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A Study of the effects of aging on the liver of healthy elderly patients

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A STUDY OF THE EFFECTS OF AGING ON THE LIVER OF HEALTHY ELDERLY PATIENTS

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Since his creation, man has sought to make his life safer and easier and, as a consequence of the success of these endeavors, has greatly increased his life span. Whereas the average member of the population of Ancient Greece had a life expectancy of 34 years, Steiglitz states that a member of our present society may anticipate a lifetime of the often quoted "three score and ten" years(5). This increased life span, due in no small part to greater medical knowledge, has produced a marked shift in the percentages of population falling within the various age groups(5). Boas stated that there were six million more people over the age of 65 in 1944 than in 1900. He also predicted a population of over 22 million in that particular age range by 1980(7). According to Steiglitz 5.4 percent of the people in the United States were over 65 in 1920. By 1950, this figure had reached 8.2% and Steiglitz feels it will exceed 14 per cent of the total population by 1980(51). The U. S. Census figures show that while the increase in the general population was 7.2% during the decade 1920-1930, the increase in the total number of persons over 65 was 35%(7).

Because this change in population is the result, in part, of better medical practice, it has become the responsibility of the medical profession to increase the emphasis on clinical investigation of the problems of the older age group in the interest of continued good medical practice.

The usual disorders of the geriatric age group, i.e., that group of persons over 65, are cardiovascular renal disease,

arthritis, metabolic disorders, and neoplasms. These diseases have received a good deal of attention in the past, especially since they have surpassed the infectious diseases as the leading killers of men[21]. Age changes in the organ systems involved by these diseases also have received thorough investigation.

The less prominent role of the liver in the overall pattern of aging and in the geriatric diseases apparently has precluded much research into its alterations, if any, in the older age group. It is the purpose of this paper, then, to attempt to determine the presence and nature of any changes in liver parenchyma or stroma which may occur in persons of reasonably good health over the age of 65. By persons of reasonably good health, we mean to include those individuals who are clinically well and who have no gross damage of liver as determined by the usual laboratory tests of hepatic integrity. An attempt will also be made to correlate any histopathological findings with any abnormalities of physiology of the liver as determined by these laboratory tests.

Biopsy specimens of human livers have been used for this study to obviate inaccuracies produced by post mortem, degenerative changes. This method has enabled us to obtain accurate information regarding the state and, to a lesser degree, the extent of any morbid processes taking place.

The first recorded biopsy of the liver for diagnostic purposes was performed in 1895 by Lucatello[33].

However, puncture of the liver for therapeutic pruposes in cases of abscess and hydatid disease was first reported by Biett[5] and Roberts[44] in 1833. Hammond[21] in 1878 and Simms[52] in 1879 also are reputed to have inserted a hollow needle into the liver for treatment of hepatic abscess. Twelve years after Lucatello's original work, Schupfer[48] reported a series of 14 biopsies of the liver for diagnostic purposes. He used a small bore needle with an aspiration apparatus on one end to obtain small fragments of hepatic tissue and blood which, when smeared on a slide and stained, were satisfactory for microscopic examination. Although the risk was low and the accuracy of diagnosis was claimed to be high, this method did not permit a study of hepatic architecture and stroma. Frola[17] reviewed the reports of aspiration biopsy of the liver in 1935 and added 66 cases of his own. Examination of his photomicrographs reveals small particles of hepatic tissue which betray the limitation of that technique. Despite its drawbacks, the aspiration method of liver biopsy in its various forms has remained popular in Europe as evidenced by the review of Topp and Lindert[58].

In 1923 Bingle[6] and Olivet[39] developed a technique which enabled them to obtain a small piece of hepatic tissue satisfactory for preparation of paraffin sections. They used a wider cannula (2mm.) and a suction type of apparatus to aspirate a plug of liver. Bingle reported 100 biopsies with two deaths due to hemorrhage. Olivet extended that series in 1926 by adding 140 more cases with another death due to peritonitis.

Iverson and Roholm[27] in 1939 modified the Bingle-Olivet technique which has since been widely adapted. They reported 160 biopsies on 114 patients with a successful result in 72.5 per cent of their cases. Two cases of intraperitoneal hemorrhage were their chief complications.

