to Pfeiffer's bacilli or *Bacillus prodigiosus,* at 650 mm. Hg negative pressure. The culture media[2] used were the Smith-Noguchi fluid medium and rabbit blood agar plates, and the cultures were incubated for 7 days under anaerobic conditions in Brown's jar—a modification of the McIntosh and Fildes apparatus.

In no instance, thus far, have any filter-passing microorganisms been cultivated from this material by the methods employed.[3]

199 (2431)

Ovogenesis during sexual maturity as elucidated by experimental methods.[1]

By GEORGE N. PAPANICOLAOU.

[*From the Department of Anatomy, Cornell University Medical College, New York City.*]

It has been repeatedly claimed that ovogenesis continues during sexual maturity but the evidence so far has not been considered entirely conclusive. On this account the more popular view among morphologists today is that ovogenesis in mammals ceases before puberty. The primary ovocytes contained in the mature ovary are not thought to be newly formed but simply stored there for later use.

The chief reason that a demonstration of post-pubertal ovogenesis has been so difficult is on account of the fact that the process in normal mature ovaries is much retarded and easily overlooked.

Recently my attention to this problem has been attracted by studying a series of ovaries from guinea pigs, in which the thy-

[2] Olitsky, P. K., *J. Exp. Med.*, 1922, xxxvi, 501; Olitsky, P. K., and McCartney, J. E., *J. Exp. Med.*, 1923, xxxviii, 427.

[3] In the case of two rabbits both methods yielded growth of a minute, gas-producing microorganism, the *Staphylococcus parvulus,* wholly unrelated to any of the bacilloid, bacillary, or vibrio-like bacteria of human origin. These cocci were filterable, under the condition of the experiment, only in the early generations.

[1] This work has been supported by the Committee for Study of the Sex Problem of the National Research Council.

roids have been treated with radium emanation. Some of these ovaries had their germinal epithelium so much activated that all stages in post-pubertal ovogenesis could be seen and demonstrated almost as well as in young immature ovaries. By concentrating attention on this point I was later able to observe the activation of the germinal epithelium by other factors. Every factor that increases the congestion and the blood supply in the ovary seems to produce such an activation. Injections of cystic fluid and of other glandular extracts may produce this effect.

This experimental stimulation of post-pubertal ovogenesis led me to study a large number of ovaries from normal guinea pigs at different life periods as well as from embryos and from the pregnant female. From this study a number of points of importance have been derived.

There is a continuous process of ovogenesis from the time of the gonadal differentiation in the embryos up to the time of cessation of sexual activity in the older female. This process decreases in activity with progressive age and almost ceases in the old female.

Ovogenesis is not entirely uniform but varies in intensity at different times. All factors that increase genital activity tend to increase this process. Favorable nutritive or seasonal conditions, estrous activities or any factor increasing the blood supply in the genital organs act in this direction. Seasonal variations and cyclic changes tend to give to it a periodical expression.

Other factors act in an inhibitory way. Unfavorable nutritive or seasonal conditions, diestrous inactivity and especially increase in developmental competition within the ovary act in an inhibitory manner. The appearance and presence of active *corpora lutea* within the ovary gives the most severe setback to the ovogenetic process. For this reason during pregnancy or during diestrum when the ovaries contain active *corpora lutea* the ovogenetic process is lowered in activity. There is, however, a slight new production of primary ovocytes continued during diestrum or pregnancy which increases with the decrease of function and activity of the *corpora lutea*—that is, during the latter part of these periods.

Therefore, at all times, even during diestrum and during pregnancy, a number of new primary ovocytes are formed and a number of these begin to develop within the ovary. There is evidence that every primary follicle must grow or die. Long persistence

of any follicles of any size within the ovary seems to be quite impossible. Any theory based on the assumption that primary ovocytes persist for long periods of time or throughout the life of the organism is untenable.

The existence of a continuous ovogenetic growth within the ovary is a very fortunate arrangement, tending to supply fresh developing ova within the ovary at all times. The actual rhythm is expressed more clearly in the large follicles and the *corpora lutea,* which are periodically destroyed and reformed.

The germinal epithelium of the ovary gives rise not only to primary ovocytes, but also to follicular cells, and interstitial groups. In young ovaries there is an excess of primary ovocytes and interstitial groups, while in mature ovaries there is an excess in production of follicles and luteal cells. The follicular cells are steadily formed and migrate into the stroma of the ovary in order to supply the growing follicles with an additional number of such cells. The primary follicles do not depend on the small number of follicular cells around them nor on their slow multiplication to provide the material for their growth, but they are steadily acquiring new follicular cells and later on luteal cells from new cells derived from the ovarian epithelium.

This migration is also participated in by the primary ovocytes, which move into the interior of the ovary and degenerate, if they have not the chance to develop further.

Only a few of the primary follicles develop further. Most of them degenerate in the primary ovocyte stage. This is especially the case with the large groups of ovocytes, which are sometimes accumulated together and covered by a single layer of follicular cells. Their poor follicular supply does not allow them to compete with other more isolated and better supplied ovocytes.

The ovocyte groups mainly appear after an active period of ovogenesis and are produced by a group-like differentiation of germinal epithelium cells. This may happen only under very favorable conditions; otherwise such groups differentiate into interstitial clumps. It seems to me that the interstitial groups are nothing more than abortive groups of primordial ovocytes showing signs of progressive fatty degeneration. One has to draw a line between the ovogenetic activity and the relative differentiatiation of the epithelial cells. At times there is a rich growth of ovocytes, while at others a higher growth of follicular and luteal cells or a richer production of interstitial groups.

The activation of the epithelium is not uniform over the entire ovary, but it is rather localized in different areas. In some places, probably on account of a better blood supply and better nourishment, the epithelial cells, which are to be considered as resting ovogonia, begin to grow and their nuclei display some of the morphological changes that characterize the growth of spermatogonia into primary spermatocytes. In other places the epithelium may be found entirely inactive.

Mitotic figures can be seen in the epithelium but they are not as common as one might expect if every epithelial cell were to divide before giving rise to a primary ovocyte. It seems, therefore, that the ovogonia of the germinal epithelium may grow into an ovocyte without a previous mitotic division.

200 (2432)

The appearance of specific antibodies in the serum of rabbits by intratracheal and intravenous injections of living tubercle bacilli.

By B. M. FRIED and E. E. PROCTOR.*

[*From the Department of Pathology, Harvard Medical School, and the Pathological Laboratory of the Boston Sanatorium, Boston, Mass.*]

The production of antibodies in animals (rabbits) by the intratracheal injection of bacterial antigens other than tubercle bacilli is considered as practically identical to the intravenous injection.

The importance of the introduction of antigens directly in the lungs has received a particular importance in relation to recent investigations concerning "autonomy of organs" (Besredka[1]) in infection and immunity. It is of interest to note Besredka's experiment in vaccination against diphtheria. A repeated intratracheal injection of killed diphtheria bacilli will protect a guinea pig against a subcutaneous injection of living Klebs Loeffler bacilli, while the control animal vaccinated subcutaneously will die from the same dose of the living bacilli.

---

* Introduced by S. B. Wolbach.

[1] Besredka, A., *Ann. de l' Institut Pasteur*, 1919, 1920, 1921.