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ADDITIONAL EVIDENCE AS TO THE INTERCELLULAR FORMATION OF CONNECTIVE TISSUE

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In a previous communication[1] to the Academy, evidence was presented to show that the connective tissues in the amphibian embryo arise by the direct transformation of an intercellular secretion rather than by a modification of embryonic cytoplasm. Thus, the process is fundamentally intercellular, rather than intracellular. It was also shown in this communication that the intercellular secretion, which constitutes the ground substance of the connective tissues, was of such a nature that, under the influence of various factors—probably both chemical and mechanical in character—it gives rise to the characteristic connective tissue fibers by means of a gradual consolidation and fusion of the minute structural elements of which it is composed. This gradual transformation of the more or less homogeneous, secreted ground substance into a definite fibrous tissue appears, from the morphological standpoint at least, to be identical with that which takes place, under certain conditions, in the transformation of the fibrin of a blood clot into a fibrous tissue, as shown by previous experiments.[2]

Since that time, Harrison,[3] in his investigations on the development of the balancer in Amblystoma, has reached the same conclusion with regard to the formation of connective tissue in this group. He says: "The balancer membrane thus affords a clear proof that connective tissue fibers take origin in an amorphous ground substance, independently of any direct action on the part of the mesenchyme cells." Furthermore, the results from studies[4] on the development of connective tissue in the chick embryo show that essentially the same process obtains there.

For some time investigations have been in progress, under a grant from the National Tuberculosis Association (Medical Research Committee), in an endeavor to determine the origin of the fibrous tissue which, as a rule, forms so abundantly in regions infected with tubercle bacilli, and which undoubtedly plays a very important rôle in the control of the infection and the healing of the affected tissues. Thus it has recently been stated[5] that "The importance of fibrosis in the healing or partial healing of a tubercle cannot be over-estimated. As the connective tissue cells lay down more and more collagen in their peripheries, the intercellular tissue spaces are encroached upon and finally closed, thereby obliterating the only channels of communication between the interior of the lesion and the surrounding tissues. Tubercle bacilli and their soluble products can no longer readily escape from the focus of infection; extension of the infection can no longer take place, and absorption of tuberculin-like bodies can no longer poison the system." It is evident from the above that the question of fibrosis in tuberculosis is a very important one. It is believed that the results herewith presented give clear evidence as to the method of formation of this material in experimentally infected testicular tissues of the guinea pig.

In the present studies[6] on the development of fibrous tissues in tubercular infections, the testes of guinea pigs have been used. The selection of the testis, which has proved to be an almost ideal field for these investigations, was made after an extensive examination of various other organs and tissues. Long[7] had already shown that the testis afforded a very favorable field for his studies on tuberculin. He found that the extent of the degenerative processes, following an injection of tuberculin into the testis, could be used as a direct indicator of the strength of the tuberculin solution. Long also studied the response of the testicular tissues to the living tubercle bacilli and noted that, along with other striking degenerative changes, an abundant formation of fibrous tissue occurred; thus giving evidence that these tissues were adapted for use in the present studies. The exposed position of the testis, making the direct inoculation of any desired region easy; their paired condition, giving a control testis for comparison; and, finally, their unique histological structure, which readily permits following and interpreting the degenerative structural changes that take place, are additional advantages which have been found to be of great importance for these experiments.

Following the general plan used by Long, pairs of male guinea pigs were chosen—the members of each pair being of approximately equal weight. One from each pair, which was termed the reinfected animal, received two inoculations of living tubercle bacilli; the first being a sensitizing inoculation, usually intraperitoneal in which bacilli of low virulence were used; the second, given three weeks later, being a direct inoculation into one testis, of a suspension of highly virulent bacilli. The other animal of each pair, which was termed the control animal, received only the direct testicular inoculation. The response of the testicular tissues in the reinfected animals to the direct inoculation is much more rapid and severe than in the control animals. Later, however, as the infection progresses, the histological picture in the testes of the two types is apparently identical.

