THE RELATION OF AUTOLYSIS TO THE HISTOLOGICAL CHANGES OCCURRING IN NECROTIC AREAS.*

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In 1900 Jacoby[1] found that if a portion of a dog's liver was ligated off and the dog kept alive for some time afterward, the necrotic isolated portion contained free leucin and tyrosin. This was the first actual demonstration that the retrogressive changes in areas of anemic necrosis are associated with, and presumably the result of, enzymotic autolytic processes. Since that time it has been generally accepted that the softening and absorption of necrotic tissues is the result of autolysis by the proteolytic enzymes contained within the degenerated cells, and numerous chemical investigations have shown that in gangrenous tissues, suppurating areas, resolving exudates, degenerating tumors, and other similar conditions the products of proteid digestion may be found. There can be little question, therefore, that autolysis is an important factor in all processes associated with the removal of areas of degeneration, but as yet there has been little consideration of the relation of autolysis to the histological changes that occur in such areas, nor has it been determined to what extent the autolysis is accomplished by (1) the intracellular enzymes of the necrosed cells; (2) by the enzymes of invading leucocytes, and (3) by the constituents of the blood plasma. Neither do we know to what extent the action of these digestive agents is modified by various conditions and substances existing within the body.

A few studies of the histological changes occurring in aseptically and antiseptically preserved pieces of organs kept for varying periods outside of the body have been made, but most of them antedate the general appreciation of the intracellular proteolytic enzymes. Meissner[2] observed many years ago that aseptically preserved organs retained their general

structural characteristics for many weeks, but the finer struct-
ure seems not to have been considered. Hauser[3] studied
the structural changes more closely, and noted the loss of
nuclear staining, while the general cell form remained relatively
well perserved; he observed particularly the appearance of
fat in the cells, and of fat crystals on the surface of the
tissues, and considered that he had found evidence of " fatty
degeneration " of proteids. Consequently, because of the
warm controversy over the problems of fatty metamorphosis,
the observations on the histological changes in autolyzing
tissues have been directed particularly towards the fatty sub-
stances that may become visible and stainable, rather than
towards the fundamental questions of cell necrosis. The first
systematic study of the histological changes in autolysis was
that of F. Kraus,[4] who considered particularly the question
of fatty changes and came to the conclusion that no actual
increase of fat occurred during autolysis (it may be stated
here, parenthetically, that the general result of the more
recent discussion of this phase of the question has been in
agreement with Kraus' results; *i.e.*, during cellular autolysis fat
that is normally invisible may become visible and stainable,
but there is no actual increase in the fat in the tissues, and
therefore no " fatty degeneration " of proteids).[5] He also
noted as an early and characteristic feature of autolysis the
disappearance of the nucleus, which occurred in the large
parenchyma cells of the glandular organs, and stated that
this change is identical with the change described in necrosis
by Cohnheim and Pfitzner (the karyolysis of Klebs), and
therefore is solely a post-mortem alteration. This was in
direct contradiction to the view of Weigert that the " Kern-
schwund " was characteristic of coagulation necrosis, and
required a vital suffusion of the tissues with blood plasma,
and aroused much opposition.[6] A new phase was then added
to the interest in the changes occurring in dead tissue, and
as a result a number of investigators compared the his-
tological alterations observed in autolyzing tissues with those
described by Weigert, Ribbert, Israel, and others in experi-
mentally produced areas of anemic necrosis. This was

particularly done by Schmaus and Albrecht,[7, 8] who found that the changes in autolysis are in the main quite the same as those observed in infarcts, including not only the usual nuclear solution (karyolysis), but also the karyorrhexis, which had been considered as a more vital process, although this last is not so prominent in vitro as it often is in anemic necrosis.