Dible, McMichael, and Sherlock[15] made an extensive study of viral hepatitis, arsenotherapy jaundice and serum hepatitis in England during World War II. Using the aspiration technique for biopsy of the liver in a series of 126 cases - later extended to 245[50] - they had two hemorrhages and one death due to massive necrosis. Their incidence of failure was only six per cent. These authors were the first to emphasize the necessity of 24 hours absolute bed rest and of the desirability of adequate facilities for whole blood transfusions in order to forestall the complication of hemorrhage.

The first series of liver biopsies in the United States was reported by Baron[4] in 1939. He performed 48 aspiration type biopsies on 35 patients. Eight of those were executed through the intercostal approach; the remainder were obtained by the conventional subcostal approach to the liver. Needle biopsy of the liver became recognized as a truly safe procedure when, in 1945, Gillman and Gillman[18] published the results of a group of 500 biopsies in which they had no fatalities.

A special needle for biopsy of tumors was developed by Silverman[51] in 1938, but it was not until 1941 that the instrument found its greatest use when Tripoli and Fador[59] utilized the

instrument for biopsy of the liver. Their report cited 14 successful biopsies in 14 attempts using the subcostal approach. Hoffbauer[24] made use of the Silverman needle in performing 65 biopsies without incident. With Evans and Watson[25] this same author published the results of 85 biopsies among which were 16 failures, but no deaths. These workers were among the first to go beyond the realm of diagnosis by liver biopsy methods when they attempted to correlate the histological pattern of their specimens with studies of hepatic function. It should be noted that they reported they found no such correlation. On the other hand, Popper,[40] using specimens obtained by liver biopsy, stated that he was able to correlate certain architectural changes seen in his sections with some of the studies of hepatic function. Terry[57] in 1952, reviewed a total of 10,500 liver biopsies. In this series he found a morbidity rate of 0.22 per cent and a mortality rate of 0.12 per cent. He found further, that the number of deaths and complications has decreased in recent years with greater knowledge of the dangers of the procedure and with greater care in choice of patients.

Success in obtaining tissue suitable for microscopic examinations varied, in the reports cited, from 75[24] to 98[3] per cent. There was nearly uniform agreement on the great value of the needle biopsy specimen of liver in diagnosing and studying the diffuse affections of that organ[3,11,12,24,31,36,46,47,62]. Reports were quite varied to its effectiveness in the problem of localized liver pathology, e.g., tumors[3,11,12,28,36].

Literature reporting histological changes occuring in the liver with age is meagre. The reasons for this seem to be several; any subtle changes may be obscured by post-mortem changes in autopsy specimens; any changes present may be overlooked because of more obvious lesions in other organ systems; and, due to the high reserve capacity of the liver, any lesion present may result in no functional aberration. Boas(7) has said that "each organ and tissue has its own time curve of aging and that this time curve depends on three factors: heredity; external environment; and internal environment of the body." He added that the liver shows few signs of age change until late and then the changes are only those of atrophy. In a study of 34 livers at post-mortem, Boyd(8) demonstrated an average weight loss of 11 per cent in livers of elderly persons when compared with average young adult livers. Howell,(26) in a general study of gross and microscopic pathology of tissues in persons over 65, showed a fairly constant deformity in the shape of the livers as well as a decrease in weight but he felt that there was no significant accompanying histological changes. Strassman and Krush(56) found a slight decrease in liver size with thickening of the capsule in persons over 60. They described an increase in fibrous tissue with round cell infiltrations in the portal tracts of these same livers. In an investigation of liver parenchyma of Wistor rats over 650 days old Andrew, Brown and Johnson(2) found numerous hypertrophied nuclei, intranuclear inclusion bodies especially in the large nuclei, and periportal infiltration with lymphocytes and connective tissue cells. These

