Age Changes in the Liver as Studied by Light and Electron Microscopy

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The subject of the intimate nature of the changes which occur during the aging process has become one of increasing interest, partly due to the very practical fact that the percentage of individuals in our population 65 years of age or over is increasingly greater. For a number of years our own research efforts have been directed toward study of the histological or tissue changes in various organs with age in pedigreed animals. In previous papers we have described the histological changes (2, 3, 4). In collaboration with workers in other laboratories we have been able also to show a correlation between the chemical and the histological changes which occur in some tissues with age (5).

More recently, we have become interested in the cellular or cytological changes with age and are attempting to study the various organelles of the cell. Our present studies are being made on mice of the C57 Black strain. Both the classical cytological methods with light microscopy and the newer method of electron microscopy are being used and we are making an attempt to correlate the two where possible.

The liver is an organ in which the age changes as seen by histological or tissue study are relatively inconspicuous. There is very little fibrotic change in the liver of a senile animal. In some other organs, as in the thyroid gland, for example, fibrosis is a conspicuous change in old age, while in the parotid gland, fatty degeneration is common (2, 3). The picture of the liver on the other hand, its multitudes of hepatic cells with their many different functions, and the hepatic trinities, the bile duct, artery and vein, is generally fairly similar in young and old animals under low powers of the microscope. The tissue changes which occur in the liver in old age are primarily those of what may be called lymphocytic infiltration or the appearance of new lymphoid tissue. This we have called ectopic lymphoid tissue or lymphoid tissue in an abnormal place.

In the present study ten pedigreed mice of the C57 Black strain, from the Roscoe B. Jackson Laboratory, were used. The individuals according to age in days and sex were as follows: 105 ♂, 105 ♀, 539 ♂, 539 ♀, 549 ♂, 549 ♀, 610 ♀, 664 ♂, 664 ♀, and 664 ♂. We have represented here, then, young adult animals, animals of "late middle age" (539-549 days) and animals which may be considered "old" or senile, over 600 days of age, since the life span of the mouse is about two years.

The animals were sacrificed by use of ether. Very small portions of liver, about 1 mm. in diameter, were fixed in Palade's fixative, osmic acid buffered with veronal acetate, embedded in a 4:1 mixture of butyl-methyl methacrylate, sectioned with a glass knife on a Porter-Blum ultramicrotome and mounted on 200 mesh grids. Only sections in the silver-gold range were used. Larger pieces of the same liver were fixed in Regaud's fluid,

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postchromated, dehydrated, embedded in paraffin, and stained for mitochondria with acid aniline fuchsin, with a counter-stain of methyl green. Still other pieces were fixed in 10 per cent formalin, prepared by the paraffin method and stained with hematoxylin and eosin.

For the electron microscope study several grids from each specimen were studied. The instruments used were of the RCA EMU-2 and RCA EMU-3E types.

With the light microscope only a rather extensive study indicates any change in the hepatic cells and this change either has to be worked out quantitatively or seen in individual aberrant cells. In other words, it is not a part of the readily observable total picture with the light microscope. A study of a number of fields of the liver reveals occasional large aberrant hepatic cells. Such cells also have tremendously large nuclei.

The mitochondria of the liver cells both in young and old animals generally are abundant. In the younger animals, however, the mitochon-

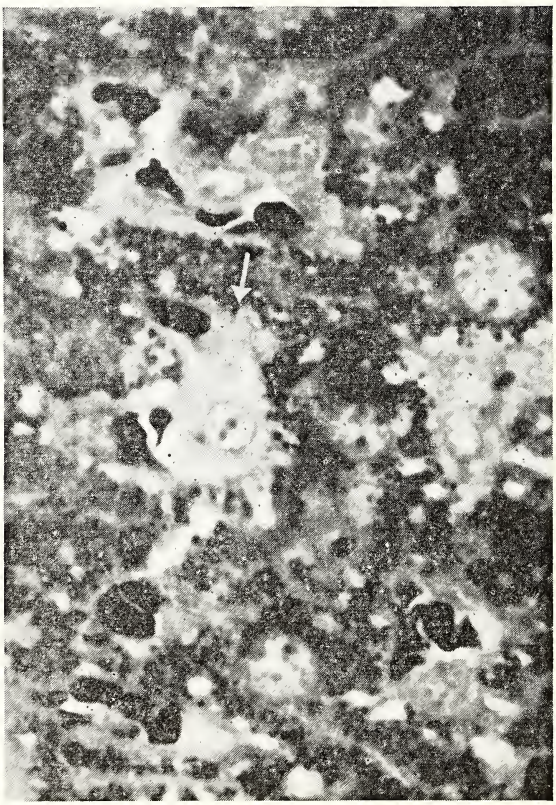

Figure 1: Liver of a senile male mouse as seen with high magnification of the light microscope. In some areas hepatic cells with very few or apparently no mitochondria are seen, such as the one indicated by the arrow. Regaud fixation, acid anilin fuchsin stain. X 1,900.

