vasopressin are Ca^{++}-sensitive[23,24]. Thus, taking into account the mode of action of vasopressin, quercetin could modify either the number and/or the functional state of the osmotic shunts (intramembrane-particle aggregates) of the apical membrane.

1 This work was supported by the Swiss National Science Foundation, grant No. 3.043-0.76.
2 R.C. de Sousa and A. Grosso, J. Membrane Biol. *40*, 77 (1978).
3 A. Grosso and R.C. de Sousa, J. Membrane Biol. *40*, 305 (1978), special issue.
4 R.C. de Sousa and A. Grosso, Nature *279*, 803 (1979).
5 Y. Kuriki and E. Racker, Biochemistry *15*, 4951 (1976).
6 M. Rüphi, R.C. de Sousa, E. Favrod-Coune and J.M. Posternak, Experientia *28*, 1391 (1972).
7 A. Grosso and R.C. de Sousa, Experientia *34*, 593 (1978).
8 I. Singer, D. Rotenberg and J.B. Puschett, J. clin. Invest. *51*, 1081 (1972).
9 A. Taylor, M. Mamelak, E. Reaven and R. Maffly, Science *181*, 347 (1973).
10 A. Grosso, F. Spinelli and R.C. de Sousa, Cell Tissue Res. *188*, 375 (1978).
11 S.D. Levine and N.C. Verna, 10th A. Meet. Am. Soc. Nephrol., 1977; abstracts, p. 112 A.
12 S.D. Levine, W.A. Kachadorian and D. Schlondorff, Clin. Res. *27*, 422 A (1979).
13 J. Bourguet, J. Chevalier and J.S. Hugon, Biophysical J. *16*, 627 (1976).
14 R.C. de Sousa, Colloque INSERM *85*, 337 (1979).
15 J. Muller, W.A. Kachadorian and V.A. DiScala, J. Cell Biol. *85*, 83 (1980).
16 R.C. de Sousa and A. Grosso, in: Membranes and the Environment: Responses of Membranes to External Agents. Ed. K. Bloch, L. Bolis and D.C. Tosteson. PSG Publishing Company, in press (1981).
17 Y. Graziani and R. Chayoth, Biochem. Pharmac. *26*, 1259 (1977).
18 A. Grosso and R.C. de Sousa, Eur. J. clin. Invest. *8*, 333 (1978).
19 A. Schwartz, G.E. Lindenmayer and J.C. Allen, Pharmac. Rev. *27*, 3 (1975).
20 Y. Graziani, J. Winikoff and R. Chayoth, Biochim. biophys. Acta *497*, 499 (1977).
21 A. Wüthrich and H.J. Schatzmann, Cell Calcium *1*, 21 (1980).
22 V. Shoshan, K.P. Campbell, D.H. MacLennan, W. Frodis and B.A. Britt, Proc. natl Acad. Sci., USA *77*, 4435 (1980).
23 M.A. Hardy, Colloque INSERM *85*, 311 (1979).
24 R.C. de Sousa and A. Grosso, J. Physiol., Paris, in press (1981).

Refeeding of mice after fasting stimulates cell renewal in the gall bladder epithelium[1]

Ph. Putz[2], L. Bazira and G. Willems

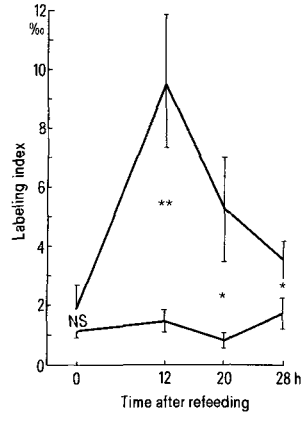
Eenheid Gastroenterologisch Onderzoek, Vrije Universiteit Brussel, Fakulteit Geneeskunde en Farmacie, B-1090 Brussel (Belgium), 17 October 1980

Summary. The effect of refeeding of mice after a fasting period on the uptake of 3H-thymidine and on mitotic activity in the gall bladder epithelium was studied by histoautoradiography. A significant increase in both DNA synthesis and mitotic activity was observed after 12 h of refeeding.