All of these observations were made before the work of Salkowski, Jacoby, and others had led to a general understanding of the essential features of cellular autolysis, and the changes observed were referred to, as a rule, simply as "post-mortem change," and ascribed generally to a dissolving of the cell components by the fluids of the plasma. More recently we find the histological changes of autolysis studied more definitely, as a result of the action of the intracellular enzymes, in the Tübingen laboratories. Dietrich and Hegler[9] examined various organs permitted to undergo autolysis, either preserved in a moist condition or in physiological salt solution, considering particularly, however, the relation to fatty changes and the appearance of "myelin" forms. They studied liver, kidney, heart muscle, and psoas muscle, and found in general much the same alterations that were observed earlier by Kraus, Goldmann, and others. The liver shows more rapid and extensive changes than the kidney, which preserves its general gross structure fifty-six days or more, while the heart muscle undergoes changes much more slowly. There seemed to be no essential difference whether autolysis occurred in salt solution or in a moist chamber. Dietrich seems inclined to question the importance of autolysis in causing the nuclear changes, referring to autolysis as a "Schlagwort." Pycnosis he ascribes to loss of water from the nucleus to the cytoplasm; if the nuclear substance is soluble in the cytoplasm karyolysis occurs, if insoluble we get karyorrhexis. He does not, however, explain the cause of the changes in the intracellular physico-chemical conditions that follow cell death. Richter[10] found that pieces of liver, kidney, and muscle placed in the abdominal cavity of an animal (either free or in collodion sacs) undergo changes

similar to those observed in similar tissues preserved asepti-
cally in the incubator, but was particularly interested in the
peripheral deposition of fat observed in these tissues.

We have, then, evidence that the changes occurring in in-
farcts and in other necrotic areas are similar to those that
occur during autolysis in vitro, but there remain a number of
interesting problems to solve. For example, if we examine
an aseptic anemic infarct of the kidney of some standing we
find that the removal of the dead tissues goes on very slowly,
and apparently solely from the periphery. Thus in a renal
infarct, the age of which could be definitely stated to be
fourteen weeks, there remained still a layer of necrotic cortex
about one millimeter thick that had not been absorbed, and
under the microscope it could be seen that the epithelial
lining of the unabsorbed dead tubules was quite as thick as
that of a recent infarct. On the other hand, in a kidney
undergoing autolysis outside the body the epithelium of the
convoluted tubules will be found in twenty days to have lost
over three-fourths of its volume, and in sixty days it will have
almost entirely disappeared. Evidently, then, cells do not
become digested as rapidly by their own enzymes in infarcted
areas as they do in vitro; indeed, there is little evidence
that in small infarcts the intracellular enzymes digest the
necrotic cells at all. On the other hand it can be readily
seen that the absorption of the infarcted area occurs from the
periphery, and is associated with a slow invasion of leucocytes
and proliferating cells. Apparently the changes that occur
in an aseptic necrotic area do not lead to the production of
chemotactic substances in any considerable amount, for the
failure of leucocytes to invade aseptic necrotic areas is rather
striking. Indeed, there is much less infiltration of such ne-
crotic tissue by leucocytes than is observed in the case of
chemically inert foreign bodies (*e.g.*, Adler's experiments
with elder pith [11]), which would suggest that possibly neg-
atively chemotactic substances may be present in the infarcts.
In support of this may be mentioned the demonstration by
Magnus-Levy [12] of lactic acid among other substances pro-
duced in autolysis, and lactic acid has been shown repeatedly

to be one of the most active substances exerting "negative chemotaxis" in the usual sense of the term.* In any event, it would seem that the absorption of aseptic dead tissues does not depend upon autolysis by the intracellular enzymes so much as has been generally assumed, but is usually the result of heterolysis by invading leucocytes. Hence aseptic areas undergo softening and solution extremely slowly unless something happens that causes an invasion of leucocytes. The failure of absorption characteristic of tuberculous areas may depend upon a destruction of the autolytic enzymes of the dead tissues (a matter that has yet to be investigated) or, more probably, upon the absence of positive chemotaxis and the consequent failure of leucocytic invasion. Certainly it is not due to the conversion of the proteids of the dead cells into an indigestible modification, as has been suggested, for whenever leucocytic invasion of caseous material is brought about through infection (or by injection of iodoform emulsion), the softening of the necrotic tissue goes on very rapidly.

