Drummond[1] has recently tested the possibility of carotin being the fat-soluble vitamine by feeding both crude and crystalline preparations of the pigment to rats, although the question may be raised as to the logic of testing the relation to fat-soluble vitamine of a substance of which is not natural to the body of the animal upon which the test is performed. Carotin is not found in the body of the rat.

The writer[2] has recently reported the fact that it is possible to raise a flock of chickens from hatching to maturity on a diet free, or at most containing the merest traces, of carotinoids. Not only did the mature hens lay eggs whose yolks were free from carotinoids, but a second generation of carotinoid-free chicks were hatched from them. Only one of two possible conclusions can be drawn from this experiment. Either the fat-soluble vitamine and the yellow plant pigments are not related physiologically or the fat-soluble vitamine requirement of fowls differs from that of mammals. The diet which we used for the successful growth of the chickens contained an abundance of fat-soluble vitamine, however, in the form of carotinoid-free pork liver.

Another interesting case of negative relation between carotinoids and fat-soluble vitamine is seen in the fact that a number of species of animals, such as sheep, swine, dogs, cats, rats, rabbits, and guinea pigs are free from carotinoids in blood[3] and adipose tissues, and nerve cells.[4] The milk fat of the mammals of these species is also colorless. How is one to make the successful raising of young on carotinoid-free milk coincide with the assumption that fat-soluble vitamine is one of the yellow plant pigments?

Still another instance of negative relation between carotinoids and fat-soluble vitamine is seen in the case of certain vegetable oils, like cottonseed oil. Fresh cottonseed oil, after being purified from resinous material, has a beautiful golden yellow color and is rich[5] in carotinoids. It should also contain an abundance of fat-soluble vitamine to be in keeping with Steenbock's assumption. Apparently this is not the case since both bleached and unbleached cottonseed oil has been found to be free from vitamine.[6] The oil from yellow corn, similarly, should contain the vitamine, but the same investigation[6] has reported failure to obtain growth with diets containing the commercial unbleached corn oil.

It is thus possible to cite a number of instances where the probable relation between carotinoids and fat-soluble vitamine breaks down. No doubt others could be found. The writer regards the instances of a simultaneous occurrence of fat-soluble vitamine and plant carotinoids as fortuitous. The similarity of certain of the properties of the two kinds of material admittedly offers a working basis for the ultimate isolation of the fat-soluble vitamine, and research in this direction offers many fascinating possibilities. The relation between the vitamine and color in the case of corn may be a genetic one, in which case it should be possible to transfer the vitamine to white corn. Further attempts, however, to establish an identity of the vitamine with one of the carotinoid pigments is not likely to lead to profitable results.

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SCIENTIFIC ARTICLES

WOUND HEALING IN EXPERIMENTAL (CELLFIBRIN) TISSUE[1]

1. If we make a defect in the skin, processes of healing set in which in time lead to a closure of the wound. Primarily, the defect

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calls forth an emigration of epidermal cells into the wound. Secondarily, cell proliferation by mitosis and a contraction of fibrous tissue takes place and these three processes contribute to the wound closure. Under certain conditions the intensity of cell migration depends upon the size of the wound; and the contraction of the wound, depending in all probability on the contraction of the fibrous tissue and the number of retracting fibers being greater in the larger than in the smaller wound, shows a certain quantitative relation to the size of the wound.

Essentially and disregarding complicating factors, the same stimulus leads to the migration of cells and to cell prolification in wound healing.[2] To understand wound healing it is necessary to study experimentally the conditions which influence the migration of the cells into the wound. The important fact in wound healing is that in a tissue which was previously at rest, the making of a defect calls forth new activities in the cells adjoining the wound.

2. In earlier investigations we have shown that after the shedding of the blood of *Limulus* the amœbocytes agglutinate and thus produce a tissue-like organization which under certain experimental conditions bears a certain resemblance to epithelial, under others to connective tissue. This agglutination of cells is not accompanied by a transformation of fibrinogen into fibrin.[3] Subsequently we observed that an emigration of cells takes place from such tissue if pieces of this "cellfibrin" are put on a slide and kept under suitable conditions.[4]

We have recently resumed these experiments and have succeeded in working out methods which permit us within certain limits to imitate in an experimental tissue composed of agglutinated blood cells processes which are characteristic of normal tissues.

[2] A more detailed discussion of these conditions will be given in a forthcoming paper on wound healing in the *Journal of Medical Research*.

[3] Leo Loeb, *Biological Bulletin*, 1903, IV., 301; *Virchow's Archiv*, 1903, Vol. 173, 35.

[4] Leo Loeb, *Biochem. Zeitschrift*, 1909, XVI., 157.

