the radioactive tracers and by vesical catheterization. Doses of about 1.5 μCi of diatrizoate-I^{131} and 3.0 μCi of hippuran-I^{125} per kg of body weight were dissolved in 500 ml of saline. A priming dose of 100 drops per min was administered i.v. to all patients for 10 min, after which the individual dosage was adjusted to give the same plasma concentration for all patients having different renal function. This was verified by checking the constancy of the body radioactivity by external counting over the precordial area. Another scintillation counter, similar to the type now being used to measure GFR by external counting[5], was collimated over the bladder to verify whether the emptying of the bladder through the catheter was total. A sufficiently constant radioactive concentration was reached 45–60 min after commencement of the venous infusion. At this moment the clearances were started. 2 basal clearances, each of 20 min duration, were carried out; thereafter in a period of 5 min 10 mg of tolamolol were injected i.v. to 5 patients and 15 mg to the other patients. 10 min after the end of the venous injection of tolamolol, 2 other clearances of the same duration were performed to assess the possible changes in renal function. Blood pressure and pulse rate were monitored every 2 min during the whole experiment. Radioactivity of plasma and urine was measured by a well-type counter and a multichannel analyzer. Urinary creatinine was determined by a Technicon autoanalyzer.

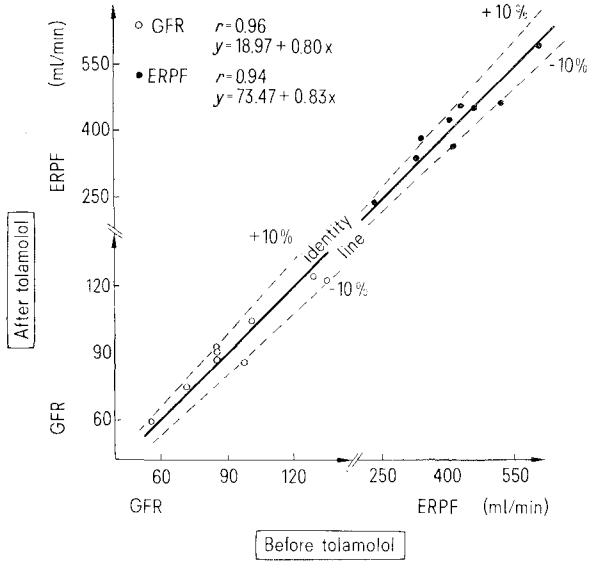


Fig. 2. Relationship between GFR and ERPF before and after tolamolol administration.

Results. The results obtained in each case are shown in Figures 1 and 2. Tolamolol induces a significant decrease of the heart rate. The mean pulse rate was 77 ± 5 SD beats/min before injection and 66 ± 6 SD ($p < 0.001$) after injection. Decrease of the heart rate was maximal 5 to 10 min after tolamolol administration. No significant variation of MAP was observed. MAP (mm Hg) resulted 129.57 ± 12.46 SD before and 128.90 ± 15.69 SD after tolamolol. A decrease of urine output and a consequent increase of urine creatinine concentration were found after using the β-blocker, in spite of the constant hydration of the patients studied. The mean urine output (ml/min) was 9.8 ± 2.3 SD before and 7.0 ± 2.5 SD after tolamolol ($p < 0.001$); the mean urine creatinine (mg/100 ml) resulted 8.7 ± 2.3 SD before and 12.6 ± 5.4 SD after ($p < 0.05$).

Finally, as far as the main purpose of this study is concerned, a remarkable stability of the renal function was observed after injection of the new drug. GFR (ml/min) was 93.69 ± 22.99 SD before and 93.75 ± 21.10 SD after; ERPF (ml/min) 410.13 ± 103.25 SD before and 413.88 ± 96.56 SD after tolamolol. No statistically significant differences were observed between the two clearances carried out after the tolamolol administration. No adverse side-effects were observed.

Discussion. After i.v. administration of tolamolol in hypertensive subjects, a significant fall of the heart rate was observed. MAP remained unchanged, as found by MILLER et al.[6] in patients with coronary heart disease. Our results seem to indicate that tolamolol induces a decrease of urine output (verified by the increase in urine creatinine concentration) without determining any variation of GFR and ERPF. This indicates an increased tubular reabsorption of water by some unknown mechanism(s). In contrast to this, other new β-blockers were recently found to cause a significant decrease of GFR and ERPF together with a marked lowering of urine output[7].

The stability of renal haemodynamic effects found after acute administration of tolamolol justify further clinical investigations on its chronic effects in relation to the renal function and arterial pressure.

[5] C. BIANCHI, in *Evaluation of Renal Function and Disease with Radionuclides* (Ed. M. D. BLAUFOX; Karger, Basel and University Park Press, Baltimore 1972), p. 43.

