The modern knowledge of development of tissues, largely lies on the famous studies of Spemann, who discovered the role of “organizers” for development of an embryo, and established the meaning of “induction phenomenon”. And later along this experimental method many results were added by Waddington(1), Needham(2), Brachet(3) or other investigators.

In 1931, Huggins(4) reported a bone formation(5) induced by sub-fascial transplantation of urinary bladder epithelium. This is an exceedingly interesting fact from the standpoint of induction theory.

In my studies I found elastic fibers were formed remarkably(6) in the node following transplantation of prostatic tissues and seminal vesicles epithelium.

Hitherto the experimental formation of elastic fibers had been considered impossible. In spite of that, there occured the changes of connective tissue accompanied by a new formation of that fibers, which should be regarded as a consequence of the transplantation. In these experiments we were guided by the suggestion of induction theory.

At present my data are not sufficient to dwell on full discussion of the problems my experimental results are sufficient to indicate new formation(7) of elastic fibers, on which I wish to report in this paper.

MATERIALS AND METHODS

Guinea pigs of about 350 g in weight were used in the experiments. As a general rule, adult tissues of prostate were transplanted into female animals and those of seminal vesicles in male animals. This combination is decided from the preliminary experiment as the most convenient for my purpose. The total number of animals was 80. Following the method of Huggins, the isolated tissues were inserted just under the abdominal fascia, then ligatures were placed.

About 10 days after the transplantation, the nodal induration became tangible at the site of operation. This node was excised out in various stages,
fixed in acetone-alcohol, embedded in paraffin, and cut into serial sections. Then hematoxylin-eosin stain, Mallory stain, resorcin-fuchs in stain (or orcein stain), PAS stain, silver impregnation and the test for acid and alkaline phosphatases were tried.

Besides the above I transplanted a tissue excised from mature fetus, and tried to compare the inductive activity in both adult and foetal tissues.

RESULTS OF THE EXPERIMENTS

The nodes above mentioned are mostly grey-colored and half as large as a grain of rice. They can be easily isolated from the surrounding tissues.

HISTOLOGICAL FINDINGS OF THE NODES

GROUP (1) comprises the cases which did not show notable changes of inserted tissues and showed nearly normal pictures. The cases were usually observed when the tissues were transplanted into male animals.

As for the prostate, the original structure with glandular lumens are kept enough. There is always proliferation of epithelium inside of lumens. There is metaplasia, too, that is to say, squamous epithelisation of those increased epithelial cells. And we can see the focus of hyalinisation which engirdles the lumen at the outer zone of epithelium lamination, like a belt, clearly bordered. Those foci are grown remarkably well especially where the proliferation is prominent.

In stroma, which is normally composed of muscular tissue and a little connective tissue, the latter element proliferates around the epithelium after the transplantation. The above-mentioned focus of hyalinisation stains blue in Mallory, red in van-Gieson, and gives a strong positive reaction in PAS stain, and is really the congregation of argyrophilic fibers. When Weigert stain is tried, elastic fibers come in sight, quite adjacent to the focus of hyalinisation in the connective tissue surrounding it. The farther from the focus, there is less elastic fibers. No elastic fibers are seen even in a whole section adjacent to the basement membrane, if there is no focus of hyalinisation around these epithelial cells. In the epithelial cells metamorphosed by metaplasia, acid phosphatase is found at the periphery of cytoplasm, alkaline phosphatase also is found in whole cytoplasm equally and the both are rich in quantity, but there is no phosphatase in the original cuboidal epithelium contrariwise. The focus of hyalinisation has no phosphatase but the fibers surrounding it contain alkaline one.

In these cases, therefore, the fibers containing alkaline phosphatase nearly
conicide with the elastic fibers.

