Anomalous stability of insulin at very high pressure[1]

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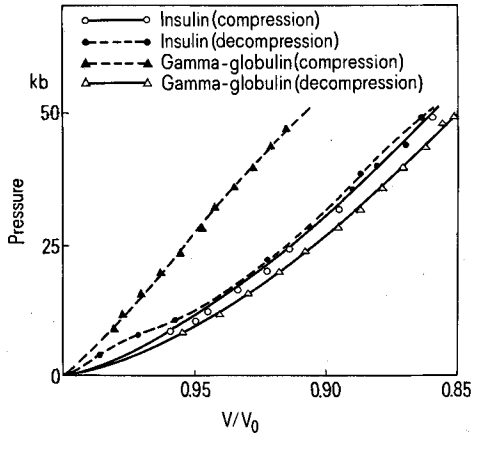
Summary. Insulin is unaffected by pressures of 48,000 b in the solid state at room temperature, as elucidated both by spectroscopic measurements and bioassay. Its compression curve is reversible. The presence of water does not alter this property. Of a number of other proteins investigated insulin appears to be unique with regard to its pressure stability. The relative rigidity of the molecule combined with its small size may account for some of these properties.

High pressures are generally acknowledged to have a denaturing influence upon biologically important systems[2,3]. The precise nature of the mechanism is still not well understood, although recent studies[4] have established the thermodynamics of the process in metmyoglobin. In no case has any protein been found which will withstand pressures in excess of 10 kb without significant physical or chemical alteration[5,6]. We wish to report the anomalous stability of insulin at 48 kb using both biological and chemical criteria to characterize the integrity of the material.

Materials and methods. Zinc insulin (Calbiochem, 22.7 units/mg) was packed tightly into a thin-walled cylindrical sample capsule of dimensions 1.00″ by 0.250″. The container was wrapped with 0.001″ indium foil to reduce friction, and was compressed in a standard piston-cylinder apparatus as has been described previously[7,8]. Briefly, pressure is generated by driving a tungsten carbide piston into a similar sample vessel with the aid of a 500-t hydraulic ram. All carbide components are massively supported with tool steel. The system is capable of producing a sustained hydrostatic pressure in excess of 65 kb. Pressure-volume measurements may be quantitated with the aid of a linear displacement transducer and an oil line pressure transducer coupled to the main ram. Reagent grade sodium chloride is used as a calibration standard.

Results. Upon compression and decompression of insulin to 48 kb it was found that the decompression curve almost perfectly matched the compression curve. The resultant pressure-volume plot is displayed in the figure. The small deviation at low pressures may be ascribed to frictional losses within the sample assembly. This apparent reversibility was not observed for methemoglobin (equine), metmyoglobin (equine), or gamma-globulins (human), all in the solid state under identical conditions. The extent of irreversible behavior is particularly striking in the latter case and its P-V plot is illustrated in figure 1 for comparative purposes.



Plot of pressure against relative volume showing the compression and decompression of insulin and gamma-globulins.

Linearization of the P-V data yields a bulk modulus of 275 kb for insulin. This value appears to be fairly typical of the other proteins investigated. Clearly this is a remarkably high bulk modulus for an organic compound. It is believed that this is a result of the true 3-dimensional structure of the protein which is not approached to a significant extent in synthetic organic polymers and molecular crystals[9].

After application of 48 kb of pressure for 1 h insulin was unaffected in its physical appearance. The color of the material was the same as the control and the compressed protein was readily soluble in 0.05 M pH 2.90 acetate buffer. The UV and circular dichroism spectra of the solution of compressed material, were identical to that of the control of equivalent concentration[10]. At this low pH insulin is unassociated[11], so conclusions cannot be drawn as to pressure induced perturbations in quaternary structure.

Insulin was bioassayed by measuring the drop in plasma glucose of the rat[12] resulting from the administration of a preparation of known activity in comparison with the compressed material. Healthy male rats of uniform age and weight were given 0.57 mg of the hormone i.m. in 1.0 ml of acetic acid, pH 3.0. After 40 min the substance caused a drop in plasma glucose[13] of 75 mg/100 ml in both groups of rats receiving the pressurized and control materials, within experimental error. After 160 min the

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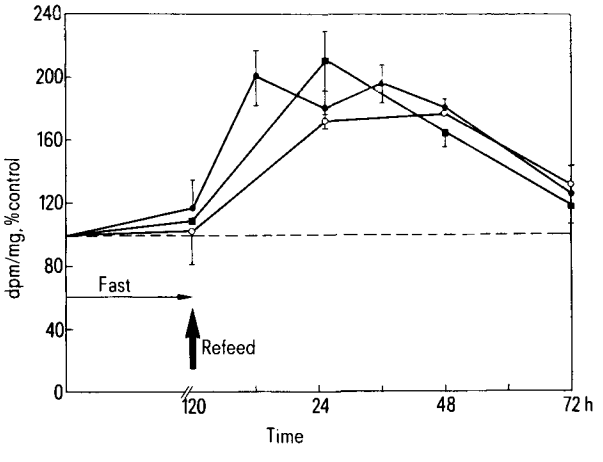
Summary. Following fasting and refeeding, the colonic epithelium of the rat exhibits a marked hyperplasia. This response is of a similar magnitude but of a longer duration to that observed in mice. This response is not affected by reducing serum calcium levels to those reported to alter normal tissue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