Histology. — *De- and Regeneration of sensible endcorpuscles in the duck's bill (corpuscles of* GRANDRY *and of* HERBST*) after the cutting of the nerve, the removing of the entire skin or the transplantation of the skin in another region.* By J. BOEKE and C. DIJKSTRA.

(Communicated at the meeting of November 26, 1932).

In the connective-tissue layer of the skin of the bill of the duck there are found two sorts of sensitive endcorpuscles, viz. the corpuscles of GRANDRY and of HERBST. They are lying underneath the epithelium of the skin, and are distributed quite regularly throughout the smooth "wax"skin of the bill. By their regular distribution and their simple, well-defined structure they are very wel adapted to an investigation of their de- and regeneration.

The chief features of the regeneration process after the cutting of the nerve were described in these proceedings (meeting of September 1922) by one of us, so we may refer the reader to that article for the general features of the regeneration process.

In the communication of to-day we are going to describe:

1. De- and regeneration of the corpuscles mentioned above after the cutting of the nerves, a: the nerves are cut only once and the regeneration is not disturbed any more; b: the nerves are cut and after a month are sectioned again, and again after two months. Then the regeneration is studied;
2. a piece of the entire skin is removed, and regeneration of the endcorpuscles is studied in the regenerated piece of skin;
3. a piece of the skin of the bill is removed and transplanted on the leg, and vice versa; regeneration is studied in both cases, after the lapse of several months (4 to 11 months).

Chief aim of the investigation was the answer to the question, whether regeneration only occurs in endcorpuscles, which remained more or less recognizable, or new endcorpuscles have to be formed, and whether in regeneration of the nerves inside a newly formed piece of skin or inside a transplanted piece of skin there occurs a formation of new endcorpuscles.

I. *De- and Regeneration of sensible endcorpuscles after the cutting of the nerve.*

In the skin of the bill small rectangular pieces of skin were circumcised, the knife being pressed to the bone, so that we may be sure that all the nerve fibers are cut. After a few days or weeks a slight degeneration of the sensitive corpuscles (both those of GRANDRY and of HERBST) is

visible; in the corpuscles of GRANDRY the tactile cells schrivel a little, in their protoplasma a degenerative granulation becomes apparent and the periterminal network disappears. The tactile disc becomes vacuolated, but even after several weeks the tactile disc remains visible, so that we may conclude in correspondence with the observations of one of us (BOEKE, 1927), that even in degeneration the tactile disc does not disappear but may be used again by the regenerating nerve fibers and reconstructed into a normal nervous disc, when regeneration sets in in time, before the whole corpuscle has disappeared.

Beside these alterations there was observed a slight thickening of the capsule by multiplication of the capsule cells.

In the corpuscles of HERBST only a slight alteration of the central core and a slight thickening of the capsule was noticed.

In order to see, whether these degenerative alterations become stronger, when degeneration lasts longer and regeneration does not set in speedily, in a number of animals the nerves were again sectioned after 4 weeks, and sometimes a third time after 8 weeks and 12 weeks, in these cases the remains of the tactile corpuscles remained without the slightest vestige of a reinnervation during four months. The preparations showed that in these circumcised pieces of skin not a single nerve fiber was to be found. Even in these pieces of skin many of the tactile cells were still visible, but of the tactile (nervous) disc in most cases not a trace remained visible. There where the nervous disc had been, between the tactile cells, an invasion of the capsule cells is found, which send their protoplasmic processes into the space between the tactile cells, occupied beforehand by the nervous disc. This brings us to the supposition, that after the original tactile disc has disappeared when regeneration did not set in, a new tactile disc is formed by and from the capsule cells; we must bear in mind, that probably the capsule cells are of lemmoblastic origin, and differentiated inside the strands of lemmoblastic cells, which according the investigations of HERINGA, build up the tactile corpuscles in the embryo.

After a second and third section of the sensory nerves and a lapse of 4 months the increase in bulk and in number of the capsule cells was more marked than after the first section.