When the epithelium of seminal vesicles was transplanted into male animals, about a half of cases showed formation of cystic cavity composed of double epithelium, which mostly proliferated and protruded papillarily into the cavity, but generally speaking, they were like normal pictures. The sub-epithelial connective tissue becomes rough, and the fibers increased a little, but the focus of hyalinisation does not appear. But in these cases, too, the appearance of elastic fiber in the connective tissue around the epithelium was noted. Phosphatases, similarly to the normal cases before the transplantation, were found in the epithelium of cystic cavities and the fibers of connective tissue around them. Metaplasia of epithelium can not be seen in these cases, in contrast with the cases using prostate. But some cases showed the narrow hyalinisation at the region corresponding to the basement membrane, though it does not come up to those which were seen in prostate in width.

GROUP (2) comprises the cases which showed notable changes of inserted tissues. There were cases where the prostate tissues were transplanted into female animals and in the other half of the cases where the epithelium of seminal vesicles were transplanted into male animals.

The former cases, moreover, can be divided into two subgroups. First, some of them, in spite of similar findings as the cases of male animals regard to the outlook of lumens, show a quite difference from them in the attitude of epithelium and connective tissue. The disposition of cells becomes irregular, and the cell itself tends to degenerate and desquamate, so there is no proliferation, no phosphatase in it. In contrast with the cases of male animals there occurs chiefly the degeneration of epithelium instead of remarkable proliferation in the other. Though localized hyalinisation as seen in the cases of male animals, characterized by strong reaction in PAS stain, the congregation of argyrophilic fibers are not seen, and hyaline foci are scattered quite extensively in the connective tissue surrounding each glandular lumen. Namely, hypertrophy of the basement membrane does not appear. These foci contain cells (like fibroblasts) here and there in them, besides they are stained differently in comparison with the cases of male animals. They are stained faintly and homogenously with eosin, and light-blue in Mallory, giving a weak positive reaction in PAS, and contain hardly any argyrophilic fibers. The more important characteristic finding is that they are nothing but a congromeration of elastic fibers, and there is no elastic fiber in the connective tissue surrounding these foci. Regarding the form of each fiber, it is in general far thicker than the ones found in the cases of male animals and some of them are winding,
some are turn to pieces, and still others show other various forms.

Secondly, the remainder of the cases of female animals shows hardly any resemblance to the original structure of prostate. In some of them there are scattered small groups of cells, apparently epithelial cells, here and there, but even such picture can not be seen in other cases. Some show the hyalination of connective tissue, but some show clear stripes of fibers. At any rate it is quite impressive that the connective tissue, after all, are very rich in elastic fibers.

In the cases where the epithelium of seminal vesicles were transplanted into male animals, excepting about a half of them which belong to group (1), the formation of several typical cystic cavities composed of simple squamous epithelium were seen. The proliferated epithelial cells are not stratified but spread to make the cystic cavity. This inclination resembles that of prostate epithelium of group (2), and differs from that of group (1). The most characteristic findings are the appearance of focus of hyalination surrounding the cystic cavity, and the appearance of elastic fibers surrounding these foci. This time, furthermore, there are quite abundant elastic fibers. They are thick, sometimes fragmentary, and among them accompanied by the granular or membraneous structures having affinity with resorcin-fuchsin and orcein.

Thus far we have described histological findings in our experiments, dividing them into two large groups, (1) and (2), but here, in order to make them more intelligible, they may be divided into four groups, A, B, C and D, from a viewpoint of the relation between the focus of hyalination and the elastic fibers.

A: Cases which show the diffuse and extensive hyalination adjacent to the epithelium and in the same place notable new formation of elastic fibers. (Prostate tissues in female animals).

B: Cases which show the evident foci of hyalination around the epithelium but show the new formation of elastic fibers outside those foci. (Prostate tissues in female animals or male animals, seminal vesicles in male animals).

C: Cases which do not show any hyalination but show the new formation of elastic fibers adjacent to the epithelium. (Prostate tissues in female animals, seminal vesicles in male animals).

