**Physiology.** — *"Effects of the Rays of Radium on the Oögenesis of Daphnia pulex"*. By Miss M. A. VAN HERWERDEN. M. D. (Communicated by Prof. C. A. PEKELHARING.)

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For a considerable time I have been prosecuting the effects of radium radiation on a race of Daphnia pulex bred in the laboratory, with whose method of reproduction I had become thoroughly acquainted during a study of rather more than 8 years. My original purpose was to evolve, if possible, parthenogenesis in the sexual period, or conversely, to impart to the parthenogenetic females the faculty of producing a sexual offspring. The importance of this experimentation was sufficiently borne in upon me in connection with the view adopted by many researchers that by radiating the organism with radium, enzymic actions are accelerated or diverted. A short or a prolonged radiation with 0,7 mgrs or 3,1 mgrs of radiumbromide, at my disposal, never resulted in any effect upon the sexual or the parthenogenetic stage, whereas after a radiation with a stronger preparation the animals succumb. [1]) Nevertheless I considered it a point of importance to continue the experiments, since they throw a peculiar light upon the resistance of the protoplasm in the several phases of the oögenesis and of the embryonic development under the influence of radium rays.

Daphnia pulex affords extremely fit material for such experiments. Besides being fairly transparent and easy to watch under the microscope, it also enables us not only to follow in the living animal the development of the parthenogenetic eggs located in the broodpouch, but also to determine the degree of maturation of the eggs in the ovary. Sometimes the amount of yolk in the maturing eggs enables us to foretell correctly to a few hours, when they will leave the ovary. There is always plenty of material for control, as several young ones can be expected at every parturition. Over and above, the rapid succession of generations grants a comprehensive survey not only of the animals under examination, but also of a large progeny.

[1]) Verslag "Koninklijke Akad. v. Wetenschappen" Deel XX p. 20.

In the experiment I placed Daphnia pulex, embedded in a drop of ditchwater, immediately on the micaplate of the radium-preparation. In the following descriptions I shall designate the preparations used, containing 0,7 and 3,1 mgrs. of radium-bromide respectively as capsule A and capsule B.

Animals belonging to different age-periods were radiated separately. It immediately appeared that the adult Daphnia is much less responsive to the radium rays than the new-born animal. An exposure of 18 hours on capsule A did not affect the animals so as to kill them. If the animal kept alive after this prolonged radiation, it was sometimes seen to succumb later on at the periodical ekdysis of the chitin shell, which was often attended by an abnormal chitin-formation. This then pointed to some injury to the ectoderm. Still, even when the animal remained perfectly healthy, it had become sterile for the rest of its life. In the case of only a few hours radiation without any yolk-rich mature eggs being noticeable in the ovary the reproductive faculty is not interfered with: the eggs leaving the ovary later on develop normally and the young generating from these eggs reach sexual maturity in the regular way and produce a healthy offspring.

*However, when radiation takes place, while the ovary bears large yolk-rich eggs, or when the eggs have only just entered the broodpouch, a radiation of* 25 *minutes on capsule A, or of some minutes on capsule B, will suffice to cause the eggs to develop abnormally,* so that they are destroyed already in the blastula-stage and are resorbed in the mother. Susceptibility varies individually, without any apparent connection with the age of the mature females. Whereas the eggs that have only just entered the broodpouch are as susceptible as, or more susceptible than before leaving the ovary (abortus sometimes occurs already after $1^1/_2$ minutes' sojourn on capsule B), the resisting power of the embryos is seen to increase during the development. Viable young were developed even after a three hours' radiation on capsule A in the gastrula-stage. Yet in these cases the brood did not seem to be always in good condition, since one of the young emerging from such a radiated gastrula — the only one of this lot that reached sexual maturity — produced a brood of an anomalous morphological structure. It appears then, that the future germ-cells of the gastrula in this case must have been injured already during the radiation.

A greater resisting power is displayed by the almost viable young, as they can stand a 20 hours' radiation on capsule *A*, without any prejudice to their future fecundity. If, however, these young ones

leave the brood-pouch during the radiation, they invariably perish, every one of them; a sojourn on capsule *A* of a couple of hours only will kill them. After an hour's radiation the cardiac action is weakened and irregular and they die soon after. In the brood-pouch they are presumably walled off by the chitin shell of the mother and by the fluid in which they are swimming, which protection, however, is not sufficient for the young embryos, which are so much more susceptible to the radium rays.

A short radiation of a female with maturing egg-cells in the ovary, leads indeed to destruction of the eggs; but it leaves the mother unhurt. Afterwards it even causes sometimes a more numerous offspring, which phenomenon is analogous to that seen in the action of various poisons on Daphnia pulex [1]), on which a small dosage of the poison acts as a stimulus. The resistance of eggs from one and the same lot sometimes differs very much, as among the eggs that are being destroyed occasionally a single normal young may be seen that does not appear to have suffered at all from the noxious influences that threatened it before the embryonic stage, and later on may possibly possess a normal faculty of reproduction. An anomaly in the structure of such a young occurs only rarely. It cannot be said to be typical for radium-radiation. Generally these monstra (with abnormal profile or defective intestine) are few and far between. As a rule, therefore, radiation yields a normal embryonic development or none at all. This is the reason why I never succeeded yet in breeding mutations of Daphnia pulex by radiation with radium, as MORGAN achieved on a large scale with the Drosophila fly. The few abnormal specimens never reached maturity, one excepted, which recovered completely and produced a normal offspring that was still healthy after four months. Indeed my eight years' experience with Daphnia pulex have convinced me that this race though highly modifiable, shows only slight mutability.