workers extended their research to aged livers in humans and found in reviewing post-mortem material that here, too, were large irregular cells with large nuclei containing increased numbers of nucleoli but no marked periportal infiltration. Andrew[1] enlarged this study in 1952 by examining the mitochondrial distribution in the cytoplasm of liver cells of senile rats and comparing it with that in young adult rats. He found marked variation in distribution and amount of mitochondria in senile rat hepatic cells. Bucher and Glinos[9] also have studied senile rat livers and concurred with Andrew et.al. in that they reported larger than normal hepatic cells, larger than normal nuclei and in addition, they described smaller numbers of binucleated cells - 35 per cent - compared with 58 per cent in young rats. A comparison of changes due to age and due to castration in rats was undertaken recently by Korenchevsky, Paris, and Benjamin[29]. The senescent rat livers which they studied showed large parenchymal cells but no change in nuclei. They also reported round cell infiltration in the portal tracts of 25 per cent of their subjects and small bile duct hyperplasia in 54%. The decreased ability of senile hepatic cells to proliferate has been postulated by these workers as the underlying cause of the hypertrophy of the liver cells. They felt that this compensatory hypertrophy was a natural process of aging. They have in essence, restated Minot's theory of atrophy in old age which states that the failure of hyperplasia to replace worn-out cells not only results in hypertrophy of the remainder of cells but results also in ultimate failure to replace total mass lost and hence, the organ in question becomes atrophic.

Frischman,[16] on the other hand, studied the effect of aging on connective tissue stroma in human livers and reported that he could find "no increase in fibrous tissue which was not in some way related to disease." He examined ten specimens ranging in age from 60 to 83 years. In another study in which he compared livers of humans from six months to 86 years of age he found no changes in the relative amounts of either collagenous or reticular connective tissue as compared with parenchyma in advancing age.

Methods and Materials.

This project consists of 19 biopsies from 19 patients. These were 14 white and 2 negro males and 3 white and no negro females. All the subjects were 65 years of age or older, all had no clinical evidence of liver disease; all were in reasonably good health.

The biopsies of liver parenchyma were obtained with a Silverman type biopsy needle. It consists of an 18 cm by 1.8 cm cylindrical cannula with a sharp bevel at one end and a knob at the other. A 20 cm. split, hollow stylet fits snugly within the cannula and can be advanced inside the cannula so that its sharp split distal end protrudes 2 cm. from the beveled end of the cannula. Other materials required are two Luer-lok syringes, one 2 cc. size and one ten cc. size, a No. 11 Bard-Parker scalpel blade with a No. 4 handle, two needles, one 25 ga. one inch long and one 23 ga. three inches long, sterile gauze sponges, three sterile towels, one percent Procaine, sterile rubber gloves, and a bottle of a 1:1000 tinted tincture of Zephiran Chloride and a bottle of ether. All but the sterilizing solutions are autoclaved beforehand.

The biopsy is done on the ward and not recommended as an office procedure(50). The patient is first given one-sixth to one-eight of a grain of Morphine Sulfate with one-one hundred fiftieth of a grain of Atropine intramuscularly 45 minutes prior to the procedure. Before the biopsy is attempted, the patient is placed flat on his back close to the right side of the bed with his right hand behind his head and his left and at his side. The upper border of the liver is determined by percussion and the site for introduction of the biopsy needle is chosen one interspace below the level of liver dullness in the anterior axillary line on the right. The skin over the chosen area is cleansed and sterilized with ether followed by the tincture of Zephiran Chloride. After the skin preparation, the operator dons sterile gloves and drapes the area with sterile towels. A skin wheal is raised over the previously chosen site with one percent procaine using the short needle and small syringe. Then the subcutaneous tissue, pleura, peritoneum and Glisson's capsule are anesthetized with approximately eight cc of Procaine, using the large syringe and long needle, while the patient holds his breath in complete expiration. Next, a 0.5 cm incision is made in the skin over the wheal with the scalpel. The Silverman needle, with the split stylet in place but not protruding from the leveled end of the instrument, is then inserted into the incision. The patient now is instructed to breathe deeply four times and to hold the fourth breath at the peak of expiration. While the patient is apneic, the needle is quickly inserted through the pleural space into the liver substance a depth of 2 cm.

The split stylet is then advanced from within the cannula ~~and~~ an additional 2 cm into the liver parenchyma. The cannula in turn is advanced around the stylet while the stylet is held in place and the original relationship of stylet-to-cannula is restored. On completing the advancement of the cannula over the stylet, the entire apparatus is turned through an arc of 90 degrees on its long axis and then withdrawn. This procedure causes a 1.5 to 2.0 cm by 0.18 cm cylinder of liver tissue to be lifted from the organ. The specimen is immediately put in fixing solution and then deep pressure is made over the biopsy site for two minutes as an effort to help establish hemostasis. Lastly, a simple dressing is put over the small incision.

