

Suramin also inhibits the fusion of phagosomes and lysosomes of mouse peritoneal macrophages[16]. However, it stimulates B-lymphocyte proliferation in the mouse in vitro[17]. The results of this study appear to indicate that a single high dose of suramin can induce renal growth in the rat, and maximum changes are taking place at 6–9 days of treatment. This time course seems to be different from that described after folate administration when maximum values are reached at 4 days[3]. However, changes induced by suramin in kidney are less pronouced than after folic acid but are still considerable. The constancy of percentage dry weight values observed in this study indicate that edema does not essentially contribute to the weight increase produced by suramin. We also failed to detect any precipitated substances in the kidney tubules which are thought to be responsible for folic acid-induced renal hypertrophy via a partial tubular blockage[18]. Histological findings suggest that regenerative processes are involved in suramin-induced renal hypertrophy which predominantly localized in the proximal tubules, whereas no changes were seen in the glomeruli or medulla. The model described in this paper seems to be a simple one, easily reproducible, that might be suitable for studying induced cell proliferation in the kidney.

7 A. Fleck and H. N. Munro, Biochim. biophys. Acta *55*, 571 (1962).
8 W. C. Schneider, Meth. Enzym. *3*, 680 (1957).
9 E. D. Wills and A. Wormall, Biochem. J. *47*, 158 (1950).
10 I. Lominski and S. Gray, Nature *192*, 683 (1961).
11 G. Weissman, P. Davies and K. Krakauer, Fed. Proc. *28*, 265 (1969).
12 M. Davies, J. B. Lloyd and F. Beck, Biochem. J. *115*, 54P (1969).
13 J. S. C. Fong and R. A. Good, Clin. exp. Immun. *10*, 127 (1972).
14 V. Eisen and C. Loveday, Br. J. Pharmac. *49*, 678 (1973).
15 P. A. G. Fortes, J. C. Ellory and V. L. Lew, Biochim. biophys. Acta *31*, *262* (1973).
16 P. Hart, D'Arcy and M. R. Young, Nature *256*, 47 (1975).
17 T. L. Vischer, Agents and Actions *6*, 479 (1976).
18 D. M. Taylor, G. Threlfall and A. T. Buck, Biochem. Pharmac. *17*, 1567 (1968).