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The Anti-Autolytic Action of Blood Serum and its Relation to Hyaline Changes in Uterine Fibroids.*

By LEITH MURRAY, M.D. (Aberd.),

Pathologist to the David Lewis Northern Hospital, Liverpool.

HYALINE change as occurring in uterine fibroids receives, as a rule, but scant attention in any description of the degenerations found in these tumours. In many analyses of degenerations in fibroids it is no more than mentioned, while in others it is described under some such name as fibrous degeneration¹. This latter is a complete misnomer if it suggests any *new* formation of fibrous tissue.

Noble,² from 2,274 cases, puts the frequency of hyaline degeneration at 3.35 per cent., while McDonald,³ in a recent article based on 700 cases, places it highest with 18 per cent. Cullen,⁴ however, goes much further: "In nearly every myoma there is hyaline degeneration in *some* part of the growth."

My own experience, based on a careful examination of over 100 specimens, is that hyaline change (very often slight in amount no doubt) is to be found in almost every fibroid. While certainly more marked in fibroids of medium or large size, I have seen it well developed throughout a small interstitial fibroid measuring $\frac{3}{8}$ in. in diameter, and even in subperitoneal seedlings. Several pieces of a fibroid may have to be cut before finding an affected area, but as a rule in one large section the typical appearances of at least the early stages can be seen. The low percentages given above probably represent the more advanced degrees.

Hyaline change may or may not be the only sign of degeneration. Œdematous fibroids usually show it to a very marked degree, especially where cysts are present. In necrobiotic fibroids a tendency in the same direction is invariable, but here in addition a certain amount of granular change is seen.

Distribution.

Any part of a fibroid may be affected, but more commonly the

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central portions show it first, or if the whole be affected then the central portions are most advanced. An exception must be made in the case of pedunculated tumours: in these the hyaline change is most marked furthest from the pedicle.

Macroscopical Appearances.

The naked-eye appearances of a fibroid affected with hyaline degeneration vary considerably. In the slighter degrees no deviation from the ordinary may be seen. A more advanced specimen will show areas with less of the usual wavy appearance, and the tumour becomes harder and more difficult to cut; while at a still later stage areas form which are almost cartilaginous to the knife and more or less resemble cartilage to the eye.

The colour, as a rule, remains whitish or yellowish-white, but occasionally a reddish tint may supervene. According to Kelly and Cullen⁵ the following tints may be observed: whitish-yellow, greyish-blue, greyish-red, yellowish-brown, pink, dark-red or reddish-blue.

Microscopical Appearances.

The slighter cases show a hyaline thickening of the connective-tissue fibres, irregular and streaky in appearance and indefinite in its limits—the affected streams of fibres tailing off gradually into the normal condition. The nuclei early lose all staining property. In this stage the muscle bundles suffer no injury and remain absolutely normal, the intra-fascicular connective tissue being unaffected. A little later this latter also becomes involved, and now the muscle bundles become gradually strangulated and finally atrophy. So that in an advanced case the appearance presented is of an almost homogeneous mass, translucent on section showing perhaps in places a vague outline of the course of fibres. No lined vessels are visible, but fairly numerous flattened or irregular lacunæ are present, often empty but sometimes containing a nucleus which is probably usually that of a wasted muscle fibre, but may represent an endothelial cell or perhaps a lymphocyte. The margin of these very advanced areas is often extremely well defined and the general contour rounded or oval. In other cases this delimitation is not attained, and a similar appearance to that just described is seen gradually fading into the slighter degrees. In one fibroid, therefore, it may be possible to trace every gradation of this very chronic process, one area showing early streaky change, another a more pronounced hyaline thickening with implication of the intervening muscle bundles, and still another the almost structureless firm mass beyond which, as I hope to show, the process *per se* does not go.

The earlier stages are by far the commoner. In about 5 per cent. only of fibroids have I seen these circumscribed areas.

In no cases have I observed hyaline change affecting muscle fibres, and I cannot believe that it occurs. Although Kelly and Cullen⁶ figure a section in which they assert it to be present, the most careful search with suitably differentiating stains, such as picro-fuchsin and picro-nigrosin, has never shown muscle tissue taking any but an absolutely passive part in the process.

Staining Reactions. With eosin hyaline areas stain moderately well at all stages.

With picro-fuchsin the streaky forms, *i.e.*, the earlier degrees, stain well with the fuchsin, and appear as a definite pink. As the process advances less and less fuchsin is retained till, in the massive forms, picric acid staining alone remains, and the affected areas stand out a distinct yellow. This last point is of especial interest in that it brings the degeneration into line with hyaline degeneration as occurring elsewhere. (Beattie and Dickson).⁷

Hyaline degeneration, furthermore, is invariably negative to iodine and to Weigert's fibrin stain.

