13 Goldberg, M., McCurdy, D. K., and Ramirez, M. A., J. clin. Invest. *44* (1965) 182.
14 Handler, J. S., Kidney Int. *19* (1981) 831.
15 Koseki, C., Hayashi, Y., Toprikai, S., Furuya, M., Ohnuma, N., and Imai, M., Am. J. Physiol. *250* (1986) F210.
16 Tikkanen, I., Fyhrquist, F., Metsarinne, K., and Leidenius, R., Lancet *2* (1985) 66.
17 Brown, J., in: New Perspectives in Cardiovascular Medicine, vol. 1, p. 9. Ed. C. Wood. Royal Society of Medicine, 1987.

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Effect of an antiglucocorticoid (RU-38486) on hydrocortisone induction of maltase-glucosamylase, sucrase-isomaltase and trehalase in brush border membranes of suckling rats

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Summary. Induction of α-glycosidases by hydrocortisone in suckling rats is inhibited by the daily administration of an antiglucocorticoid (RU-38486). Conversely, RU-38486 injected daily in 15-day-old rats for 7 days does not prevent the spontaneous development of α-glycosidases.
Key words. Antiglucocorticoids; hydrocortisone; maltase-glucoamylase; sucrase-isomaltase; trehalase; brush border; development.

Trehalase, maltase-glucoamylase and sucrase-isomaltase are integral membrane glycoproteins of brush border membranes[1]. Several studies have led to the conclusion that development of neonatal α-glycosidases is not absolutely dependent on glucocorticoids in the intestine[2-4], and not at all in the kidney[5]. Nevertheless, during the first two postnatal weeks, glucocorticoids have unquestionable effects; administration of hydrocortisone to suckling rats[6], rabbits[5] or mice[7] causes their precocious and simultaneous biosynthesis in the intestinal microvillous membrane. But these effects do not prove that glucocorticoids act as the final effectors; glucocorticoids may produce changes in other hormones or substances which in turn may be the true effectors. RU-38486 is a potent antiglucocorticoid, which is capable of fully antagonizing the effects of dexamethasone in vitro as well as in vivo at the level of its receptor[8]. RU-38486 has a strong binding affinity for the glucocorticoid receptor and lacks agonist activity[8]. In this paper, we describe the action of RU-38486 on trehalase, maltase, glucoamylase, sucrase and palatinase activities, prematurely induced by hydrocortisone during the second week of development of the rat. Evidence is given that during the first two postnatal weeks, exogenous glucocorticoids act as a physiological trigger.

Materials and methods. Rats of the Wistar strain, of either sex, were used. All rats were fed ad libitum and had unrestricted access to water. Three sets of littermates were used. The first set was divided into 4 groups; one group was given a s.c. injection of hydrocortisone acetate (Roussel Uclaf,

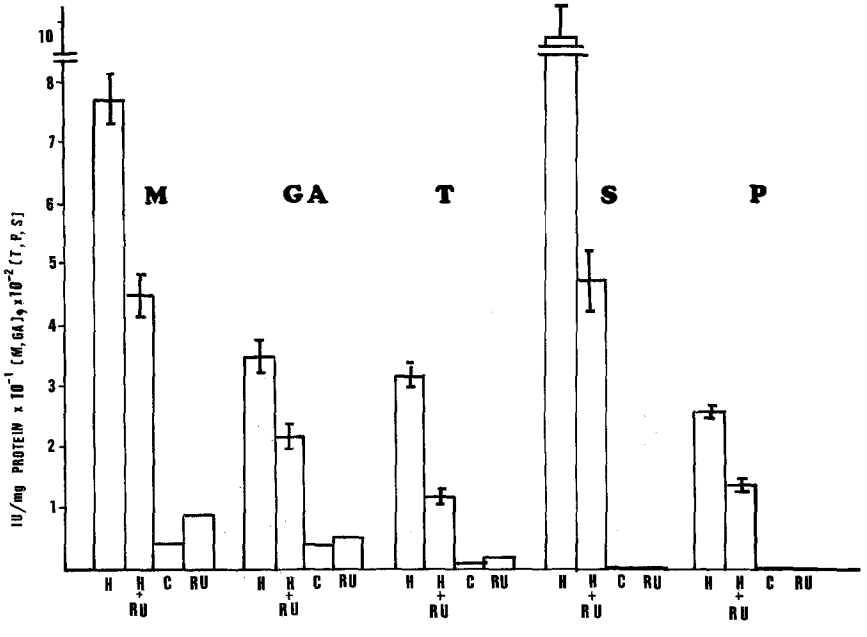

Figure 1. Effects of RU-38486 (25 mg/kg) on α-glycosidases induced by hydrocortisone in the suckling rat. 10-day-old rats were injected with (H) hydrocortisone; (H + RU) hydrocortisone + RU-38486; (RU) RU-38486 as described in the text. (C) control rats; (M) maltase; (GA) maltase-glucoamylase; (T) trehalase; (S) sucrase; (P) palatinase. Results are presented as the mean ± SE of 6 experiments for C and RU and 10 experiments for H and H + RU. Statistical differences between H and H + RU: $p < 0.001$.