Regeneration (after a single section of the nerves) sets in about four weeks after the operation, and 6—8 weeks after the section of the nerves the young regenerating nerve fibers reach their destination, the sensory corpuscles. The tactile cells regain their normal volume, the shrivelling disappears, the entire corpuscle returns to its normal aspect. The regenerating nerve fiber reaches the protoplasmic tactile disc and branches to form a new neurofibrillar structure. As it was described years ago (these Proceedings, Sept. 1922) by one of us, in connection with the branching and outgrowing neurofibrillar strands a new intraprotoplasmic network is formed inside the protoplasma of the tactile cells, this network first showing itself round the end-branches (end-reticulations and end-

knots) of the ingrowing nerve fibers and then it appears to extend gradually over the whole extent of the flat tactile cells.

In accordance with this description it could be stated, that besides a regeneration of the old existing sensory corpuscles a great number of new corpuscles is formed, especially GRANDRY's corpuscles, in which process sheathcells (lemmoblasts) grow larger and become tactile cells, as HERINGA (1920) has established as to embryological development (BOEKE, l.c.p. 320). From a study of a number of cases it followed, that in the preparations the number of newly formed corpuscles of GRANDRY is at least as great as that of the regenerated old corpuscles. It is the question however whether these newly formed corpuscles remain all of them; it is possible that a number of them disappear again after the regeneration-impuls ends, as is known for the exuberant motor innervation after regeneration. It was impossible to get a definite answer to this question from the preparations.

II. *Regeneration of the nerves and reappearance of sensory corpuscles after removal of the entire skin.*

In the same species used for the first experiments, the duck, small pieces of the skin of the bill (of the same size as in the first series) were circumcised and removed entirely, the connective tissue underneath the epithelium being taken away as thoroughly as possible, until the periost of the bone of the bill is exposed. The wound was covered with a solution of collodium. After a few weeks a new skin is formed, the epithelium and the connective subcutaneous tissue growing in from the borders of the wound, and after a time the newly formed piece of skin is only recognizable from its surroundings by its colour and in section by its structure. In all the cases studied the pieces of newly formed skin were to be distinguished with absolute certainty from the surrounding normal skin, the border lines of the scar could be traced easily in the sections. In the young newly formed tissues of the scar there was found an exuberant regeneration of the nerve fibers, which reached its culmination point 4 to 6 months after the removal of the skin. Not only was the regeneration of the nerve fibers throughout the whole extent of the scar, but there appeared new endorgans too, the first traces of which were found about 4½—5 months after the operation. Both corpuscles of GRANDRY and of HERBST are formed anew, but the corpuscles of HERBST in general appeared somewhat later than those of GRANDRY. Just as was the case in the first series of experiments, there are formed a number of endorgans, which disappear again after a few months; 7½—8 months after the removal of the piece of skin most of them have disappeared. Those which remain intact have developed further during those months, so that they appear now as normally built large corpuscles, which are not to be distinguished from the normal corpuscles in the surrounding skin. The number of regenerated nerve fibers too lessens after a time, just

J. BOEKE and C. DIJKSTRA: DE- AND REGENERATION OF SENSIBLE ENDCORPUSCLES IN THE DUCK'S BILL (CORPUSCLES OF GRANDRY AND OF HERBST) AFTER THE CUTTING OF THE NERVE, THE REMOVING OF THE ENTIRE SKIN OR THE TRANSPLANTATION OF THE SKIN IN ANOTHER REGION

Fig. 1.

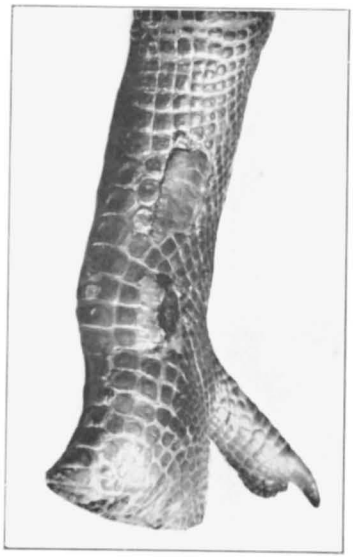

Fig. 2.

