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Hepatocyte Functional State

Quantitative Evaluation with I^{131} Rose Bengal in Lepromatous Leprosy Patients[1, 2]

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Relatively little interest is evident in the study of visceral manifestations of leprosy, in spite of their clinical importance ([1]). The liver might be expected to be the organ most frequently affected; it is responsible for plasma protein alterations, e.g., effective albumin reduction and globulin increase.

Histologic reports show that hepatic lesions in lepromatous leprosy are characterized by the transformation of Kupffer cells and Kiernan's space histiocytes into Virchow cells. There is miliary granuloma dissemination, and degenerative and proliferative alterations occur, such as amyloidosis, fibrosis and cirrhosis. These lesions seem to occur simultaneously with cutaneous lesions ([3]).

Hepatic biopsy puncture is little used, although it has the advantage of identifying the location of the invasive processes of disease. Biopsy puncture shows not only the disease's last evolutive stage but also its eventual involvement in any stage of the disease. It shows that the liver can be the seat of histio-lympho-plasmocyte granulomatous change, and Kupffer cell hyperplasia, even when it does not show any signs of involvement clinically ([4]).

The restriction in use of liver biopsy puncture in any stage of lepromatous leprosy is due to the patient's resistance. For that reason, the degree of disturbance in the numerous liver functions is being studied through other tests.

In spite of many difficulties in biopsy puncture, it is necessary to perform a comparative, histologic and functional study of the liver with the clinical data of the organ's involvement.

In order to attain this objective a radioactive tracer technic was used in the present investigation. It showed the possibility of determining the hepatocyte functional state in any evolutive stage of the disease and, after repeated tests, of constructing a curve representing an effective functional organ profile.

The purpose of this paper is to show the value of a safe and technically easy test, which may be repeated as many times as necessary, and, once the value of the method has been proved, to carry out a comparative study making use of this hepatic function test, with I^{131} labeled rose bengal, simultaneously with histologic findings obtained with the aid of hepatic biopsy puncture.

Determination of the functional efficiency of the liver by any laboratory method is

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difficult. One reason is that in a hepatic disturbance the multiple liver functions may be affected differently. In some cases only certain functions suffer alterations, and some functions are more acutely affected than others.

A set of tests, including flocculation or thymol clouding, cholesterol concentration, colloidal gold, zinc sulfate turbidity and many others, are called hepatic function tests. However, only a specific state of blood serum protein balance is demonstrated by these tests. Globulin fractions interfere quantitatively, and suffer a qualitative alteration, sometimes because of their lipid components. The reticuloendothelial system is concerned in the liver and other organs, without organ specificity (2).

The hepatic parenchyma presents a complex group of biologic activities, which do not permit an easy and simple evaluation as a whole with the usual laboratory methods.

Moreover, the liver might be the seat of extensive morphologic change, notwithstanding good functional capacity. On the other hand the functional capacity might be reduced in the absence of evident morphologic disturbance.

With these facts in mind, a good knowledge, of considerable clinical value, of the liver's functional capacity may be obtained by uptake and excretion tests (8) with a radioiodine-labeled rose bengal. In 1955 Taplin *et al.* (7) obtained radioactive rose bengal by replacing stable iodine atoms by I^{131} in the molecule of tetra-iodo-tetrachloro-fluorescein potassium.

Since I^{131} atoms emit gamma rays that may be measured externally, the continuous uptake of I^{131} rose bengal and excretion measurement may be carried out without the necessity of repeated venipunctures for blood sampling. By the use of I^{131} rose bengal data may be obtained on liver polygonal cells, on liver blood volume, and on bile duct permeability.

The polygonal liver cells specifically absorb the I^{131} rose bengal circulating in the blood; the intensity of this absorption process depends on the functional state, on the hepatic blood flow, and on the permeability of the bile ducts. Any alterations of these systems act decisively on dye uptake and excretion by the liver. The persistency of one of these alterations may cause secondary hepatocyte functional lesions.

In acute infectious hepatitis the hepatic cells are injured, but the blood circulation in the organ and the bile excretion may remain intact. Fibrotic processes, however, give place to reduction in intrahepatic circulation, which causes secondary parenchymatous lesions. The I^{131} rose bengal test may help in finding the basic lesion and give some idea of the extent of the lesion and resultant dysfunction.