[6] R. R. MILLER, L. A. VISMARA and D. T. MASON, Clin. Pharmac. Ther. *17*, 713 (1975).

[7] P. ZECH, N. POZET, M. LABEEUW, J. BERNHEIM, M. PELLET and J. TRAEGER, Kidney internat. *8*, 132 (1975).

Inhibited Hormonal Induction of Hepatic Phosphoenolpyruvate Carboxykinase in Poly I:C Treated Mice, an Endotoxin-Like Glucocorticoid Antagonism[1]

R. N. MOORE and L. J. BERRY

Department of Microbiology, The University of Texas at Austin, Austin (Texas 78712, USA), 8 June 1976.

Summary. Corticosteroid induction of mouse hepatic phosphoenolpyruvate carboxykinase was inhibited by prior injection of poly I:C. Mice challenged with a lethal dose of endotoxin 4 h after administration of poly I:C could not be protected by a concurrent injection of hydrocortisone.

Gram-negative bacterial endotoxin and the synthetic double-stranded RNA polyriboinosinic-polyribocytidylic acid (poly I:C) are known to elicit similar biological effects, both toxic [2–4] and apparently beneficial[5–8]. Several of these effects are mediated by substances produced by cells within the treated animal.

Poly I:C and endotoxin are also known to elicit metabolic alterations in mice such as inhibition of corticosteroid induction of hepatic tryptophan oxygenase (TO)[9–10] and depletion of liver glycogen[10,11]. Endotoxin also inhibits stress induced synthesis of hepatic phosphoenolpyruvate carboxykinase (PEPCK)[12]. This metabolic im-

pairment is obviously related to the hypoglycemia and inhibited gluconeogenesis observed in endotoxin poisoning[13-14] since PEPCK is the major limiting enzyme of the gluconeogenic pathway[15]. More recently a mediator of this effect of endotoxin has been detected in the serum of endotoxin-poisoned mice[16]. This mediator has been tentatively named glucocorticoid antagonizing factor (GAF), and phagocytic cells of the reticuloendothelial system (macrophages) have been identified as its source[16]. Experiments performed to assess the effect of poly I:C treatment on hormonal induction of PEPCK are reported in this communication. The relationship between altered hormonal response in poly I:C pretreated mice and sensitization to endotoxin challenge[17] has also been investigated.

Materials and methods. Pathogen-free HaICR mice, bred in our research facilities, were employed. Only female mice, 6 to 8 weeks old, were used.

PEPCK activity in whole liver homogenates was determined by the method of PHILLIPS and BERRY[18]. Enzyme activity determined as μM of phosphoenolpyruvate (PEP) formed per g of dry liver per 6 min is expressed as percent of induced control activity. Enzyme induction was initiated by s.c. injection of 1 mg of hydrocortisone acetate (Sigma Chemical Co., St. Louis, Mo.) suspended in 0.2 ml of sterile nonpyrogenic saline (Travenol Laboratories, Deerfield, Ill.) containing 0.0025% Tween-80 (Sigma). In all cases, enzyme activity was determined 4 h after hydrocortisone injection. Each experimental group contained a minimum of 7 animals. The mean induced control PEPCK activity for 24 mice was 263 μM PEP/g dry wt. liver/6 min.

Poly I:C (Sigma) was injected i.v. at a concentration of 50 µg in 0.1 ml of sterile nonpyrogenic saline. The endotoxin used in these studies was extracted from *Salmonella typhimurium*, SR-11, by the Boivin trichloroacetic acid method[19], and was injected via the i.p. route in 0.5 ml of saline. Statistical significance was determined with the Wilcoxon rank sum test[20].

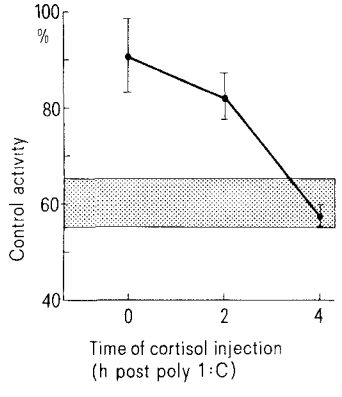
Results. The effect of poly I:C administration on hormonal inducibility of PEPCK is shown in the Figure. Enzyme induction was slightly inhibited, though not significantly, in mice given 50 µg of poly I:C either at the same time or 2 h prior to injection of 1 mg of hydrocortisone. Poly I:C administration 4 h prior to hydrocortisone, however, resulted in a total inhibition of enzyme induction. PEPCK activity determined 4 h after injection of the hormone was not only significantly lower than induced control values ($p \leq 0.01$) but also not significantly elevated over noninduced control activities. BERRY et al[10] previously demonstrated that poly I:C inhibits hormonal induction of TO within this same time interval required for impaired induction of PEPCK.

