RESULTS OF INTRA-OCULAR INOCULATION OF TRACHOMA

(On the question of the "follicle-building agent" of von Szily)

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I

IN order to clarify the question of how far the appearance of typical lymph-follicles is the result of infection with the virus of trachoma and not merely the usual reaction of the conjunctiva to such, von Szily published a series of articles giving the results of his experiments in the intra-ocular and intracerebral inoculation of trachoma on rabbits, guinea-pigs, and chickens. The starting-point of his experiments was formed by the reflection that there is normally no lymphatic tissue in the interior of the eye and that, as in his experiments in inoculation in sympathetic ophthalmitis, the production of lymph-follicles in the interior of the eye would make the specificity of the reaction-product the more evident. He did indeed, in a series of intra-ocular inoculations with infectious material from human conjunctivitis trachomatosa, succeed in producing, when the inoculation took, a repeatedly typical and identical result: namely, the appearance of typical lymph-follicles with germinal centres and all the usual histological and cytological characteristics in the choroid and the corpus vitreum. The histological pictures so obtained agree in every way with the well-known appearance of the conjunctival follicle of trachoma and are therefore spoken of as true trachoma follicles by von Szily. Besides, as characteristic of the positive result, is considered the further development of the intra-ocular process—considerable neoformation of tissue in the corpus vitreum, spontaneous healing with cicatrization and new follicular outgrowths side by side, just as one is used to seeing in true trachoma. The end-result of the process is phthisis bulbi.

The specific reaction begins in three to four weeks, after the disappearance of the first inflammatory shock-reaction and the resorption of the primary injected substance. Good results are, therefore, not to be expected before two to three months. The anterior chamber of the eye does not take part in the process.

The fact that follicular development is entirely independent of the primary injected mass—which has already degenerated—allows

Dr. Rutishauser, of the Institute of Pathology, was so kind as to examine a series of sections with me, for which I express my sincere thanks to him here.

von Szily entirely to reject the assumption that the process might be a sort of cellular culture in the interior of the eye. Just so does he deny the possibility of an overgrowth from the entirely uninvolved anterior part of the bulb. These views are essentially supported, further, by the results of intracerebral inoculation of the trachoma virus, which always occurred without the accompaniment of the nervous tissue and always led to the follicular development on the inner surface between dura and arachnoid. As a whole his results point to a toxin which actively spreads and excites its typical reaction, lymph follicles, in all its sites of inoculation. A certain difference was noted, in so far as the follicle showed a tendency to formation in the neighbourhood of blood-vessels in the interior of the eye, whereas the meningeal follicle showed no such tendency, practically no relationship to the vessels.

According to von Szily, the pathogenesis of the follicle is such that at first nodules form at the site of inoculation of the virus; their cells undergo active mitotic division and become the germinal centre of the now freely-developing follicle. Since all his attempts at inoculation with non-trachomatous material led to entirely negative results—the results of the experimental inoculation of sympathetic ophthalmitis forming a certain exception, but differing characteristically in important points from the results in trachoma—and a clearly-defined causal agent of trachoma could not be shown, the aetiology of the process will be assumed to lie in a “follicle-building” or else in a “germinal-centre-building” agent.

II

In several experiments we used as basic substance pieces cut from human trachomatous conjunctiva, which were finely ground up in a mortar with a little physiological salt-solution, according to the method of von Szily. After puncture of the anterior chamber, 0.1 c.c. of the resulting mixture was injected with a lying needle intravitreally into each of six eyes of four rabbits. According to our experience, one must be careful that the paracentesis is not made in the region of the limbus, but rather somewhat more corneal, as otherwise an annoying prolapse of the iris, which might jeopardize the result, can easily occur.

Of the six eyes enucleated at different intervals (between five and six weeks post injectionem) five gave an absolutely positive result, while the sixth showed changes not characteristic enough for us to count it with the positive cases.

As a whole, the findings of von Szily could be entirely confirmed, especially his finding that truly good and characteristic results could not be expected before the end of the second or during the third month.

The histopathological examination of the series of sections gave the following important results:—

Choroid. The younger the process is, that is the sooner after inoculation of the infective material, that the eye is examined the more characteristic the result is. However, even by the fifth week, in addition to the diffuse lymphocytic infiltration, quite typical follicles are found in the choroid as well as in the corpus vitreum. At this time the volume of the choroid is usually increased to several times its normal size, because of oedema as well as massive cellular infiltration. The vessels are often gapingly wide and empty, at other spots filled ad maximum; however, an intravascular increase in lymphocytes can practically never be discovered. The earliest appearing small follicles have an undoubtedly close relationship to the vessels (Fig. 1), which, however, does not necessarily mean that there is a primary relationship between those vessels and the origin of the follicles, but rather that the primary relationship might, to a certain degree, be purely to the structure or the wealth of vessels of the particular tissue. For the not too small collection of cells in the choroid, must have some relationship to its abundant blood-vessels, without having to admit *a priori* that these vessels have a special function in the origin of the follicles.