dria generally are more elongated. Study of the large aberrant cells in the liver of senile animals shows relatively little difference in the mitochondrial picture for such cells. There are, however, areas in the senile liver, particularly in regions where there is rather marked sclerosis of the blood vessels, where the mitochondrial picture is quite varied and where some cells are almost completely lacking in these cytoplasmic elements (fig. 1).

The details of structure of the hepatic cells and of other features of the liver as studied with the electron microscope have been presented in papers by Dalton et al. (8), Bernhard et al. (6, 7) and Fawcett (9).

Our initial studies with the electron microscope have included observations on hepatic cell nuclei and on mitochondria. In figure 2 we see the

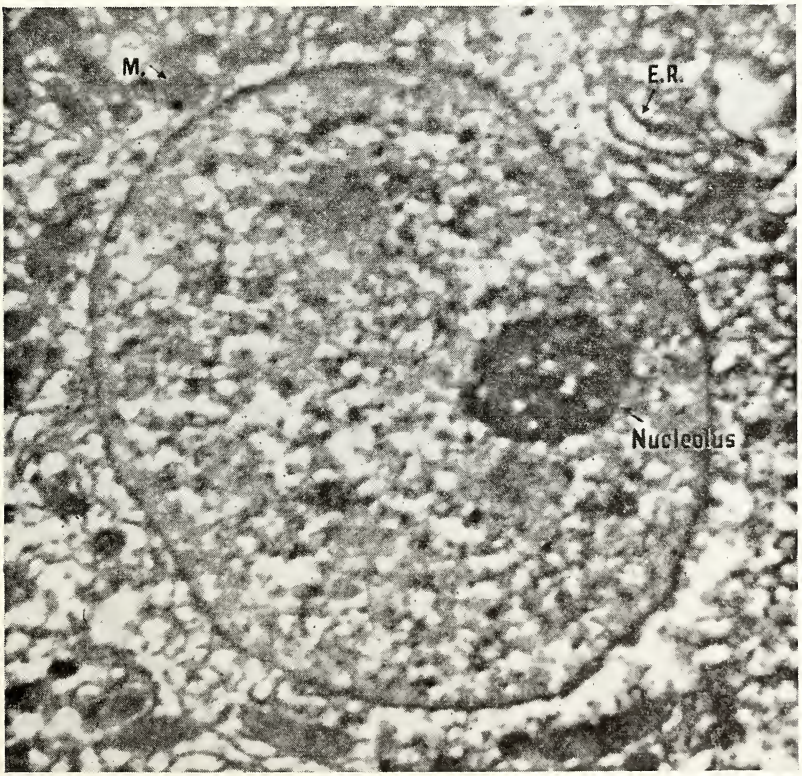

Figure 2: Electron micrograph of nucleus and part of the cytoplasm of an hepatic cell of a young adult male mouse. The nucleolus is large and conspicuous. E.R. = endoplasmic reticulum; M. = mitochondrion. X 26,000.

nucleus of an hepatic cell of a young mouse, 105 days old. The nuclear membrane is smooth. The interior of the nucleus shows a coarse network with some portions, denser than others, probably representing the desoxyribonucleoprotein. The nucleolus is conspicuous and shows a definite internal structure. In fig. 3, from a mouse of 664 days, we see the nucleus

