1 This study was supported by funds from the Veterans Administration and by NIH research grant NS 06820.
2 We thank Mr C.C. Smathers, Jr, for his technical help.
3 J.F. Hirsch, J. Buisson-Ferey, M. Sachs, J.C. Hirsch and J. Scherrer, Electroenceph. clin. Neurophysiol. *21*, 417 (1966).
4 P. Gloor, G. Ball and N. Schaul, Neurology, Minneap. *27*, 326 (1977).
5 A. Wikler, Proc. Soc. exp. Biol. *79*, 261 (1952).
6 F. Rinaldi and H.E. Himwich, Archs Neurol. Psychiat. *73*, 396 (1955).
7 P.B. Bradley and J. Elkes, Brain *80*, 77 (1957).
8 N. Schaul, P. Gloor, G. Ball and J. Gotman, Brain Res. *143*, 475 (1978).
9 G. Moruzzi and H.W. Magoun, Electroenceph. clin. Neurophysiol. *1*, 455 (1949).
10 R. Spehlmann and K. Downes, Brain Res. *74*, 229 (1974).
11 F. Rinaldi, Prog. Brain Res. *16*, 229 (1965).
12 J.C. Daniels and R. Spehlmann, Electroenceph. clin. Neurophysiol. *34*, 83 (1973).
13 R. Spehlmann, Electroenceph. clin. Neurophysiol. *27*, 201 (1969).
14 H.H. Jasper and C. Ajmone-Marsan, A stereotaxic atlas of the diencephalon of the cat. The National Research Council of Canada, Toronto 1954.

Lack of effect of cortisone, thyroxine and insulin on the developmental pattern of mouse intestinal glucose-6-phosphatase activity[1]

D. Ménard and C. Malo

Unité de Biologie Intestinale, Département d'Anatomie et de Biologie Cellulaire, Faculté de Médecine, Université de Sherbrooke, Sherbrooke (Québec, Canada J1H 5N4), 19 March 1981

Summary. Daily administration for 3 days of cortisone (25 µg/g b.wt), thyroxine (1 µg/g b.wt) or insulin (12.5 mU/g b.wt) to 8-day-old suckling mice does not induce a premature decrease of the phosphohydrolase activity of intestinal glucose-6-phosphatase.

Glucose-6-phosphatase (G-6-Pase), a multifunctional enzyme[2], is the only principle enzyme of sugar metabolism associated with endoplasmic reticulum and is mainly found in liver, kidney and small intestine. At the intestinal level, G-6-Pase activity appears at the end of gestation[3]. After birth the activity increases during the 1st week, remains stable during the 2nd week and decreases to adult level in all intestinal segments during the 3rd week[4]. We have shown that its ultrastructural localization in the enterocytes is similar during the postnatal development[5] and in adult mouse[6]. The decrease of G-6-Pase activity observed during the 3rd postnatal week occurs when important enzymatic changes are taking place at the brush border level; sucrase activity appears, and the other brush border hydrolytic activities such as trehalase, glucoamylase, alkaline phosphatase and leucylnaphthylamidase increase to reach adult levels[4,7,8]. Since the enzymatic modifications occurring at the brush border membrane level are under control of glucocorticoids, thyroxine and insulin[7-13], we questioned in the present investigation whether the normal development of intestinal G-6-Pase is also controlled by these hormones.

Materials and methods. At 8 days of age, Swiss ICR mice received 1 injection per day during 3 days of the following hormones: 1. cortisone acetate suspension (Merk, Sharp & Dohme) diluted in saline, injected i.p. at a dosage of 25 µg/g b.wt/day; 2. DL-thyroxine (Sigma) dissolved in 0.005 N NaOH injected i.p. at a dosage of 1 µg/g b.wt/day and, 3. NPH insulin suspension (Connaught Laboratories Ltd., Ontario) diluted in saline injected s.c. at a dosage of 12.5 mU/g b.wt/day. Control animals were injected with equivalent volumes of saline. At the end of the experimental period, the intestines were removed immediately following decapitation, measured and cut into 3 equal parts. Each intestinal segment was weighed and homogenized in 99 vol. of ice-cold redistilled water. The phosphohydrolase activity of G-6-Pase was assayed as previously described[4,6] and the proteins were determined according to Lowry et al.[14]. The G-6-Pase activity was expressed as µmoles of phosphorus liberated per min · g of protein. Differences between the experimental groups were analyzed using Student's t-test.

