ENDOMETRIUM IN SUB-FERTILITY

(A Histochemical Study)

by

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The range of histological changes, the normal endometrium passes through, during the various phases of the menstrual cycle, is too familiar. The purpose and innate significance of these structural alterations must be found in the underlying biochemical changes, concomitantly taking place. The histochemical study of the normal endometrium has established the close inter-relationship between the morphological variations and their physiological basis. In the investigations for sterility and related problems, our attention must be focussed on the histochemical behaviour of the enodmetrium as much as on its morphology.

Fats, proteins (like nucleo-proteins), carbohydrates (like glycogen), enzymes (like alkaline and acid phosphatase), minerals and vitamins have all been proved to be concerned in the intrinsic metabolism of the uterine mucosa. The work of Page et al. and Stuermer as also the observations of McKay et al. (1956) have established a definite cyclic pattern for most of the metabolic constituents in the normal endometrium. It is also known that carbohydrate metabolism is intimately bound with the process of implantation; the enzyme alkaline phosphatase plays a major role in this phase of activity. Besides, our knowledge of these factors is more precise and definite. So the presence and extent of glycogen and alkaline phosphatase was selected for study in cases of sub-fertility.

102 consecutive unselected cases of sub-fertility, admitted into the Gynaecological wards of Government Hospital for Women and Children, Madras, form the subject of this investigation. Of these, 81 were cases of primary sterility, 11 of relative sterility and 10 of habitual abortions. The age of these patients varied from 19-32 years and the duration of primary sterility, computed from the date of marriage, varied from 2-19 years. Specimens of endometrium were obtained by the usual procedure of D & C; the timing of the procedure was between the 15th and 30th day of the cycle

to ensure the obtaining of the sample during the expected advanced secretory phase. The material was fixed in chilled formalin, embedded in paraffin and sections were prepared in the routine manner as for histology. Glycogen was stained by the Periodic Acid Schiff McManus Method; this material takes a purple blue colour. The Calcium-cobalt Method of Gomory was employed to stain the alkaline phosphatase, in which the enzymes take a deep brownish black colour. Since the depth and intensity of the staining reactions indicate the amounts of glycogen as well as alkaline phosphatase present in the tissues, the results were read as "Good" and "Low".

In three cases, the results were inconclusive due to inadequate endometrial tissue. Of the remaining 99 cases, ovulation was not evident in 25 instances, the endometrium was proliferative in 14 cases and hyperplasic in 11 cases. The findings in this group are summarised in Table I.

It can be seen that over 80% of cases show good enzyme activity and low glycogen content, as is to be expected in the non-secretory endometrium.

Glycogen makes its appearance in the glandular epithelium shortly before ovulation and increases progressively in quantity during the secretory phase of the endometrium. It shifts into the lumina of the glands along with the secretion. It is also present in the secretion escaping from the surface of the endometrium immediately before menstruation. There is a slight reduction in the amount of glycogen in the epithelia of the glands at this time, but small amounts are now present in some of the large stromal cells and intercellular oedema fluid. A gradual increase of alkaline phosphatase is noticed in the epithelium lining the glands during the proliferative phase; the enzyme reaches its peak concentration shortly before ovulation. During the luteal phase there is a reduction of this substance in the glands, but appreciable amounts are noticed in the secretions inside the glands. In the pre-menstrual endometrium, however, the endothelial lining of the spiral arterioles shows fair amounts of alkaline phosphatase. The stromal cells remain poor in this enzyme throughout. These fluctuations are not seen in the basal layer of endometrium. The distribution of glycogen and alkaline phosphatase among 74 cases

TABLE I

Non-secretory Endometrium; Glycogen and Alkaline Phosphatase

	No.	Glycogen		Alkaline phosphatase	
		Good	Low	Good	Low
Proliferative endometrium	14	3	11	13	1
Hyperplasic endometrium	11	2	9	8	3
Total	25	5	20	21	4

of secretory endometrium is shown in Table II.

TABLE II

Glycogen and Alkaline Phosphatase Distribution in Secretory Endometrium

	Total	Good		Low	
		No.	percentage	No.	percentage
Glycogen	74	41	55.4	33	44.6
Alkaline phosphatase	74	61	81.5	13	18.5

Among 41 cases with good glycogen content, 7 cases show poor alkaline phosphatase and of 33 cases with low glycogen content, 6 showed low enzyme distribution. The alkaline phosphatase activity appears to be good in more than 80% of the cases both in the secretory and in the non-secretory groups; nor is there any appreciable difference, among the secretory endometria, related to their glycogen content. A good enzyme level is present in all growing epithelial cells and its presence in adequate amounts indicates "growth" and not "differentiation", the latter a functional phenomenon, taking place in the endometrium after ovulation.

The presence, in small quantities, of glycogen in the non-secretory endometrium proves that this by itself does not constitute evidence of ovulation. That among 74 cases of secretory endometrium 44.6% (33 cases) showed glycogen deficiency is significant.

The biochemical changes run parallel to the histological changes that we commonly observe in the endometrium; they are as much influenced and regulated by the ovarian hormones. Experimental evidence has shown that the basic pattern of histochemical behaviour can be reproduced in the ovariectomised rats by injections of oestrogen and progesterone. The absence of these changes in post-menopausal endometrium also points to this hormonal control.

The glucose circulating in the blood is converted into glycogen and stored in the glands as glycogen, which represents the most convenient and readily utilisable form of storage. The high concentration of alkaline phosphatase in the glands before ovulation is necessary in effecting this storage. At the time of implantation this is reconverted into a simple monosaccharide like glucose that could serve as an excellent nutritional basis for the blastocyst, before its actual implantation in the endometrium. Thus something analogous to the Cori's cycle in the muscle is taking place in the carbohydrate metabolism of the endometrium. Though a number of factors are concerned in this glycogen storage and conversions, the enzyme alkaline phosphatase is known to play an important role. The storage of glycogen is facilitated by the alkaline phosphatase already present in the endometrium during the proliferative phase; the presence of glycogen in the luminal and surface secretions and the re-appearance of the waning enzyme activity in the secre-

tions, both occurring near the end of the cycle, seem primarily designed to meet the requirements of the fertilized ovum both before and after implantation. The glycogen deficiency seen in this series must be an important contributory factor in the causation of sterility and abortion. How precisely this histochemical defect operates in making the endometrial environment unfavourable for the early ovum is at present speculative. Whether the endometrium is primarily at fault or there is a quantitative deficiency in the secretion of the hormones, pituitary and ovarian, that control the metabolism, is equally uncertain.

The accepted belief that a good glycogen content in the late secretory endometrium denotes its normal functional capacity has been recently questioned. Annoret and Latour, reporting their histochemical observations on endometria from 18 infertile women, found that 2 women in whom glycogen deficiency was observed, subsequently became pregnant. That quantitative biochemical estimations are more accurate and reliable than histochemical findings based on density and distribution of stainable material; and that a glycogen lack observed in one cycle does not hold good for other cycles are two other criticisms against this approach. Still at present it is widely believed a good glycogen level in the pre-menstrual endometrium is always favourable for successful implantation.

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