The liver cell functional state is measured by the increase of radioactivity in the liver and by the reduction in blood radioactivity in relation to time. The increase rhythm, plus the time interval before evidence of radioactivity in the intestine, furnishes a measure of bile duct permeability.

The I^{131} rose bengal test presents the following advantages over other liver function evaluation methods: (1) The function of the polygonal cells is measured directly. (2) The dye uptake and the time necessary for its elimination by the liver are registered graphically. (3) The dye is not toxic, even in cases with liver cell lesions or biliary duct obstruction. (4) It provides data on liver circulation and on the state of the bile ducts. (5) It is more sensitive than any nonradioactive dye test.

The technic of the test is extremely simple. A scintillation counter is placed on the antero-lateral section of the patient's chest over the upper margin of the liver, as determined by percussion. The detector must have a wide solid angle and a reduced collimation. It must be placed in such a position that radiations proceeding from the gallbladder are not registered.

The dose of radioactive dye is 0.3 μC/kg. injected intravenously in a patient whose thyroid gland has been blocked previously by Lugol solution.

The patient must remain in a reclining position for the duration of the test, avoiding changes in the position of the detector on the chest wall. Movements of the extremities are permissible. The patient may

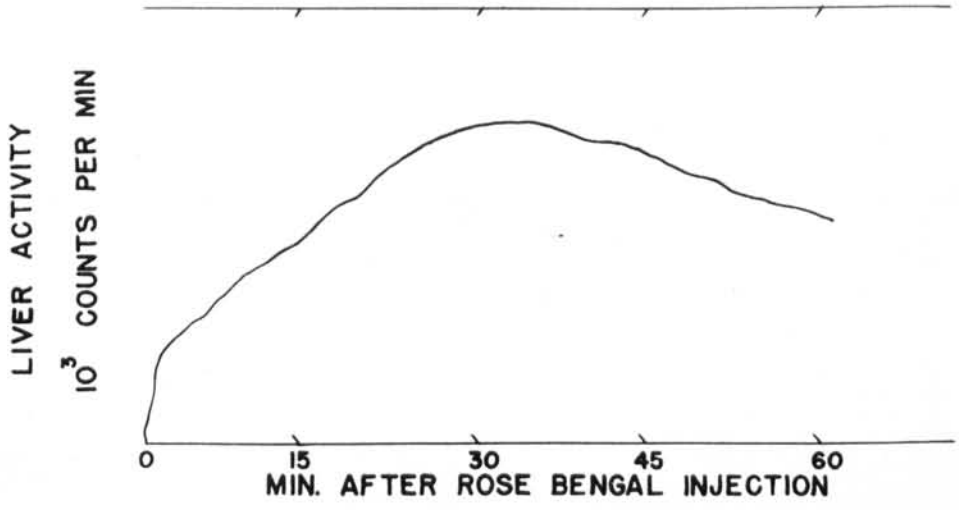

FIG. 1. A normal I^{131} rose bengal uptake excretion curve. Each record was continued until the peak activity was passed and the counting rate was well on the down slope.

be fasting or not. The tracing is registered during the 60-90 minutes until stabilization occurs in the excretion curve. Figure 1 illustrates a "hepatogram," (the designation used by Taplin). It is traced from the data obtained after subtraction of background radioactivity.

A method for quantitative analysis of the pattern of uptake and excretion was described by Loewenstein ([6]).

The curve is traced with three constants: (1) Uptake half time or interval of time in which there is a liver uptake of half the amount of dye circulating in the blood. (2) Excretion half time or interval of time in which a half part of the dye taken up is excreted by the bile ducts. (3) Percentage relationship between the liver blood volume and the total blood volume.

These constants may be obtained through a semilogarithmic plot of the uptake-excretion curve (Fig. 2).

A straight line is drawn from the ordinate through the descending line: this is the excretion line (E). The uptake line (U) is obtained by plotting differences between E and the rising portion of the curve, and drawing another straight line from the ordinate through these points. Half Uo is the uptake half time. Half Eo is the excretion half time. Eo represents the amount of radioactive material injected before any uptake, and Uo represents the amount of radioactive material in the liver before any excretion. The liver blood volume is equal to the difference between Eo and Uo.

Since the liver blood volume is calculated in relation to the total blood volume, to obtain the percentage it is necessary to divide the total blood volume by Eo and to multiply the result by 100. Clinical studies have shown that these three values are highly significant for the differential diagnosis of hepato-biliary lesions.

