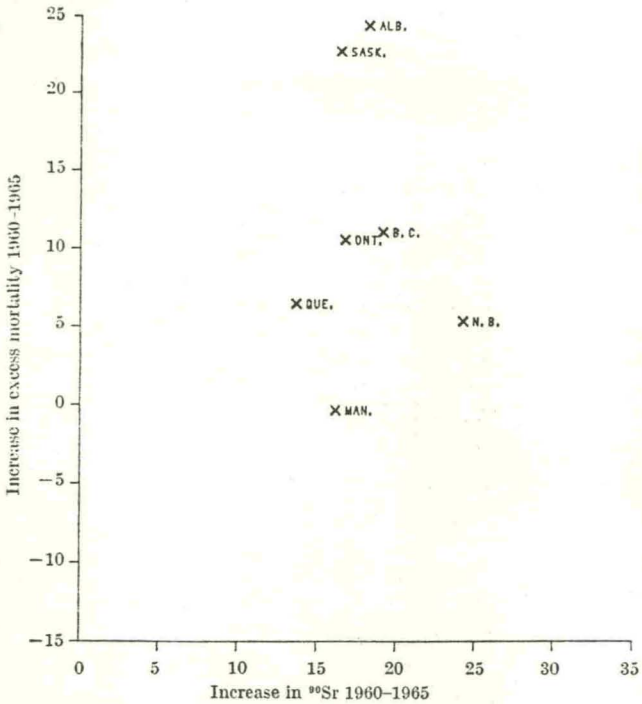

Fig. 2. Change in infant mortality and change in strontium-90 level, calculated using 1946–55 as the basis for the least-squares line. (Least-squares estimate of slope is $b = -0{\cdot}12$.)

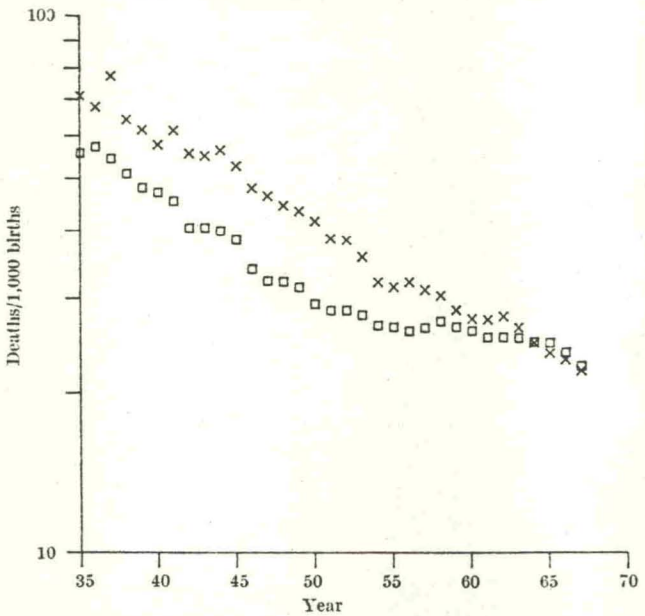

Fig. 3. Infant mortality in Canada and the US. ×, Canada; □, US.

We thank Mr R. G. McGregor for providing the Canadian data on strontium-90 and Dr A. Irwin for assistance in locating and interpreting both these and mortality data.

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Influence of Hydrostatic Pressure on the Reversible Polymerization of Fibrin Monomers

The conversion of fibrinogen to fibrin, which is the last stage of the complex process of blood coagulation, is composed of three successive reactions[1]: an enzymatic hydrolysis by thrombin of four arginyl-lysyl bonds per molecule of fibrinogen, yielding a fibrin monomer; a spontaneous polymerization of monomers to form a gel or fibrin clot; and an enzymatic transamidation by fibrin stabilizing factor (factor XIII or fibrinase) between ε-amino groups of lysyl and glutaminyl side chains. The mechanism of the reversible polymerization of fibrin monomers remains largely unknown, although it can be studied separately because fibrin monomers can be stabilized in acidic media of high ionic strength such as 1 M NaBr, *p*H 5·3[2].

