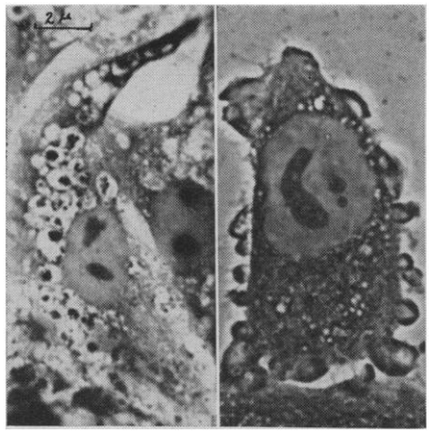

Fig. 1 (left). Cytoplasmatic vacuoles in human aortic intimal cells produced by trivalent hexamminecobaltic chloride. (Right) Intranucleolar granules in human aortic intimal cells produced by hexavalent hexol nitrate.

Half of the cultures were incubated for 12 hours after the drug was added, rinsed twice in Hanks' solution, and maintained in normal culture medium for the rest of the incubation time. As is shown in Table 1, different morphological nuclear and cytoplasmatic changes were observed in the aortic intimal cells and in HeLa cells at various concentrations of the salts. Trivalent hexamminecobaltic chloride at concentrations of 5 μg/ml produced in aortic intimal cells large cytoplasmatic vacuoles containing metachromatic granules after 12 hours of incubation, but no nuclear changes (Fig. 1, left). Such effects were not observed in the HeLa cells under similar conditions. Both cell types show growth inhibition and typical cytotoxic effects at concentrations of 100 μg/ml.

Table 1. Effect of various salts on human cell cultures.

Concn. (μg/ml)	Human aortic intimal cells		HeLa cells	
	Cytoplasmatic	Nuclear	Cytoplasmatic	Nuclear
	$Co(NH_3)_6Cl_3$			
1	±	−	−	−
5	+	−	−	−
25	++	−	−	−
50	+++	−	−	−
75	+++	−	−	−
100	+++	±	±	±
	$Co(OH)_6(Coen_2)(NO_3)_6$			
1	−	±	−	−
5	−	+	−	−
25	±	++	−	−
50	++	+++	−	±
75	+++	+++	±	+
100	+++	+++	+	++
	$CoCl_2$			
250	±	−	±	−
	$Ni_2(NH_3)_6Br_2$			
200	±	−	±	−

Hexavalent hexol nitrate (5 μg/ml) produced intranucleolar granules but no cytoplasmatic changes in human aortic intimal cells. At concentrations of 50 μg/ml these granules were very abundant and were accompanied by formation of large bubbles in the cell membrane but no vacuolization (Fig. 1, right). The nucleolar changes persisted for up to 120 hours of cultivation if these cells were exposed to the salt for 12 hours. The salt did not inhibit their mitotic index or prolong their generation time. In HeLa cells this salt produced nuclear picnosis, growth inhibition, and cytolysis, at concentrations of 100 μg/ml.

Control tests carried out with cobaltous chloride at similar concentrations showed growth inhibition with 250 μg/ml in both cell types without any of the morphological changes described above. Nickelous ammonium bromide produced similar results at concentrations of 200 μg/ml.

The effects of trivalent hexamminecobaltic chloride in aortic intimal cell cytoplasm can be interpreted as the induction of dilated vacuoles of endoplasmic reticulum with metachromatic granules. They are of interest because such findings have not been observed in HeLa cells, which in our experience have never shown production of mucopolysaccharides in tissue culture. No mitotic changes like those described for cobalt nitrate and other sulfhydryl reagents (*10*) were observed in any of these two cell lines. The effects of the hexavalent salt in the nucleolus and its persistence after removal of the salt from the culture medium are particularly pertinent considering the very low concentrations of this salt required to react in vitro. The action of these cobalt salts seems to be different from those observed by Levy *et al.* (*11*) with cobaltous sulfate in bacteria (*Proteus vulgaris*), which was able to arrest protein synthesis without halting RNA production.