In considering the manner in which infarcts are absorbed, as outlined above, a number of recently described features of autolysis were found to apply to the conditions observed in the infarcts. First of all may be mentioned the observations of Hedin, Wiener,[13] and Schryver,[14] that a certain degree of acidity is favorable to autolysis, which process goes on relatively very slowly if the reaction is kept neutral, and is completely checked by so slight a degree of alkalinity as that of a 0.2–0.4 per cent solution of NaOH. When tissues undergo autolysis in vitro considerable amounts of organic acids are produced (Magnus-Levy), but in the body these acids presumably diffuse out and also are neutralized by the alkalies of the plasma, which are perhaps sufficient to impede if not to check autolysis. In support of this hypothesis may be mentioned that, in large areas of aseptic anemic necrosis, softening occurs much more rapidly and

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*It has been shown by Hektoen (Jour. Amer. Med. Assoc., 1906 (xlvi) 1407) that substances of this nature appear to cause their chemotaxis-inhibiting effect by producing an alteration in the opsonins. It would be interesting to ascertain the effect of the products of autolysis upon the opsonins.

chiefly in the center, which may, perhaps, be due to an accu-
mulation of the organic acids in the central part rendered
possible by the distance from the alkaline plasma of the cir-
culating blood. Secondly, we have the observation that
normally the blood plasma contains anti-enzymes, not only
for the enzymes of the digestive secretions, but also for the
intracellular autolytic enzymes. These have been studied
particularly in relation to the autolysis of exudates by Opie,[15]
who found that the serum of inflammatory exudates has a
marked inhibiting effect upon the autolytic action of the
leucocytic enzymes. Furthermore, he found that the leuco-
cytic enzymes differ from those of the fixed tissue cells (ex-
cepting bone marrow, which resembles the leucocytes)[16] in
being most active in an alkaline medium, although the action
of the anti-enzymes is destroyed by acids. Schryver[17] has
found that the serum also retards autolysis of liver tissue.
It would seem quite probable that these anti-enzymes may be
the cause of the failure of autolysis of necrotic areas, and
also of their slow peripheral digestion by the leucocytes,
except in the case of an excessive accumulation of leuco-
cytes out of proportion to the amount of inhibiting plasma
present at the same time. The more rapid digestion of the
central parts of large necrotic areas might be referred to
this lack of inhibiting substance, as well as to the develop-
ment of an acid reaction — both factors working con-
jointly in favor of central and against peripheral autolysis.

In order to test the relation of the inhibiting action of the
serum upon autolysis, studies were made of tissues allowed
to stand for varying lengths of time in serum that had
been heated, and compared with tissues from the same
animal placed in fresh serum (serum and tissues were
obtained from the same animal to render conditions as nearly
natural as possible, the bleeding of the animal at the same
time reducing the amount of residual blood in the organ to
be examined). As most investigations of this kind have
been made with tissues kept in physiological salt solution,
organs kept in this medium were also studied, and, as will
be shown later, gave quite different results from those

obtained with the tissues kept in serum. This might well be expected in view of J. Loeb's demonstration of the profound modification of physiological processes by an excess of one variety of ion, and is but one of a number of evidences that in many experimental studies the assumption that physiological salt solution is an inert substance, not modifying the results of the experiment, is unwarranted. As the histological changes of autolysis are most striking in the kidney, because of its variety of functionating cells, the chief results of an experiment with this organ are given below.

A large dog was made unconscious with morphine, and bled to death. The blood was whipped free from fibrin, and the serum separated by centrifugalization. One-half was heated for thirty minutes at 80° C. and separated from the resulting coagulum by filtration and expression. Pieces of tissue of suitable size for sectioning (about 1.5 centimeters square and one centimeter thick) were taken from the same dog, and placed in : (A) sixty cubic centimeters of heated serum plus five cubic centimeters of toluol ; (B) sixty cubic centimeters of unheated serum plus five cubic centimeters of toluol; (C) sixty cubic centimeters of 0.85 per cent NaCl solution plus five cubic centimeters of toluol.* These stood in stoppered bottles at 20° C. for varying periods of time, and were hardened for examination in Zenker's fluid. Staining was by hematoxylin and eosin. The results were as follows :

Eight hours. Fresh serum. — Epithelium of the convoluted tubules slightly swollen, nuclei stain a trifle deeper and more diffusely. All other structures appear normal, including the red corpuscles.

