THE FUNCTIONAL VALUE OF NEWLY FORMED CONNECTIVE TISSUE.

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Experimental tuberculosis, viewed broadly, presents a characteristic picture not to be confused with any other except that of the spontaneous or natural disease. Experiments devised to analyze the picture into its component parts have usually failed to develop information of decisive value. If, for example, parallel inoculations are made with living tubercle bacilli and with those killed by heat, the lesions produced are much alike in the beginning and remain so for some time. Finally, those due to the living bacilli are progressively reduplicated by the further invasion of the tissues, while those due to the dead bacteria are gradually healed. It is difficult again to say in what essential particular the lesions due to dead tubercle bacilli differ from those caused by other mildly irritant foreign bodies introduced into the tissues.

The lesions above mentioned have in common an initial acute inflammatory reaction and a subsequent deposit of more or less perfectly formed fibrous connective tissue. At the point of divergence this connective tissue in the case of the living tubercle bacillus undergoes degenerative changes; it is broken down. But this divergence in the type of reaction is not universal. With the living bacteria, wherever either locally or generally the infection is resisted or overcome the connective tissue develops more or less steadily until the products of the earlier inflammation are replaced. It then tends to be reabsorbed. If the mass is small it may completely disappear with time; if the mass is large it remains as a scar in no essential particular to be distinguished from a scar due to any other cause.

This reaction of the connective tissue, intimately associated as it is in favorable cases with the healing of tuberculosis, may in the abstract be viewed either as a process dependent in its entirety on other factors in the disease or it may be considered to be a factor of prime importance in the recovery or partial recovery of the animal. Facts are lacking on which to base the adoption of one or the other of these concepts. For the purposes of this paper, and merely as a working hypothesis, we have adopted the view that the reactive qualities of the connective tissue may be the decisive factor in determining the outcome of a tuberculous infection.

In order to proceed from this point we require some knowledge not at present available of the connective tissue and its reactive possibilities. Is the connective tissue in this sense a constant or a variable for any species of animal? If connective tissue is to be held responsible for variations in reaction to the tubercle bacillus it can only be on the assumption that it has a variable capacity for reaction. Such an assumption is, of course, in accord with the presumption on the broadest biological principles, most, if not all, functions which can be measured being variants in either direction from a typical mean value. In the search for a measure of the capacity of connective tissue to react we have turned to the facts and experiments relating to the healing of wounds.

The repair of any solution of continuity of tissues other than the purely epithelial surfaces involves the production of connective tissue, and attempts have been made to study this reaction quantitatively, particularly, of recent years by Carrel and his associates.[1] A careful consideration of the conditions of these studies makes it seem unlikely that the values determined have any direct relation to the reaction of connective tissue.

As an example we may select an incised wound made through the skin into the soft parts and allowed to gape during the healing process. The gaping wound will fill with exudate which will then become organized with granulation tissue, and this will subsequently be replaced by connective tissue. When the gape or cavity has become well filled with granulations the epithelium will begin to cover the wound by extension from the margins. It is the rate of growth of the epithelium which has been the subject of the quantitative studies above mentioned. Granted that this growth of the epithelium is dependent

[1] Carrel, A., du Noüy, P. L., and Carrel, A., Cicatrization of wounds. IX, *J. Exp. Med.*, 1917, xxvi, 297.

on the state of the underlying connective tissue or granulation tissue as the case may be, it is yet most unlikely that the rate of epithelial growth indicates either the rate of the preceding repair of the subcutaneous tissues or its functional perfection. If conditions could be precisely defined it is probable that in the whole process of wound healing the period when the deep defect was being replaced by potential connective tissue would take its place as a latent period in the reaction of the epithelium.