The *suprachoroid* shares to a large extent in the process, it is greatly swollen by an enormous oedema and its fibrous framework is extremely torn apart by the secondary progressive shrinkage ("Auflamellierung" of von Szily). We could not establish a special tendency towards the origin of follicles in the neighbourhood of the suprachoroid in our cases, which does not mean, however, that this tissue remained entirely untouched.

FIG. 1.

Primary follicle in the choroid. Suprachoroid extremely torn apart (auflamelliert). (Five weeks post injectionem.)

FIG. 2A-C.

Development of a follicle at the level of the pigment epithelium.

The *pigment epithelium* was also strongly affected by the extreme infiltration of the choroid. A far-reaching scattering of the pigment cells toward the bulb as well as the choroid takes place, which can at several points lead to a noticeable proliferation of these cells.

An impoverishment of pigment could nowhere be established—it can, however, be easily simulated by an oedematous swelling with displacement of the pigment to the borders. Should the enlarging follicle develop under the pigment epithelium, the result will be not only an outpocketing of the latter but also gradual rarification and, as end-stage, degeneration of the pigment epithelium so that the follicle, about ready to perforate the interior of the bulb, will be covered merely by the intact capsule of the corpus vitreum. At other places there can result such appearances as we see from time to time in the case of the rupture of an uveal sarcoma through

the choroid, that is, there appear mushroom-like figures, whereby the pigment epithelium has broken through at only one small spot and the follicle, now freed from tissue pressure, spreads over the pigment epithelium. It is barely necessary to state that it happens not too seldom that pigment epithelium is purely mechanically taken along at the time of rupture and therefore the finding of pigment epithelium within the follicle is not especially surprising. It is worth while to note that it is just the choroidal initial follicles which prefer to originate in the neighbourhood of pigment epithelium, in a surrounding tissue that is barely or relatively little changed, whereas there, where diffuse infiltration occurs, the alteration of the fundamental tissue is very great.

FIG. 3.

Primary follicle in the corpus vitreum ;
cicatricial tissue.

FIG. 4A and B.

Giant follicle in the corpus vitreum, tendency to confluence.

FIG. 4c.

Multiple formation germinal centres (Figs. 2-4, nine weeks post injectionem).

Probably the most striking changes take place in the *corpus vitreum*. This becomes more or less intensely a sometimes delicate but more often coarse cicatricial tissue, rich in blood-vessels and in which lymphatic tissue develops extraordinarily abundantly. In accordance with the lack of structure of the corpus vitreum, the development of the lymphoid tissue therein is quite different from that in the choroid. A diffuse growth takes place here, according to our previous experiments. It is extraordinarily hard to say whether we are here confronted with a confluence of small primary nodules or whether single follicles become secondarily detached from the diffuse mass (daughter-nodules). One thing, however, is quite definite; at the time of the most active lymphoid growth, there is, in the neighbourhood of the original injection-canal, no or practically no sign left of the original injection-mass. In general the only sign of it is the tissue debris which stains badly. Especially noteworthy is the behaviour of the light spots in the lymphoid tissue of the so-called germinal centres. While their appearance in the choroidal follicles is related only to the well-defined typical nodules, set off from the diffuse infiltration, we see them appearing, on the contrary, quite irregularly in the interior of the lymphatic cell-masses. It is quite probable that we can assume these germinal centres to be the primary sites of inoculation (von Szily) and that the development of the lymphoid follicles occurs concentrically to them. We can perhaps assume a confirmation of this view in the fact that the most greatly developed and typical germinal centres are on the periphery of the lymphatic masses, whereas they become smaller (younger?) the more they approach the centre. The older the structures in the corpus vitreum become the greater do they tend to discard the

original more or less constant globule for a long-oval shape, whose curve approaches more and more the posteriorly convex posterior lenticular surface, aided by a corresponding arrangement of the connective tissue fibres of the vitreal cicatricial tissue.

Besides these specific changes in the eye tissue, the probably unspecific reaction of the *retina* is of considerable interest. While there is practically never any follicular formation in the nervous

FIG. 5.

(Transient) iris nodule in *living* rabbit-eye.

FIG. 6.

Follicular formation in the choroid and corpus vitreum following inoculation with material from conjunctivitis vernalis (eight weeks post injectionem).

substance, the retina, in so far as it does not totally degenerate in the lymphatic proliferative process, responds to the development of these neoformations with a growth of its non-ganglionic elements that crosses over all borders. In other words, the result here is a true gliomatous neoformation conditional upon inflammation, since the proliferating retinal elements are composed almost entirely of spongioblasts. Mitoses are copiously met in this tissue. A second point is worth mentioning: where the lymphoid tissue comes in direct contact with the retinal elements, the latter take up very fine punctal granules, distributed in the protoplasm and forming groups here and there. Whether these granules have any relation to the inclusions of Prowaczek-Halberstaedter must be left undecided for the moment; in this connection overstraining of the sections after the method of Giemsa-Romanowsky gave no noticeable result which under the present conditions must therefore be considered negative.