Heated serum. — Epithelium of the convoluted tubules at the surface of the specimen seems cleft, nuclei often distinctly pycnotic or in some cases pale or quite unstained ; deeper in the tissue the changes are similar but less marked. Other tissues unchanged.

Salt solution. — Appears quite different, in that near the periphery the epithelium seems to have been dissolved out to a great extent, leaving a granular material filling the lumen of the tubules. Where protected by fat tissue in the capsule this change has not occurred, showing that it is due to a solvent action of the salt solution. Deeper in there is less change, but some of the epithelium of the convoluted tubules stains deeply with eosin, resembling coagulation necrosis, or sometimes stain diffusely pale purple, as if containing dissolved nuclear material.

Twenty hours. Fresh serum. — Epithelium of convoluted tubules generally much swollen ; cytoplasm coarsely granular and stains well with eosin ; most of the nuclei have disappeared, those remaining being for the most part small and staining deeply and diffusely ; in a few tubules a

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*There is no evidence that toluol causes appreciable modifications in the tissues.

diffuse, faint, blue-staining substance in the lumen. The epithelium of the collecting tubules and the limbs of Henle's loop show some pycnosis. All other structures are practically unchanged.

Heated serum. — Changes are all much more marked than in the fresh serum specimens. Convoluted tubules have lost all of their nuclei, the cytoplasm is granular and cleft, and there is a diffuse blue stain in the contents of the lumen. Collecting tubules show much pycnosis, but in the medullary rays there is often found a diffuse blue staining as if the nuclear substance had diffused through the cytoplasm; the same changes are observed in the limbs of Henle's loops. Glomerular tufts in many instances show some loss of chromatin substance and in the renal capsule the connective tissue nuclei are faint or absent, but the endothelium remains well stained. All staining is faint immediately about the periphery, but there is no such dissolving out of cell substance as in the salt solution specimens.

Salt solution — The dissolving-out process has extended deeply into the specimen, but affects only the larger forms of epithelial cells; apparently just before undergoing solution the epithelium swells and stains deeply with eosin, and then the central portion is dissolved, leaving a faintly stained granular débris in the lumen, while a thin margin of deeply stained cytoplasm remains near the basement membrane.

Forty-eight hours. Fresh serum. — Condition very similar to that of the specimen in heated serum twenty hours, except that there is no disintegration in the cytoplasm. Because of the preponderance of the changes in the convoluted tubules the specimen resembles the so-called " differential necrosis " observed in acute intoxications.

Heated serum. — Only a few nuclei remain in the cortical structures, the limbs of Henle's loops and the collecting tubules retaining about half of their nuclei in a pycnotic condition, while each glomerular tuft has but three to six nuclei remaining; even the nuclei of the stroma and the capsule are gone. In the pyramids the epithelial nuclei are very small and dark-staining, as also are those of the stroma. The nuclei of the vessel walls and endothelium still stain well.

Salt solution. — Differs from the heated serum specimen chiefly in the extensive dissolving out of cell substance; the nuclear changes are quite similar in most respects.

Seventy-two hours. Fresh serum. — Changes stand between those seen in the specimens kept in heated serum twenty-four and forty-eight hours. In the cortex the nuclei of the stroma and the smaller tubules still remain, although pycnotic, and the glomerular nuclei are nearly all present.

Heated serum. — In the cortex an occasional nucleus can be found in the limbs of Henle's loops. In the pyramids a part of the nuclei remain in a pycnotic condition, and often fragmented. About the periphery there is a zone from which all stain has been lost.

Salt solution. — Differs from the above chiefly in the greater loss of cell substance.

Ninety-six hours. Fresh serum. — Less disintegration than in heated serum specimen of forty-eight hours, about half of the nuclei still remaining in the glomerules and in Henle's tubules, and the nuclei of the basement membrane still stain well.

Heated serum. — Only a few nuclei left in the collecting tubules, and in the endothelium and muscular tissue of the larger vessels.