We have found one account in the literature of an experiment made to measure the effectiveness of connective tissue repair. Sir James Paget[2] (1853) writes as follows:

"The strength, both of the new substance itself, and of its connection by intermingling with the original substance, is worthy of remark. To test it, I removed from a rabbit an Achilles tendon, which had been divided six days previously, and of which the retracted ends were connected by a bond of the size and texture usual at that period of the reparative process. I suspended from the half-section of this bond gradually increased weights. At length it bore a weight of ten pounds, but presently gave way with it: yet we may suppose the whole thickness of the bond would have borne twenty pounds. In another experiment, I tried the strength of a bond of connection which had been ten days forming: this, after bearing suspended weights of twenty, thirty, forty, and fifty pounds, was torn with fifty-six pounds. But surely the strength it showed was very wonderful, when we remember that it was no more than two lines in its chief diameter; and that it was wholly formed and organized in ten days, in the leg of a rabbit scarcely more than a pound in weight. With its tenacity it had acquired much of the inextensibility of the natural tendon. It was indeed stretched by the heavy weights suspended from it, yet so slightly that I think no exertion of which the rabbit was capable would have sufficed to extend it in any appreciable degree."

While these experiments were directed toward a study of the repair of tendons a superficial review of the conditions suffices to show that in the early days following tendon section it must be a value for connective tissue which is determined. This connective tissue may or may not be wholly representative, but it is difficult to devise a better experimental test for the reactive capacity of connective tissue. We therefore adopted the general method of Sir James Paget's experiments as a basis for further work. Our work falls naturally into

[2] Paget, J., Lectures on surgical pathology, London, 1853, i, 271.

two phases: (1) an effort to determine the rate of replacement of the tendon as measured by the strength of the connective tissue laid down between the cut ends; (2) an attempt to use the method in the study of the action of certain chemicals locally or generally applied and for the examination of that phase of the tuberculosis problem previously discussed.

Method.

Adult rabbits were anesthetized with ether. With various degrees of aseptic precaution the Achilles tendon was exposed and sectioned. At various intervals thereafter the animal was killed and the leg amputated at the hip and suspended by the knee through a strong wire loop as an intermediary to a stout chain hung from the ceiling. With a similar wire loop a pail was suspended from the hock. Into this pail clean sand was run from a funnel at a uniform rate and from a uniform height. When a break occurred the flow of sand was stopped, the pail, its contained sand, and all the apparatus coming away with the foot were weighed, the result being recorded in grams as the tensile strength of the newly formed tissue.

At the time the leg was amputated the tendon was dissected free from the surrounding tissue and covered with gauze wet with salt solution. After the leg was hung up, as a last step before the sand was run in, the tibia and the remaining soft parts were cut through with bone forceps and scissors. In this way the new tissue was protected against any irregular strain during the time of preparation.

In general the method can be said to have served its purpose. The difficulties and irregularities for the most part seem to be connected with the surgical procedure. Infection must be avoided as the presence of small pockets of pus in the connective tissue naturally weakens it. Hemorrhage at the time the tendon is cut cannot always be controlled and when the test is made the instances in which evidence of hemorrhage persists must be excluded. The tendon sheath must be carefully sutured at the time of operation. If this is not done the exudate extends through the opening and infiltrates the surrounding fascia. This then organizes and forms adhesions which may involve all the structures from the newly formed matrix of the tendon outward to the skin. On dissection it then is impossible to isolate the representative tissue. The new connective tissue seems to spring from the old tendon sheath as well as from the cut ends of the tendon, or at least adhesions to the old tendon sheath are universal. If when the dissection is done an attempt is made to remove the last traces of old connective tissue, splits and shreds will be run down into the new tissue which would naturally weaken it. With care, however, the old tissue can be reduced to a thin film without damage to the new. It is not difficult to avoid the gross sources of error, and the variations due to minor faults probably do not affect the results in the main.

Experiments Bearing on the Healing of Wounds.

In the first sets of experiments, those intended to determine the general feasibility of the method, surgical cleanliness was sought through the use of antiseptic solutions. A coal-tar disinfectant of the creolin type was used in solution of appropriate strength for cleansing the skin, keeping the hair wet, and cleansing the hands and instruments. Ordinarily care was taken to touch the field of operation with instruments only and these were freed from excess of antiseptic. Protocols selected as being representative are summarized in Tables I and II.

