inoculated each with 0.02 ml of the test materials before incubation. Duplicate groups were tested for each sample material. Control groups were inoculated with sterilized potato dextrose broth, chloroform extract of potato dextrose broth, or water and chloroform extract of uninoculated potato slices. Analysis of variance was made and means were compared according to Tukey's ω-procedure[9]. Honest significant difference (HSD) was used to judge the significance between control and treated.

Results and discussion. Table 1 shows that none of the extracts and culture filtrates from growth of 9 races of *Phytophthora infestans* on living potato tissue and potato dextrose broth had any toxicity to check embryos. This was shown by the nonsignificant difference in hatchability between control and extract treated fertile eggs. Four out of the 10 isolates of *Alternaria solani* showed some toxicity to chick embryos (table 2). These included isolate A-1, C-3, Mi-6 and Ma-9. Among these, chloroform extract of A-1 grown on potato dextrose broth was the most toxic. It is interesting to know that only chloroform extracts from *Alternaria solani* isolates grown on potato dextrose broth showed some toxicity. Culture filtrates of potato dextrose broth and chloroform and water extracts of *Alternaria solani* infected living potato tissue did not have toxicity to chick embryos. Surface sterilized potato tissue supported luxury growth of *Alternaria solani*. Apparently, some inhibitor(s) in the living potato tissue inhibited the formation of toxin(s) by *Alternaria solani* and the inhibitor(s) must have been heat-labile. Also, the toxin(s) must have been water insoluble since only chloroform extracts showed toxicity. Chicken egg air sac inoculations[8] were generally used for screening of mycotoxins produced by fungi. The results together with the finding by other researchers[3-7] suggest that consumption of blighted potaoes is unlikely to create a hazard as far as public health is concerned.

9 R. G. D. Steel and J. H. Torrie, Principles and procedures of statistics. McGraw-Hill Book Co., Inc., New York, N.Y. 1960.

An escape phenomenon from water and sodium retention induced by propranolol in rats

J. M. López-Novoa, S. Casado and L. Hernando[1]

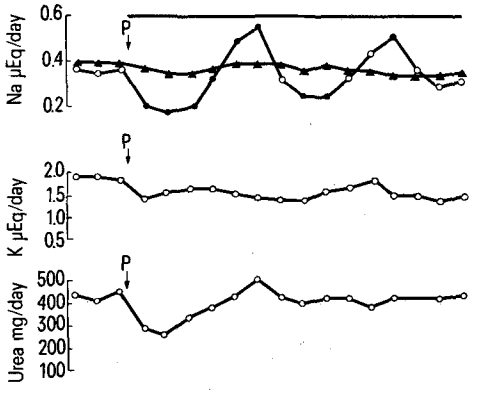
Servicio de Nefrología, Fundación Jiménez Díaz, Avda Reyes Católicos 2, Madrid 3 (Spain), 6 July 1977

Summary. Rats given a daily dose of propranolol 45 mg/kg b.wt retain water and sodium for 4 days, escaping during the 5th and 6th days in which their excretions are larger than basal values. Afterwards, in the period studied, they make a new retention and clearing is less accentuated. No relationship could be found between these retentions and plasma renin activity or renal renin content.

Propranolol (Sumial®, ICI-Farma, Pontevedra), a known β-blocker, has been proved to be able to lower the secretion of renin from the juxtaglomerular apparatus[2,3]. Also an effect of propranolol (P) in reducing diuresis and Na and urea excretion has been recently observed[4].

Female Wistar rats (weight 242 ± 16 g, average ± SEM) were placed into individual metabolic cages and allowed free access to standard rat food and tap water. During 4 days (basal period), all rats received an i.m. injection of 0.25 ml glucose 5% twice a day. From the 5th day (day 0 of experiment) water was substituted by 20 ml (average of water intake on basal days) of a solution containing P 9 mg/kg b.wt and a i.m. injection of P 18 mg/kg b.wt was given twice a day. Every day water and food consumption were recorded and clean urine collected under mineral oil. Urinary volume was measured and Na and K contents analyzed by flame photometry (IL 143, Instrumentation Laboratory, Boston, Mass. USA). Urinary urea was measured with an AutoAnalizer (Technicon).