Salt solution. — Except for the excessive solution of the cytoplasm resembles the heated serum specimen.

Later changes. — After six days the fresh serum specimen is somewhat better preserved than the specimen kept in heated serum for but seventy-two hours; whereas the heated serum specimen of six days shows only a few pycnotic and fragmenting nuclei in the collecting tubules in the centers of the pyramids. After eleven days there are still some nuclei in the cortex of the fresh serum specimen, both in the stroma and in the smaller tubules. After twenty days not a nucleus can be found in the specimens kept in either heated serum or in salt solution, and the epithelium of the convoluted tubules has suffered a great reduction in volume by a dissolving out from the center. In the fresh serum specimen there is still little loss of cell substance, and an occasional pycnotic nucleus can be seen in the pyramidal epithelium.

We see, then, that there is a striking difference in the rate of autolysis in the specimens kept in heated and in those kept in unheated serum, which, in our present state of knowledge, can be best ascribed to the presence in the latter of thermolabile inhibiting substances, *i.e.*, anti-enzymes. The effect of the anti-enzymes is merely retarding, the changes in structure produced by the autolysis pursuing the same course, but requiring about double the time to reach the same stage (this time ratio of two to one seems to be quite constant so long as the nuclear changes can be used as an index). The tissues preserved in physiological salt solution show that this fluid has a marked solvent effect upon cellular constituents, particularly the cytoplasm, the central portion of the epithelium of the convoluted tubules being dissolved out early, especially near the surface of the specimens; otherwise the process of autolysis, so far as the nuclear changes are concerned, proceeds at about the same rate as in the specimens kept in heated serum. In the specimens kept in fresh serum the changes are much more similar to those observed in infarcts than in the specimens kept in either heated serum or salt solution, in that the nuclear changes

far outweigh the cytoplasmic changes, which consist chiefly
of a coarse granular alteration (recalling the "tropfige Ent-
mischumg" of Schmaus and Albrecht), with little evidence
of digestion or solution of the proteid constituents until after
a period of three weeks, and then relatively very slowly.

The nuclear changes are various — they occur first in the
large epithelial cells of the convoluted tubules, and last in
the stroma, vessels, glomerules, and the epithelium of the
limbs of Henle's loops. In the convoluted tubules little can
be observed but a simple solution of the staining elements,
leaving the structure of the nucleus still visible in an un-
stained condition. Occasionally in these cells there occurs a
preliminary decrease in size of the nucleus, associated with
a diffuse and greatly increased affinity for basic dyes, *i.e.*,
pycnosis. The pycnosis is, however, much more marked in
the nuclei that persist longest, especially in the epithelium of
the collecting tubules, and occasionally in these tubules a
considerable degree of karyorrhexis may be observed in the
later stages. These changes differ but little from those
described by Weigert, Ribbert, and others as occurring in
experimental infarcts, especially if we compare only the
specimens kept in fresh serum, which, of course, approach
most closely the conditions in infarcted tissues. Sometimes
the dissolved nuclear material seems to diffuse slightly before
it has entirely lost its staining power, so that a diffuse basic
stain may be seen in the lumen of the tubules, especially in
the ascending limb of Henle's loop. This is not marked,
however, and is almost entirely absent in the tissues pre-
served in fresh serum.

In comparison with the above statements we find that the
changes occurring in the other organs and tissues of the
body differ from those observed in the kidney only in rate of
occurrence, and not at all in the nature of the histological
changes. These may be summarized as follows:

Of the other organs the *liver* is by far the most rapidly affected, marked
changes occurring in eight hours (in salt solution at 20° C.) and after
thirty-four hours no nuclei whatever are left except in the walls of the
larger vessels. The changes of cytoplasmic disintegration are also most