D: Cases which show no formation of glandular lumens but an extensive appearance of elastic fibers accompanied by the foci of hyalination. (Prostate tissues in female animals).
NEW FORMATION OF ELASTIC FIBERS

In addition, the same experiments were repeated using the prostate and seminal vesicles obtained from a fetus. The result was that new formation of elastic fibers was generally so slight that the classification above mentioned could not be applied to them, though the nodes of transplantation had almost double in the size. Regarding the comparison with the cases using the tissues obtained from adult animals, we will later touch upon them again in the paragraph of discussion.

In the next, we will describe the findings in the early stage of new formation of elastic fibers. The results of time-difference observations of the cases which were expected to be A type (prostate tissues transplanted in female animals) are as follows.

In 4–6 days after transplantation, the section shows still the original structure of prostate, and gradual degeneration may be presumed from the lowering of stainability, but no elastic fibers yet are seen.

In 7–10 days the section begins to show evidence of appearance of the
fibers having affinity with resorcin-fuchsin or orcein. Therefore, it may be said that it requires about 7–10 days for the formation of elastic fibers in our experiments. In that stage about a half of epithelial cells still keep their normal state, but the others show evidences of degeneration and desquamation. The hyalinisation occurs at the surrounding muscular and connective tissues. The cells of the connective tissues (containing the muscular cells partly) are deprived of their nuclei. But there are neither granular proliferation nor cell infiltration. Fine elastic fibers appear in bundles or network at the transitional region between the normal connective tissue and the parts of hyalinisation. It is difficult to say about the disposition of fibers, but their long apices are pointed towards the center of hyaline region from the margins.

Though the new formation of elastic fibers often surround the fragments of nuclei, or seen as if they were joined to the orceinophilic granules which lie in the cytoplasm, the cell itself probably has no direct relation with the formation of elastic fibers. When the hyalinisation occur strongly, there are hardly any elastic fibers in the central part (just adjacent to the epithelium) of it. The fibers, anyhow, appear in manifold manners. When there is no hyalinisation, the thick elastic fibers appear none the less, membraneously adjacent to the basement membrane. They are not stained equally; stain most densely near the epithelium, and the farther from it, the more faintly. Such faint stainability are seen in the cases where the focus of hyalinisation are in the previous stage of completion of type A, and does not yet show the evident figure of elastic fibers.

DISCUSSION

In our experiments, the transplanted epithelium of genital glands and the focus of hyalinisation, the changes of connective tissue caused by the transplantation, played the chief role in the formation of elastic fibers. This is very significant. Of course they are not the remainder of the fibers which existed in the transplanted tissue or abdominal muscle from the observations in the early stage.

The common findings through 4 types are that elastic fibers always surround the epithelium and lie in or outside the region of hyalinisation. The first subject to be discussed is the attitude of epithelium. In the type B and type C, the degeneration and destruction of epithelium, which are evidently seen in type A and type D, can hardly be found. The transplanted epithelial cells of prostate proliferates remarkably and are laminated into the glandular lumen. In many cases there is squamous epitherisation. On the other hand, some cases
show no stratification but make one layer of typical cystic epithelium, engirdling a fairly large cavity. Judging from these findings, the former cases can be called metaplasia, but the latter should be called histological accommodation. The original epithelium has no phosphatase, but on the contrary, the metaplastic epithelium is rich in phosphatase, so some changes of intracellular mechanism may be assumed. The findings about the metaplastic epithelium are similar to the Bern's description, but do not coincide with his views, regarding the participation of mesodermal cells in the process of metaplasia or such appearance of “basal cells” as he described.

Considering the relation of hyalinisation to the appearance of the fibers containing phosphatase, and besides the mechanism of formation of the elastic fibers together, it is more difficult to discuss the problems of connective tissue than the findings of phosphatase in epithelium.