A hepato-cellular lesion is revealed by the uptake-excretion delay. Obstructive processes cause excretion delays. Circulatory deficits lead to reductions in uptake rate and liver blood volume.

Loewenstein ([6]) gives the following values as normal:

Uptake half time	8 to 10 minutes
Excretion half time	40 to 100 minutes
Liver blood volume	± 18% of total blood volume

Through the determination of these values an attempt is made to study the functional state of the liver polygonal cells in cases of lepromatous leprosy, in which the liver cells are believed to be unaffected or rarely affected secondarily by the lepro-

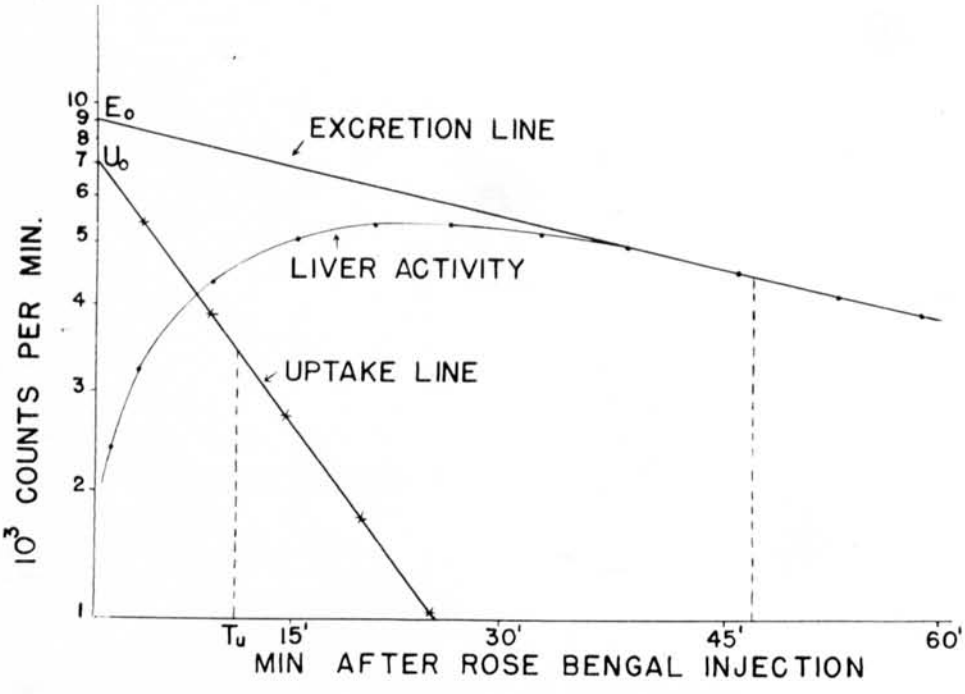

FIG. 2. Graphic analysis of the curve in Figure 1. After background activity is subtracted the curve is transposed to semilogarithmic paper and analyzed.

matous invasion process, which develops in the portal spaces. It is generally accepted that a functional deficit is observed only when structural alterations and degenerative phenomena in all the liver cells are verified, and that focal lesions do not cause evident alterations in liver function.

In lepromatous leprosy, however, some authors have observed a specific histo-functional correlation, which is not recognized in most other liver diseases ([5]).

We shall try to establish this correlation in a second phase of this study, when biopsy puncture and histologic studies will be carried out in addition to the I^{131} rose bengal test.

CASE REPORTS

The functional state of the liver polygonal cells was studied in a group of 29 cases of lepromatous leprosy by quantitative measurement of the I^{131} rose bengal test. Table 1 presents clinical data on these cases.

The 29 patients were subdivided into two groups: (1) patients presenting evidence of functional lesions of the liver cells, as determined by this test, and (2) patients not presenting evidence of functional lesions.

Other subdivisions were made, according to (1) the presence or absence of excretory dynamic disorders, (2) the size of the liver, (3) the reduction or increase in liver blood volume, (4) the evolutive phase of the disease, and (5) the response to treatment. These subdivisions are illustrated in Tables 2 and 3.