The experiments recorded in Tables I and II were, as stated above, carried out depending upon an antiseptic solution for surgical cleanliness. In Table III is shown the result of an experiment in which the operative work was done with rigid aseptic precautions. All material used was previously sterilized in the autoclave. Gloves were worn and at the same time especial precautions were taken to keep dust and bits of hair from the skin of the animals from contaminating the wound. In this experiment dichloramine-T is again introduced.

It is not our present purpose to discuss the results of the three experiments presented so far with any pretense to finality.

The results of the second series are in accord with the expectation we entertained when benzene was administered to part of the rabbits. Benzene is known to cause an extensive aplasia of the lymphoid tissue and to give rise to an extreme leucopenia. It was expected to influence in a deleterious way the exudation processes involved in the repair or replacement of the tendon. The experiment, if its results are accepted at their face value, and we see no reason why they should not be so accepted, indicates that the method is adequate in that it is capable of revealing the action of extraneous influences brought to bear on the reparative process through systemic channels.

It will be noted that there is a large difference between the average tensile strength found in Tables II and III and that that in

TABLE I.

The Achilles tendons of rabbits were cut by open operation under ether anesthesia. The tendon sheath and skin were sutured separately. In the dichloramine-T series $7\frac{1}{2}$ per cent dichloramine-T dissolved in chlorinated eucalyptol, specific gravity 1.2, was put into the tendon sheath and the remainder of the wound at the operation. A small amount remained when the sutures were tied. Breaking strength determined 10 days after section of tendons.

Untreated.	Dichloramine-T.
gm.	*gm.*
4,120	5,470
6,320	7,170*
6,620	7,520
7,750	9,100
7,900	9,370
8,050	10,520
10,070	12,200
10,600	
Average........ 7,678	8,764

* In this case the connective tissue did not break. The tendon pulled out of the muscle and the recorded strength is considerably too low.

Table I is intermediate to these. The low place of Table II is in part to be explained by the fact that when the first of the series were tested the operator made more effort to trim away all traces of the original connective tissue than has seemed practicable. In order that the experiment might not be lost the method was persisted in even though it seemed somewhat faulty. It is probably true also that there was in this series in general more evidence of slight persistent hemorrhage and low grade infection than in the others. The internal varia-

TABLE II.

Operation as in Table I. The rabbits in the benzene series had 5 cc. of 50 per cent benzene in olive oil administered subcutaneously on the day after operation. Breaking strength determined 7 and 10 days after operation.*

Untreated.		Benzene.	
7 days.	10 days.	7 days.	10 days.
gm.	gm.	gm.	gm.
2,270	3,900	1,900	
2,920	4,140	2,380	
3,120	4,400	2,430	
3,450	5,050	2,540	2,240
3,650	5,350	2,650	2,540
3,850	5,700	2,720	3,350
3,950	5,970	2,950	3,390
4,200	6,120	3,420	3,970
4,950	6,200	4,170	5,750
5,620	6,650	4,270	6,200
Average. 3,798	5,348	2,943	3,917

* This whole experiment was carried out by Mr. R. H. Kennicott, then a special student in the Medical School of the University of Pennsylvania.

TABLE III.

Operation aseptic; otherwise as in Table I. In the dichloramine-T series the statements made in Table I about similar series also apply. Breaking strength determined at 10 days.

Untreated.	Dichloramine-T.
gm.	gm.
4,920	4,350
6,450	5,350
7,150	6,650
7,700	7,300
8,170	7,600
9,150	7,650
10,000	7,850
11,170	8,800
12,750	10,000
13,750	12,800
Average........ 9,121	7,835

tion in Table II is, however, no greater than the others, if it is as great.

The increased strength attained in 10 days in Table III contrasted with Table I is, we believe, accounted for by the improvement in operative technique—a careful aseptic method having been substituted for a rather crude procedure in which reliance was placed in an antiseptic solution. The fourth experiment (to be presented later), also done aseptically and after more experience, shows a still further increase in the average tensile strength.

