Results from urinary balance studies are shown in the figure. The table shows the results of blood determinations and RRC. PRA and RRC decrease progressively and plasma creatinine is modified according to the water retention.

From these data an 'escape' phenomenon from the salt and water retention induced by P can be deduced. The reabsorption ability seems to be limited by the level of extracellular volume expansion. When a certain degree is exceeded, the kidney discharges partially the overload, perhaps through an increased GFR[4].

Neither PRA nor RRC seem to be involved in these mechanisms because their values decrease continuously without relation to the state of extracellular volume expansion or water excretion.

Propranolol as blocker of β-adrenergic receptors in the blood vessels can modify the size of their lumen and alter the dynamics of glomerular ultrafiltration, lowering the renal plasma flow without changing the glomerular filtration rate through a rise in the filtration fraction. This situation would involve an increase in proximal reabsorption because of the rise in peritubular oncotic pressure. Although this hypothesis is compatible with the observed changes in sodium and water excretion, direct measurement by micropuncture or other technique seems necessary for verification of this hypothesis.

Changes in half life of propranolol chronically administered[8] could explain the attenuation in the intensity of the 'escape' mechanism. The 'escape' itself could be mediated by the different action of P in blood vessels receptors at different doses[9].

Values of plasma creatinine, plasma renin activity (PRA) and renal renin content (RRC)

Day	ARP (ng AI/ml h)	RRC (μg AI/ml h g.k.w.)*	Creatinine (mg/100 ml)
0	118 $\pm$ 23	830 $\pm$ 50	0.63 $\pm$ 0.01
4	59 $\pm$ 4	680 $\pm$ 21	0.55 $\pm$ 0.02
	$p < 0.05$	$p < 0.01$	$p < 0.005$
7	57 $\pm$ 4	626 $\pm$ 42	0.62 $\pm$ 0.01
	$p < 0.05$	$p < 0.01$	NS
10	54 $\pm$ 2		0.50 $\pm$ 05
	$p < 0.05$		$p < 0.0005$
13	52 $\pm$ 2		0.61 $\pm$ 0.001
	$p < 0.01$		$p < 0.05$

Mean $\pm$ SEM are represented. * g.k.w. = gram of fresh kidney weight.

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The metabolism of methadone by cultured mammalian cells[1]

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Summary. Rat hepatoma tissue culture cells and mouse leukemic cells were found to metabolize [1-^{3}H] methadone to at least 2 unidentified radioactive compounds. These results suggest that cultured cells may be useful models for studying methadone metabolism by specific cell types.

Studies of drug metabolism generally have been performed in vivo although experiments in vitro have been used to determine metabolic contributions by specific tissues. Unfortunately most tissues are made up of many cell types and the contribution of each to drug metabolism may be difficult to evaluate. This question may be answered by employing cultured cells where the cultures can be cloned, if necessary, to achieve a homogeneous cell population.

It was the purpose of this study to determine if 2 common cell lines metabolize methadone. Methadone was used because the metabolism has been thoroughly characterized in vivo[3-7] and in vitro[8-10]. Rat hepatoma tissue culture cells (HTC), derived from a minimal deviation hepatoma[11], were used for the initial experiments since methadone is metabolized by the liver[5,10]. The L5178Y mouse leukemic cells (lymphoma) were used as a contrasting cell line since other types of cells may metabolize opiates[12].

Materials and methods. The HTC cells were cultured in monolayer at 37 °C in Swims S-77 medium (Grand Island Biological Co.) containing 10% (v/v) fetal calf serum and 5% calf serum[11]. The lymphoma cells were cultured in suspension at 37 °C using Fischer's medium with 10% (v/v) horse serum[13]. The cells were cultured using established techniques with periodic examination to assure freedom from contamination by bacteria, fungi, or mycoplasma[14]. Solutions of l-(–)-[^{3}H]methadone hydrobromide (New England Nuclear Corporation, 92.3 Ci/mole^{-1}) and dl-methadone hydrochloride (S.B. Penick Co.)were sterilized by filtration through 0.22 μm filters (Millipore Corp.) and were added to logarithmic cultures at the start of the incubation period. Concentrations

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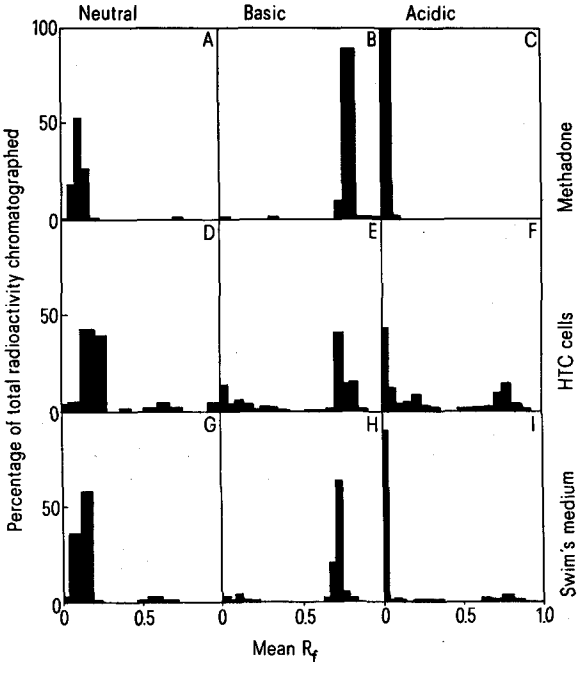

Fig. 1. TLC of Swim s medium and HTC cell extracts. The chromatograms depicted in A, D and G were developed with the neutral solvent system, those in B, E and H with the basic solvent system, and C, F and I using the acidic solvent system. Authentic [1-^{3}H] methadone, the HTC cell extract, and the Swim's medium extract were chromatographed on A, B and C; D, E and F; and G, H and I respectively.