Sudan III. nearly always demonstrates the presence of fatty acids, but as a rule scarcely more in the hyaline areas than in the fibroid as a whole. Often the fatty change is much *less* marked than in the surrounding fibroid, and in some cases is practically absent. Although in a few instances distinctly more fatty deposit is present in the hyaline areas than in the neighbourhood, yet, generally speaking, it can not be said that the two processes go hand in hand. When present, the fatty globules are found as irregular streaks throughout, and especially in and around the walls of the vessels.

Vessels. In general, the appearances presented are those of senile vessels. They show the sclerotic hyaline changes typical of such, either in the inner, outer or in advanced cases in all three coats with strangulation of the muscle fibres of the middle coat and a certain amount of fusing of the coats as a whole. The lumen is encroached upon, and in a considerable number of the smaller vessels the endothelium is moniliform, the cells sometimes standing almost perpendicularly to the lumen. This latter, it may be, is a post mortem retractive change, as Eden⁸ has suggested with regard to a similar appearance in sclerosed placental vessels, but in any case it can only be a sign of altered elasticity.

These vascular changes vary in intensity *pari passu* with the degeneration of the fibroid.

As noted above, fatty changes are usually found in the vessel walls.

In a small proportion of cases the degeneration apparently commences directly around the arterioles, the hyaline vessel wall merging into the surrounding tissue. The endothelium of the vessel implicated seems in such cases to persist much longer than in the

more ordinary forms of the condition. This peculiarity has also been noted by Kelly and Cullen.⁶ It may, however, be due simply to the larger size of vessel usually affected. The appearance under a low power of such a fibroid is highly characteristic, yellow rounded areas with little nuclear staining and a central canal studding the field.

In the great majority of cases, however, no such intimate vascular association is obvious; but the rounded or oval areas are of course highly suggestive. I have not seen any hyaline areas definitely wedge-shaped at the margin of a fibroid, but then the degeneration is never very advanced towards the periphery.

One case alone, where hyaline degeneration was the sole change in the fibroid, showed thrombosis, and this was in a uterus with a submucous hyaline fibroid complicated by carcinoma of the endometrium. Only one or two rather small vessels showed the fibrin network.

Cyst Formation in Association with Hyaline Degeneration.

Marked hyaline degeneration and œdema frequently co-exist in a fibroid. Cullen describes the boggy fibroid simulating pregnancy as being usually associated with hyaline degeneration, and further describes the thinning out of the hyaline material into clear straw-coloured fluid with spaces traversed by capillaries. Again, he says that "œdematous cystic areas in a fibroid are the result of a marked hyaline transformation with subsequent liquefaction." That this is the usual sequence I have strong reason to doubt. On one hand, a fibroid may be extremely œdematous, either of the locular variety or of the so-called myxomatous type, and yet show very little hyaline change—merely the usual irregular streaks; on the other hand, a fibroid equally œdematous may show very extensive hyaline change indeed, usually best marked around the spaces, and especially the larger ones. I do not consider that the thin fluid cavities in fibroids are ever directly the result of solution or "thinning out" of the hyaline material. For, in the first place, the vascularity occasionally found within the hyaline borders of the cavity is quite against the cavity having originated within a hyaline area; and, secondly, the experiments I am about to describe indicate the probability of the extensive hyaline change having followed the œdematous degeneration. It is, however, possible that hyaline thickening by obstructing the lymphatics may lead to some œdematous infiltration.

Again, fibroids almost completely hyaline are very commonly without a trace of œdema.

Apart from this, however, breaking down does occur in the centre of the larger masses of hyaline degeneration. This breaking down is of a fatty nature, and appears as a brownish or yellowish semi-fluid mass, often containing cholesterolin crystals, in an irregular

cavity. The variability in amount of the fatty change in hyaline areas as a whole would lead one to infer that the two processes are not directly dependent, but rather co-existent. Just as in vessels so in these tumours, the fatty degeneration exists along with, and not either as a cause or a result of, the hyaline transformation.

Pathogeny.

The firm consistence of hyaline areas combined with practically complete absence of nuclear staining led me to study the action of blood serum on pieces of excised fibroids.