France) on postnatal day 10 (50 mg/kg b. wt). The second group was injected with saline and served as control. The third group was given a single dose of hydrocortisone (as in the first group) and s. c. injections of RU-38486 (25 mg/kg) on postnatal days 10, 11, 12 and 13. The fourth group was given only RU-38486 as in the third group. RU-38486 (17 β-hydroxy-11 β, 4-dimethyl-aminophenyl-17 α-propynylestra-4, 9-diene-3 one) was a gift from Roussel-Uclaf, France. A second set of littermates was used, in order to study the effect of increasing doses of RU-38486 on sucrase activity and to test possible toxical effects of RU-38486 using lactase activity. 10-day-old rats were injected with a single dose of hydrocortisone 50 mg/kg and with RU-38486 (12.5, 25, 50, 75, 100, 150, 200 mg/kg) every day for 4 days. All suckling rats were killed at 14 days. A third set of littermates was used in order to study the effects of RU-38486 on postnatal normal development. One group of the littermates was injected every day during 7 days with RU-38486 (25 mg/kg) starting at 15 days; the second group was injected with saline instead of RU-38486 and served as control; all rats were killed at 22 days. Immediately following decapitation, the entire small intestine was removed, flushed with cold saline solution, weighed and homogenized in 10 volumes of ice-cold 10 mM phosphate buffer pH 6.0. The homogenate was centrifuged at 27 000 g for 30 min and the precipitate was used for enzymic determinations. Trehalase, sucrase, palatinase, maltase and lactase activities were assayed according to Dahlquist[9]. Glucoamylase was measured according to Schlegel-Haueter et al.[10]. Proteins were determined according to Lowry et al.[11]. Enzyme specific activity was expressed as µmoles of substrate hydrolyzed $mn^{-1} \cdot mg\ protein^{-1}$.

Results and discussion. Specific activities of α-glycosidases were measured in a 27 000 g precipitate because, as demonstrated by Galand and Forstner[12] high levels of cytosol acid maltase, neutral maltase and glycoamylase interfere with the determination of the same activities in the brush border membrane. As shown in figure 1, low levels of maltase, glucoamylase and trehalase were present in 14-day control rats; sucrase and palatinase were undetectable. Hydrocortisone injections in 10-day-old rats caused a precocious increase in the specific activities of maltase [18 ×] glucoamylase [9 ×], trehalase [35 ×], sucrase and palatinase. Daily injections of RU-38486 (25 mg/kg for 4 days) in 10 day-old rats injected with hydrocortisone, inhibited induction of sucrase (− 56%), palatinase (−46%), maltase (−41%), glucoamylase (−37%) and trehalase (−62%) (fig. 1). But, as shown in figure 2, doses of RU-38486 about ten times higher (200 mg/kg) were needed to inhibit almost completely the induction of sucrase activity. To rule out toxic effects of high doses of RU-38486, lactase was used as a marker. In contrast to α-glycosidases, lactase is high at birth and decreases during weaning. Lactase-specific activities of 14-day-old control and hydrocortisone-injected rats were 0.12 ± 0.01 and 0.08 ± 0.01 IU/mg of protein (4 experiments), respectively. Lactase specific activity of rats which were injected with