Results and discussion. Daily administration of cortisone, thyroxine and insulin during 3 days at dosages known to induce precocious enzymatic modifications at the brush border membrane level of suckling mice and rats[7,13] does not provoke a premature decrease of G-6-Pase activity. Indeed, as shown in the table, G-6-Pase activity remains unchanged or even increases slightly following the different hormonal treatments. One has to remember that during the suckling period this activity is 4 times the adult level[4]. The specific metabolic functions of intestinal G-6-Pase have not been clearly defined. Even though the intestinal mucosa is not considered to be a gluconeogenic tissue, the possibility that it might be an important source of glucose production in certain circumstances has not been excluded, as reported for starved guinea-pigs[15]. The higher-than-adult values reported for the phosphohydrolase activity of G-6-Pase during the suckling period could be related to a gluconeogenic function of the mucosa during this period. The high G-6-Pase activity observed during the first 2 postnatal weeks has been associated, in part, with an important

Influence of cortisone, thyroxine and insulin on intestinal phosphohydrolase activity of glucose-6-phosphatase in suckling mice

	Number of animals	Small intestinal segments 1/3 P	1/3 M	1/3 D
Controls	6	40.3 ± 6.6	83.7 ± 5.8	47.8 ± 2.7
Cortisone	8	51.6 ± 4.3^{NS}	97.6 ± 5.2***	54.0 ± 2.9^{NS}
Thyroxine	9	61.8 ± 4.3*	83.9 ± 2.4^{NS}	56.9 ± 2.9**
Insulin	6	61.8 ± 3.6*	93.7 ± 2.2^{NS}	57.2 ± 2.2**

Glucose-6-phosphatase activity is reported for the proximal thirds (1/3 P), middle thirds (1/3 M) and distal thirds (1/3 D). Results are expressed as the mean of each group ± SEM. The hormonal treatments started at 8 days of age and the animals received 1 injection/day for 3 days of cortisone (25 µg/b b.wt), thyroxine (1 µg/g b.wt) and insulin (12.5 mU/g b.wt). The animals were sacrificed 24 h after the last injection. Levels of statistical significance of differences between control and experimental groups: * $p < 0.01$; ** $p < 0.025$; *** $p < 0.05$; NS, not significant.

proliferation of the smooth endoplasmic reticulum[5]. The present study suggests that the decrease of intestinal G-6-Pase activity will occur along with the normal decrease of the amount of endoplasmic reticulum[5] instead of being the result of a control exerted by thyroxine, cortisone or insulin. However, even though these hormones are unable to induce the decrease of G-6-Pase, they are still able to provoke regional increases of activity as shown in the table. The physiological significance of these small but still significant increases is still unknown. This is the first report concerning the study of a possible implication of hormones in the regulation of the post-natal decrease of G-6-Pase activity in the small intestine. Recently, it has been shown that thyroxine (1 µg/g b.wt) administrated to developing rats decreases hepatic G-6-Pase activity[16]. On the other hand, it has been reported that hydrocortisone and insulin have no effect on this activity in pre- and postnatal liver[17]. In conclusion, even though the G-6-Pase activity decreases when the brush border hydrolytic functions increase, the hormones known to influence or even control the postnatal development of brush border membrane-bound enzymes are not involved in the regulation of the post-natal decrease of the endoplasmic reticulum membrane-bound G-6-Pase activity of the intestinal epithelial cells.

1 Supported by grant MA-5969 from the Medical Research Council of Canada (D.M.). C.M. is a recipient of a studentship from MRC of Canada. D.M. is a 'chercheur-boursier du Conseil de la recherche en santé du Québec'.
2 R.C. Nordlie, Life Sci. *24*, 2397 (1979).
3 R. Calvert, D. Malka and D. Ménard, Histochemistry *63*, 209 (1979).
4 D. Ménard and C. Malo, Devl Biol. *65*, 508 (1978).
5 D. Ménard, Histochemistry *67*, 53 (1980).
6 J.S. Hugon, D. Maestracci and D. Ménard, J. Histochem. Cytochem. *19*, 515 (1971).
7 F. Moog, E.H. Birkenmeier and H.S. Glozier, Devl Biol. *25*, 398 (1971).
8 F. Moog, A.E. Denes and P.M. Powell, Devl Biol. *35*, 143 (1973).
9 S. Henning and N. Kretchmer, Enzyme *15*, 3 (1973).
10 C. Malo and D. Ménard, Comp. Biochem. Physiol. *65B*, 169 (1980).
11 C. Malo and D. Ménard, Experientia *35*, 874 (1979).
12 D. Ménard and C. Malo, Devl Biol. *69*, 661 (1979).
13 K.Y. Yeh and F. Moog, J. exp. Zool. *200*, 337 (1977).
14 O.H. Lowry, N.F. Rosebrough, A.L. Farr and R.J. Randall, J. biol. Chem. *193*, 265 (1951).
15 J.W. Anderson and A.F. Rosendall, Biochim. biophys. Acta *304*, 384 (1973).
16 A.K. Paul and A. Dhar, Horm. Metab. Res. *12*, 261 (1980).
17 O. Greengard, Biochem. J. *115*, 19 (1969).