Longcope⁹ has shown its marked power of inhibiting softening and plasmolysis of tissue in the case of exsanguinated dogs' liver and kidney immersed in sterilized homologous or heterologous (horse; human) serum. The process is one independent of the fresh element in serum as heating to 65°C. for 50 minutes (which destroys all complement and amboceptor) does not impair this power; it is rapidly lost on diluting the serum; it is not due to the neutralization of the acids of autolysis, as neutralized serum is still active; but evidently resides in the albuminous fraction,—egg-white diluted with an equal quantity of salt solution having a well-marked preventative power.

Cubes of liver $\frac{1}{2}$ –1 cm. in diameter placed in normal saline and incubated at 36·5°C. become soft after 12–24 hours and deposit a sediment; no nuclear staining is visible after 12–18 hours; and after 2–3 weeks only a friable shrunken mass remains, buried in a copious sediment.

Similar pieces in serum show a firm zone round the margin after 24 hours; in from 8–14 days the whole thickness is firm and pale, and by 2–3 months the consistence is distinctly rubbery; nuclear staining remains for 43–68 hours and occasionally longer.

In the following experiments pieces of fibroids fresh from operation were cut in the shape of oblongs about 1 by 1 by 3 cm., with all precautions for maintaining sterility. These oblongs, which were taken from near the surface, were cut in three, each central portion being sectioned as a control against the presence of hyaline change in the area chosen. The other pieces were placed in sterile tubes containing blood serum and saline (0·85 per cent.) solution respectively. The tubes were sealed with paraffin wax and incubated at 36·5°C. At first 5 per cent. of toluol was added to dispose of any idea that the serum was furnishing nourishment to living cells; but this precaution was soon found to be quite unnecessary and was given up.

To begin with, human serum was employed, but the inconvenience of obtaining this in sufficient quantity led to the substitution for it of stale normal horse serum which is neither hæmolytic nor cytolytic to human cells and only occasionally slightly agglutinative. The results obtained with both were identical.

Pieces were removed from time to time up to eight months in several cases, and after sterility had been again confirmed were placed in formalin fixative and sectioned by freezing and paraffin microtomes.

In considering the results, two points must be borne in mind: firstly, that the tissue already contained some serum, and therefore the pieces in saline were not under absolutely serum-free conditions; secondly, that a fibroid is incomparably more difficult to penetrate than such a soft structure as liver. As a result of this toughness, it was found that fibroids retained their shape moderately well for months in saline, although uterine wall (in nature more porous) was invariably reduced to a brown sediment within two months, and placenta in a rather shorter time.

Although retaining its shape fairly well, the fibroid tissue in saline presented certain quite definite features distinguishing it from the tissue in serum. The consistence of pieces removed after two or three months was pulpy, and became increasingly so as a whitish sediment slowly developed. By the end of six or eight months the tissue was exceedingly soft, but still, although shrunken, retained roughly the original outline. Microscopically, nuclear staining was found to disappear much earlier than in the case of the same tissue in serum; occasionally a little of the hyaline change to be described as occurring in the latter was seen after a week or two, but this later on disappeared, and was in all likelihood due to the serum or lymph present in the tissue to begin with. As time went on the appearance became more and more porous till, by the end of a few months, one could readily detect in sections, even with the naked eye, a loose network of wavy fibres. (Fig. 4.)

The staining affinities for picro-fuchsin were constant. The longer the tissue remained in saline the better was fuchsin taken up. Sections after four months stained a red colour without a trace of picric acid colouration.

Fibroid tissue in serum, on the other hand, behaved quite differently. A typical instance—one of many—may be given in detail:—

19th Nov., 1908: cubes of fibroid placed in serum at 36·5°C.; from a patient, æt. 42; tumour 5 cm. in diameter, interstitial in the anterior wall; control showed a typical undegenerated fibroid. (Fig. 1.)

25th Nov., 1908: firm and pale; connective-tissue:—nuclei have disappeared, distinct swelling of fibres, fuchsin moderately well taken up; muscle fibres:—somewhat shrivelled, nuclei stain sharply. (Fig. 2.)

1st Dec., 1908: firm and pale; connective-tissue:—general homogeneous appearance with fusion and a lack of differentiation

Fig. 1. Fibroid as removed at operation.

Fig. 2. The same fibroid after six days in normal horse serum at 37°Cent.

Fig. 3. The same fibroid after twelve days. (It was found impossible to photograph the later stages of the process as the tissue becomes completely homogeneous and hyaline.)

Fig. 4. The same fibroid after eight months in saline solution.

of the fibres, coloured by fuchsin; muscle:—scattered nuclei visible, especially towards the margins. (Fig. 3.)