3. In such experimental "cellfibrin" tissue the processes of wound healing and tissue grafting can be imitated, as far as the primary process in wound healing, namely the formation of layers of regenerating tissue through migration, is concerned. A defect in this artificial tissue, measuring about 6–8 square mm. can be closed in the course of two to three days, and a piece of tissue grafted into a defect can be seen to unite with the host tissue through regeneration taking place in the host as well as in the graft. We have every reason to believe that the essential factors underlying these healing processes in the skin of a mammal and in such experimental cellfibrin tissue are very similar. In both cases a tissue which has been in a resting condition is made to migrate into a wound under the influence of the wound stimulus.

4. In order to produce cellfibrin tissue, we collect in a stender dish a certain quantity of blood of a large *Limulus* under conditions which preserve the blood cells as much as possible. The latter form several layers on the bottom of the dish. The cells are glued to each other as well as to the bottom of the dish and thus form a compact even layer of tissue. With a scalpel we can make wounds of various sizes in this tissue and then readily follow with a low power of the microscope the different stages of wound healing. At the border of the wound we may recognize the outgrowth of the regenerated tissue even with the naked eye. In this defect we can transplant tissue of the same kind and follow the union between host and graft.

We may furthermore cut out a very small piece of tissue, place it on a cover glass, add a drop of blood serum or other fluid, and fix it with vaselin on a hollow slide, in the same way as in the case of other tissues growing in vitro. We can thus follow the radial outgrowth of the tissue. The pictures obtained correspond closely to those seen in the vitro culture of other tissues.

5. We have begun an analysis of the conditions determining wound healing in this

experimental cellfibrin tissue; we shall mention here a few of the results obtained so far.

(*a*) The influence of the temperature is very marked. The temperature coefficient seems to be such as might be expected, if wound healing depended upon chemical processes. While regeneration takes place steadily even in the ice chest at a temperature of from 6–10°, the outgrowth is much more rapid at a temperature of about 20°. Here however also secondary changes take place much more rapidly in the outgrowing cells.

(*b*) The depth of the layer of blood serum covering the wound or piece of cellfibrin does not seem to influence the rapidity of the healing process. This seems to indicate that the quantity of oxygen supplied is sufficient, even if a layer of serum about 10 mm. deep separates the tissue from the oxygen of the atmosphere. The amount of free oxygen was still further diminished in experiments made by Miss Clinton. Hydrogen passed through the blood serum for one hour previous to the introduction of the tissue into the serum. This was followed by a second period lasting fifteen minutes in which again hydrogen was carried through the serum. Even under these conditions outgrowth took place from pieces of cellfibrin previously placed on cover glasses.

(*c*) In a third set of experiments we compared the intensity of tissue movements in tissue growing in or against the direction of gravity. The tissue was held in a vertical position on the cover glass. We found that the tissues can grow out against the direction of gravity as well or almost as well as in the opposite direction. The average intensity of outgrowth is probably somewhat greater in the direction of gravity than in the opposite direction.

(*d*) If we observe tissue growing towards each other from different parts of a wound, or from two separate pieces of cellfibrin placed near each other, we find that the cells coming from opposite directions intermingle quite freely with each other. There is apparently no repellent action exerted by one sheet of tissue upon the movements of the others. It is evidently not the products of metabolism of certain cells which induce the cells to become active and to leave the position in which they had been at rest.

(*e*) By using our method it is possible to alter experimentally the base on which the tissue moves. Thus we can substitute a surface of paraffin, vaselin, coagulated egg or agar for glass or cellfibrin tissue. It is of considerable theoretical interest to determine the character of ameloid movements on substances like paraffin. We find that even on paraffin and vaselin an excellent outgrowth of tissue can take place, although the physical properties of these substances modify in some respects the behavior of the tissue cells. On coagulated egg and agar outgrowth takes place likewise but secondarily osmotic or chemical factors may come into play and injure the cells.

(*f*) We have begun the study of the effect of various inorganic substances, particularly of constituents of the blood and seawater on the movement of cellfibrin tissue in wound healing, and on ameboid movement in general. According to their effect on the tissue movements, we can arrange the various substances in the following order: (1) 2/3–1/2 *m* NaCl, (2) 2/3–1/2 *m* KCl, (3) 1/2 *m* $CaCl_2$, (4) *m*/3 Na_2 HPO4, (5) 5/8 *m* N H_4Cl, (6) *m*/3 Na H_2 P O4, (7) H_2O. NaCl is the least and N H_4Cl, Na H_2 P O.4 and H_2O are most injurious. In the latter solutions no distinct outgrowth takes place. How far certain variable factors as the amount of blood serum adherent to the tissue or bacterial infection may modify the results will have to be determined in further experiments.

Dilution of the solution within certain limits is not incompatible with outgrowth. Thus outgrowth can be readily obtained in a solution of 5 c.c. 5/8 *m* NaCl + 3 c.c. H_2O; addition of as much as 0.5 c.c. of a *m*/100 HCl or NaOH solution to 5 c.c. 5/8 *m* NaCl likewise permits frequently the outgrowth of tissue.

We wish to express our thanks to Mr. Julian P. Scott, who assisted us in these experiments.

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