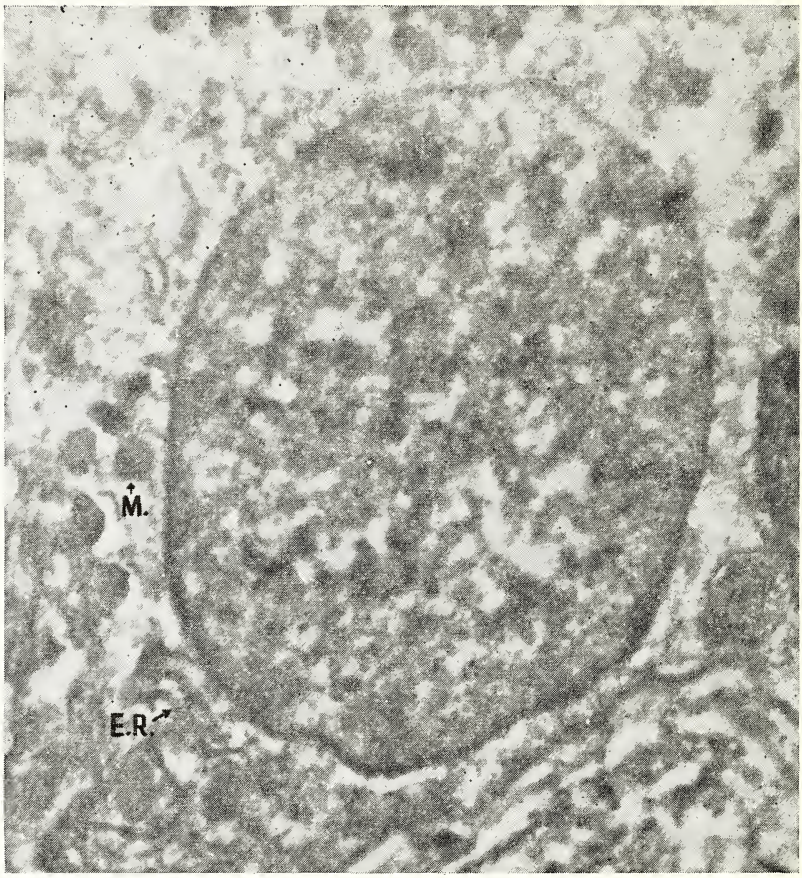

Figure 3: Nucleus and part of the cytoplasm of an hepatic cell of an old female mouse. Nucleoli in older animals are less conspicuous than in younger. E.R. = endoplasmic reticulum; M. = mitochondrion. X 26,000.

of an hepatic cell in which the nucleoli, several of which are shown, are not as conspicuously marked off from the rest of the nucleus.

The hepatic cells show intranuclear inclusions in some pathological and experimental conditions. We have described such intranuclear inclusions as seen much more frequently in mouse and human liver in old age than in youth or "middle age" (4). An electron microscope study of such inclusions in the livers of six rats treated with thioacetamide and one human individual, a woman of 65 years suffering from cirrhosis of liver, has been made by Kleinfeld, Greider and Frajola (10). They have shown that these inclusions arise by an invagination of the nuclear membrane, a process which traps a portion of the cytoplasm. Thus the composition of the inclusion may be complex and include lipid, glycogen, and even cytoplasmic organelles.

In the present study we have noted that invagination of the nuclear membrane is of frequent occurrence in the liver of old mice (fig. 4), while

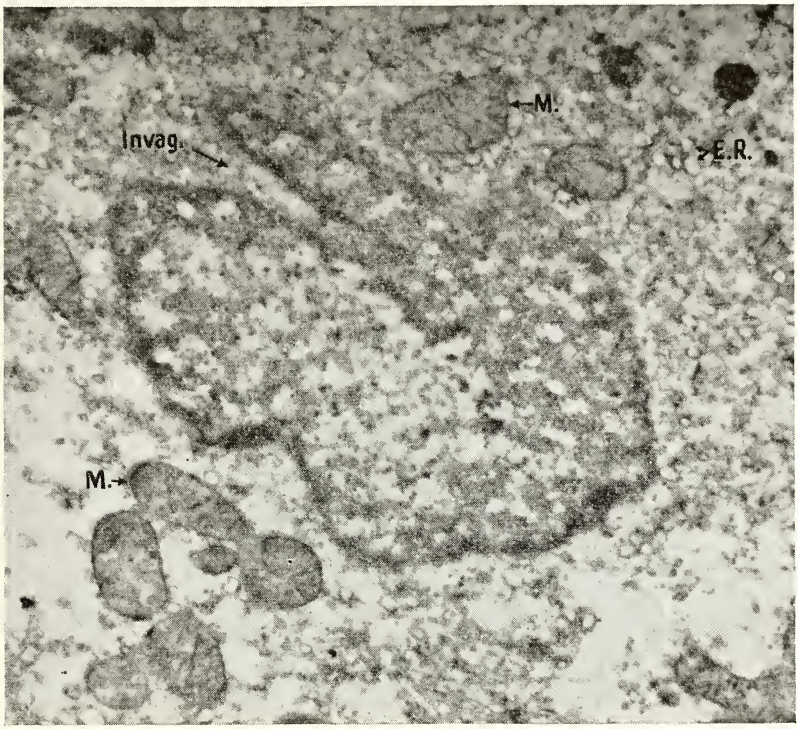

Figure 4 : Nucleus and cytoplasm of an old male mouse. Many nuclei in old mice show deep invaginations of the nuclear membrane. E.R. = endoplasmic reticulum ; invag. = invagination of nuclear membrane ; M. = mitochondrion. X 26,000.

we have not as yet seen it in our young animals. Such invagination would of course make the production of intranuclear inclusions more frequent.