The hypothesis of hydrogen bonding between ionizable groups, in which histidyl functions as acceptor and tyrosyl or lysyl as donors, as proposed by Sturtevant *et al.*[3], accounts for the depolymerizing effect of dilution, of slight temperature increase and of salts and urea. It also explains the *p*H range of polymerization (between 5·5 and 10·5), the *p*H evolution during the reaction (proton liberation below *p*H 7·5) and the inhibiting effect of chemical blocking of histidyl and tyrosyl residues. The only real shortcoming of this hypothesis is its failure to account for the magnitude of the heat evolution during polymerization[4]. It seems that additional heat evolving reactions such as hydrogen bonding between non-ionizable groups, ion pair bonding, hydrophobic bonds or conformational changes should also be involved. Up to now, however, no sufficient independent evidence for either of those additional reactions has been presented.

The origin of the heat evolution in reversible fibrin polymerization has become an interesting problem since a number of other cases of exothermic protein polymerizations, including flagellin, β-lactoglobulin and lactic dehydrogenase, have been discovered.

A study of the influence of hydrostatic pressure on the polymerization equilibria gives the sign and magnitude of the volume increment, which is dependent on the nature and the number of the interactions involved in polymerization. Volume changes during polymerization have been noted before, for example in tobacco mosaic virus protein[5] and in myosin[6].

Bovine fibrinogen was prepared according to the method of Blombäck and Blombäck[7]; fibrin monomers were prepared according to Donnelly[2] as modified by Endres[4].

A technique for observing association and aggregation reactions of proteins by means of light scattering measurements under high pressure has recently been developed by Putzeys and Vaneghem (unpublished thesis). We were able to use the apparatus and technique in the Laboratory of Biochemistry I. Light scattering measurements were taken at 90° and under pressure up to 3,200 kg/cm². Temperature variations resulting from application of pressure were prevented by a water jacket, maintained at 25° C.

Fibrinogen and fibrin monomer solutions showed no change in light scattering under pressure up to 3.200 kg/cm² except for a small stepwise change during the application of pressure, caused by deformation of the windows in the optical path (Fig. 1A).

Intermediately polymerized fibrin monomer solutions of 10 mg/ml. in 1 M NaBr, 0·05 M acetate buffer, with a *p*H varying from 5·75 to 6·15, showed a large decrease in light scattering under pressure (Fig. 1B–E) and were apparently already completely depolymerized under a pressure of 2,500 kg/cm², for after equilibration a further increase did not change the amount of scattered light. The polymerization–depolymerization reaction was always completely reversible. The influence of the concentration

