

sure) was siphoned through jars in which were placed similar sacs filled with the same fluid. No bubbles whatsoever appeared in any of the sacs, and when these same sacs with their contents were transferred after several days to untreated tap water, in order to control the possibility that these particular sacs were impermeable, the appearance of the first bubbles was much delayed (from three to five days), but after the bubbles began to form the rate of increase was as before. When sacs which had become partially or completely filled with gas from the ordinary tap water were transferred to jars of running "degassed" tap water (faucet filter-pump treatment) the bubbles gradually disappeared. In three days a sac which had been quite tense with gas became almost completely collapsed. Upon returning it to ordinary tap water it again filled with gas and became tense.

The actual amount of pressure developed by the accumulated gas has been measured in only one sac, and this was done during June, at which time other sacs did not fill as quickly or seem as tense as during April and May, when I believe the values obtained would have been somewhat greater. The sac, filled with tap water and surrounded by running tap water in a suitable container, was connected through a perforated stopper to a water manometer with a leveling bulb arrangement for adjustment of the column height without disturbance of the gas pressure to be measured. In ten days (middle of June) the sac had become completely filled with gas; during the next four days the pressure gradually rose until it reached a maximum that supported $49\frac{1}{2}$ inches of water (a 5-mm diameter tube was used to minimize the surface tension factor). For several days then there was a fluctuation of from two to three inches, apparently correlated with temperature variations in the issuing tap water. Whenever many other faucets on the line were open so that the water was cooler there occurred a decrease of considerably more than can be accounted for by the temperature change of the gas itself. It seems reasonable to assume that the pressure was correlated also with the degree of saturation of the water with gas when freshly released from the piping system at different temperatures. After making such readings for several days tap water which had been submitted to the action of a faucet filter-pump for two hours (and which was warmer than any of the direct tap flow) was run through the container around this sac. The pressure of the gas in the sac fell to zero in ten hours (six gallons of water were used in this time). Then the direct tap connection was again made and in three days the pressure increased to its previous level, thus proving that the fall in pressure was not due to leakage developing coincident with the use of the "degassed" water.

Rigid control of each possible factor, including the study of other membranes, fluids and gases, is needed to determine the laws governing this phenomenon and to work out the true explanation of it. I am not in a position to conduct or to direct such a study, and therefore can only express the hope that physical chemists or others will carry on. When worked out these purely physicochemical laws concerning inert membranes and gases in solution will probably be found of much assistance in understanding some of the phenomena of living matter, such as the gaseous exchanges in respiration and the so-called secretions of gas in various forms.

The significance of these observations as regards distortions of the membranous labyrinth is obvious, and will be dealt with in detail elsewhere; it is equally obvious as regards all the other fields of histologic work in which cavities and delicate tissues are associated and where gas bubbles frequently cause serious damage (embryology, for instance). For the benefit of others who may be troubled with gas bubbles in their tissues a few applications of these observations will be briefly indicated. It is practicable to reduce by filter-pump evacuation the dissolved gas content not only of the water used, but also of many fixing fluids and of the lower strengths of alcohols, and from my own experience to date I am quite certain that by means of this simple procedure (carried out with each fluid just before use, not after the specimen is in the fluid) it is possible not only to prevent the formation of internal bubbles but even to withdraw bubbles that are present without pricking into or cutting valuable specimens. The rate of reabsorption from the atmosphere is sufficiently slow (unless the container is violently agitated) that it is not necessary to cover the surface with oil if a reasonably deep layer of fluid is used and changed daily and the specimen placed well below the upper surface. Even freshly distilled water contains so much air, from absorption during the condensed film stage, that it does not prevent formation of some bubbles and has little or no tendency to absorb bubbles already present unless it has been freshly "degassed." Formalin solutions or fluids containing formalin give off much formaldehyde when acted upon by a filter-pump, but bubble formation is diminished if the water (or the Zenker's or Müller's fluids, for instance) is "degassed" just before adding the formalin required.

STACY R. GUILD

THE OTOLOGICAL RESEARCH LABORATORY,
THE JOHNS HOPKINS UNIVERSITY

DOES REGENERATION FOLLOW COMPLETE OVARIOTOMY IN THE ALBINO RAT?

SINCE the time when August Weismann first made a clear distinction between the soma and germ-plasm

and introduced the now familiar idea of the uniqueness and continuity of germ-cells, the origin and history of the definitive germ-cells have become subjects of active investigation.