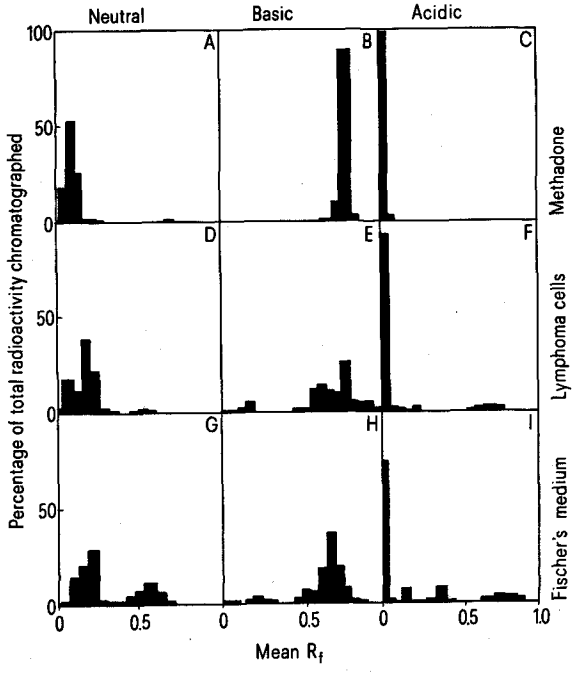

Fig. 2. TLC of Fischer's medium and lymphoma cell extracts. The chromatograms showns in A, D and G were developed with the neutral solvent system; those in B, E and H with the basic solvent system, and C, F and I using the acidic solvent system. Authentic [1-^{3}H] methadone, the lymphoma cell extract and the Fischer's medium extract were chromatographed on A, B and C; D, E and F; and G, H and I respectively.

were chosen to avoid cytotoxicity[15,16]. Approximately 1×10^8 HTC cells were incubated with 4 μM methadone for 24 h and about 3×10^8 lymphoma cells were incubated with 6.6 μM methadone for 29 h. At the end of the incubation period, the HTC cells were detached[17] and collected by centrifugation. The lymphoma cells were harvested by centrifugation and washed once with 10 ml physiological saline. The washed cels were lysed with 30 ml of distilled water. The media samples mixed with the wash solutions were lyophilized and the resulting material was suspended in 100 ml of 10 mM HCl. The cell lysates and the media solutions were adjusted to pH 10 with Na_2CO_3 and extracted with 10% methanol in chloroform (CM). The aqueous phases, which contained less than 6% of the total radioactivity, were discarded and the organic phases were filtered through Whatman No. 1, flash-evaporated and suspended in a small volume of CM. The CM extracts were analyzed by TLC using silica gel strips (Eastman Kodak Co., number 6061) with the following solvent systems: *neutral*, t-amyl alcohol-n-butyl ether-water (14:7:1)[18]; *basic*, ethyl acetate-n-butanol-ethanol-concentrated ammonia (70:10:15:1); and *acidic*, petroleum ether-diethyl ether-glacial acetic acid (90:10:1)[19]. After development the strips were cut into small segments and the radioactivity was measured with a scintillation spectrometer (Packard Instrument Co., Model 3310) using a toluene-based counting solution[20]. The chromatography of [1-^{3}H]methadone was performed in the same manner as with the extracts. No significant effect on the mobility of methadone in any of the solvent systems was observed when the drug was spotted with either purified lipids or an extract of untreated cells. The chromatographic behavior of methadone was also unaffected by pretreatment with culture medium.

Results and discussion. Figure 1 illustrates the results obtained with the HTC cells. It can be seen by comparing the chromatograms of the cell and media extracts with those of methadone that a substantial amount of the radioactivity does not migrate the same as authentic methadone. The percentage of methadone metabolized was estimated from the total amount of radioactivity present at greater than R_f 0.1 in figure 1I. This estimation is based upon the observation in figure 1C that methadone does not migrate in this system. It was found that

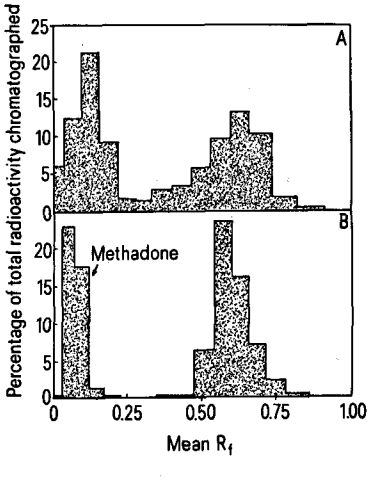

Fig. 3. Neutral TLC of Fischer's medium extracts previously chromatographed with the basic solvent system. Peak A from figure 2H ($R_f < 0.4$) contained ~ 1200 dpm and Peak B ($R_f > 0.4$) contained ~ 14,000 dpm.

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.

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Suramin stimulates renal growth in the rat[1]

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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

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