The localized hyalinisation adjacent to the epithelium, which is seen in type B, can be regarded as the hypertrophy of the basement membrane judging from its locality and the results of silver-impregnation. These belt-like regions of hyalinisation contain no phosphatase, but in the rough connective tissue surrounding them there are fibers having a little phosphatase, which coincide with the appearance of elastic fibers. Such fibers appear in type A, or D, too, outside the focus of hyalinisation. But the relation between such fibers and the elastic fibers in type A is entirely opposite to that of type B. This is a difficult subject to understand. The discussion concerning this will be done once more, later.

In type A and type D, there are remarkable degeneration and destruction of epithelial cells, and no stratification of them is seen even in the cases having evident glandular lumens. And in the connective tissue, too, hyalinisation occurs diffusely and extensively. Here, the focus of hyalinisation is not so homogenous and amorphous as that of type B. Something resembling fibroid structure can be seen.

In type B and type C, as was shown repeatedly, the proliferation of transplanted epithelial cells occurs into the glandular lumen, and is accompanied by the hypertrophy of the basement membrane, which is clearly bordered against the circumferential stroma, in which there is no hyalinisation, and the new formation of elastic fibers is rather slight. All these are seen when the genital tissues of male animals are transplanted into male animals.

Most of the cases in type A and type D, which show different pictures, are presented when the same tissues are transplanted into female animals. It reminds us, therefore, of sexual hormons, which may be the most important
factor that causes such difference between the two. In the latter cases the epithelial cells tend to degenerate and fall down, and consequently accompanies no hypertrophy of the basement membrane, which joins with the surrounding connective tissue without a clear border. Besides, the widespread hyalinisation which is seen in the stroma and the replacement of itself by abundant elastic fibers characterizes them.

It is difficult to know the function of cells, because the epithelial cells are special tissues (sexual glands) and in special environment (transplantation). But I recognized at least a fact that the transplanted epithelium did cause the new formation of elastic fibers in the stroma.

The epithelium should change its ordinary metabolism in such foreign environment. It is known by the examination of phosphatase, too. Those epithelial cells take two ways; one is the way of proliferation, the other is the way of degeneration and destruction.

As the formation of elastic fibers is remarkable in the latter cases, there must be a close relationship between it and the destruction of epithelium. It is imaginable that some substances contained in the cells would be thrown out because of such destruction. Hyalinisation that appears in the stroma may be due to the co-operation of such substances and the heterosexual hormons, as the changes of connective tissue are always bounded within a node. Now, the phenomenon called "hyalinisation" is regarded generally as one of so-called "regressive changes". But since there is the fact that elastic fibers appear in parallel with such changes of connective tissue, the definition that hyalinisation is degeneration reaction does not hold in the cases demonstrated in our experiments.

On the other hand, the appearance of elastic fibers surrounding the hyalinisation (or hypertrophy of the basement membrane), which are seen in type B, are mostly not so marked as in type A quantitatively, no growth was seen by time-difference. Moreover, the hyalinisation is not extensive. For that reason, it may be assumed that the condition of collagen (prae-collagen) fibers takes up a tendency to precipitate elastin.

But from the stand point of the theory of induction, various results obtained in our experiments may be explained as a mere question of degree of excitation of epithelial cells. The formation of elastic fibers may be destined to proceed till the sub-epithelial cells are formed and only in type A and type D this process may have been almost completed. This idea is not incompatible judging from the fact that elastic fibers always begin to appear at the margin of focus of hyalinization.
NEW FORMATION OF ELASTIC FIBERS

The course taken by the epithelium after transplantation differs depending upon various influences, among which that of hormones is apparently the greatest and the time of appearance of elastic fibers in indefinite. At any rate, it is quite interesting that the elastic fibers are much more abundant when the tissue of prostate is transplanted into female animal, and this fact shows the close relation of sexual hormones to the formation of elastic fibers. It was also shown that the epithelium which were under the influence of active sexual hormones caused far more remarkable formation of elastic fibers as shown in our experiments in which prostate of a fetus was used, and the results of which were only slight new formation.