The technique just described is recommended by Kumpe, Gall and Schiff[31] and, if properly performed, causes the patient no discomfort; The actual biopsy of the liver requires approximately 10 seconds from the introduction of the needle to withdrawal of the tissue.

Routine post-biopsy orders consist of absolute bed rest for 24 hours, hourly checks of pulse and blood pressure for eight hours, and ten grains of aspirin with one-half a grain of codeine every four hours, orally, as needed for pain[31]. One unit of blood properly matched ahead of time is available on the floor to be used if symptoms of intraperitoneal hemorrhage develop, i.e., shock and abdominal pain in the right upper quadrant[50].

We have followed the criteria of Kumpe,[31] Sheloch[50], Maddook[34], and Terry[57] for contraindications to biopsy of the liver.

These include: (1) prolonged prothrombin time or other bleeding tendencies; (2) failure or inability of the patient to cooperate; (3) hepatic congestion; (4) infection in the right lower lobe of lung or right upper quadrant of the abdomen; (5) ascites; and (6) absence of liver dullness.

While peroussion of the upper border of the liver may seem inaccurate and dangerous it has been shown that one tends to err on the side of underestimation of liver size[20,43].

By using the intercostal technique and by paying close attention to the contraindications and post-biopsy follow-up, Cogswell[12] has been able to report a series of 403 biopsies on 345 patients resulting in suitable specimens in all but eight attempts and in only one fatality. According to Kumpe[31], the commonest complication of the operation is vague, dull, epigastric pain on the day following. Other less frequent complications have been, tenderness at the biopsy site, perihepatic friction rib, subcutaneous emphysema, and severe right upper quadrant pain with splinting of the right diaphragm and referral of pain to the right shoulder. These data are closely corroborated by Terry[57], Scott[49], and Koch[28]. The most serious complications are hemorrhage and bile peritonitis. Rubenstone[45] stated that bile peritonitis can be prevented by avoiding biopsies on patients with obstruction of the biliary system. Hemorrhage can be prevented to a large extent, by not using patients with hepatic congestion or bleeding tendencies[50].

Two of our biopsies were performed utilizing the subcostal

approach which Koch[28] feels is fraught with fewer complications. The technique is quite similar to that just described. We were unable to see any difference in effect on the patient between the two. The overall mortality in 10,500 biopsies has been 0.12 per cent[59].

Christian[11] recently was able to study the effect of the trauma produced by the Silverman needle in biopsied livers. He had 14 patients who died within five days of biopsy from other causes. The site of entrance of the needle into the liver, he found, showed slight extravasation of blood into the surrounding tissue with beginning fibroblastic infiltration of the area indicating prompt tissue repair.

In our series of 19 cases we had no deaths, no hemorrhages and only two cases of severe epigastric pain after biopsy. Two patients developed a friction rub around the site of biopsy, the day following the procedure, which disappeared the next day. Six out of our 19 patients had dull, post-biopsy pain at the operative site for 24 hours.

Biopsies of living liver parenchyma were taken for this study to eliminate the changes which occur in the liver post-mortem. Popper[41] demonstrated a quite different morphologic pattern between autopsy and biopsy specimens. He further showed by comparing specimens obtained from subjects who died suddenly with specimens from patients with a long terminal course, that agonal periods influence the morphologic picture of hepatic tissue[41].

The validity of using a small piece of liver tissue from the periphery of one lobe as representative of an entire large organ has often been questioned. Hinsworth[23] from his studies of liver disease deduced that all basic changes in liver involve the functional unit, the lobule. Furthermore, Stone[55] stated that "the needle biopsy is well suited to examination of histological changes in liver because the functional unit is only a few millimeters square in area and thus several parts of several units are available in one biopsy specimen." It would, therefore, seem logical to carry out this study using needle biopsy specimens of liver.

The accuracy and correlation of biopsy findings in liver disease compared with autopsy findings have been thoroughly investigated. Waldstein[53] took 10 biopsies from various regions of each of 15 cadaver livers and compared the biopsy and anatomical findings. He found that "epithelial cell injury and fatty metamorphosis were uniform throughout the livers studied," and that, on the other hand, there was a discrepancy of about 10 per cent between biopsy and autopsy findings in cases of fibrous and inflammatory changes. Molle and Kaplan[36] in their overall series, which included non-diffuse liver disease, e.g., metastatic carcinoma, found their biopsy material was accurate in 85 per cent of cases. Surgical and autopsy findings were compared with needle biopsy findings in a group of cases reviewed by Koch[28] and he reported "good correlation." Even in a study of 53 patients with neoplasms, Safdi[46] was able to make the correct diagnosis 85 per cent of the time. Wagoner[62] has recorded an accuracy of approximately 88 per-cent in 84 biopsies of all types of liver disease.