The gall bladder as well as the pancreas are well known target organs of cholecystokinin (CCK-PZ). Besides its effects on pancreatic secretion, on the muscular contraction of the gall bladder and on the secretion of glycoproteins by the gall bladder epithelium[3], this hormone exerts a significant growth promoting effect on both organs in the adult animal. Cholecystokinin and also caerulein, a synthetic decapeptide analogue, stimulate DNA synthesis[4,5] and increase total DNA content in the pancreas[6]. Recently, we have observed a potent stimulation of the DNA synthesis activity in the gall bladder epithelium of mice after acute administration of caerulein[7]. The physiological significance of this trophic effect remains unknown, however.

Feeding is one of the physiological stimuli for the release of gastrointestinal hormones, namely of cholecystokin. As far as we know, the influence of a meal on pancreatic or gall bladder growth has never been investigated. In the present study, the effect of feeding on cell proliferation in the murine gall bladder epithelium was studied by autoradiography.

Material and methods. 80 C57 black mice weighing 21-23 g were randomly subdivided into 2 equal groups. The animals in the 1st group were allowed free access to food after a 48-h period of fasting. The animals in the 2nd (control) group were allowed free access to food throughout the experiment without any preliminary fasting period. Ten animals from each group were killed simultaneously by neck dislocation at 0 h, 12 h, 20 h and 28 h, after refeeding the mice of the fasting group. The total food intake by each group was measured during these periods. The hours of killing were 12.00 h (0-h interval), 24.00 h (12-h interval), 08.00 h (20-h interval), and 16.00 h (28-h interval). All the animals were given an i.p. injection of 1 µCi/g b.wt of tritiated thymidine (Radiochemical Center, Mol, Belgium, sp. act. 16 Ci/mM) 1 h before killing. Cholecystectomy was performed after ligation of the cystic duct with



Mean labeling indices (± SEM) in the gall bladder epithelium of mice at intervals after refeeding and in control mice killed at the same hour. Labeling index values were higher after refeeding the animals: * $p < 0.05$; ** $p < 0.01$.

0000 silk. Each ligated gall bladder was fixed for 4 h in 10% neutral formalin. Thereafter, the gall bladder was opened, and after an additional 24-h fixation at 4 °C the specimens were embedded in paraffin. Transverse sections, 3–4 µm thick, were taken from the midportion of each gall bladder. Sections were covered with photographic emulsion (Ilford K5), developed after 16 days exposure at 4 °C (Kodak Dektol Developer) and stained with haematoxylin-eosin. In each gall bladder, the percentages of labeled cells (labeling index) and of mitoses (mitotic index) were counted on a total of 2000 consecutive epithelial cells.

Analysis of variance was used to test the statistical significance of the data.

Results. There was no difference in body weight between both groups of mice before the experiment was started. A significant loss of weight after 48 h of fasting was noted, whereas the body weight increased after free access to food. The weight of food intake of the fasting animals during refeeding was 67% higher than this value in the controls.

At the 0-h mark, the difference in labeling indices between fasting and control animals did not reach statistical significance. After refeeding, a considerable increase in DNA synthesis activity was found at 12 h ($p < 0.01$), and this effect was still significant at 20 h ($p < 0.05$) and at 28 h ($p < 0.05$) (fig.). Mitotic indices (table) were also higher in the refed group, but the difference was significant ($p < 0.05$) only at the 12-h mark.

Discussion. Our data indicate that proliferative activity in the gall bladder epithelium is stimulated by refeeding mice after fasting; not only DNA synthesis activity but also mitotic indices were increased during refeeding. The fact that no significant difference in the kinetic parameters was found between normally fed mice and fasting animals suggests that the observed postprandial burst in proliferative activity may not occur in normal circumstances, when physiological eating habits are maintained. Food intake by the animals after 48-h fasting period was higher than in the controls; the stimulus was more intense than in the controls, and it was more intense than it was after a normal meal. The rapid increase in DNA synthesis may also indicate that a number of cells in a resting phase of the cell cycle were probably synchronized to enter the DNA synthesis phase after refeeding.

