EXPERIMENTAL[1]

2-Ethyl-3-methyl-5-morpholinomethyl-4-keto-4,5,6,7-tetrahydrothionaphthene Hydrochloride (II)—A mixture of 3.0 g. (0.0155 mole) of 2-ethyl-3-methyl-4-keto-4,5,6,7-tetrahydrothionaphthene (4), 0.47 g. (0.0155 mole) of paraformaldehyde, 1.92 g. (0.0155 mole) of morpholine hydrochloride, 1 drop of concentrated hydrochloric acid, and 20 ml. of ethanol was refluxed for 4 hr. A solid precipitated upon cooling and was removed by filtration. After recrystallizing three times from ethanol, 3.1 g. (61%) of product, m.p. 223–223.5° (dec.) remained; $\lambda_{max.}$ (KBr) 2,450 and 1, 660 cm.$^{-1}$; NMR (D_2O), 1.01–1.40 (3H, t), 2.29–2.42 (3H, s), 2.55–2.98 (2H, m), 3.05–3.38 (4H, m), 3.40–3.65 (6H, m), 4.00–4.30 (5H, m).

Anal.—Calcd. for $C_{16}H_{24}ClNO_2S$: C, 58.25; H, 7.33; Cl, 10.78; N, 4.25; S, 9.72. Found: C, 57.96; H, 7.18; Cl, 11.05; N, 4.40; S, 9.78.

[1] All melting points were taken on a Thomas-Hoover melting point apparatus and are corrected. IR spectra were taken on a Perkin-Elmer 137B IR spectrophotometer using potassium bromide pellets. The NMR spectra were determined on a Varian A-60A spectrophotometer using tetramethylsilane as an internal standard. Chemical shifts are recorded as δ values (s = singlet, m = unresolvable multiplet, t = triplet).

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* Present address: Geigy Chemical Corp., McIntosh, Ala.

Quantitative Determination of Deoxyribonucleic Acid (DNA) in Normal and Abnormal Human Liver

K. G. BHANSALI*, J. A. CLIFTON, and J. L. LACH

Abstract □ Normal and abnormal human livers from autopsy and biopsy specimens were analyzed for DNA content. It was found that the DNA content of young normal livers as well as cirrhotic livers was elevated above that obtained for normal adult livers.

Keyphrases □ Deoxyribonucleic acid (DNA) determination—normal, abnormal human livers □ Age, cirrhosis—liver DNA contents

The normal value of DNA content of human liver as well as animal liver is reported to be relatively constant (1). However, Leslie (2) has shown an elevation in DNA content in young livers of rats and other animals. Therefore, it was of interest to perform the quantitative determination of DNA from normal and abnormal human livers.

The determination of DNA can be based on the analysis of one of the three components; namely, the phosphoric acid, the purine or pyrimidine bases, or the sugar. The reactivity of the functional carbonyl group of the pentose has been utilized as the method of choice (3). Moreover, in cytochemistry the carbonyl group of 2-deoxyribose of DNA has been utilized in the feulgen staining reaction which is widely used in conjunction with microphotometric instrumentation to estimate the amount of DNA in nuclei (4).

EXPERIMENTAL

Human liver specimens were obtained from autopsy as well as biopsy from the State University of Iowa General Hospital and Veterans Administration Hospital of Iowa City, Iowa.

Liver specimens were stored in the freezer as soon as they were removed from the cadavers and from the patients, and the specimens preserved in cold showed reproducible results. In general procedure, DNA of the whole tissue is extracted with trichloroacetic acid (3) but in this study to isolate mitochondria, liver tissue was first homogenized in 0.88 *M* sucrose solution with a Teflon tissue homogenizer (5). The nuclei of the liver cells were separated by centrifuging the liver homogenate at 600 × *g* for 20 min. at 4°. The nuclear pellet was hydrolyzed in 20 ml. 5% trichloroacetic acid on a boiling-water bath for 30 min. using a sealed ampul bulb as a condensor. The hydrolyzed nucleic acid solution was cooled and filtered into a 25-ml. volumetric flask. It was diluted to volume with 5% trichloroacetic acid. A 2-ml. aliquot of this solution containing about 150 mcg. DNA was assayed by the method of Webb and Levy (3), which is a colorimetric procedure based on the reaction of 2-deoxypentose moiety of DNA with *p*-nitrophenylhydrazine. The concentration of DNA was determined by using as a reference standard calf thymus DNA (Mann Research Co.). The results of the analysis are shown in Table I and Table II. Statistical evaluation was by the Student *t* test.