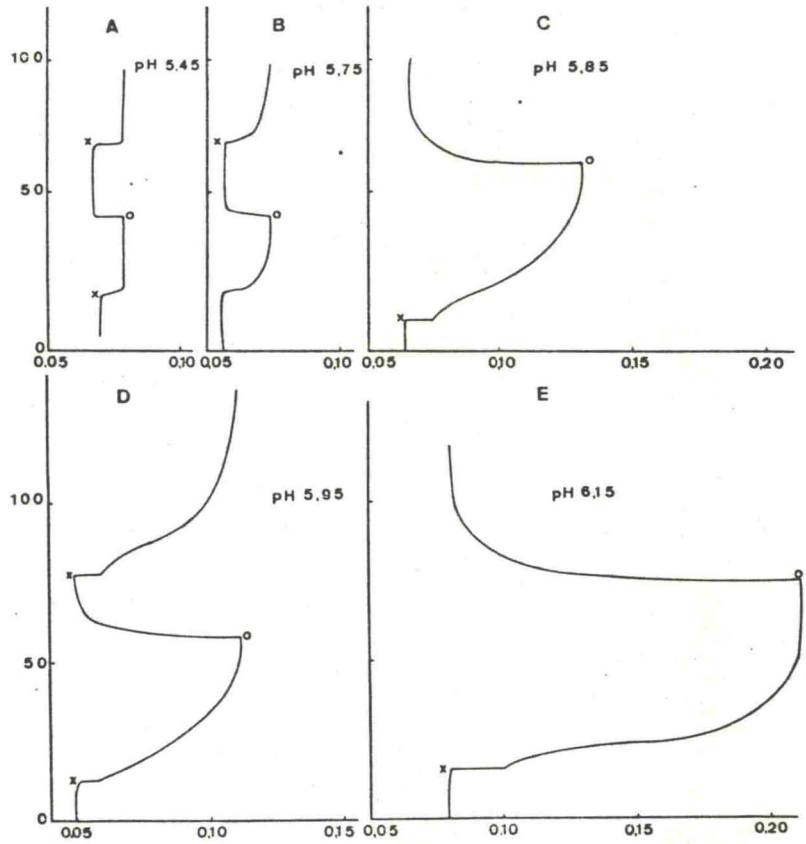

Fig. 1. Influence of pH on the polymerization of fibrin monomers in 1 M NaBr; abscissa, photomultiplier current in μA; ordinate, time in min; ○, application of pressure of 2,500 kg/cm²; ×, release of pressure. The solutions were initially depolymerized by application of pressure.

of protein on the light scattering changes under pressure is shown in Fig. 2. Turbid clots[8], prepared by 20 fold dilution of 10 mg/ml. fibrin monomer solutions in phosphate buffer ($I = 0{\cdot}2$, $p\mathrm{H} = 6{\cdot}0$), depolymerized equally well under a pressure of 2,500 kg/cm².

Because the polymerization is very pH sensitive and the pH of the acetate buffer decreases under pressure (about 0·4 pH units at 3,000 kg/cm²) it was tested whether the depolymerizing effect of pressure could be ascribed to pH changes. The experiments were therefore repeated in a 0·05 M ammonium acetate buffer (decrease of about 0·1 pH units at 3,000 kg/cm²) and in 0·05 M MES (N-morpholino - ethane - sulphonic acid) buffer (increase of about 0·25 pH units at 3,000 kg/cm²). In all cases an apparently complete depolymerization was obtained at 2,500 kg/cm².

Finally, it was tested whether the pressure effect could be ascribed to an alteration of the protein during the preparation of monomers. Native plasma, diluted with buffer to a concentration of 1 mg fibrinogen/ml. and clotted with thrombin, behaved identically under pressure.

On the basis of the complete reversibility of the depolymerization and polymerization reactions and in view of the fact that the phenomenon is the same in buffers with different pH dependence on pressure, we conclude that the polymerization is accompanied by an increase in volume. This volume increase is not explained by the hypothesis of hydrogen bonding between histidyl and tyrosyl or ε-amino groups as the only polymerization mechanism for this reaction in the pH range investigated should be accompanied by a volume decrease (resulting from hydrogen bonding and from electrostriction around the liberated protons).

In fact, our results indicate that during polymerization an additional interaction occurs with a significant volume increase sufficient to overcome the volume decrease due to hydrogen bonding and electrostriction. This seems to point in the direction of the existence of additional hydrophobic bonding or ion pair bonds (salt linkages)[9]. The role of hydrophobic bonding seems to be small in view of the depolymerizing effect of temperature and electrolyte concentration. Attempts to show the presence of hydrophobic sites on the surface by binding the ligand