He emphasized that only one case of diffuse hepatic pathology was misdiagnosed in the entire group. The consensus of opinion seems to be that needle.type biopsy of the liver is satisfactory for study of hepatic pathology because it gives a good percentage of diagnosis and allows one to gain a dynamic picture of changes taking place in the liver without subjecting the patient to a major surgical procedure(62).

Liver function tests were run prior to biopsy on all patients studied in our series for two reasons: first, to rule out gross liver disease; and second, to avoid any patients with a prolonged prothrombin time. Lichtman(32) has said that a comprehensive laboratory determination of the status of the liver parenchyma should include one test of each of the following functions: carbohydrate, metabolism, secretory and excretory activity; protein metabolism; and lipid metabolism. A test of carbohydrate metabolism was not used in this series because Crellin(14) and others have shown that persons in the geriatric age group have altered oral glucose tolerance. The battery of tests utilized included bromsulfothalien excretion, cephalin-cholesterol flocculation, total serum proteins with albumin, globulin ratio, serum cholesterol and cholesterol esters, serum bilirubin and prothrombin time. According to Mateer(35), Neefe's method(37) for cephalin-cholesterol flocculation represents the best screening test and the bromsulfalien excretion test is the best screening procedure in study of liver function. Popper's(40) criteria for abnormal values of these tests were followed.

The minimum for each is: bromsulfalein excretion 94 per cent in 45 minutes; cephalin-cholesterol flocculation one-plus in 48 hours; albumin-globulin ratio greater than one-to-one; cholesterol esters at least 60 per cent of total cholesterol; total serum bilirubin of less than 1.2 mg. per cent; and a prothrombin time greater than 85 per cent. On several of our subjects, these criteria were not strictly adhered to in that one of the tests showed an abnormal value. While such a procedure may seem arbitrary, especially in the light of Ivy's statement that there is rarely a functional decrease in liver with age;[13] Ratzky et. al. in 1938 demonstrated alteration of one or more of the liver function tests in question in 97% of a series of 48 patients over 65 years of age[42]. We feel justified, therefore, in comprimising as we have done.

Several attempts have been made to correlate histological changes with altered laboratory tests of liver function and to predict one from the other. Zimmerman and his group were unable to demonstrate any such correlation in diabetics[64]. Popper[41] reported slight correlation between the two in "diffuse cell damage and in distorted reconstruction of the lobules." The findings of Norcross[38] were equivocal although he did feel that correlation with architectural changes was better than with parenchymal changes. For these reasons little effort was made to find any correlations in our work.

Sections were prepared with paraffin and stained with eosin and methylene blue in 14 biopsies.

In addition to hematoxylin and eosin, the first 14 tissues were also stained by a modified Prussian Blue method(30) to determine the presence or absence of iron.

The remaining five biopsies were fixed in Champy's fluid(30) as recommended by Andrew(1). This fixative is rapid in its action and is therefore supposed to be of value for fixing tissues in which it is desirable to demonstrate mitochondria. Phosphotungstic acid hematoxylin, the iron pigment stain, Altman's amtine acid fuchsin, and hematoxylin and eosin were all used to stain sections of each of these specimens in an attempt to demonstrate mitochondrid. The fixative made the biopsy specimens so brittle that sectioning was technically extremely difficult. Staining of these tissues also was a problem. However, after resectioning and restaining, material suitable for examination was obtained. Fat deposits in the cells were nicely delineated but the mitochondria failed to take the stain.

Experimental findings:

The results of the laboratory work are presented in Table I. The key to the abbreviation in the table is as follows: S.B. = serum bilirubin, expressed as the total amount reacting in 30 minutes, in milligrams per 100 c.c; P.T. = prothrombin time; T.S.P. = total serum protein, followed by serum albumin, A., and serum globulin, G., all expressed as grams per 100 cc. of serum; Chol. = cholesterol which is reported in milligrams per 100 cc. of serum as are the esters of cholesterol; Ceph. floc. = cephalin-cholesterol flocculation which is reported 1+, 2+, etc.