Other researches in experimental embryology also show that eggs from the same lot vary as to their resisting power to noxious effects. I here call attention to the researches of PEARL [2]) on the difference in degree of resistance evinced by embryos of the domestic fowl to intoxication with alcohol, in which case also the eggs, liable to reach full development bring forth normal chickens.

It might be objected that in the radiation experiments the eggs are differently exposed. This may occur with a numerous brood, in which the inner eggs are shielded from the noxious rays by the

[1]) l.c. p. 1.

[2]) Proceedings of the National Acad. of sciences. U. S. A., Vol. II, p. 380.

external eggs. But the exposure will presumably be the same for the Daphnia moving about freely in the waterdrop on the radium capsule, when, as in our case, there are only 4 or 5 eggs located in the brood-pouch.

What is the cause of this unequal resistance of the Daphnia eggs? A reduction-division of the chromosomes, which might be responsible for the unequal distribution of paternal and maternal hereditary units does not occur in this parthenogenetic development; the egg retains the number of chromosomes of the mother. A similar difference in the reaction of the eggs to the noxious influences I previously detected, when treating the Daphniae with phenylurethan [1]).

Whereas with a short radiation of a female with mature egg-cells only the first brood succumbs, and the succeeding broods are normal (even very abundant), with a longer radiation also the eggs in a younger stage of development are seen to be damaged, until ultimately the Daphnia becomes completely sterile. After one single radiation a Daphnia may, after many abortus, produce quite unexpectedly normal young again, if namely the younger oögonia are not damaged. Such an after-effect I observed up to the 6th of January 1917 of a radiation for some hours on capsule *A* on the 23d of November 1916. After the first date again young were born that were completely normal.

My experience that eggs of Daphnia pulex in the last stage of maturation are most susceptible to radium rays, and that only after a prolonged radiation also the younger egg-cells and at last the oögonia are injured, accounts for PACKARD's [2]) experience that after radiation of the Drosophila larvae, the young flies become sterile for some weeks, and afterwards become fertile again. If we bear in mind that results with mammals also favour the theory that especially the mature egg-cells are very susceptible, we are justified in presuming that this holds for the whole animal kingdom.

How to account for the fact that the maturing eggs are more susceptible to the radium rays than the immature and the remaining cells of embryonic and maternal organism? Again, what molecular transformations occur in the protoplasm under the influence of radium radiation? The view adopted by several researchers that enzymic actions are accelerated or diverted, prompted me to compare the embryonic development of radiated and non-radiated sisters, which as to temperature and diet had been bred under the same conditions.

[1]) l.c. page 1.

[2]) Journal of exp. Zoology. XIX, p. 332.

Up to the moment the eggs left the ovary these sisters were kept in the same culture glass, animals being selected for this experiment whose eggs entered the brood-pouch in the same hour. As soon as the eggs began to develop one Daphnia was radiated for fifteen minutes on capsule *A*.

After this the conditions were made equal for either animal. In case a normal brood was developed after this radiation, embryogeny was neither retarded, nor accelerated. At the same hour the heart's pulsation became visible in the brood of the two animals; at the same time the development of the limbs commenced and the first eye-pigment developed itself; in the same hour the young left the parental organism. A similar observation was made at a second radiation. If only the radium action does not pass the physiological boundary there is neither acceleration nor retardation of development.

Is it the alpha-, the beta-, or the gamma-rays to which the egg-cells of the Daphnia are particularly responsive. In radiating on the capsule with radium-bromide the alpha-rays are screened out by the mica-plate of the capsule, which they cannot penetrate and consequently they do not reach the animal. When separating the Daphnia from the radium-preparation by a leaden platelet of 3 m.m. thickness, the beta-rays do not reach the Daphnia, while the secondary beta-rays are allowed to resorb through a mica-platelet of 50 $\mu$ thickness on which the Daphnia is placed. In this way Daphniae with maturing eggs in the ovary could stand a radiation with the gamma-rays from 0,7 mgrs. of radium-bromide for 24 hours, without abortus, which proves the harmlessness of the gamma-rays. When applying the radiumpreparation of 3,1 mgr., which, as has been seen, will destroy the eggs within a few minutes, a radiation of 24 hours with the exclusion of the beta-rays, could be borne without deleterious influences. In a few of the latter experiments, however, the first brood is aborted. It is, therefore, possible that to a stronger concentration of the gamma-rays (not obtainable with these preparations) the Daphnia egg-cells prove to be sensitive, a sensitivity, however, that is not to be compared with that to the beta-rays.