Biliary excretion of sulfobromophthalein in isolated perfused livers from normal and spironolactone-treated rats[1]

J.M. Pellegrino, A.D. Mottino, J.V. Rodriguez and E.A. Rodriguez Garay[2]

Departamento de Fisiología, Facultad de Ciencias Bioquímicas, Universidad Nacional de Rosario, Suipacha 531, 2000 Rosario (Argentina), 14 April 1981

Summary. Bile flow and biliary excretion of sulfobromophthalein (BSP) was examined in isolated perfused livers from normal and spironolactone(SP)-treated rats. BSP biliary excretion contributed to the bile production in both groups. Moreover SP increased BSP biliary excretion but transfer of dye from plasma into liver was not affected.

Sulfobromophthalein (BSP) has been widely used in the evaluation of liver function and its handling by the isolated liver was considered useful to test the applicability of the model in the study of drug metabolism[3]. Transfer of BSP from the plasma to the bile has been shown to consist of 4 separate steps: a) translocation from plasma into the liver by a carrier-mediated process[4,5], b) binding to a cytoplasmic protein denominated ligandin[6], c) conjugation with glutathione[7], d) excretion into bile mainly in the conjugated form[8]. Bile flow is also an important parameter in determining the rate of excretion of BSP into the bile[9], but decreased bile flow after administration of large doses of BSP has been reported[10]. Biliary excretion of BSP may be increased by microsomal enzyme inducers. The effect of these compounds might be due to their ability to increase bile flow[11], whereas ligandin seemed not to be involved in the phenomenon[12]. Spironolactone (SP) has been shown to be an important inducer of bilirubin metabolism in the rat liver[13,14], and to increase bile flow in this species by enhancing the bile salt-independent fraction of canalicular bile[15]. This steroid accelerates the elimination of BSP in living rats not only by increasing bile flow but also by enhancing enzymic BSP-glutathione conjugation[16]. Since BSP, to a certain extent, enters all tissues of the body[17], it was of interest to avoid the potential problems of extrahepatic dye distribution. Therefore, in this study we examined bile flow and biliary excretion of BSP in isolated livers obtained from normal and SP-treated rats, using the model applied to single dose incorporation[18].

Materials and methods. Adult male Wistar rats weighing 300–350 g were used as donors of livers. A group of rats was injected with SP dissolved in propylene glycol at a daily dose of 240 µmoles/kg (100 mg/kg) i.p. for 3 consecutive days. Control rats were injected with 1 ml of propylene glycol. The rats were allowed free access to water and saline solution during treatment, and were maintained ad libitum on a standard laboratory pellet diet. The perfusion medium consisted of heparinized rat blood mixed in a solution of Krebs-Ringer-bicarbonate buffer (pH 7.4, total volume 110 ml) with a hematocrit value of 11%. Albumin concentration in the supernatant was determined by the Bromocresol Green method[19], and averaged 4.8 ± 0.2 mg/ml. The preparation was essentially that described by Brauer et al.[20] with some modifications[3]. A constant pressure of 14 cm of water was maintained at the portal vein level. Flow rate through the liver (Q) determined by a direct measurement[20] 2 ± 2 ml/min. After 30 min of equilibration, a single dose of BSP of 16.2 ± 0.4 µmoles (13.5 ± 0.2 mg) dissolved in 6 ml 0.9% NaCl solution, was introduced into the reservoir. The ratio BSP to albumin was well below the binding capacity of albumin estimated for BSP[4]. Perfusate samples were obtained from the prehepatic and posthepatic circuits, at 5 min after the injection, and then every 2–3 min for 20–25 min. Bile was collected at 10-min intervals for 60 min. The volume of bile was estimated by gravimetry. The concentrations of BSP in samples of perfusate (after centrifugation), and bile (diluted 1:100 with distilled water) were determined by colorimetry after alkalination with