Table I. Results of Liver Function Tests

Patient	Age	Sex	Race	S.B.	P.T.	T.S.P.	Alb.	Glob.	Chol.	Esters	C.F.	B.S.P.	Hb.	R.B.C.	U.A.
L.N.	65	M	W	0.20	94%	6.00	3.47	2.53	—	—	1/	None	12.3	6,300	Neg.
C.C.	65	M	W	0.30	95%	5.50	3.47	2.03	-	-	1/	NOne	16.2	9,200	Neg.
C.A.	65	M	W	0.20	98%	6.7	3.78	2.92	-	-	Neg.	None	16.6	7,600	Neg.
E.D.	72	F	W	0.20	95%	5.8	3.02.	2.78	140	85	1/	None	10.7	9,900	Neg.
E.J.	80	M	W	0.55	98%	5.5	2.90	2.60	120	72	1/	7%ret.	11.0	2,900	Neg.
C.L.	78	M	W	0.45	100%	4.8	3.24	1.60	120	66	1/	4%ret.	9.0	6,400	1/ alb.
T.P.	78	M	N	0.20	100%	6.7	3.78	2.92	112	51	Tr.	one	6.6	6,700	Neg.
F.S.	84	F	W	0.20	100%	5.65	2.99	2.66	178	115	2/	None	11.8	7,800	Neg.
A.L.	77	M	W	0.65	100%	4.8	2.99	1.81	118	79	Neg.	3%ret.	13.8	7,300	3/ alb.
H.H.	74	M	W	0.40	90%	6.15	3.78	2.37	200	70	1/	None	13.4	6,200	Neg.
C.T.	72	M	N	1.05	92%	6.3	3.24	3.06	380	155	Neg.	None	14.2	5,600	Neg.
F.G.	72	M	W	0.20	100%	5.5	3.27	1.63	120	76	Tr.	None	11.6	7,200	Neg.
S.[illegible]	76	M	W	0.6 5	96%	5.35	2.90	2.45	130	72	1/	None	10.6	9,000	Neg.
F.R.	68	M	W	0.45	100%	5.8	2.90	2.90	100	68	1/	None	13.2	9,200	Neg.
J.M.	84	M	W	0.40	100%	6.2	2.6	3.6	137	46	1/	None	9.4	9,400	Neg.
J.K.	77	M	W	0.75	100%	6.85	3.21	3.64	16 0	96	Neg.	one	11.4	8,800	Rbc.s
C.S.	72	M	W	0.50	90%	6.65	3.80	2.85	166	70	1/	None	14.4	9,800	Neg.
D.U.	88	F	W	0.50	93%	6.40	3.30	3.10	180	115	Neg.	None	13.6	4,500	Neg.
C.G.	83	M	W	0.40	93%	5.85	3.70	2.15	118	59	1/	None	12.8	9,900	Neg.

according to the degree of turbidity present in 48 hours; B.S.P ret. bromsulfalien retention and is reported as the percentage of injected dye retained in the serum 45 minutes after injection of 5 mg. per kilogram of body weight; Hb = hemoglobin in grams per 100 cc. of blood; W.B.C. = leukocytes per cubic mm. of blood; and U.A. = urinalysis.

In this series, only one patient had a serum bilirubin over one mg. per cent. While only two patients had a T.S.P. of less than 5.5 gm. per cent, it is of note that 13 or 63% of the group had an albumin:globulin ratio of less than 1.5:1. This finding has been quite consistent in our observation of a large number of geriatric patients, Serum cholesterol was less than 150 mg. per cent in 53 per cent of the individuals. More significant, however, is the fact that cholesterol esters were less than 60 per cent in 11 cases or 69 per cent. Cephalin-cholesterol flocculation was 2/ in only one instance and there were only three cases of bromsulfalien retention. The greatest of these was 7 per cent retention in 45 minutes. While some of these results are abnormal for young adults, they apparently fall within the range of normal for geriatric patients according to the criteria of Ratzky(42). Ninety-five per cent of the group had an abnormality of one or more of the liver function tests when measured by ordinary standards.