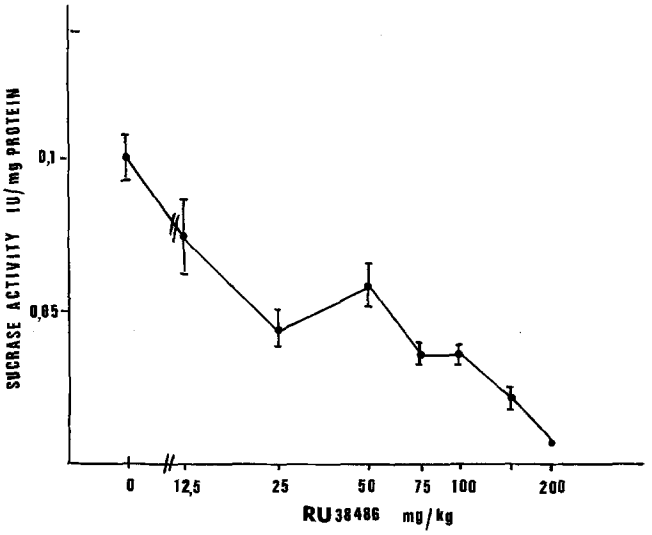

Figure 2. Inhibition of sucrase activity as a function of increasing doses of RU-38486. Sucrase activity was induced by a single dose of hydrocortisone (50 mg/kg) at 10 days; these rats also received one injection of RU-38486 per day for 4 days and were killed at 14 days. Results are presented as the mean ± SE of 4 experiments, except one with 2 experiments (200 mg/kg).

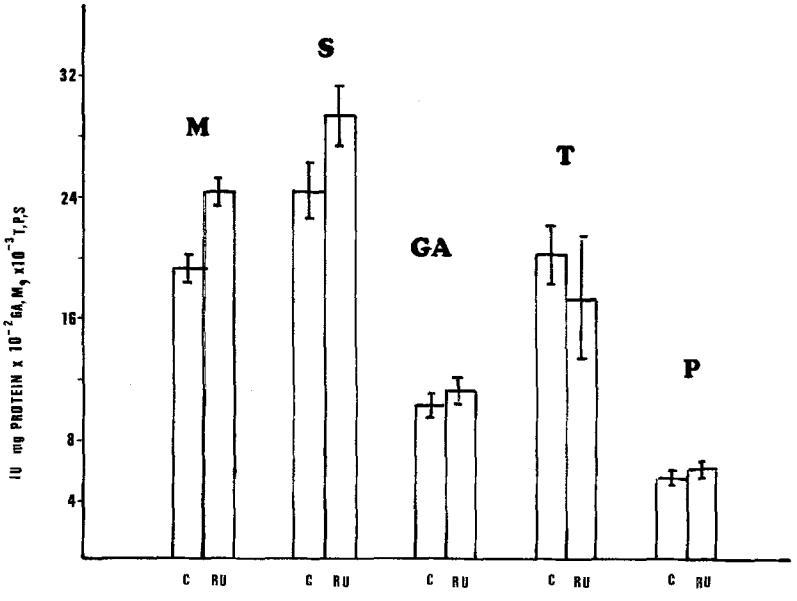

Figure 3. Effects of RU-38486 (25 mg/kg) on spontaneous development of α-glycosidases. 15-day-old rats were injected daily for 7 days and killed at 22 days. Symbols as in figure 1. Results are presented as the mean ± SE of 6 experiments. Differences between C and RU are not statistically significant.

hydrocortisone and RU-38486 (50 or 150 mg/kg) were not significantly different (0.11 ± 0.04 and 0.11 ± 0.02 IU/mg of protein, respectively) from control rats.

Conversely, as shown in figure 3, α-glucosidase-specific activities were not significantly different in 22-day-old normal rats and 22-day-old rats previously injected with RU-38486 (25 mg/kg) for 7 days.

These results suggest that: 1) before 14 days, hydrocortisone induces α-glucosidases by a direct interaction with its own receptor; 2) during the third postnatal week, the spontaneous rise in α-glycosidase activities is not prevented by RU-38486. This absence of an effect of RU-38486 between 15 and 22 days is in agreement with the progressive loss of glucocorticoid responsiveness by α-glycosidases during this period [13]; at the end of the fourth week, adrenalectomized and sham operated rats have identical α-glycosidase specific activities [2, 14]. Several studies support the hypothesis that normal development of α-glycosidases during the third week is not dependant upon glucocorticoids, but is regulated by an intrinsic timing mechanism. Sucrase and maltase develop normally in fetal rat or mouse intestine that is transplanted into the stable hormonal environment of an adult animal [15 – 17]. The most conclusive evidence was given by Kwo-Yih Yeh and Holt [3] who showed that day 5 isografts expressed sucrase activity in 13-day-old hosts; at a time at which the host intestine did not. Our experiments give additional evidence that during the third week of postnatal life the normal development of trehalase, sucrase-isomaltase and maltase-glucoamylase is most probably independant of glucocorticoids.