The study of sections shows that the inoculation of living tubercle bacilli into the testis results very shortly in an almost complete degeneration and elimination of the germinal cells from the seminiferous tubules, so that the latter are soon emptied and appear shrunken. Coincident with these changes in the tubules, an abundant exudate appears in the intertubular areas of the testis. In well-preserved material, it is clear that the seminiferous tubules lie, for the most part, in quite close contact so that the intertubular areas are normally quite small, but they soon become greatly enlarged; due, apparently, to the shrinkage of the tubules and the presence of the exudate. Along with the exudate in the intertubular areas varying degrees of cellular infiltration may occur. In many cases, however, large areas of the exudate are practically cell free when first observed.

We may now turn our attention to the origin of the fibrous tissue which, as a rule, is formed so abundantly that within a few weeks after the inoculation, the histological picture of the testis is almost completely changed, the germinal tissues being largely replaced by masses of fibrous tissue. What has been the method of formation? It may be said that the present studies on the infected testicular tissues give clear evidence that the process of fibrosis results from a direct transformation of the exudate present in the intertubular areas. The permanent preparations present a striking and consistent series which is easily interpreted on this basis.

The exudate when first noted appears, for the most part, quite homogeneous or with a fine fibrillation. The later stages show gradual stages in the direct transformation of this material into a typical fibrous tissue, together with the formation of heavy bundles of wavy fibers which stain very beautifully and typically with a Mallory stain. This fibrous tissue encapsulates and infiltrates the tubercles which have been forming in the infected areas, and usually develops in such great abundance throughout the infected areas, as noted above, that the testicular tissues are largely transformed into fibrous tissue.

The transformation of the exudate is not dependent upon direct cellular connections, and is seen to best advantage in intertubular areas in which cells are few in number or entirely lacking. From the morphological standpoint, at least, the process which results in the formation of fibrous tissue from the exudate in the intertubular areas of a testis infected with tubercle bacilli appears to be identical with that which takes place in the development of connective tissue in the embryos of the frog and the chick, and in wound healing in the skin of the adult frog.

Thus it seems safe to conclude from the several lines of evidence now at hand that connective tissue formation is essentially an intercellular process in which certain cell secretions undergo a direct morphological transformation by a process apparently of the same character as that which takes place in the transformation of a plasma clot.

[1] Baitsell, G. A., *Proc. Nat. Acad. Sci.*, **6**, 1920, p. 77; *Amer. J. Anat.*, **28**, 1921, p. 431.

[2] Baitsell, G. A., *J. Exp. Med.*, **21**, 1915, p. 455; *Ibid.*, **23**, 1916, p. 739; *Amer. J. Physiol.*, **44**, 1917, p. 109.

[3] Harrison, R. G., *J. Exp. Zoöl.*, **41**, 1925, p. 412.

[4] Baitsell, G. A., *Quart. J. Micr. Sci.*, **69**, 1925, p. 571.

[5] Baldwin, Petroff & Gardner, *Tuberculosis, etc.*, Lea & Febiger, Philadelphia; 1927, p. 104.

[6] A preliminary report of these studies was presented at the 22nd annual meeting of the National Tuberculosis Assoc., Washington, D. C., Oct., 1926.

[7] Long, E. R., *Amer. Rev. Tuber.*, **9**, 1924, p. 215; Smith, M. I., *Jour. Med. Res.*, **43**, 1922, p. 45.

RESULTS OF HETEROPLASTIC TRANSPLANTATIONS OF THE HEART RUDIMENT IN AMBLYSTOMA EMBRYOS

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Adults of the two species of salamanders, *Amblystoma punctatum Linn.* and *Amblystoma tigrinum Green*, differ considerably in size, adult tigrinum salamanders reaching a length of about 25 cm. (in extreme cases 32 cm.) while adult punctatum individuals measure about 16 cm. in length. However, early embryos of the two species are of approximately the same size; at the time of complete yolk resorption the tigrinum embryos may even be smaller than those of punctatum. These two species, therefore, provide suitable material for transplanting an organ rudiment which at the time of operation and during early larval life is of a similar size in the two species but which becomes markedly different in magnitude in later development.

Transplantations of the fore-limb rudiment between the two species have been described by Harrison,[1] who has found that a tigrinum limb transplanted to a punctatum embryo grows much more rapidly than it would have grown if left in place, and that even after ten or twelve weeks, when the tigrinum larva is much larger than the punctatum of corresponding age, the grafted limb is larger than the control limb of either species. On the other hand, the fore limb of punctatum grafted to a tigrinum embryo, although getting an initial start ahead of its normal tigrinum