In examining the histological pattern of the tissue sections, architecture, portal tracts, and parenchymal cells were all carefully studied for alterations. The degree of anisonucleosis, bile pigmentation and iron pigmentation, and the numbers of vacuolated nuclei

Table 11. Histological Findings on Formalin-fixed, H&E Stained Tissues

Patient	Architecture	Portal Tracts	Liver Cells	Anisonucleosis	Nuclear Vacuoles	Bile Pig.	Iron Pig.
L.N.	ormal	Central sinus -oids prominent	Normal	1-4x	None	1/-2/ Centrilob	None
C.C.	Normal	Normal	Normal	1-3x	None	Tr.	1/ Midzon.
C.A.	Normal	ormal	Ocassional fatty vac.	1-3x	None	Tr.	one
E.D.	Normal	Normal	Granular	1-3x	None	1/- Centrilob	None
E.J.	ormal	Normal	Occasional binuc. cell	1-4x	None	Tr.	None
C.L.	Lobular pat. distorted	Marked hyaline thickening of portal vessels	Normal	1-4x	Occasional	1/ Centrilob	Minimal
T.P.	Normal	ormal	Normal	1-5x	None	None	None
F.S.	Normal	Portal vessels thickened	Occasional fatty vac.	1-5x	Large no.	1/ Centrilob.	None None
A.L.	Normal	Normal	Normal	1-4x	Mod. no.	Tr.	None
H.H,	Sinusoids in- -filtrated by mononuc. cells	Portal v essels thickened	Occasional binuc. cell	1-5x	Occasional	Tr.	Tr. Midzonal

Table 11. (continued)

Patient	Architecture	Portal Tracts	Liver Cells	Anisonucleosis	Nuclear Vacuoles	Bile Pig.	Iron Pig.
C.T.	Normal	Normal	Occasional fatty vac.	1-5x	Occasional	Tr. Centrilob.	None
J.G.	Normal	Normal	Normal	1-3x	Occasional	2/ Centrilob.	None
S.M.	Normal	Portal vessels thickened	Normal	1-4x	Normal	None	None
F.R.	Sinusoids dilated, no congestion	Normal	Normal	1-5x	Numerous vac.	1/ Centrilob.	Tr. Tr.

Table III. Histological Findings on Tissue Fixed in Champy's Fluid

Patient	Architecture	Portal Tracts	Hepatic Cells	Karyonucleosis	Nuclear Vac.	Iron Pig.	Bile Pig.
F.B.	Normal	Normal	Normal	1-3x	one	None	None
J.K.	Normal	Normal	Normal	1-3x	None	None	one
C.S.	Normal	Occasional fatty vac.	Normal	1-3x	None	None	one
T.U.	Section too small to determine architecture		Normal	1-4x	None	one	None
C.G.	Normal	Normal	Normal	1-3x	None	None	None

were especially noted. The findings in the formalin-fixed, hematoxylin and eosin stained, tissues are shown in Table II. It can be seen immediately upon looking at this table that anisonucleosis was the most prominent feature of this particular group of tissues. The degree of nuclear variability is reported as the number of times the largest nucleus exceeds the smallest nucleus in area and any one highpower, microscopic field. The next most common alteration is vacuolization of the nuclei which according to Chipps and Duff[10] represents glycogen. This occurred to a greater or lesser extent in 6 of the 14 sections. This is a percentage of 43 as compared with an overall incidence of 39 per cent in a series of 315 consecutive autopsies reported by Chipps and Duff[10]. Another pertinent finding was deposition of iron pigment varying from a trace to one-plus when compared with a section of liver from a case of hemochromatosis used as a four-plus standard. Iron pigments were found in 4 biopsies for a percentage of 28.6. Unfortunately, no standard for iron pigmentation in the liver has been established[19]. There was one case of mild, inactive portal cirrhosis(C,L.).

The histological findings on the last five biopsies are presented in Table III. They are essentially the same as the previous 14 tissues. No increase in fatty deposits was encountered in this group and no vacuolated nuclei were seen. Anisonucleosis similar to that previously found was also evident in this group. There was no iron pigmentation demonstrated.

Discussion:

Himsworth[23] stated that "a consideration of the immediate

effects of injury upon composite organs reveals that, in general, those cells which are most highly differentiated and which endow the organ with its characteristic function are also those which are susceptible to damage." The liver contains three highly differentiated types of tissue - parenchyma, biliary ducts, and cells of Kupfer. Of these, the parenchymal cells are usually injured first. It is in the parenchymal cells then, that a search for histopathology should begin.