The activities of cells may be determined by measuring the phosphatase. According to Robinson et al., the phosphatase content of the bone relates to its growth but not to its function, while that of the kidney or prostate relates to their functions but not to their growth. Their graphic pictures, show phosphatase contents of various organs in different stages. Its amount in the bone various great deal in fetus but after birth it shows usually low values. On the contrary, the phosphatase content of the kidney or prostate shows a sudden increase after birth, in spite of its low values in the fetal period, specially in the prostate it attains the highest value in the period of adolescence, during this period the activities of the gland are vigorous. It has already been shown that the formation of elastic fibers with fetal epithelium is less active in comparison with the cases with the epithelium of adult animals. Thus it is clearly seen that the formation mechanism is stimulated distinctly by activated cell-function. This formation, as was described already, seems to begin with the appearance of fine fibrils or network composed of linked granules in the most cases.

Kobayashi et al., observed experimentally a new bone formation, including mallow tissues, induced by urinary bladder epithelium transplanted in sub-fascia. According to them, the cells in the connective tissue participate directly in the formation, in this process there appeared the focus of hyalinization, but no elastic fibers could be found in any stage. This is also very interesting experiment concerning metaplasia.

Hitherto no observation on the new formation of elastic fibers, except that of fetus, has been reported, nor studies on their formation mechanism. Recently, with an electron-microscope, the scene of elastic fibers digested by elastase was reported, and the investigation of their delicate structures is still continued, but the mechanism of their formation is still in the dark.

The elastic fibers which appeared in my experiments take various forms, such as fibrous, granular, membraneous, fragmentary forms and so on. But
the figure shown in "degeneration of elastic fiber" also can be seen depending on the changing environment in some stages, and it can not be constantly distinguished from those young forms (16).

Lastly, no significant fact is shown by the histochemical examination of the focus of hyalinization which should play an important role in the formation of elastic fibers.

CONCLUSION

The results of my experiments are summarized as follows:

1) The formation of elastic fibers begins with the hyalinisation of connective tissue.

2) Elastic fibers appear within the focus of hyalinisation in one group of cases, but appear outside, engirdling, and adjacent to it in the other.

3) The above-mentioned foci of hyalinisation are induced by the transplantation of a part of prostate or seminal vesicles.

4) When the prostate tissues are transplanted into female animals, those foci appear most extensively, and the elastic fibers rise most abundantly.

5) It requires about 7~10 days till the appearance of elastic fibers after transplantation, in my experiments.

6) The figure of elastic fibers at the new formation looks like linked granules, or network of fibrils.

REFERENCES

NEW FORMATION OF ELASTIC FIBERS


Fig. 5 Type A, H.E. stain.

Fig. 6 Type A, Resorcin-fuchsin stain. Section shows formation of elastic fibers surrounding several glandular lumens in the upper part. Those regions look like hyaline in ordinary stain. The epithelium shows degeneration and destruction. In the lower part there are scarcely lumens, but widespread elastic fibers.

Fig. 7 Type B, Resorcin-fuchsin stain. Section shows foci of hyalinisation which appeared around several cystic cavities composed of the epithelium of seminal vesicles, and elastic fibers surrounding the foci. There is no degeneration or destruction of epithelium. In the left-upper and lower parts, the abdominal muscle of the host can be seen. Transplantation-node is clearly bordered around.

Fig. 8 Type C, Resorcin-fuchsin stain.

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Fig. 9 Type A, Silver impregnation. The foci of hyalinization surrounding glandular lumens contain no argyrophilic (reticulin) fiber. (Refer to Fig. 5 and 6)

Fig. 10 Type B, Silver impregnation. Subepithelial foci of hyalinization contain argyro-philic fibers. The outside light zone contains abundant elastic fibers. (Refer to Fig. 7)

Fig. 11 The new formation of elastic fibers. (Resorcin-fuchsin stain)

Fig. 12 The new formation of elastic fibers (Resorcin-fuchsin stain). 10 days after transplantation. Elastic fibers appear at the margin of those hyaline regions. Note of the hyalinosis around several masses of degenerated epithelial cells.