figures in the case of the controls were 103 mg/100 ml and the pressurized were 115 mg/100 ml. It is clear that compression at 48 kb in the solid state does not materially affect the biological activity of insulin.

The presence of water has been suggested to have a profound influence upon the course of denaturation of proteins at high pressure. In order to test the possible influence of this phenomena upon the present system, insulin in concentrated solution (pH 2.90, acetate buffer) was compressed for 1 h at 50 kb and ambient temperatures. After this treatment it was found that the hormone was fully active as measured by the rat assay as described above.

We believe insulin to be a unique example of a protein which is able to withstand very high pressures without apparent effect. All other biological macromolecules are denatured by such treatment, even in the solid state. The relative rigidity of the molecule in spite of its substantial degree of association combined with its small size may account for these properties.

Effect of parathyroidectomy on the fasting-refeeding response in the rat colon[1]

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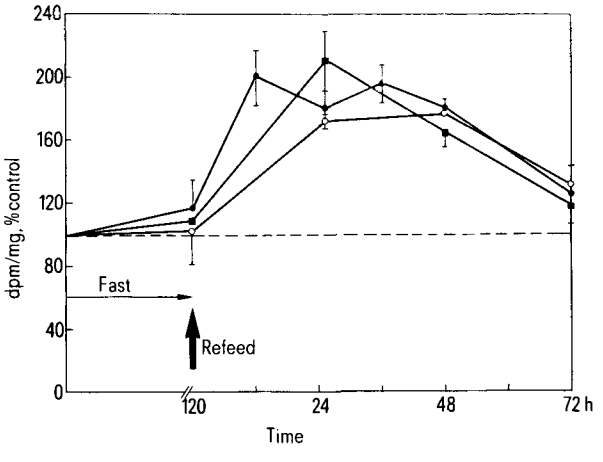
Summary. Following fasting and refeeding, the colonic epithelium of the rat exhibits a marked hyperplasia. This response is of a similar magnitude but of a longer duration to that observed in mice. This response is not affected by reducing serum calcium levels to those reported to alter normal tissue proliferation in vivo.

Previous studies have shown that cell renewal of the colonic epithelium is markedly sensitive to dietary manipulation. Following a period of fasting, colonic cell production in the mouse is reduced by a factor of 2, with subsequent refeeding producing a 4fold increase in S-phase cellularity[3]. This response requires both physical[4] and nutritional[5] factors in the refeeding diet. Among th latter, a distinct requirement for dietary minerals is noted. Animals refed with nutritionally complete, but mineral free, diets fail to undergo the colonic hyperplasia which is seen with the refeeding of a mineral replete diet. These non-injurious alterations of intestinal cell production present a unique tool to examine control mechanisms governing cell production in this system. Previously reported observations have implicated serum calcium as a regulator of cell proliferation in both normal and irradiated bone marrow[6–8] and thymus[8–10] and the liver following a partial hepatectomy[11,12]. These observations, and the mineral requirement of the colonic refeeding response, suggest a potential role for calcium as a regulator of this response, as well as intestinal cell renewal. The purpose of the present study was 2fold: a) to examine the colonic fasting-refeeding response in the rat; b) to evaluate the role of altered serum calcium levels, as established by a parathyroidectomy procedure as a regulator of this response.

Materials and methods. Sprague-Dawley/Zivic-Miller female rats (125–150 g) were used throughout (Zivic-Miller, Pittsburgh, PA). All animals were housed singly in metabolism cages to prevent the consumption of bedding and/or fecal material. Parathyroidectomized rats, with intact thyroids, and sham-operated animals were obtained from Zivic-Miller 24 h after surgery, and allowed an additional 48–72 h acclimation period following delivery. During this period, all animals received laboratory chow (Purina) and distilled water ad libitum.

Food was removed from the animals, beginning at 14.00 h, for a period required to reduce the b.wt by 20% (120 h), with water supplied throughout the fast. Animals were then refed, ad libitum, with laboratory chow. At times after refeeding, colonic prolifative activity was determined employing the technique of Hagemann et al.[13]. Briefly, the animals received a single i.p. injection of 50 μCi tritiated thymidine (^{3}H-TdR) $^1/_2$ h prior to sacrifice by



Effects of fasting and refeeding in the colon of control (●), parathyroidectomized (■) and sham-operated (○) rats. Mean of 10 rats per point ± 1 SE.