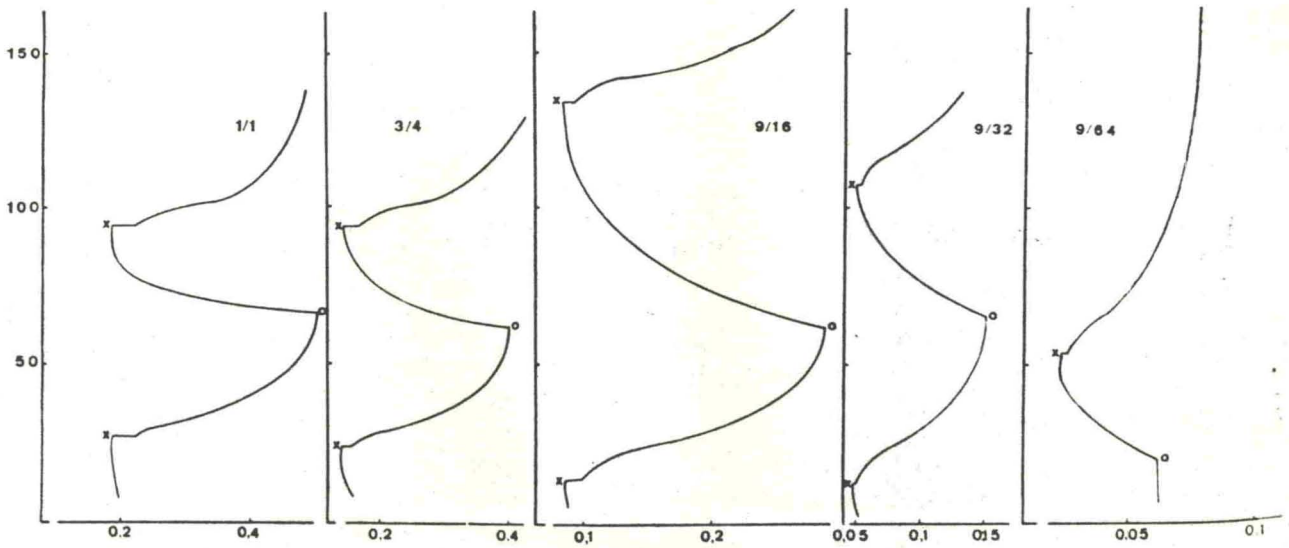

Fig. 2. Influence of concentration of protein on the polymerization in 1 M NaBr, pH 6·1. Abscissa, photomultiplier current in μA; ordinate, time in min; ○, application of pressure of 2,500 kg/cm²; ×, release of pressure. The solutions were initially depolarized by application of pressure. Dilution 1/1 = 10 mg fibrin monomers per ml.

ANS (1-anilo-8-naphthalene sulphonate)[10,11] were unsuccessful. Further studies to estimate the volume increase per link by quantitative determination of the effect of pressure on the successive polymerization constants will be undertaken.

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In vitro Production of Nucleolar Channel System by Progesterone in Human Endometrium

Electron microscope examination of normal human endometrium during the midsecretory phase of the menstrual cycle demonstrates that the nucleolus of the epithelial cell differentiates into an organelle variously termed a nucleolar canalicular system[1], a nucleolar basket[2] or a nucleolar channel system[3]. Such a structure appears if ovulation has occurred. Nucleolar baskets have been seen as early as the sixteenth day of a normal 28 day cycle. After this they may increase in number, reaching a maximum around the twentieth day and then disappear, not being found later in the cycle. They have not been seen in the proliferative phase of the cycle or in early pregnancy.

This system seems to be a unique form of nucleolar differentiation having been observed in human glandular endometrial cells and in no other tissue. It consists of an involved labyrinth of channels coursing through a more dense homogeneous matrix (Fig. 1). Electron opaque 150 Å particles are arranged in a regular fashion around the periphery of the total channel mass but may also surround the individual channels (our unpublished results).

Previous attempts to demonstrate the specific physiological effect of progesterone by means of organ culture were frustrated by the induction of secretory changes in the endometrial cell in the control culture[4]. Electron microscope examination of such organ cultures failed to show nucleolar channel systems[5]. Using organ culture of proliferative human endometrium it has recently been shown[6,7] that progesterone does act directly on the epithelium to convert proliferative to secretory endometrium. Thus the effects observed *in vivo* could be mimicked *in vitro*. This conversion was specifically due to progesterone and was not produced in the control cultures, in cultures with oestrogen alone or with cortisol. We undertook this study to find out whether nucleolar differentiation can be induced by progesterone in organ cultures of proliferative endometrium.