Fig. 3.

as could be stated for the motor nerve fibers in regeneration (BOEKE, 1916, 1921). This fact may be brought in connection with the physiological fact, that the sensibility of the scar tissue, which is generally very high during the first stages of regeneration, lessens after a time. This may be due not to a lessening sensibility of the regenerated endorgans, but to the smaller number of endorgans remaining after the first exuberant regeneration-processes.

In the newly formed connective tissue of the scar the formation of the new endorgans appeared to be in exactly the same way as during the embryological development of them, as it was described by SZYMONOWICZ and HERINGA.

III. *Degeneration of the sensory endorgans after transplantation of the skin and regeneration of them in the transplanted piece of skin.*

In 15 animals (ducks) small rectangular pieces (4 in number) of the skin of the bill were cut away; the same operation was performed on the legs and the pieces of the skin of the bill were transplanted on the leg and vice versa. When properly treated the pieces of skin healed perfectly, and after 11 days the dressing (gauze and collodium) could be taken away and the pieces remained intact and healthy. As is shown in the photographs (Fig. 1 and 2), the transplanted pieces of skin from the legs may be recognized very easily from those from the bill by their curious chequered rhombiform appearance (scales). When the transplanted pieces of skin heal properly, this pattern remains perfectly visible, even 11 months after the transplantation it was visible in the pieces of skin from the leg as well as in the normal leg. The pieces of skin from the bill transplanted on the leg (Fig. 2) remain as perfectly smooth as in their normal position. In microscopic sections the structure of the transplanted pieces showed itself to remain entirely as it was. The borderline of the implanted pieces was recognizable everywhere as a distinct line in the sections. The pieces were cut out and studied ½, 1½, 3, 5, 6, 8 and 11 months after transplantation. Structure and aspect both of the epithelium and of the connective tissue of the transplanted pieces remained absolutely the same even after 11 months. The pieces of skin on the bill all healed perfectly, from the pieces of skin transplanted on the leg about one third of the total number of transplants did not heal well (the technical difficulties were here of course much greater than in the other series), but in 60 to 70 % of the operations performed the results were as described above. Because the borderlines of the transplanted pieces could be seen in the sections accurately, it was possible to distinguish in every case in the sections the endcorpuscles formed inside the transplanted piece from those in the scar and those in the surrounding normal skin; so it seems to us, that the study of the sections and the deductions drawn from that may be relied upon.

Microscopic investigation showed in the first place, that in the

transplanted pieces of skin the endcorpuscles, which were present before transplantation, degenerate entirely and disappear. Four weeks after transplantation both the corpuscles of GRANDRY and of HERBST have degenerated entirely. Of the corpuscles of GRANDRY only traces of the capsule cells are still recognizable; six weeks after the operation these vestiges too have entirely disappeared. The corpuscles of HERBST degenerate somewhat later, and 6—8 weeks after the transplantation their capsules are still visible, but in an altered condition. In the capsules we see a number of blood capillaries; apparently they are being organized by the surrounding connective tissue.

In the normal skin of the leg there are present only a few corpuscles of HERBST (they are very rare) and here and there tactile cells of MERKEL. Corpuscles of GRANDRY are not to be found in the skin of the legs. Both the corpuscles of HERBST and the MERKEL-cells degenerate and disappear entirely within four weeks after the transplantation. After 3—4 weeks not a trace of them is to be found. In this stage the transplanted piece of skin is entirely devoid of nerve fibers; the old nerve strands have entirely degenerated, and new nerve fibers have not developed yet. It is only $2\frac{1}{2}$—3 months after the transplantation, that we find the first traces of regeneration of the nerve fibers. So a re-innervation of the old endcorpuscles is absolutely impossible. When there is found in the transplanted piece of skin a real endcorpuscle, we have to regard it as a newly-formed one.