Interest in this field has been particularly active during the past dozen years. Many cytological contributions have appeared during the interval. Some favor continuity of germ-cells from generation to generation, and, by inference, at least, support the hypothesis that all germ-cells now in existence go back in an unbroken line to the germinal materials of the first living thing upon the earth; others find evidence supporting the view that there is a discontinuity of germ-cells between the generations, each crop of definitive germ-cells arising *de novo* from the peritoneum of the gonads. Therefore, according to this school, soma-cells proliferate germ-cells.

The subject-matter of this preliminary note deals, however, not with the cytological aspect of the origin of germ-cells, but with an attack upon the problem from an entirely different angle.

Davenport,¹ spaying mice, found that, after a lapse of from eight to forty-five weeks, regeneration of the ovaries occurred in 64 per cent. of his animals. Complete removal of the ovary was claimed, but cytological evidence in support of this was not secured. Macroscopic examination of masses of tissue at or near the site of operation was the criterion for regeneration of the ovary.

Haterius² secured four cases of regenerated ovarian tissue in mice out of seventy-six operations, and attributed these to incomplete ovarian extirpation. Parkes, Fielding and Brambell³ had 121 cases of double ovariectomized mice. After ovariectomy the oestrous cycle (vaginal smear method) ceased. But in eleven cases spontaneous oestrous subsequently occurred, and this was taken to indicate regenerated ovarian tissue. In eight of these eleven cases the presence of new ovarian tissue was demonstrated histologically.

Hanson and Heys⁴ added data of a similar nature from the albino rat. Both ovaries were removed from 105 rats and each ovary preserved for sectioning. The animals ranged in age from ten to two hundred days at time of operation. The period allowed for regeneration ranged from ninety to one hundred and eighty days. There were eight cases of ovarian regeneration as determined by sectioning the regener-

ated masses of tissue. The eight original ovaries removed from these sites of regeneration were then sectioned to determine whether the whole ovary had been removed.

In two of the eight original ovaries incomplete removal was demonstrated cytologically, while in six cases extirpation was apparently complete. This gives a regeneration rate of 3.5 per cent. in the rat.

It was observed by Hanson and Heys that no rats under forty days of age showed regenerated ovaries, and the suggestion was made that the chance of success in complete removal of the ovary increases as the age at which animals are spayed decreases. This is due to the fact that the immature ovary is a small ovoid body, freely movable and not yet embedded in the fat body. On the other hand, the mature ovary is surrounded by the fat body and very irregular in shape due to the presence of numerous corpora lutea, hence the difficulty of complete removal.

With these facts in mind a second experiment with rats under forty days of age (range ten to forty days) was performed. One hundred and eight rats were operated upon. Both ovaries were removed. As expected from the results of the first experiment there was no regeneration at all in rats under forty days of age. In this experiment a few operations were performed on sexually mature rats as a check on those under forty days of age. After operating upon over 213 rats some skill in removing the entire ovary was attained, yet so great are the difficulties in getting out the adult ovary from surrounding fat that again three cases of regenerated bodies were found in these older rats.

CONCLUSION

These two experiments seem to show that in the rat it is easily possible to be certain of getting out the entire ovary in young rats (under forty days old) and that upon complete removal no regeneration occurs.

It also seems plausible to believe that in older animals the regeneration that occurred in these experiments, as well as in those of other workers, was due wholly to the difficulties attendant upon a perfect ovariectomy.

In so far as these experiments have a bearing upon the problem of the origin of the germ-cells they lend no support to those cytologists who trace definitive germ-cells to a peritoneal source.

Complete experimental data with a critical discussion of the cytological and experimental evidence will be published elsewhere.

FLORENCE M. HEYS

WASHINGTON UNIVERSITY

¹ C. B. Davenport, *Jour. Exp. Zool.*, 42: 1-11, 1925.

² H. O. Haterius, *Proc. Soc. Exp. Biol. and Med.*, 24: 784-786, 1927.

³ A. S. Parkes, W. Fielding and F. W. R. Brambell, *Proc. Roy. Soc., Series B.* CI, 328-354, 1927.

⁴ Frank Blair Hanson and Florence Heys, *Proc. Soc. Exp. Biol. and Med.*, 25: 183-184, 1927.