A SUGGESTION AS TO THE PROCESS OF OVULATION AND OVARIAN CYST FORMATION[1]

A PRELIMINARY REPORT

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It is generally conceded, that pressure atrophy of the ovarian stroma is the chief means by which the extrusion of the ovum becomes possible. During the growth and maturation of the ovum, the cells of the Graafian follicle, after increasing greatly in number, begin to disintegrate and liquefy. From then onward, due it is thought to the different chemical composition of the liquor, thus forming in the follicle, or of the general content of the follicle, an endosmosis seems to be induced by which the liquor folliculi increases to a far greater amount than is thought possible to result from the liquefaction of the follicular cells. The follicle so distends that, following the direction of least resistance, one side of it approaches the free surface of the ovary, producing a bulging in this surface, dispersing the ovarian stroma, thinning its tunica albuginea and the overlying epithelium, and results in a compression of the blood capillaries intervening between it and the surface of the ovary. Clark has shown that the capillaries in the summit of the bulging are practically obliterated by the pressure. It is supposed that nourishment thus cut off from the ovarian stroma under compression, the stroma atrophies till its resistance is less than the pressure exerted by the distending follicle and the content of the follicle bursts into the body cavity. The liquefying of the follicular cells having continued till the ovum is free within the follicle, the ovum is extruded into the body cavity with the discharge of the liquor folliculi.

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The question was suggested by Dr. Irving Hardesty as to whether the liquor folliculi does not exert some special digestive action upon the resisting tissues thus aiding in the process by which the ovum is extruded. The idea carried with it the action of the liquor folliculi in the phenomena of menstruation.

Accordingly, a series of experiments were made, the result of which may be of interest. The work was undertaken with the view to determine:

1) Whether the liquor folliculi has a digestive action and if so does it possess a specific enzyme that can be demonstrated by dialysis or other tests?

2) If it possesses such action, under what conditions is it altered? Is it decreased in pathological conditions?

3) If possessed, will a quantitative estimate of its strength indicate the amount of it present.

A review of the literature has failed to suggest any special chemical action of the liquor folliculi, and nothing of its chemical composition except that it is a para-albumen.

MATERIALS AND METHODS

Obviously only small quantities of the liquor can be obtained at best. Human material is not available sufficiently fresh and in sufficient abundance. Therefore, it was necessary to obtain it from ovaries of animals available in large numbers and freshly killed.

Ovaries of the sexually mature hog (sus Scrofa) were used, as these could be very readily obtained in the slaughter houses of New Orleans. All histological observations indicate, that the process of the production of the liquor folliculi in the Graafian follicle and the process of extrusion of the ovum are the same in the human as in the hog; and it is logical to assume that the liquor plays the same rôle as in the human ovulation and that any results indicating its physiological action in the hog must be similar to the human. In the experiments, amniotic fluid and fluid from ovarian cysts of the hog were used for comparison with liquor folliculi, and also fluid from human ovarian cysts.

The Graafian follicles may be readily distinguished in the surface of the ovary from the corpora lutea and ovarian cysts by their reddish color and manifest turgidity. The corpora lutea are yellowish spherical protrusions of firm consistency, and the cysts are translucent and usually larger. The liquor folliculi was obtained by inserting into the large follicles a fine needle of small, dry, thoroughly sterilized, all glass syringe. All material was collected at the slaughter house under rigid aseptic precautions from ovaries of freshly killed animals. The hog being one of the animals, which give birth to litters of young, ovaries may be obtained containing a number of mature follicles. Obviously but a few drops of the liquor could be obtained from a single follicle. All ovaries were taken warm just as the viscera were removed. It was noticed that if allowed to cool only a very small quantity or none could be obtained, due probably to coagulation.

The experimental technique used in this work is based on the principles of Abderhalden's dialyzation reaction with its modification by Grützner. It may be briefly summarized: 1) in preparation of material to be tested; 2) process of obtaining the liquor folliculi; 3) diffusion tubes; 4) the test; 5) dialyzation; and 6) the controls.

In preparation of the material for the digestive experiments, blood was obtained, defibrinated, and the fibrin thoroughly washed in cold water until all blood was removed from it. The pieces of fibrin containing the faintest tinge of pink were discarded to eliminate whatever reaction any blood or plasma themselves might give. Pieces of fibrous connective tissue, muscle, and ovary were also used. These were boiled in distilled water for five minutes and the filtrate tested for substances reacting with ninhydrin and with the biuret reaction. This was repeated until the filtrate failed to give a reaction with one cubic centimeter of ninhydrin on boiling one minute. The fibrin in Grützner's test was prepared according to the method devised by him, which must be omitted as time and space will not permit.

Schlercher and Schull, No. 579 dialyzing tubes were used. These were first carefully tested with albumen to insure imper-

meability to it. They were then boiled for five minutes just before each test.