Our results suggest that at very low concentrations trivalent cobaltous salts act upon cytoplasmatic RNA and sulfated mucopolysaccharide while the hexavalent salt reacts more specifically with nuclear RNA. These results support the hypothesis that complex cations capable of reacting with polyanions in solution exert morphological effects upon living cells at sites where polyanions are present, their degree and site of action depending on the cell type used and on the ability of the cells to produce mucopolysaccharides in vitro (*12*).

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Hypothesis Concerning the Role of Follicular Contractions in Ovulation

Abstract. Autotransplants of ovarian tissue in the anterior chamber of the eye may be studied in the lightly anesthetized rabbit. Individual follicles in such implants, consisting of two to five follicles, have been observed to contract after subcutaneous injection of urine of pregnant women, after application of rat pituitary homogenate to the cornea, and 9 to 10 hours after cervical stimulation.

Although the presence of smooth muscle in the theca externa of the ovarian follicle of the rabbit was first reported by Thomson (*1*), neither he nor Guttmacher and Guttmacher (working with the sow) (*2*) succeeded in demonstrating functional activity in this muscle. The Guttmachers in 1921 attempted to induce the smooth muscle of the sow's ovarian theca externa to contract by means of electrical excitation and application of acid, alkali, and barium chloride solutions. These stimuli, where successful, were unphysiological, and although these workers suggested that the follicular muscle coat might be involved in the mechanism of ovulation, they failed to substantiate their hypothesis.

The observations reported here define a physiological role for the smooth muscle present in the theca externa. Such a role has been largely discounted because of two sets of experimental observations. Friedman (*3*) demonstrated that ovulation could occur in ovarian implants in the rectus muscle, and Hinsey and Markee (*4*) found that ovulation occurred in the denervated ovary.

Autotransplants of small numbers of ovarian follicles in the anterior chamber of the eye may be studied in the

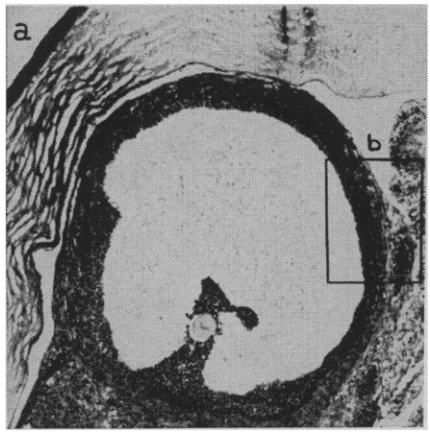

Fig. 1. Mature ovarian follicle in the anterior chamber of the rabbit eye. (*a*) Section through cornea; (*b*) section of wall shown in Fig. 2 (Gomori trichrome, about × 75).

lightly anesthetized rabbit. Implants were made into both eyes of several animals. Contractions were observed in one eye in each of two animals. The contractions involved individual follicles and caused changes in shape from the spherical to the elipsoidal form.

Implanted follicles grow, become filled with fluid, contain an ovum, and remain turgid for several months (Fig. 1). Figure 2 shows the organization of the follicular wall and the presence of spindle-shaped cells. We assume that these cells are the smooth-muscle cells that give rise to the contractions.

Contractions may be induced by several methods. Subcutaneous injection of 2 ml of ether-extracted urine obtained from pregnant women during their first trimester induced the appearance of contractions from 8 to 180 minutes after administration of the urine. The frequency of contraction was between 1 and 5 per minute. It is well established that ovulation in the rabbit occurs 9 to 10 hours after either mating or cervical stimulation (*5*). Nine hours after electrical stimulation of the cervix contractions appeared and persisted for approximately 2 hours. Marked contractions followed the application of a homogenate, prepared from an acetone-extracted rat pituitary, to the surface of the eye. This response is not obtained by direct application to the cornea of one drop of 1:100,000 epinephrine, one drop of 0.1-percent solution of acetylcholine, or one drop of 1 USP unit (oxytocic activity) of posterior pituitary extract. Hence it is very likely that the force responsible for the delivery of the ovum from the follicle is the series of contractions that pass over the ripe ovarian follicle, and that these are induced as a result of the release of luteinizing hormone.