Since corticosteroids, both endogenous and exogenous, are potent antagonists of endotoxic effects, failure of these regulatory enzymes to respond to hormone administration should be symptomatic of metabolic derangements predisposing to endotoxic lethality. Results shown in the first 2 lines of the Table demonstrate that this is the case. Animals injected with 50 µg of poly I:C 4 h prior to challenge with endotoxin were sensitized to endotoxin lethality when compared with saline pretreated controls. This observation confirms an earlier report by HUANG and LANDAY[17]. Even more impressive results are shown in lines 3 and 4 of this Table. Simultaneous injection of 1 mg of hydrocortisone with 200 µg of endotoxin reduced mortality in control mice from 92% (line 2) to 31%. Pretreatment with poly I:C, however, inhibited this protective effect of the hormone. In this group, 83% of the mice

Endotoxic lethality in poly I:C pretreated mice

Pretreatment (4 h)	Hydrocortisone[a]	No. dead/total injected[b] with endotoxin dose (µg)		
		25	50	200
50 µg poly I:C	–	12/20	12/13	–
0.1 ml saline	–	4/20	7/16	12/13
50 µg poly I:C	+	3/7	–	15/18
0.1 ml saline	+	0/10	–	9/29

[a] 1.0 mg injected s.c. at time of endotoxin challenge.
[b] 48 h survival.



Hormonal inducibility of PEPCK in mice injected with 1 mg of hydrocortisone at specified time intervals after injection of 50 µg of poly I:C. Enzyme activity is denoted as percent of control induced activity ± SE. The shaded area represents the nontreated control mean (10 mice) ± 1 SD.

[1] This investigation was supported by U. S. Public Health Service research grant No. AI-10087 from the National Institute of Allergy and Infectious Diseases.

[2] M. ABSHER and W. R. STINEBRING, Nature, Lond. *223*, 715 (1969).
[3] H. L. LINDSAY, P. W. TROWN, J. BRANDT and M. FORBES, Nature, Lond. *223*, 717 (1969).
[4] R. H. ADAMSON and S. FABRO, Nature, Lond. *223*, 718 (1969).
[5] E. A. CARSWELL, L. J. OLD, R. L. KASSEL, S. GREEN, N. FIORE and B. WILLIAMSON, Proc. natn. Acad. Sci., USA *72*, 3666 (1975).
[6] P. ALEXANDER and R. EVANS, Nature New Biol. *232*, 76 (1971).
[7] A. K. FIELD, A. A. TYTELL, G. P. LAMPSON and M. R. HILLEMAN, Proc. natn. Acad. Sci., USA *58*, 1004 (1967).
[8] W. R. STINEBRING and J. S. YOUNGNER, Nature, Lond. *204*, 712 (1964).
[9] L. J. BERRY and D. S. SMYTHE, J. exp. Med. *118*, 587 (1963).
[10] L. J. BERRY, D. S. SMYTHE, L. S. COLWELL, R. J. SCHOENGOLD and P. ACTOR, Infect. Immun. *3*, 444 (1971).
[11] L. J. BERRY, D. S. SMYTHE and L. G. YOUNG, J. exp. Med. *110*, 389 (1959).
[12] D. F. RIPPE and L. J. BERRY, Infect. Immun. *6*, 766 (1972).
[13] R. E. MCCALLUM and L. J. BERRY, Infect. Immun. *7*, 642 (1973).
[14] J. P. FILKINS and R. P. CORNELL, Am. J. Physiol. *227*, 778 (1974).
[15] R. W. HANSON and A. J. GARBER, Am. J. clin. Nutr. *25*, 1010 (1972).
[16] R. N. MOORE, K. J. GOODRUM and L. J. BERRY, J. reticuloendothel. Soc. *19*, 187 (1976).
[17] K. HUANG and M. E. LANDAY, J. Bact. *100*, 1110 (1969).
[18] L. J. PHILLIPS and L. J. BERRY, Am. J. Physiol. *218*, 1440 (1970).
[19] A. M. STAUB, in *Methods in Carbohydrate Chemistry V* (Eds. R. L. WHISTLER, J. N. BEMILLER and M. L. WOLFROM; Academic Press, New York 1965), p. 92.
[20] C. WHITE, Biometrics *8*, 33 (1952).

succumbed to endotoxin challenge. Even with an endotoxin challenge dose of 25 μg, pretreatment with poly I:C inhibited complete protection by hydrocortisone.

Discussion. Endotoxin and poly I:C elicit synthesis and/or release of potent biologically active mediators. Three of these mediators are interferon[7,8], colony stimulating factor[21], and tumor necrosis factor[5]. A fourth mediator, endogenous pyrogen, is also probably released in response to poly I:C, as it is in response to endotoxin, since both substances are pyrogenic in rabbits[3]. Results presented in this report imply that poly I:C also stimulates production of GAF, the inhibitor of PEPCK induction in endotoxemic mice[16].