A group of 10 animals was studied during 19 days (4 basal + 15 P) in order to control water and salt balance. A second group of 25 animals identically treated were sacrified by lots of 5 animals previously to the P administration the days 4 and 10 (maximal retention) and 7 and 13 (maximal excretion). They were lightly anesthetized with sodium pentobarbital (Nembutal, Abbot) and after renal pedicle ligation, blood samples were taken by aortic puncture for Plasma Renin Activity (PRA[5]) and creatinine[6] measurements. Kidneys were removed, weighted and Renal Renin Content (RRC) was determined[7].



Na, K and urea excretion on each day of the experiment. Propranolol (P) is administered from the 4th day (arrows) to the end of the experiment. Each point is the average of 10 animals. ▲, Intake; ○, excretions; ●, excretions significantly different from the intake (in Na excretion figure only).

1 Acknowledgments. The authors thank Mrs Inmaculada Millás for excellent technical assistance and Maria Isabel Navajos for secretarial help.

2 F. R. Bühler, J. H. Laragh, E. D. Baer, Jr, and H. R. Brunner, New Engl. J. Med. *287*, 1209 (1972).
3 A. M. Michelakis, R. G. McAllister, J. clin. Endocr. Metab. *34*, 386 (1972).
4 F. J. Ortega, J. M. López-Novoa, M. A. Rengel and L. Hernando, submitted to Eur. J. Clin. Invest.
5 E. Haber, T. Koesner, L. Page, B. Kliman and A. Purnode, J. clin. Endocr. *29*, 134 (1969).
6 P. D. Doolan, E. L. Alpen and G. B. Theil, Am. J. Med. *32*, 65 (1972).
7 J. Menard and K. J. Catt, Endocrinology *90*, 422 (1972).

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.

8 G. H. Evans and D. G. Shand, J. clin. Pharmac. Ther. *14*, 487 (1973).
9 H. J. Waal-Manning, Drugs *12*, 412 (1976).

The metabolism of methadone by cultured mammalian cells[1]

P. C. Will[2] and W. D. Noteboom

Department of Biochemistry, School of Medicine, University of Missouri, Columbia (Missouri 65201, USA), 13 July 1977

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

1 This study was supported by United States Public Health Service Research Grant No. MH 15622. The illustrations were prepared by Diane J. Kastelic.
2 Present address: Department of Anatomy, School of Medicine, Case Western Reserve University, Cleveland, Ohio 44106, USA.
3 A. H. Beckett, J. F. Taylor, A. F. Casy and M. M. A. Hassan, J. Pharm. Pharmac. *20*, 754 (1968).
4 A. L. Misra, S. J. Mule, R. Bloch and N. L. Vadlamani, J. Pharmac. exp. Ther. *185*, 287 (1973).
5 D. L. Roerig, A. T. Hasegawa and R. I. H. Wang, Biochem. Pharmac. *24*, 355 (1975).
6 H. R. Sullivan, S. E. Smits, S. L. Due, R. E. Booher and R. E. McMahon, Life Sci. *11*, 1093 (1972).
7 H. R. Sullivan and S. L. Due, J. med. Chem. *16*, 909 (1973).
8 N. C. Jain, W. J. Leung, R. D. Budd and T. C. Sneath, J. Chromat. *103*, 85 (1975).
9 O. Schaumann, Naunyn-Schmiedebergs Arch. Path. Pharmak. *239*, 311 (1960).
10 T.C. Spaulding, L. Minium, A. N. Kotake and A. E. Takemori, Drug. Metab. Disp. *2*, 458 (1974).
11 E. B. Thompson, G. M. Tomkins and J. F. Curran, Proc. nat. Acad. Sci. USA *56*, 296 (1966).
12 M. Liesse, G. Lhoest, A. Trouet and P. Tulkens, Archs int. Physiol. Biochim. *84*, 638 (1976).
13 G. A. Fischer, Ann. N. Y. Acad. Sci. *76*, 673 (1958).
14 J. Paul, Cell and Tissue Culture, 5th ed., p. 243. Churchill Livingstone, London 1975.