A wide series of gastrointestinal hormones is known to be released after the meal, and cholecystokinin is only one of them. Caerulein, its synthetic analogue, has been recently shown to stimulate DNA synthesis in the epithelium of the gall bladder[7]. This observation may lead to the hypothesis that cholecystokinin is the hormone determining the postprandial increase in proliferative activity. However, the influence of other hormones cannot be excluded by the present data. Gastrin[8], secretin[8], and pancreatic polypeptide[9] are known to exert a trophic influence on the pancreas. The effect of these polypeptides on the gall bladder epithelium is unknown at the present time. On the other hand, feeding provokes a vagal stimulation, and the effect of vagal stimuli on cell proliferation has not been investigated. It is probable that a complex system of humoral and neurogenic influences is involved in the postprandial stimulation of cell renewal in the gall bladder.

Mitotic index values (‰) in gall bladder epithelium of mice refed after fasting (group 1) and of non fasted, normally fed controls (group 2)

Time after refeeding	Mitotic index (‰) Group 1	p - value	Group 2
0 h	0.05 ± 0.05	NS	0
12 h	0.55 ± 0.17	< 0.05	0.05 ± 0.05
20 h	0.35 ± 0.20	NS	0
28 h	0.20 ± 0.08	NS	0.10 ± 0.07

1 Acknowledgments. This work was supported by the 'Fonds voor Geneeskundig Wetenschappelijk Onderzoek' and by the 'Algemeene Spar- en Lijfrentekas'.

2 Address for reprint requests: Department of Surgery, Akademisch Ziekenhuis, V.U.B., Laarbeeklaan, 101, B-1090 Brussel (Belgium).

3 T. Whalin, G.D. Bloom and A. Danielsson, Cell Tissue Res. *171*, 425 (1976).
4 V. Erspamer, Gut *11*, 79 (1970).
5 T.E. Solomon, M. Petersen and M.I. Grossman, Scand. J. Gastroent. *13*, 172 (1978).
6 D.L. Mainz, O Black and P.O. Webster, J. clin. Invest. *52*, 2300 (1973).
7 Ph. Putz, L. Bazira and G. Willems, Experientia *36*, 429 (1980).
8 A.B. Dembinski and L.R. Johnson, Endocrinology *106*, 323 (1980).
9 G.R. Greenberg, Mitznegg, S.R. Bloom, Experientia *33*, 1332 (1977).

Independence of circulating insulin levels of the increased glucose turnover in shivering dogs

Y. Minaire, J. Forichon, M.J. Jomain and G. Dallevet

Laboratoire de Physiologie (L.A. CNRS 181), Faculté de Médecine Lyon Nord, F-69373 Lyon Cédex 2 (France), 25 September 1980

Summary. In dogs, selective insulin deficiency induced by simultaneous somatostatin and glucagon infusion does not alter the high rate of glucose utilization provoked by acute cold exposure. However, both in resting and in shivering dogs, lowering of plasma insulin decreases plasma glucose metabolic clearance significantly.

Shivering is usually not mentioned in papers devoted to the influence of insulin upon glucose utilization by muscular activity[1]. However, shivering, which plays a major part in cold-induced thermogenesis in large animals, shows many similarities to running, with respect to glucose production and utilization[2]. Recently, Therminarias et al.[3] suggested that a non-increase in plasma insulin concentration was responsible for depressed shivering and hypothermia observed in dogs immersed in cold water; they suggested that in spite of hyperglycemia, glucose uptake by shivering muscle might be limited because of an epinephrine-induced inhibition of insulin secretion. Actually, little information on glucose uptake can be obtained by just measuring plasma concentrations. Using labelled glucose, we pre-