The anterior portion of the globe, that is the iris, cornea, and anterior chamber, remained unaffected in all our cases. It must not be left unmentioned that we regularly found a diffuse nodular formation in the iris of the living eye; these nodules, however, disappeared just as regularly during the third week, without leaving any traces in the living eye or the histological section. It is probably a matter of local oedema irregularly distributed over the iris-tissue, which possibly has some connection with the regional swellings of the ciliary body. (Herrmann and Kiewe described in another connection, more or less transitory ciliary oedema, which could lead to a partial flattening of the anterior chamber.) A linear interstitial keratitis found in some eyes, may be considered traumatic, since no traces of nodular formation could be discovered. A certain tendency to nodular formation in the neighbourhood of the angle of the iris cannot forthwith be compared to the specific reaction-products, as we meet them in the interior of the eye, since the rabbit-eye responds to a whole series of entirely heterological interventions completely unspecifically with an angular lymphocytic outpouring that remains local. How far single perivascular collections of round cells found in the eye-muscles, have a specific aetiology, must for the present remain undecided until we have more material.

III

The origin of the material and histo-cytological characterization of the experimentally produced intra-ocular neoformations leave hardly any doubt that we are concerned here with true lymph-follicles and in the special case with trachoma-follicles. The claim made by von Szily that the so-called germinal centres were specially rich in mitoses, could not be confirmed. In our

cases only quite isolated mitoses could be shown in addition to the mainly polynuclear leucocytes and so-called microcytes, whose relations to the mitoses are not quite clear (von Albertini). Nor could we find a fibrous structure in the interior of the follicles. In older cases there is indeed a certain tendency for the connective tissue to lie on the surface of the follicle in concentric layers of fibres (that is, a sort of capsule formation), without our being able however to find any formation of radial fibres above the highest cellular layers of lymphocytes or a framework reticulum. In order to study this point more closely we used a silver-impregnation method of Bielschowsky in addition to van Gieson's stain, which could but confirm our previous negative result.

The question of the origin of the leucocytes was unequivocally decided by von Szily in favour of their derivation from the choroidal system of blood-vessels. We have already taken the opportunity above of casting certain doubts upon the tenability of the view as far as the choroid is concerned and we believe that these doubts must be further emphasized in view of the tremendous development of lymphoid tissue in the corpus vitreum. The capillary neoformation in the vitreal cicatricial tissue which he so strongly emphasizes seems to be quite insufficient. Since it is unquestionably a progressive process, although we find the vessel-lumina practically free of lymphocytes, we must consider both lymphocytes derived *in loco* from histocytic elements (cicatricial tissue) and to a certain extent a sort of distant chemotactical influence of the inoculated "virus." With this last view could be reconciled the appearance of follicles in the meninges of guinea-pigs as noted by von Szily. Further in accordance therewith would be often the appearance of multiple peripheral germinal centres in diffuse lymphoid corpus vitreum tissue just behind the lens, that is at foci where the existing connective tissue is minimal or even absent and visible blood-vessels are practically non-existent. Agreeing with von Szily we reject as improbable and unsupported the view that the whole process might be a sort of intra-vitreous cell-culture, especially in view of the fact that the originally inoculated material is practically destroyed and the actual pathognomonic process begins only after several weeks and then is progredient.

We would prefer not to decide the question of the *specificity* as unrestrictedly as von Szily who categorically affirmed it, because of the negative results of his controls. Of course our experiments also support the thesis that by inoculation of undoubted trachomatous material from human conjunctivitis the changes found in the animal experiments are to be considered typical trachoma-follicles. On the other hand, we are in possession of an undoubtedly positive case of true lymph-follicle formation in

the uvea and corpus vitreum of the rabbit, after inoculation of material from human conjunctivitis vernalis. Since further experiments with other types of material are being pursued, we should prefer not to hazard any further opinion on this question for the moment, but rather to await later publication, should the results warrant it. However, it seems just, even now, to question the absolute specificity of the described findings for trachoma and, with certain modifications, for sympathetic ophthalmitis. We are, as is von Szily, far from taking as the result of our experiments a "unitarian" standpoint in regard to the supposed agent of the disease in question; we believe, however, that the zone of influence of the "follicle-building" or "germinal-centre-building" agent will have to be widened.

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ABSTRACTS

I.—ANATOMY AND PHYSIOLOGY

- (1) **Stilo (Messina).**—The development and anatomical relations of the fascia of the orbit in man. (Contributo allo studio dello evoluzione della fascia del bulbo oculare nell'uomo). *Rass. Ital. d'Ottal.*, January-February, 1935.

(1) **Stilo** has examined the developing orbital fascia in foetuses of various ages and in children as well as in the adult. In the earliest foetus examined (46 mm.), he states that between the walls of the orbit and the eye there is abundant mesodermal tissue, which represents a continuation of the intra-cranial perimeningeal tissue. This has all the appearances of embryonic connective tissue. It is specially dense in the neighbourhood of the orbital walls, the sheath of the optic nerve and the separate muscles and around the eye. There is no sign of orbital fat.

The outer part becomes attached and forms part of the periosteum of the orbit, the part round the optic nerve forms the outer layer of the dural sheath, and the part in the region of the globe becomes Tenon's capsule and the supporting fasciae of the