The observation that poly I:C pretreatment inhibits hormonal protection of mice challenged with endotoxin may have direct bearing on the nature of irreversible endotoxemic shock. Corticosteroids protect mice from endotoxic lethality only when administered prior to or concurrently with a lethal dose of the toxin. If hormone is withheld, the animal rapidly enters a state of irreversible shock in which a delayed injection of hormone is no longer protective[22]. Pretreatment with poly I:C appears to simulate this condition. These mice display antagonized protective responses to hormone therapy upon endotoxin challenge at a time following pretreatment when PEPCK and TO[10] are no longer responsive to hormal induction. Failure of these two enzymes to respond to corticosteroid induction is most probably representative of metabolic derangements contributing to this increased susceptibility to endotoxin lethality.

[21] F. W. RUSCETTI and P. A. CHEWENICK, J. Lab. clin. Med. *83*, 64 (1974).

[22] L. J. BERRY in *Microbiological Toxins V* (Eds. S. KADIS, G. WEINBAUM and S. AJL; Academic Press, New York 1971), p. 165.

Effets de la concanavaline A sur la morphologie et le comportement de cellules embryonnaires d'Urodèles en différenciation in vitro

Morphological Effects of Concanavalin A on Amphibian Embryonic Cells Differentiating in vitro

J.-P. LAMON et ANNE-MARIE DUPRAT[1]

Laboratoire de Biologie générale, Université Paul-Sabatier, 118, route de Narbonne, F-31077 Toulouse Cedex (France),
3 juin 1976.

Summary. Concanavalin A exerts an irreversible inhibitory effect on the differentiation of cultured embryonic amphibians cells in vitro. Cytological changes and disturbances of cell attachment, and spreading to the culture support occur in parallel. Polykaryons have been noticed in treated cultures.

La concanavaline A, glycoprotéine végétale, possède, outre ses propriétés hémagglutinantes[2], celle de provoquer des fusions cellulaires (cellules embryonnaires de Drosophile[3,4]).

Les effets de la concanavaline A (Con A) ont été étudiés sur des embryons d'Amphibiens in toto par MORAN[5] qui a constaté une inhibition dans le développement chez *Ambystoma maculatum*. D'autres expériences de O'DELL et coll.[6] sur l'Amphibien Anoure *Xenopus laevis* ont également montré une inhibition du développement du tube nerveux.

Le but de ce travail est *d'analyser à un niveau cellulaire* les effets morphologiques de la Con A, d'une part sur la différenciation de diverses catégories de cellules embryonnaires d'Amphibiens cultivées in vitro et, d'autre part, sur divers organites, tant nucléaires que cytoplasmiques. Les cellules d'Amphibiens Urodèles se prêtent bien à ce genre d'étude par leur taille, la bonne visibilité des divers organites qu'elles renferment et leur comportement in vitro[7]. Il était également intéressant de vérifier si les cellules embryonnaires de Batraciens réagissent vis-à-vis de la Con A d'une manière comparable à celle des cellules embryonnaires de Drosophile en formant des polycaryons.

Matériel et méthodes. Les embryons utilisés proviennent de pontes de *Pleurodeles waltlii* (Michah) et d'*Ambystoma mexicanum* (Shaw). Au stade neurula (stade 14 de la table chronologique de GALLIEN et DUROCHER[8], la plaque neurale associée au chordomésoblaste sous-jacent est prélevée, dissociée dans le milieu de Steinberg[7]. Les cellules sont ensuite cultivées à 18 °C, dans la solution de

Tableau 1. Nombre de populations de 125 cellules par type de traitement et par effectif de polycaryons (avec proportion et proportion transformée des polycaryons)

Nombre de polycaryons par population (125)		1	2	3	4	5	6	7	8	9	10
Proportion correspondant à ce nombre: p[a]		0,008	0,016	0,024	0,032	0,040	0,048	0,056	0,064	0,072	0,080
p transformée en $x = 57{,}3\sqrt{p}$		5,125	7,247	8,877	10,250	11,460	12,554	13,560	14,496	15,375	16,207
Nombre de populations présentant p par type de traitement	Témoins (1)	4	4	1							
	Jusqu'au changement (2)		2	6	7	4	1				
	Après le changement (3)		7	11	9	1	1	1			
	Traitement continu (4)				1	1	3	3	2	1	1

[a] Exemple: il y a 4 populations de 125 cellules qui présentent 5 polycaryons dans le traitement 2 (jusqu'au changement de milieu). $p = 5/125 = 0{,}040$; $x = 57{,}3\sqrt{0{,}040} = 11{,}460$.