RESULTS AND DISCUSSION

The data for 47 liver specimens are shown in Tables I and II. Table I shows the data from the analysis of young normal livers and adult normal livers. The data from the cirrhotic abnormal livers and noncirrhotic abnormal livers, regardless of age factor are shown in Table II. In reporting DNA content neither the sex (male or female) nor liver specimen (autopsy or biopsy) has been taken into consideration. Since any normal liver specimens between 20–30 years of age were not available, it was decided to use this period of age as a dividing line between young and adult normal human liver. It is very difficult to state that a given specimen of biological material is normal. However, by common agreement, the histological appearance of biological material is used to establish normal and abnormal categories. In this study, the patients were divided into these two major groups on the basis of the histological appearance of the liver specimens. Those cases in which liver cells were either

Table I—Normal Human Livers

N	Subject	Age	Sex[a]	DNA, mcg./g. Liver	Liver Specimen[b]
		Young Human Livers			
1	R.D.	28 days	M	1966	a
2	S.Mc.	2.5 years	F	1829	a
3	D.C.	4 years	F	2170	a
4	S.V.	5.5 years	M	3355	a
5	L.J.	8 years	F	3098	a
6	J.W.	15 years	F	3064	a
7	L.H.	18 years	M	3967	b
8	C.H.	19 years	M	3048	a
				Mean, 2700	
				SD: ±565	
		Adult Human Livers			
1	T.B.	31 years	M	1560	a
2	A.T.	41 years	M	1468	a
3	M.G.	46 years	F	1480	a
4	M.B.	47 years	M	2318	a
5	M.K.	47 years	F	2019	a
6	C.J.	48 years	M	1745	a
7	O.M.	49 years	M	2326	a
8	R.D.	49 years	M	1630	a
9	W.P.	57 years	M	1924	a
10	M.S.	58 years	M	1869	a
11	S.R.	59 years	F	2154	b
12	B.G.	59 years	M	2238	a
13	R.S.	60 years	M	1640	a
14	C.W.	63 years	M	1320	a
15	S.M.	64 years	M	2026	a
16	C.B.	65 years	M	1540	a
17	E.W.	66 years	M	2448	a
18	A.Mc.	68 years	F	2543	a
19	L.B.	69 years	M	1780	b
20	W.T.	70 years	M	2512	a
21	M.S.	75 years	F	1962	b
22	C.T.	76 years	M	1887	a
23	C.D.	78 years	M	1975	a
24	E.Mc.	84 years	M	1792	b
25	M.T.	86 years	M	2328	a
26	P.E.	94 years	M	1529	a
				Mean, 1923	
				SD, ±332	
				$p < 0.05$	

[a] M = Male, F = Female. [b] a = autopsy, b = biopsy.

Table II—Abnormal Human Livers

N	Subject	Age	Sex[a]	mcg./g. Liver	Liver Specimen[b]
		Cirrhotic Human Livers			
1	D.B.	9 years	F	2870	b
2	D.V.	14 years	M	2900	a
3	R.B.	26 years	F	3400	b
4	E.O.	54 years	M	5135	b
5	E.H.	62 years	F	2722	b
				Mean, 3405	
				SD, ±895	
		Noncirrhotic Abnormal Human Livers			
1	C.W.	32 years	M	1578	a
2	F.Z.	44 years	M	1860	a
3	J.D.	63 years	M	2193	a
4	L.C.	64 years	M	1384	a
5	G.W.	65 years	M	2230	a
6	P.W.	77 years	M	2095	a
7[a]	C.B.	62 years	F	1282	a
8[a]	P.P.	31 years	M	1275	a
				Mean, 1890	
				SS, ±317	
				$p < 0.05$	

[a] Excessive amount of bile cysts were observed in liver specimen and are excluded in calculating the mean value.

not damaged or not damaged severely were called "normal." Thus, cases with mild congestion of the liver from red blood cells, white blood cells infiltration, and mild fatty degeneration were included. Those cases with cirrhosis and severe liver cell damage from any cause were classified "abnormal."

The normal value of DNA content of liver is reported to be 2,100 mcg./g. (1). The DNA content of young normal liver is elevated by 40% up to 19 years of age in humans when compared to adult livers. Since the DNA content per cell remains constant, the increased DNA content might be a direct function of the larger number of cells per gram of liver in normal liver. This may reflect the growth period in which cellular activity is greater. Even though the experimental procedure was carefully rechecked and no error detected, it seems prudent to hold in abeyance any conclusions regarding this finding until additional samples in this age group can be analyzed. Hepatic DNA content remains relatively constant after age 31. However, a wide variation of DNA content from normal value has been observed. This might be due to subtle biochemical changes which might have occurred in some of these specimens as a result of primary diseases from which the patient died. The DNA content of the noncirrhotic abnormal livers, falls within normal values. However, cirrhotic livers have a large number of white blood cells (WBC) and these of course have DNA-containing nuclei (6). Regeneration of liver cells may be progressing at a rapid pace in the cirrhotic livers possibly as a defense mechanism. Moreover, the number of hepatic cells might also have been increased. The relative contribution of these two fractions to the total liver DNA is unknown, *i.e.*, WBC *versus* hepatic cells.

This study shows that the DNA content of young normal liver and adult cirrhotic livers is elevated over that of the adult liver. In the case of young normal liver, this may be due to the increased metabolic demands of growth, while the increased DNA content of cirrhotic livers may be due to regeneration of liver cells or to increased WBC infiltration.

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* Present address: School of Pharmacy, Texas Southern University, Houston, TX 77004