Our study of the mitochondrial picture with the electron microscope has enabled us to make some correlation with light microscope findings and has revealed some additional facts. We have found the same kind of areas of "mitochondrial loss" in the sections studied by this method as we had seen in other senile animals with light microscopy (fig. 5). In the electron micrographs the cytoplasm usually shows a small number of rod-shaped or filamentous structures with the cristae or lamellar partitions which electron microscopists have come to consider as characteristic of mitochondria. Some of the ovoid or spheroid bodies also show such cristae, but many of them do not show them.

It has been pointed out by Fawcett (9) and by earlier authors that in fasting animals the liver mitochondria tend to pass from a rod-shaped or filamentous type to a granular or spheroidal form. It seems to us probable that such a change is occurring in the areas in which there is a tendency

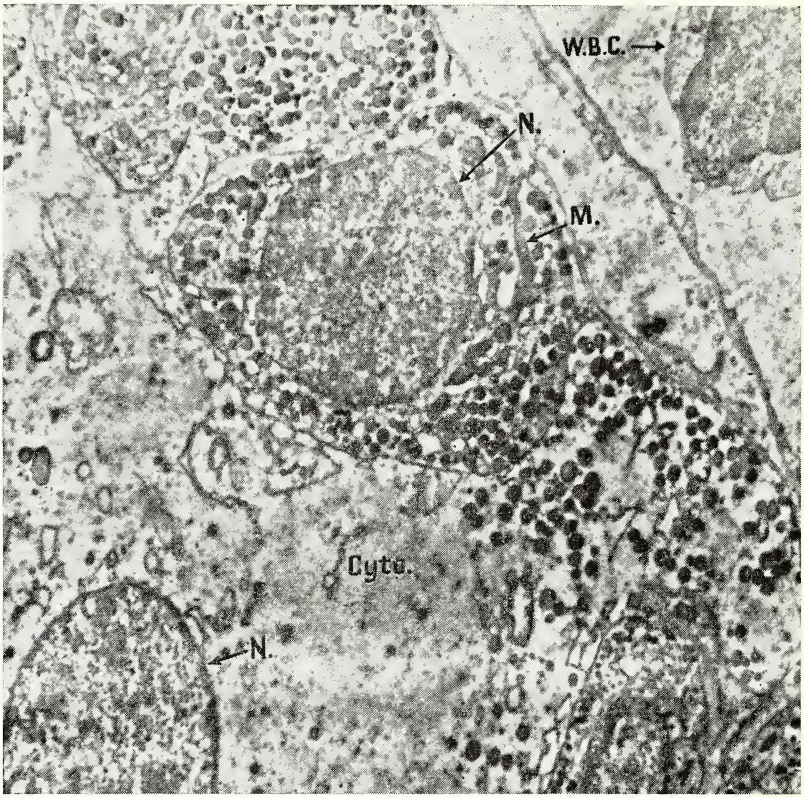

Figure 5: Low power electron micrograph of liver of an old female mouse. The unevenness of distribution of mitochondria in different liver cells is shown. Cyto. = cytoplasm; M. = a filamentous mitochondrion (most of the mitochondria shown are almost spheroidal); N. = nucleus; R.E. = reticulo-endothelial cell; W.B.C. = white blood corpuscle. X 12,600.

to actual disappearance of mitochondria, and that we are seeing here in addition some loss of internal structure in these organelles prior to their disappearance.

A contribution to the much discussed question as to how the mitochondria reproduce may well be expected from any study in which a careful and detailed observation of these organelles has to be made. We have seen a number of instances in the liver cells both of young and old animals in which mitochondria are constricted at or near the central portion and it has seemed probable to us that these do represent stages in division. We have not found any of the figures, so clearly shown by Fawcett (9) in which a partition extends across a mitochondrion to divide it into halves. It is known, however, that nuclei in amitotic division may divide either by constriction or by the formation of a partition, with later separation of the halves as daughter nuclei (1). It seems not unlikely to us that mitochondria also may have more than one method of division.

In conclusion, we may say that electron microscope study of age changes in one organ, the liver, seem to correlate well with light microscope study, to give added information, and to promise an opportunity for new and significant findings in the future.

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