Specimens were obtained aseptically from normal women by endometrial biopsy on the tenth day of the menstrual cycle. A small piece was processed for electron microscopy and another was examined histologically. The remainder of the endometrium was cut into pieces 1–2 mm³. Each of six pieces was placed on 'Millipore' filter paper resting on agar enriched with TC 199 medium to which 10 per cent calf serum and 10 μg/ml. insulin had been added. In addition to control cultures, cultures containing progesterone (1 μg/ml. and 10 μg/ml.), medroxyprogesterone acetate (1 μg/ml. and 10 μg/ml.), oestradiol (10 μg/ml.) or oestradiol (10 μg/ml.) plus progesterone (10 μg/ml.) were prepared. These were incubated at 37° C in a moist atmosphere of 95 per cent oxygen and 5 per cent CO_2. Specimens were examined both histologically and by electron microscopy after 48 and 96 h. For electron microscopy, a small portion of the tissue was fixed in 3 per cent glutaraldehyde in phosphate buffer (*p*H 7·0) at room temperature for 1 or 2 h. After re-fixation for 1 h in 1 per cent veronal buffered osmium tetroxide and subsequent dehydration, the specimens were embedded in 'Epon 812'. Thin sections were double stained with uranyl acetate and lead citrate and examined in an Hitachi HU-11B electron microscope. At least two tissue blocks containing representative samples of endometrium were examined from each specimen.

The histological appearance of the control cultures resembled that of the original proliferative endometrium. Oestrogen produced no significant change. The addition of progesterone produced the changes characteristic of secretory endometrium, first subnuclear deposition of glycogen, then secretion of the glycogen into the lumen of the glands.

Electron microscope examination of the original specimen showed the characteristic appearance of proliferative endometrium (ref. 8 and our unpublished data). No nucleolar channel systems were seen. Extensive search failed to reveal nucleolar channel systems in the control cultures and none were found in the cultures exposed to oestradiol. Characteristic nucleolar channel systems were produced in the cultures containing progesterone (Fig. 2). They were found after 48 and 96 h at both progesterone concentrations but it was not possible to quantitate the results in terms of length of exposure to, or concentration of, progesterone. Although our results suggest that differentiation may be accelerated *in vitro*, such a conclusion is not justified without further study. Nucleolar baskets were also produced in the culture

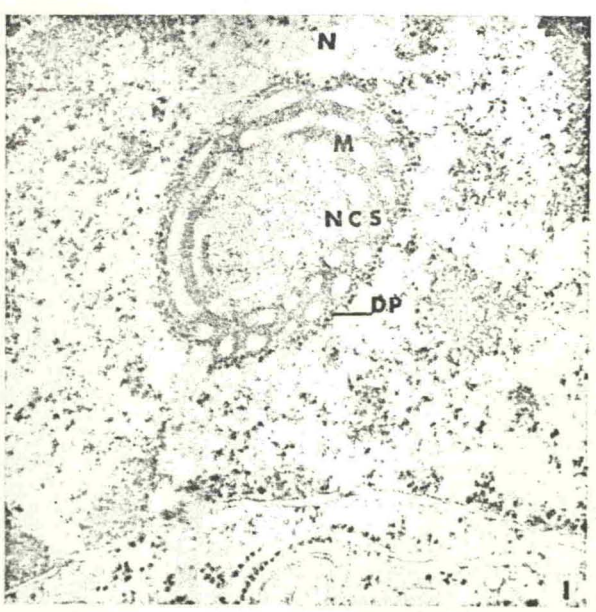

Fig. 1. Electron micrograph of endometrial epithelial cell from a biopsy specimen taken on the eighteenth day of a normal 28 day cycle. A nucleolar channel system (NCS), characteristic of secretory stage endometrium, is seen within the nucleus (N). The channels course through a dense matrix (M). Dense particles (DP) are seen at the periphery of the nucleolus. (×24,375.)