marked in the liver both macroscopically and microscopically, the cells becoming greatly reduced in size and number, and after thirty days the single lobules are reduced to a very small size. Next in order of rapidity of change comes the *spleen*, which shows some pycnosis of the nuclei, and in twenty hours a diffuse tingeing of all the tissues by nuclear stain has occurred, associated with loss of stain in the pulp and in the periphery of the Malpighian bodies. In thirty-four hours all nuclei have lost their stain except those of the reticulum, trabeculæ, and vessels, and a few in the centers of the Malpighian bodies. In the *pancreas* the changes occur in certain foci, as if the trypsinogen had become activated in some areas before in others, and the resulting changes are quite different from those of simple autolysis in that all structures except the stroma are destroyed and severely disintegrated wherever the changes occur at all. After thirty-four hours practically all nuclei have disappeared, while the stroma exhibits the peculiarity often observed in autopsy specimens, that it stains strongly with the basic stain.* The first change observed in the *thyroid* kept in salt solution is an apparent partial solution of the colloid, associated with the formation of coarse granular flocculi, which persist for a long time. Nuclear changes in the epithelium appear first after forty-eight hours, and consist chiefly of pycnosis and irregularity of form; the nuclei do not disappear until from the fourth to the sixth day; the stroma nuclei last from ten to eighteen days, and the volume and density of the dead epithelium become reduced very slowly. The *lung* changes more rapidly than the thyroid, possibly because of the more superficial character of its structure in relation to the fluid in which it is preserved. The nuclei of the alveolar epithelium disappear in about thirty-six hours, as also do those of the bronchial mucosa. The nuclei of the cartilage and the vessels remain from six to ten days. *Muscle* tissue undergoes changes very slowly, especially in its cytoplasmic structures. In the *myocardium* the nuclei fade from the fourth to the tenth day, but the striations are still distinct after thirty days; striated muscle undergoes changes still more slowly. In some respects the *brain* is the most slowly changed of all the organs, for even after thirty days the nuclei of the cortical cells are still distinct, although not well stained. The cytoplasm of the ganglion cells seems to suffer much loss after two to three days, but their nuclei stain well up to eighteen days, at which time a large proportion of the nuclei of the white matter have disappeared. *Squamous epithelium* of the skin and hair follicles persists from ten to eighteen days, while the nuclei of the subcutaneous and fat tissues disappear in from six to ten days in the same specimens.

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* *Intestine* undergoes a rapid digestion commencing with the lumen and working outward, therefore due apparently to the action of its contents. In one specimen examined an intestinal worm was present which showed severe nuclear and cellular alterations within eight hours, when the lining epithelium of the intestine was still intact. This is interesting in connection with the observations of Weinland and others concerning the presence of antibodies for trypsin in the intestinal parasites.

By far the most resistant cells of the body seem to be those of the endothelium, and similar cells found about the walls of the larger vessels, probably the endothelium of the perivascular lymph channels; these persist in some tissues in a well-stained condition for thirty days or more. Apparently these and the other elements of the stroma and vascular system are not so much affected by their own enzymes, but are attacked by the constituents of the parenchymatous cells in their vicinity, for they disappear in the various organs in proportion to the rate at which the chief cells of these organs are themselves affected; thus in the liver none survive in a stainable condition for forty-eight hours, while in the subcutaneous tissue they last for a month or more. Schryver[18] has studied by chemical means the rate of autolysis in different organs and found that the liver and spleen digest themselves with about equal rapidity and nearly twice as fast as the kidney; next in order coming the myocardium, and then the voluntary muscle. These results are quite in accord with the histological changes as observed above.

According to the histological changes previously alluded to, the solution of the tissue elements in the necrotic areas in the living body seems to be accomplished largely by the leucocytes and little, if any, by the intracellular enzymes of the dead tissue cells; and conversely, the nuclear changes characteristic of necrosis seem to depend largely upon the intracellular enzymes and little upon the leucocytes. For the purpose of establishing this experimentally a number of experiments were performed for me by Mr. J. E. Tyree, to whom I wish here to express my indebtedness, which corroborated the results obtained by me in connection with another investigation. These consisted in implanting into the abdominal cavities of rabbits pieces of spleens obtained from other rabbits, or from dogs and sheep; in half of the experiments the tissues had been heated quickly to 100° C. in an Arnold sterilizer to destroy the autolytic enzymes, while in the other half the tissues were implanted in a fresh, aseptic condition. After varying lengths of time the tissues were removed and studied histologically, the results being briefly as follows:

The heated tissues undergo no changes whatever for a surprisingly great length of time.   At the end of three weeks not only are all the nuclear and tissue structures absolutely unchanged, but even the red corpuscles appear well preserved.   About the periphery is a narrow zone that stains faintly, this change affecting all elements alike.   There seems to be absolutely no invasion by leucocytes, although a delicate capsule of connective tissue has been formed about the periphery.   By the end of six weeks this pale zone has widened to a depth equal to from two to four times the thickness of the capsule of the spleen.   In this area the nuclear stain is absent at the periphery, gradually appearing by gentle transitions as the deeper tissues are approached which stain with normal intensity.   In the middle of the pale zone may be seen numerous hematoxylin-staining granules that are apparently the result of karyorrhexis; pycnosis is not observed.   Between the encapsulating connective tissue and the spleen substance is a narrow zone where the latter seems to have been partly digested out by leucocytes that are present here in small numbers.   In the unstained zone can be found also an occasional leucocyte, although so few that one questions whether they are responsible for the loss of staining.   Yet, on the other hand, in a heated specimen that became infected, and as a result infiltrated with leucocytes, the nuclear stain had entirely disappeared in less than ten days (and *in vitro* experiments have shown that infection, at least with ordinary putrefactive organisms, does not greatly affect nuclear staining).   Apparently, then, the leucocytes are able to cause solution of the chromatic substance of the heated nuclei, which does not seem to be accomplished by infiltrating plasma alone.   The eventual solution of such heated tissues is accomplished as in aseptic infarcts, by leucocytes acting upon the peripheral portions, but not entering into and acting upon the deeper tissues.

In striking contrast to the heated specimens, the *fresh tissues* undergo rapid changes, just as they do *in vitro*, so that in the course of a few days the nuclear staining is lost.   At the end of ten days the nuclei have all disappeared except those in the capsule, which remain almost unchanged at a time when the nuclei of the trabeculæ deeper in the organ have disappeared.   This may be due either to an inhibiting effect of the plasma upon the autolysis, or to the presence of a sufficient amount of nourishment to sustain their vitality.   The spleen pulp shows a diffuse basic staining, most marked at the site of the Malpighian bodies, which is apparently due to diffusion of nuclear substance.   Granular brownish masses, apparently blood pigment, are also present, although shadows of blood corpuscles also persist; by the seventh week the pigment is more crystalline, peripheral leucocytic invasion by leucocytes has begun, and all traces of nuclear stain are lost from the spleen elements.

From these experiments we learn that nuclei of cells that have been killed by heat may remain for extremely long periods of time in the animal body without undergoing any

appreciable changes, after nine weeks such tissues show only
a slight peripheral loss of stain, the central nuclei remaining
unaltered.   At least two interpretations may be made of this
fact: first, that the changes in the nuclei observed in areas of
necrosis are brought about by the intracellular enzymes,
which are destroyed by heating; second, that the heating
so alters the nucleoproteids that they are not attacked by
the agents that make them unstainable under ordinary con-
ditions.   Against the latter interpretation, however, may
be advanced the following observations: (1.)   As a rule,
proteids that have been heated are more readily digested
than are fresh proteids, either because of a change in molec-
ular configuration, or because of the destruction of an
antibody, or perhaps both. (2.)   If the heated tissues
become infected so that an invasion of leucocytes takes place
the nuclear stain disappears promptly, showing that the
nucleoproteids are still in a condition to be attacked by the
leucocytic enzymes.   It therefore seems that the most prob-
able explanation of the results is that the disappearance of
the nuclear stain that occurs in dead tissues depends upon
the intracellular enzymes of the necrosed cells.

SUMMARY.