The antagonistic action between uranium and radium, demonstrated by ZWAARDEMAKER[1]) for the frog's heart, induced me to radiate Daphnia pulex in a drop of uranylnitrate and to determine whether resorption of the eggs stayed away in this process. A concentration of 600 mgrs. of uranylnitrate pro L. is tolerated for some hours without inhibiting the development of the brood; with

[1]) These Proc. XIX p. 1043.

a higher concentration the Daphnia itself succumbs in less time. When a Daphnia with maturing eggs in the ovary is placed in a solution of 500 mgrms of uranylnitrate pro L. and after half an hour again in a drop of the same fluid on the radium capsule of 3,1 mgrs, the radiation may be continued for $\frac{1}{2}$ to 4 hours in a series of experiments without causing the brood to abort, while, under the same circumstances, the eggs, when placed in water, are fatally injured already after some minutes. Sometimes, however, the protecting influence of uranylnitrate was not at all discernible. Up to the present I have not been in a position to account for these various results. This also applies to the lower concentration of uranylnitrate.

More than fifty Daphniae were examined in microscopic sections and compared with non-radiated specimens. *Normal* maturation of eggs in Daphniae has already been described by KÜHN [1]). Broadly speaking my findings for the normal eggs are in agreement with his. A prolonged radiation did not enable me to detect in the maturing eggs any change either in the chromosomes, or in the nuclear body, or in the egg-plasma. Only in one polar spindle (for the formation of the first polar body) was the number of chromatin rods larger than could be anticipated with twice the number of chromosomes. Any possible alteration in the shape of the chromosomes is difficult to detect owing to the small dimensions.

Not before the blastula-stage, that is about the time when also in the living animal under the low-power microscope the embryos are seen to succumb, well-marked alterations take place in the nuclei, characterised by a collapse of the chromatin into coarse granules. The injury to the eggs, however, has been done long before the aided eye can detect it.

Though microscopic examination did not put us in a position to ascertain whether the noxious action of radium-rays has initially affected the nucleus, the cell-plasma or both, the high degree of susceptibility of the egg in a period when also considerable evolutions take place in the nucleus (formation of the polar spindle and decomposition of the large nuclear body) is indicative of a noxious effect of the beta-rays, especially on the nucleus. The fact that the first cleavage proceeds regularly and only at the close of it degeneration manifests itself, may be explained, when we call to mind BOVERI's [2]) investigations, which demonstrated that with the Sea-

[1]) Arch. f. Zellforschung, Vol. I, p. 538.

[2]) Jenaische Zeitschr. Vol. 43, 1907.

urchin it is only at the close of the blastula-stage that the various properties of the chromosomes manifest themselves.

The period of maturation, in which the egg is so extremely susceptible to the radium rays, also proves to be the critical period for a poison as phenylurethan in a certain concentration, as discussed by me in an earlier paper [1]).

Just as some eggs of the brood sometimes escape death after radiation with radium, and develop into perfectly normal young, a Daphnia was occasionally developed after treatment with $\frac{1}{12000}$ $n$ phenylurethan. When transmitted to water it produced a normal offspring. This again proves that the resisting power of the eggs to the danger, threatening them from the outer world, was occasionally very different, even with these parthenogenetic animals. But if they succumb in the struggle, the method of reaction in the two series of experiments is widely different. Whereas with a treatment with radium radiation this reaction leads irrevocably to degeneration at the close of the blastula-period, a treatment with phenylurethan evolves fully developed monstra, in consequence of a deleterious influence, exerted in the same period of susceptibility. These monstra, however, are not viable after birth; they are not resorbed, but are expelled from the parental organism.

*Summary.*

The egg-cells of Daphnia-pulex are most susceptible to radium radiation in the last stage of maturation. The resisting power increases in the embryonic stage.

In one and the same brood individual differences of susceptibility to the rays of radium is frequently noted. The egg that resists the deleterious influence often develops into a perfectly normal animal, which itself becomes fertile. The rare samples with morphological abnomalies seldom become adults. Only once did we succeed in breeding from such an abnormal young a stock without morphological anomalies.

A long-continued radiation from 0,7 mgrs of radium-bromide does not endanger the life of the sexually mature Daphnia, but only its fertility. It depends on the duration of radiation whether only the maturing eggs, the oöcytes, or also the oögonia are injured. Large progenies being easy of observation afford an opportunity to study this in every special case.

[1]) L. c. p. 1.

Prior to maturity Daphniae resist radium radiation for a long time. Only after a sojourn of many hours on the capsule with 0,7 mgrs of radium bromide the future ripening of the oögonia is also endangered.

A microscopic examination of the ovary and the embryos reveals that the deleterious effect of radium manifests itself only towards the close of the blastula-stage by an abnormal behaviour of the chromatin, also when the egg-cells were affected when lying still in the ovary.

If the beta-rays are eliminated through filtration the deleterious effect of radium is arrested or highly diminished, which proves the beta-rays to be mainly responsible for the destruction of the eggs.