15th Feb., 1909: firm and pale, very homogeneous, no nuclear staining visible, both fuchsin and picric acid taken up, but especially the former.

17th April, 1909: firm and pale, absolutely homogeneous; sections stain yellow with picric acid, no trace of fuchsin staining.

19th June, 1909: tissue very firm and homogeneous; stains a bright canary yellow; no shrinking has occurred.

This sequence occurred in each of the eight undegenerated fibroids investigated, although the time taken to develop varied. Nuclear staining was detected as late as 17 days after incubation, but as a rule disappeared within 10—12 days. Distinct hyaline change was visible after 6—18 days.

Fibroids in which the control showed that hyaline change was already present were more rapidly affected than others; while, strange to say, permeable structures such as uterine wall developed it very slowly, and only after all nuclear staining had disappeared. In every case the absorption of picric acid to the exclusion of fuchsin was apparent.

Nothing could be more marked than the contrast presented after a few months by sections of the tissue from serum and from salt: the one bright yellow and homogeneous, the other a shrunken, pink, stringy network. It will be noted at once that these appearances are identical with those occurring naturally in fibroids and in vessel walls.

Negative results with iodine and Weigert's fibrin stain are confirmatory of the essential similarity between the experimental condition and the natural. Sections stained with Sudan III. were also negative.

Some further interesting points were brought out with regard to œdematous fibroids. It may be asked: Why do these not show in every instance the most marked development of hyaline change? At first thought it would appear that the tissues in such cases are under precisely the same conditions as in these experiments *in vitro*. There are, however, the following points of difference:—

1. The fact that hyaline change is less rapidly produced in a permeable structure like uterine wall, and, in the case of fibroids, is usually most marked in the centre, *i.e.*, furthest from the blood supply, would indicate a limitation of the conditions under which the degeneration is liable to occur. It would appear that the most favourable environment is one where the serum is not present in excess, and can only slowly infiltrate the tissues as a more or less stale fluid. An attempt was made to determine whether only stale serum possesses this property. Very small pieces of fibroid were

placed in human serum which was daily replaced by a fresh supply, but hyaline change appeared as usual.

2. The fluid in œdematous fibroids is not the same as blood plasma, but corresponds rather to a dilution. I have found that the percentage of albumen is greater and the clot firmer in the larger loculi where the nutritional changes in the vessels are probably more advanced. In the smaller spaces the fluid more often remains unclotted and contains less albumen.

As opportunity occurred small pieces of the same fibroid were incubated with each of these (stale) fluids, and it was found that the more highly concentrated specimen had a much greater power of converting the connective-tissue into hyaline material.

Further experiments consisted in incubating sterile necrobiosed fibroids with serum. Three specimens were investigated, and the point of most note was the preservation of the form and consistence. That stagnant blood serum in such fibroids *does* exert some action is, I think, evidenced not alone by the consistence but also by the pronounced way in which they absorb picric acid. The more necrobiosed a fibroid the yellower it stains. Experimentally, pieces of necrobiosed fibroid in serum continue to show this colour, but similar pieces in saline within a short time tend to lose this property and stain with fuchsin instead, as in the case of unde-generated fibroids.

Conclusions.

These results have a general bearing, but as regards fibroids the conclusions to be drawn are clear.

1. Hyaline change is a degeneration and not an infiltration.

2. It affects the connective-tissue only—changes in the muscle fibre resulting passively from strangulation. There is no *overgrowth* of the connective-tissue, but a swelling of that already present. It follows on a gradual but serious diminution of nutriment, either focal or general, and the affected tissues are undergoing a process of embalming in more or less stale fluid. This diminution is not the result of any widespread thrombosis, but is due to the sclerotic condition of the vessels, the thickening of these in turn being probably the result of a similar process in the vessel wall. On this point I am at present engaged.

3. Nuclear staining is no absolute criterion of the vitality of a fibroid. Parenthetically I might mention that this fixative action of serum has also to be borne in mind in cases of retention of dead products of conception. Placental tissue placed in serum showed areas of nuclear staining for as long as two weeks.

4. The hyaline layer lining clear fluid spaces in fibroids is secondary to the development of the cystic degeneration, and is in

no way productive of the fluid by liquefying. There is of course the possibility that the hyaline thickening may occasionally obstruct veins and lymphatics and so produce a vicious circle.

5. Necrobiosed fibroids show evidence of this same embalming process in their consistency and staining reactions.

6. Fatty degeneration in a hyaline fibroid is merely co-existent—both processes being related to diminished circulation.

I am obliged to Dr. Gemmell and Dr. Wallace for the greater part of the material examined.

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