Putting together the results of the above experiments, we
find evidence that in typical areas of anemic necrosis, such
as infarcts, etc., the changes that occur are as follows:

1.   There is first a decomposition of the nucleoproteids of
the nuclei, which is probably brought about by the intra-
cellular enzymes of the starved cells.   Ordinary autolytic
enzymes resemble trypsin, at least in the major effects pro-
duced, and trypsin has the power of decomposing nucleopro-
teids only to the extent of splitting off the proteid groups,
leaving the nucleic acid in a free state (Sachs).   If the
autolytic proteolytic enzymes of the cells behave in the same
way we may find here an explanation of the process of
pycnosis, as follows: The density with which a nucleus
stains seems to depend upon the extent to which the nucleic
acid is saturated with proteids.   Unsaturated nucleic acid is

strongly acid, and therefore has a marked affinity for basic dyes; the more the nucleic acid is saturated with proteids the less will be its affinity for basic stains.[19] Hence a decomposition of the nucleoproteids of the nuclei should, theoretically, cause an increased intensity of basic stain, which would be more diffuse than normally, as is the case in pycnotic nuclei. The cause of the decrease in size is probably the loss of nuclear substance through this splitting off of proteids. (Dietrich's attempt to explain pycnosis as due to an exosmosis of water from the nucleus into the cytoplasm may be well questioned on the ground that cell membranes seem to lose their semipermeable character immediately after the death of the cell.) The nucleic acid is subsequently so altered that it does not stain any more, which may be due either to its neutralization by the alkalies of the plasma, or to a further splitting, which last seems probable in view of Schmoll's[20] observation that in caseous material there is surprisingly little phosphorus, indicating that the nucleoproteids become so decomposed that the phosphorus is liberated in a readily diffusible form, which soon escapes from the necrotic area. Jones[21] has shown the existence of enzymes in the thymus and adrenal that have the power of decomposing nucleic acids into their constituents, phosphoric acid and the xanthin bases. Sachs[22] found a similar enzyme in the pancreas and called it "nuclease."* The view supported by numerous earlier histological investigators, that the chromatic nuclear substance is simply dissolved out by the blood plasma, is probably incorrect; it must first be decomposed into its soluble constituents. Apparently the leucocytes contain similar nucleic-acid-splitting enzymes, at least they seem to destroy chromatin substance in both fresh and heated substances very actively.

2. In infarcts the intracellular proteolytic enzymes seem to attack the proteid structure of the cell but little and very slowly, except in the center of large necrosed areas. It would seem that this depends chiefly upon the presence in

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* The subsequent changes that occur in the xanthin bases have also been further studied by Jones with Partridge and Winternitz, and by Schittenhelm.[23]

the blood plasma of antibodies or of some heat-susceptible substances which check the action of the autolytic enzymes. Possibly the alkalinity of the plasma also exerts a retarding influence.

3. Chemotactic substances do not seem to be formed in aseptic dead tissues; indeed the absence of leucocytic infiltration is so marked that it seems possible that substances with a negative chemotactic effect are present, such as lactic acid, which is known to be formed during autolysis (Magnus-Levy).

4. The slow absorption of infarcts, etc., seems to be accomplished almost entirely through a digestive action of the leucocytes acting slowly from the periphery; *i.e.*, the digestion is not so much through autolysis as through heterolysis (to use the term proposed by Jacoby[24] for the digestion of one sort of cell by the enzymes derived from another sort). The leucocytic proteases act best in an alkaline medium (Opie), which possibly explains their ability to attack substances impregnated with alkaline blood plasma. Leucocytes seem also to contain enzymes decomposing nucleoproteids, as shown by their effect on heated tissues in the experiments cited.

5. The rapidity with which autolytic changes occur in different organs and tissues, as indicated by the disappearance of nuclear staining, seems to be about as follows: (1) Liver, kidney (epithelium of convoluted tubules); (2) spleen, pancreas; (3) kidney (collecting tubules, straight tubules, glomerules); (4) lung (alveolar and bronchial epithelium); (5) thyroid; (6) myocardium; (7) voluntary muscle; (8) skin (epithelium); (9) brain (cortical cells). Stroma cells seem to be attacked chiefly by enzymes (or products) from the parenchyma cells, since they disappear from various organs in direct proportion to the rapidity of autolysis in the parenchymatous elements. Of all cellular elements the endothelium of the vessels seems to undergo the least autolysis and to be least subject to heterolysis by enzymes from other cells.

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