The usual types of cell damage seen are fatty infiltration and cellular necrosis. These changes occur in the presence of noxious factors, dietary deficiencies, severe vascular changes, metabolic upsets and biliary disorders[23]. In the healthy, elderly individual, none of these overt factors are in operation so one must look for more subtle changes which might result from lesser vascular damage or metabolic disorders or from degenerative changes of age if he is to find any histological change at all.

This study has revealed a few such slight changes of hepatic parenchyma, all involving only the liver cell. These changes include nuclear vacuolization, anisonucleosis and iron deposits within the cell.

Chipps and Duff[10] have stated that there is no relationship between age and intranuclear vacuolization of hepatic cells. We found an incidence of 31.6 per cent of such vacuolization in our series while they found 39 per cent of livers in 315 consecutive autopsies showed this type of change. Assuming their premise, that such intranuclear vacuoles always represent glycogen, it

would seem logical to expect a larger number of persons in the geriatric age group to exhibit this type of change. Crellin[14] has shown that geriatric individuals have impaired glucose tolerance and it is usually such patients with poor glucose metabolism in whom intranuclear glycogen develops.

Because no standard of nuclear size in liver cells has been established, less can be said about the significance of anisonucleosis. Wide variability in size of hepatic cell nuclei has been reported by Korenchevsky et.al.[29] in senile rats, and by Andrew et. al.[2] in senile rats and elderly humans. In our series, the largest cell nuclei exceeded the smallest nuclei, in area, by at least three times and often by four to five times. This finding was consistent in every specimen.

Iron deposits within cells were observed in four of our cases. It varied in amount from a trace to a moderate quantity. Here again, since the normal for iron pigmentation of liver has never been established, no importance can be attached to this finding. Gillman and Gillman[19] have found that iron within parenchyma of liver varies inversely with number of mitochondria. If iron deposits are increased in old age, then one would expect a decrease in mitochondria - an idea diametrically opposed to the statements of Andrew[1]. Gillman and Gillman believe that the etiology of iron deposits is nutritional deficiency.

Few parenchymal vacuoles appeared in the sections of our series and in the Champy-fluid fixed group, no vacuoles were seen which did not stain black. This was interpreted to mean that all

the vacuoles were fat. Fat was not present in abnormal amounts in any of the tissues studied.

Due to technical difficulties, Andrew's[1] finding of increased mitochondria and nucleoli could not be confirmed. We were unable to corroborate the presence of decreased numbers of bi-nucleated cells reported by Korenchevsky's group[29]. This does not mean to repute those findings, however.

No correlation between structural changes and functional alterations could be determined. Norcross[38], in attempting to make such a correlation, found that no consistent changes occurred in tests of liver function with cytological abnormalities alone.

Conclusions:

Evidence of definite abnormality of the histological pattern in liver parenchyma of persons over 65 is inconclusive. Further evaluation of anisonucleosis, nuclear vacuolization and iron pigmentation will require investigation of these histological patterns in liver of young adults.

While laboratory tests cannot be related to the structural changes, seen, they still show signs of impairment of function in the group studied, especially in partition of cholesterol and esters and in metabolism of proteins.

Summary:

A study of liver function and histology of healthy individuals over the age of 65 years was undertaken to determine if any degenerative changes occur in the liver along with the degenerative

changes in other organ systems. Needle biopsies of liver were done on 19 patients, 16 via the intercostal approach and three via the sub-costal approach. No serious complications were encountered during or after the biopsy and a segment of tissue adequate for microscopic examination was obtained in each case.

Laboratory function tests revealed an albumin:globulin ratio of less than 1.5:1 in 63 per cent of the group, cholesterol of less than 150 mg. per cent in 53 per cent of the group and cholesterol esters of less than 60 per cent in 69 per cent of the group. No correlation between laboratory tests and histological changes was found.

Study of tissue sections revealed three main alterations: anisonucleosis in 100 per cent , nuclear vacuolization in 31.6 per cent and iron deposits in cytoplasm in 21 per cent. The significance of these findings cannot be determined at the present time because of the lack of knowledge of the extent of these same changes in normal livers.

Results of this investigation are inconclusive at this time but further study of the problem, both in the realm of normal histology and of pathology, with particular reference to histochemical techniques, should elucidate its various manifestations.

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