In contrasting these experiments emphasis has been laid on the averages. A scrutiny of the tables shows that the averages are surprisingly representative. Where the average is higher it is not due to the presence of exceptionally high figures for individuals.

The dichloramine-T results are not such as to permit satisfactory interpretation. They might be taken to indicate that this material helped in the case of an imperfectly carried out antisepsis and hindered healing when asepsis was better. This substance is, in any event, not one which would be recommended to be left in wounds which are closed tightly as it is capable of causing an aseptic inflammation in the solvent used. Our purpose in including these figures at present is to make available all our data.

As a general method for the quantitative study of a fundamental aspect of wound healing we cannot feel that we have contributed anything in finished form. The wide variation in values found necessitates work on a statistical basis and if there prove to be no other limitation the method is obviously laborious.

Experiment on Tuberculosis.

Having in mind the possible place of the reactivity of the connective tissue in relation to the healing of tuberculosis as presented in the introductory paragraphs of this paper, we sought information by the application of the method as developed to the problem along the following lines. The fact that the length of life of animals of a given species inoculated with anything less than an overwhelming dose of virulent tubercle bacilli varies widely has recently been emphasized by the work of our laboratory. Whatever the reason may be the strength of the newly formed connective tissue as determined above also varies over a wide range. We sought to determine whether the variations in the two cases were parallel or otherwise. With this purpose in mind the following experiment was carried out.

Young well grown rabbits, weighing about 1,500 gm., were chosen. The Achilles tendon of one leg was sectioned. On the 10th day the leg was amputated at the hip and the tensile strength of the reparative tissue was tested as described. The amputation was carefully conducted under ether anesthesia. Time additional to that needed

TABLE IV.

The rabbits weighed approximately 1,500 gm. The tendons were cut between October 1 and November 15, 1917, under ether anesthesia. Leg amputated and tensile strength of connective tissue determined on the 10th day. March 1, 1918. Inoculated all surviving animals with 0.02 mg. of tuberculosis culture, Bov. C., intravenously.

Length of life after inoculation of tubercle bacilli.	Tensile strength.	Length of life after inoculation of tubercle bacilli.	Tensile strength.
days	*gm.*	*days*	*gm.*
43	8,220	62	10,500
45	6,420	62	8,200
45	12,500	62	6,850
45	9,600	85	7,550
48	12,600	88	14,000
51	11,400	92	13,100
52	11,550	92	14,700
55	8,300	92	8,800
57	9,070	113	11,200
58	13,100	113	9,900
Average strength of first half to die..................	10,276	Average strength of second half to die.............	10,480

for healing of the operation wound was allowed for the perfect recovery of the animal from the effects of this precedure. The animals were then inoculated intravenously with a suspension of virulent tubercle bacilli (0.02 mg. of the growth on glycerol agar). Every effort was made to attain uniformity in this inoculation. The animals were then retained until they succumbed to the tuberculosis. In Table IV the length of life of the rabbits in days after the inoculation with tubercle bacilli is set over against the tensile strength of the connective tissue in grams. Calculation shows that the average tensile strength of the connective tissue formed by the first ten rabbits to die is practically identical with that formed by the last ten animals.

It is apparent that no correlation appears, that consequently we have by this experiment demonstrated no relation between the reactivity of connective tissue in a normal reparative process and the general resistance of the animal to experimental infection with *Bacillus tuberculosis*. In fact the experiment seems sufficiently decisive to warrant the tentative conclusion that no relation exists between any dominant factor in the laying down of connective tissue in response to traumatism and natural resistance to tuberculosis.

SUMMARY.

1. A method has been revived which enables the connective tissue factor in wound healing to be studied quantitatively.

2. It has been found that the functional value of connective tissue formed in response to traumatism as represented by its tensile strength varies widely in different animals.

3. It is suggested that the method may find application in the study of extraneous influences which may affect wound healing either through local or systemic application.

4. It has been determined that there is no parallelism between an active connective tissue response to traumatism and natural resistance to inoculation tuberculosis in the rabbit.