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1 Semenza, G., Rev. cell. Biol. *2* (1986) 255.
2 Martin, G. R., and Henning, S. J., Am. J. Physiol. *246* (1984) G 695.
3 Kwo-Yih Yeh, and Holt, P. R., Gastroenterology *90* (1986) 520.
4 Diamond, J. M., Nature *324* (1986).
5 Galand, G., Comp. Biochem. Physiol. *85 A* (1986) 109.
6 Galand, G., and Forstner, G. G., Biochem. J. *144* (1974) 293.
7 Malo, C., and Menard, D., Comp. Biochem. Physiol. *65 B* (1980) 169.
8 Philibert, D., in: Adrenal Steroid Antagonism, p. 101. Ed. M. K. Agarwal. Walter de Gruyter and Co., New York 1984.
9 Dahlquist, A., Analyt. Biochem. *7* (1964) 18.
10 Schlegel-Haueter, S., Hore, P., Kerry, K. B., and Semenza, G., Biochem. biophys. Acta *258* (1972) 506.
11 Lowry, O. H., Rosenbrough, N. E., Farr, A. L., and Randall, J., J. biol. Chem. *193* (1951) 265.
12 Galand, G., and Forstner, G. G., Biochem. J. *144* (1974) 281.
13 Henning, S. J., and Leeper, L. L., Am. J. Physiol. *242* (1982 G 89.
14 Galand, G., and Jacquot, R., C. r. Acad. Sci. Paris *271* (1970) 1107.
15 Ferguson, A., Gerskowitch, V. P., and Russel, R. I., Gastroenterology *64* (1973) 292.
16 Kendall, K., Jumawan, J., Koldowsky, O., and Krulich, L., J. Endocr. *74* (1977) 145.
17 Montgomery, R. K., Sybicki, M. A., and Grand, R. J., Devl. Biol. *87* (1981) 76.

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Kinetics of chemo-attraction of polymorphonuclear leukocytes towards N-formyl peptide studied with a novel polycarbonate (Nuclepore) membrane in the Boyden chamber

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Summary. The motile responses of human polymorphonuclear leukocytes (PMN) to N-formyl-methionyl-leucyl-phenylalanine (FMLP) in the Boyden chamber using a new sparse-pore polycarbonate membrane (pores 3 μm in diameter and occupying 0.1 % of surface area) were compared with those demonstrated by using a standard polycarbonate (Nuclepore) filtration membrane (pores 3 μm in diameter and occupying 5 % of surface area). Motility of PMN in gradients of FMLP using the new membrane was not influenced by chemokinetic effects of the factor, and the 'background' migration of the cells was minimal. However, motility of PMN in gradients of FMLP using the standard membrane was found to be influenced by chemokinetic effects of the chemotactic factor, and the 'background' or 'control' migration (in the absence of chemotactic factor) of the cells was substantial. Greater directional migration of PMN according to steepness of the gradient of chemotactic factor was demonstrated with the use of the new membrane. The new membrane may be of considerable value in the further study of the chemotactic responses of PMN.
Key words. Polymorphonuclear leukocytes; chemotaxis; N-formyl peptide.

N-formyl peptides [1], especially N-formyl-methionyl-leucyl-phenylalanine (FMLP) [2] are chemotactic for polymorphonuclear leukocytes (PMN) and the latter substance has become a widely-used factor for assessing the motility and chemotactic activity of these cells. The technique used by most authors for testing chemotaxis of leukocytes towards FMLP has been the Boyden chamber [3], according to which, the chemotactic factor is placed in solution in the lower compartment of a two-compartment chamber and a suspension of PMN is placed in the upper compartment. The cells are allowed to sediment onto, and then migrate into or through, a cellulose-ester filtration membrane separating the two compartments and motility is determined according to the number of cells migrating to the lower surface of the membrane [1, 3] or the distance which the 'leading front' of cells migrate into the membrane [4 – 6] in given incubation period.

Chemotaxis is usually measured as the difference between such assessments and the corresponding cell motility in chambers containing no chemotactic factor ('background' or 'control' migration).

Nevertheless, when assessed by Boyden chamber techniques, the 'control' migration of PMN in the absence of chemotactic factor can be increased by non-chemotactic factors such as albumin in the medium [7 – 9]. This phenomenon of increased random migration of PMN under the influence of a chemical factor has been referred to as 'chemokinesis' [10, 11] and FMLP, when present on both sides of the membrane in equal concentrations (i.e. no gradient) has been found to have such chemokinetic effects [12].

In an attempt to overcome the problem of distinguishing chemotaxis from chemokinesis, a technique of 'chequerboard' analysis has been described [4, 6, 9] in which the migra-