In the test, small quantities of liquor folliculi were introduced into the diffusion tubes together with a small piece of muscle, fibrous connective tissue, and ovarian tissue, prepared as above; separate tubes being used for each. Care was taken that all tissues were below the surface of the liquor folliculi to prevent any possible error from decomposition of pieces. The diffusion tubes were next placed in larger tubes of sterile distilled water. A layer of xylene was placed upon the fluids within and without the dialyzer to prevent the growth of bacteria and to prevent evaporation.

Controls were made with exactly the same technique using amniotic fluid, normal saline, and cystic fluid, instead of liquor folliculi. Cultures were made from the liquor folliculi and cystic fluid used; to rule out any possibility of digested protein due to bacterial action.

The period of incubation was twenty-four hours, as some difficulty was experienced in tests of shorter periods of incubation due to weak reactions. Abderhalden also employed and later advised this modification as to time. The temperature of incubation was 38°C. The filtrate or the fluid in the tube surrounding the dialyzers were tested with ninhydrin and the biuret test.

The results obtained may be tabulated as follows:

1. Abderhalden's dialyzation reaction

	OVARIAN TISSUE	MUSCLE	LIGAMENT	FIBRIN
Liquor folliculi	+++	++	++	+++
Cystic fluid (small cysts)	++	+	+	+
Amniotic fluid	−	−	−	−
Normal saline	−	−	−	−

2. Grutzner's fibrin test

Liquor folliculi	+++			
Amniotic fluid	− − −			
Normal saline	− − −			

Sixteen tests were made with the dialyzation method which was repeated three times, making a total of forty-eight tests to which may be added the tests of Grützner's Method.

As indicated in the table, the liquor folliculi gave a positive reaction with fibrin, fibrous connective tissue (lig. nuchae), muscle and especially a strong reaction with ovarian tissue. Slight positive reactions were obtained with cystic fluid from small cysts, and negative results with larger ones. The controls of amniotic fluid and normal saline were found to be uniformly negative with the exception of slight reaction with cystic fluid, (from small cysts) which to my mind is the strongest point in favor of proper technique.

Before drawing any conclusions from the above it might be well to briefly summarize the theories of ovulation.

By ovulation is meant the discharge of a mature ovum from its Graafian follicle. The study of the process involves a consideration of the development of the follicle and its rupture.

In ovulation the earlier stages have received more attention than the late stages, though it is the latter in reality that must explain the rupture of the Graafian follicle. It is admitted by all that the most mature follicle, very probably aided by the condition of premenstrual congestion, rapidly swells to the size of a large pea, due to the accumulation of the liquor folliculi, and produces a hemispherical protrusion of the surface of the ovary.

In considering the forces involved in the formation and increased production of liquor folliculi, the changes occurring in the circulation must be considered.

If the great increase of the liquor folliculi were considered the result of a mere transudation into the follicle, it must be realized that the ordinary blood pressure in the capillaries cannot be the only important factor. It is obvious that the pressure within the enlarged follicle is greater than the blood pressure within the capillaries since the capillaries are compressed by the follicle and even constricted in the summit of its protrusion of the surface of the ovary. The question arises whether this increase of tension of the tissues may increase or decrease the

flow of blood through the tissues and thereby possibly increase or decrease transudation. Compression of veins would result in congestion of the veins behind the region of compression with increased transudation; compression of an area of capillaries must result in congestion of the contributing arterioles and capillaries not affected by the pressure with increased transudation. It is reasonable to assume, however, that an equilibrium would soon be established in which the blood pressure in the veins concerned on the one hand, or the arteries on the other would be no greater than the common blood pressure. The result would be a decreased in the blood supply, in the immediate vicinity of the follicle, beginning in the earlier stages of accumulation of the liquor folliculi. However, the approach of ovulation, or the later stages of the enlargement of the follicle, is accompanied by a marked congestion of the general ovarian and uterine vascular system, and this congestion must result in a greater transudation from the vessels into the tissue spaces. Therefore, it is very probable that the distension of the follicle by the accumulation of its liquor folliculi must be due to causes other than increased blood pressure and resultant transudation from the vessels into the follicle.

It is suggested then that the liquor folliculi must accumulate by a process similar to that of secretion, that the chemical nature of the cells of the Graafian follicle or the product of their liquefaction, or both, may induce an endosmosis into the antrum of the follicle, producing its distension to the pressure greater than that of the tissue about it or of the blood in the ovarian vessels. It is quite conceivable that the chemical composition of the liquor folliculi may vary at different periods, in the process under the agency of the increased number and liquefaction of the follicular cells, as well as the amount of the liquor. Also the layer of cells in the stratum granulosum may act as a diffusion membrane whose control of osmosis may vary at different periods, increasing the endosmotic current in the later stages of enlargement. The congestion of the ovarian vessels is probably induced by the pressure irritation of the enlarging follicle. The chemical character of the follicle may induce it.