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cervical luxation, under ether anesthesia. Samples of the ascending colon were removed, weighed, fixed in Clarke's solution to remove unincorporated thymidine and the samples counted employing standard liquid scintillation techniques. The resultant data was expressed as disintegrations per min per mg of tissue (dpm/mg). This technique is based on the observation that over 95% of the cells which incorporate ^{3}H-TdR in detectable amounts are the S-phase proliferative cells of the intestinal mucosa[14]. The dpm/mg parameter thus provides a reliable estimate of S-phase cellularity. Individual serum calcium levels were determined from samples taken via the jugular vein immediately prior to sacrifice, employing standard atomic absorption procedures.

Results and discussion. The colonic response to fasting and refeeding in rats begins within 12 h after refeeding, reaching a maximum between 24 and 48 h and regaining control levels by 72 h. This contrasts with the response in the mouse[3], where maximal response is observed between 12 and 24 h with a duration of 36 h. Additionally, the rat exhibits no significant depression in thymidine incorporation per mg tissue after fasting, as is the case with mice[3].

Total serum calcium content in sham-operated and parathyroidectomized (PTX) rats during dietary manipulation (mg%) ± 1 SE.

	Sham	PTX
Control	10.09 ± 0.18%	4.06 ± 0.23%
120 h fast	9.37 ± 0.46%	4.23 ± 0.25%
24 h refeed	9.75 ± 0.05%	4.17 ± 0.07%
48 h refeed	10.35 ± 0.09%	4.44 ± 0.22%
72 h refeed	9.80 ± 0.10%	4.14 ± 0.18%

The parathyroidectomy procedure employed here significantly reduced the serum calcium levels (table), while the sham-operated animals showed no difference from control. Animal survival in PTX-animals exceeded 80% following fasting. Parathyroidectomy did not in itself alter colonic cell proliferation when compared to sham-operated control mice.

Parathyroidectomized rats exhibited a refeeding response equivalent in both duration and magnitude to that seen in sham-operated and control rats. The serum calcium levels attained via parathyroidectomy in the present study were equivalent to those which restricted bone marrow, thymus and liver proliferation in vivo[7,9,11]. This suggests that the colonic response is not mediated through serum calcium. The exact nature of the dietary mineral requirement in the colonic refeeding response is currently under investigation.

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Oxidative activity during the sexual cycle of the central nervous system, adrenal glands and ovaries in the hamster (*Mesocricetus auratus*)

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Summary. The results indicate significant increases of the oxidative metabolism in the oestrus of the ovaries, hypothalamus and the posterior cortex, while in the amygdala this increase occurs in the phase of diestrus.

In the rat it is known that the hypothalamus regulates the secretion of the anterohypophysis and that there exists a close relationship between the oxidative metabolism of the hypothalamus and the secretion of gonadotrophines[1,2]. We know, the participation of the limbic system in the axis hypothalamus-hypophysis-gonad[3–5] and that the gonads show cyclical variations in their oxidative metabolism[6]. Recently[7], it has been shown that in the rat there seems to exist a possible participation of the posterior cortex (latero-occipital) in the control of the sexual cycle. There are numerous works[8,9] which note that the neuroendocrinal processes of the hamster seem to be different from those of the rat and other species of vertebrates. This information moved us to verify if the previously mentioned structures, which in the rat experience cyclic changes in their consumption of O_2, in the hamster experience the same changes as an index of their participation in the regulation of the sexual processes.

Material and methods. 30 female hamsters, whose weight varied from 130 g to 146 g, were used. They were fed 'ad libitum' the standard diet of the Interfacultative Department of Physiology of the University of Oviedo, with free access to drinking water. The light (12 h light, 12 h dark), temperature (23 ± 3 °C) and absolute humidity were controlled. The selection of animals was made following the study of the vaginal cytology, and only those which had complete cylces of 4 days were used. They were decapited and the following materials were dissected in accordance with Hoffman and Robinson[10]: hypophysis, hypothalamus, amygdala, posterior cortex (latero-occipital) and the septal area. Additionally, the ovaries and adrenal glands were extracted and weighed. The glucose level in the blood was determined by glucose oxidase method.

One oxidative metabolism (consumption O_2) was determined by Warburg's Manometric Method[11]. This method was used because of the abundant evidence which shows the close relationship between the oxidative metabolism of the areas of the CNS and the ovaries with the secretion of gonadotrophines[1,4]. The statistical treatment of the results was done in accordance with the test 't' of Fisher and Yates[12].

Results. Table 1 shows the results of the oxidative metabolism of the different structures studied. As we can see, the ovary, the hypothalamus and the posterior cortex suffer a significant increase in the phase of estrus, while amygdala experience it in the phase of diestrus; however the hypophysis, septal area and the adrenal glands did not experience changes through the sexual cycle. Table 2 reflects the values of the weight of the ovaries and adrenal glands (mg) as well as the glucemias in the phases of estrus and diestrus. Taking into account that there is no significant difference in the total weights of