A complete hypothesis of ovulation probably involves (i) release of the ovulation-inducing hormone, tentatively assumed to be luteinizing hormone (*6*); (ii) modification of the germinal epithelium by proteolysis (*7*); (iii) rapid swelling caused by increased secretory activity or depolymerization, or both, of the constituents of the liquor folliculi with a consequent increase in osmotic pressure (*8*); and (iv) contractions of smooth muscle in the theca externa to assist the rupture of the thecal wall at the stigma and facilitate the ejection of the intrafollicular contents. Such a mechanism appears necessary to account for the continued ejection of the intrafollicular contents after the pressures caused by osmotic forces are neutralized after rupture (*9*).

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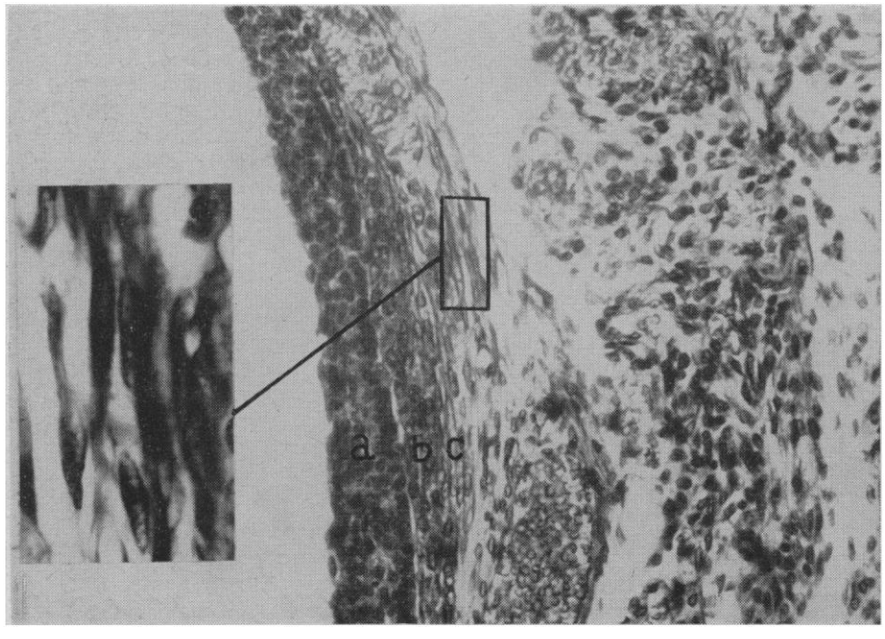

Fig. 2. Segment of thecal wall containing three layers: (*a*) granulosa; (*b*) theca interna; (*c*) theca externa (Gomori trichome, × 380). Inset shows *c*, at magnification of 1000, composed chiefly of spindle-shaped smooth-muscle fibers.

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23 December 1959

Finite Radiocarbon Dates of the Port Talbot Interstadial Deposits in Southern Ontario

Abstract. Three new finite radiocarbon dates suggest that (i) the thermal maximum of the Port Talbot interstadial occurred prior to 47,000 years before the present and (ii) the interstadial deposits were overridden by a glacial advance approximately 44,000 years before the present. To facilitate correlations with other areas, new rock-stratigraphic names are proposed for the Port Talbot type section.

New stratigraphic divisions of the last ice age, several of them older than the classical Wisconsin glacial stage, have been proposed by Dreimanis (*1*) since 1957. Unfortunately the radiocarbon dates of the principal new unit, the Port Talbot interstadial, were not finite. These dates (samples L-185A, L-217A, L-370A, L-440, W-100, S-7, and S-46; see *1*, *2*) ranged from older than 25,000 to older than 40,000 years. Therefore, several readers of the articles cited (*1*) and participants of the Friends of the Pleistocene 1959 field conference (*3*) have expressed doubt that this interstadial is younger than the last, or Sangamon, warm interglacial.

H. de Vries considered it worth while to try to obtain new radiocarbon dates, beyond the previous range of dating, at the Radiocarbon Laboratory of the University of Groningen. We collected gyttja from the Port Talbot interstadial site in the summer of 1958, but