This congestion results in a greater transudation from the vessels into the tissues, and the increased fluid in the tissue spaces; but this means a greater amount of fluid for the forces within the follicle to act upon, resulting in the greater enlargement of the follicle and this again irritating to a greater congestion, and so on till the follicle may burst and the cause removed. It would be logical to expect that, during the period of congestion, characteristic of the premenstrual period, a more vigorous elaboration of the products of the cells of the follicle (ferments or enzymes) would be made possible by the increased transudation or nutrition in the tissue spaces.

It is, of course possible, that other forces may contribute to the process of ovulation. We have no evidence that the nervous system has any direct influence. The sympathetic is of course, concerned in the altering of a caliber of the ovarian vessels (vaso-dilators), but this is no doubt a reflex, the impulses, which are aroused by the condition of the ovary itself, affecting the terminations of the nerve fibers in it.

From the comparative results with amniotic fluid, cystic fluid, and normal saline, it is suggested that the digestive action of the liquor folliculi is no mere matter of chance, but is a definite factor depending in some way at present unknown upon the particular nature of the liquor. It is a matter of common knowledge that different enzymes are specific in their action, that is to say, each enzyme acts only on one class of material and acts on it in a determined manner, producing certain specific substances as a result of that action, and that extra-cellular as well as intra-cellular enzymes act only when completely freed from the cell body. As to the enzymic action of the liquor folliculi, the general law which applies elsewhere, may be applied here. The rate at which digestion goes on varies with temperature, with the reaction, the concentration of the digesting substance, and the condition of the material digested. The forces involved may be considered accelerated or aided by the increased temperature in the region of the ovary during the congested stage, more probably accelerated as enzymic action is greater at a temperature slightly above body temperature. The chemical

reaction of the liquor folliculi was found to be alkaline to litmus and phenolsulphopthalein. Practically all the digestive agents of the body act best in the presence of an alkaline reaction. In the experiments here, the liquor folliculi was used in its normal concentration and, as noted above, at a temperature 38°C. or slightly above the normal body temperature.

THEORIES OF RUPTURE OF FOLLICLE

The purely mechanical theory of Hensen who ascribed the rupture of the Graafian follicle to be due solely to pressure atrophy, due to the increase of the liquor folliculi can no longer be held tenable, as Naegel has shown that an increased pressure causes a thickening of the tunica interna of the theca folliculi. The thickening of the theca folliculi about the enlarging follicle is very evident in all sections, and may be explained as due to two causes:

1. The mere fact that the liquor folliculi does increase in amount, and the follicle increases greatly in size must result in a packing off, or compression of the ovarian stroma about the follicle and thus in a gradual increase in the thickness of its theca.

2. It is well known that in certain other conditions in the body any gradually increasing pressure leads to a proliferative fibrosis, and thus the thickening of the elements of the theca interna (an increase in size and in number of the connective tissue corpuscles with a subsequent increase in the fibrous tissue of the theca interna) and, therefore, may be in part but an expression of one of our simple laws of physiology, namely, that all irritation causes proliferation.

According to the earlier views of de Graaf, von Baer, His, and Waldeyer, that the ovum may be extruded through rupture of the Graafian follicle resulting from the formation of a local area of necrosis or macula pellicuda due to a distinctive preformed non vascular area has been shown to be incorrect by the excellent monograph of Clark dealing with the blood supply of the ovary and its changes during ovulation.

In the words of Dr. Clark: "There is undoubtedly a deeper lying cause than the mere growth and pushing forward of the

follicle until, by erosion of the tunica albuginea of the ovary it empties itself." In his excellent monograph, Dr. Clark summarizes his views of the final epoch in the evolution of the follicle to be due to the arrangement of the vessels and the phenomena of congestion of the internal genital organs. He states, "A follicle may reach maturity through the influence of the normal circulation, but it requires the increase of arterial pressure due to the menstrual wave to induce rupture and extrusion of the ovum."

It is evident that we must go still farther in elucidating the problem of the rupture of the Graafian follicle and determine, if possible, the correct basis on which to build the argument relating to the causative factors for its rupture.

In interpreting the menstrual wave, it has been assumed by many to be due to the congestion of the internal genital organs. From this point of view then, it would seem that any congestion brought about would induce rupture of the follicle. As a matter of fact the stage of preparation, premenstrual congestion begins for some days before ovulation or actual bursting of the follicle (stage of destruction) and capillary pressure gives us an inadequate explanation.

It is suggested that it may be the period at which time the liquor folliculi reaches its maximum amount and the enzyme is liberated in the liquor.

SUMMARY

The rupture of the Graafian follicle is due in part to the digestion of the theca folliculi by a proteolytic ferment or enzyme in the liquor folliculi.

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