As it was mentioned already, it is only after $2\frac{1}{2}$—3 months, that we find regenerated nerve fibers in the transplanted pieces of skin. These regenerating nerve fibers, which could be found both in the connective tissue and in the epithelium, partly appear to have followed the course of the old degenerated nerve strands, partly to have followed new paths through the connective tissue, so that a new terminal system of nerve strands is built up in the transplanted skin.

It followed from the study of the microscopic sections (partly fixated in a solution of corrosive sublimate and HERMANN's solution *aa*, and coloured with iron-haematoxylin, partly fixated in neutral formol 12 % and impregnated after the method of BIELSCHOWSKY), that in the transplanted pieces of skin taken from the leg, even 11 months after the operation no traces of sensory corpuscles were to be found, even in perfectly re-innervated pieces full of nerve strands. In the pieces of skin from the bill transplanted on the leg a number of newly regenerated endcorpuscles were to be found.

The first traces of these regenerating endcorpuscles were found in the pieces of skin studied 5 months after the transplantation. Seven months after the transplantation full-grown GRANDRY-corpuscles were found in large numbers.

The newly-formed endcorpuscles appear to be built perfectly normally; most of them are of the two-cell type, as it is generally found in the normal skin, sometimes we found even larger ones, consisting of three or four cells

(as is shown in the microphoto of fig. 3). Beside these GRANDRY-corpuscles we found here and there newly-formed HERBST-corpuscles, but built a little abnormally, and therefore not always easily recognizable.

Thus we find after the transplantation of a piece of skin from the bill on the leg a formation of typical sensory corpuscles characteristic for the skin of the bill and not for the leg, as for example KADANOFF supposed, when he transplanted a piece of the hairy skin of the snout (in mammals) on the sole of the foot, and when a piece of the skin of the leg is transplanted on the bill, the ingrowing regenerating nerve fibers are not able to impel the tissues of the transplanted skin of form endorgans, which are not found normally in the skin of the leg.

The formation of sensitive endorgans in the transplanted skin appears thus to be in correspondence with the origin of the piece ("herkunftsgemäss") and not in correspondence with the nature of the ingrowing regenerating nerves and the nature of the surrounding skin.

It remains possible of course, that after a long time in connection with the influences and impulses acting upon the transplanted piece of skin in its new surrounding a reorganization of the innervation and a formation of new endcorpuscles of the nature of those present in these surroundings normally, may occur; we can only say, that in the cases studied by us and in the long time (11 months) allowed for regeneration after the transplantation, no trace of such a reorganization of the innervation was met with.

Mathematics. — *Involutionen auf der trinodalen biquadratischen Kurve* Von Prof. JAN DE VRIES.

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§ 1. Die Punkte einer rationalen ebenen Kurve t^4, vierter Ordnung, seien in die Gruppen einer Involution I^n geordnet. Diese hat ersichtlich $3(n-1)$ Paare gemein mit einer durch einen Strahlenbüschel erzeugten, zentralen, Involution I^4. Demnach hüllen die Träger der Punktepaare der I^n eine Kurve der Klasse $3(n-1)$ ein (*Involutionskurve* τ).

Der Träger eines Punktepaares X_1, X_2 trifft t^4 noch in zwei Punkten P, P', welche einander zugeordnet sind in einer symmetrischen Verwandtschaft $[2(n-1)]$. Denn die zentrale I^3 auf den Strahlen durch P hat mit I^n $2(n-1)$ Paare gemein. Die $2(n-1)^2$ gemeinschaftlichen Paare von I^n und $[2(n-1]$ liegen zu zweien auf $(n-1)^2$ *Doppeltangenten* der *Involutionskurve*.

§ 2. Ausser diesen hat τ eine Anzahl *dreifache Tangenten*; diese entsprechen den *linearen Tripeln* der I^n.