On the basis of these observations it was determined that 23 cases were affected by liver cell lesions, viz., 6 with discrete lesions, up to 30 per cent above the normal; 17 with evident lesions, above 30 per cent of the normal; 16 with excretory dynamic disturbances; 3 with discrete disturbances between 101 and 130 minutes; 13 with evident disturbances above 131 minutes; 29 with variations in the liver blood volume (LBV); 16 with liver blood volume increase; and 13 with liver blood volume reduction.

TABLE 1. *Clinical data on 29 cases.*

Initials of patients	Age in years	Classification on registration	Present status	Bacilloscopy				Duration of disease L type	Reactional history	Treatment	
				During treatment		Present				Regularity of	Response to
				N.M.	C.L.	N.M.	C.L.				
J.P.S.	43	Lm	R	Neg.	++	Neg.	Neg.	3 d	2	b	II
A.B.	44	La	A	Neg.	+++	Neg.	+	2 d	2	a	III
C.R. (II)	41	La	A	+	++	Neg.	RB	2 d	3	b	III
N.L.S.	44	Lm	I	+	+	Neg.	Neg.	2 d	2	a	I
J.M.L.	42	La	I	+++	+++	Neg.	Neg.	3 d	1	a	I
J.A. (II)	33	La	I	+	++++	Neg.	Neg.	2 d	2	a	I
D.P. (II)	34	La	I	++	+++	Neg.	Neg.	2 d	2	a	I
H.M.	37	La	A	++++	+++	Neg.	+	1 d	2	a	II
G.S.	36	La	R	RB	++	Neg.	Neg.	1 d	1	a	I
I.B.	36	La	I	+	+++	Neg.	Neg.	1 d	1	a	I
B.R.F.	38	La	A	Neg.	++	Neg.	+++	2 d	2	b	II/III
L.J.P.	49	La	A	RB	+++	Neg.	+++	3 d	2	b	III
W.F.S.	31	La	A	++++	++	+	+++	2 d	2	b	III
M.P.M.	36	La	A	Neg.	+++	Neg.	+	3 d	2	a	III
A.S.F.	53	La	A	+++	+++	Neg.	+++	3 d	1	b	III
P.M.	53	La	A	+	+	+	+	3 d	2	b	III
J.F.M.	38	La	A	++++	++++	Neg.	+++	3 d	3	b	IV
J.V.	40	La	A	+	++++	Neg.	++	3 d	2	a	III
S.P.	47	La	A	+	++++	RB	+++	3 d	2	b	III
A.M.	58	La	A	Neg.	+	Neg.	+	1 d	2	a	IV
T.R.	40	La	R	++	++	Neg.	Neg.	3 d	3	b	II
D.O.	59	La	I	+	+++	Neg.	Neg.	3 d	2	a	I
J.F.C.	39	La	I	++	+++	Neg.	Neg.	2 d	2	a	I
J.C.	43	La	I	++	+++	Neg.	Neg.	2 d	1	a	I

TABLE 1. *Clinical data on 29 cases. (Cont'd)*

Initials of patients	Age in years	Classification on registration	Present status	Bacilloscopy				Duration of disease L type	Reactional history	Treatment	
				During treatment		Present				Regularity of	Response to
				N.M.	C.L.	N.M.	C.L.				
P.V.	67	La	I	+++	++	Neg.	Neg.	3 d	3	a	II
A.B.	47	Li	A	+	++	Neg.	++	2 d	3	b	III
M.T.L.	41	La	A	+	++	Neg.	+	3 d	1	b	III
B.M.	35	La	A	+	+++	Neg.	+	3 d	2	a	III
J.P.C.	60	La	A	Neg.	+++	Neg.	+	1 g	2	a	II

La = Advanced lepromatous
Lm = Moderate lepromatous
Li = Incipient lepromatous
A = Activity
R = Regression
RB = Rare bacilli
I = Inactivity
1 d = Disease duration up to 5 years
2 d = Disease duration up to 10 years
3 d = Disease duration up to 15 years
1 = L.R. (lepromatous reaction) absence
2 = Little L.R. outbreak
3 = Numerous L.R. outbreaks
a = Regular antileprotic treatment
b = Irregular antileprotic treatment

I = Clinical and bacilloscopic regression in satisfactory time and without reincidence (good response)
II = Delayed clinical and bacilloscopic regression with few and short duration reincidences (regular response)
III = Frequent and long duration reincidences, no regression (bad response)
IV = Clinical and bacilloscopic activity due to the recency of treatment (new patients)
N.M. = Nasal mucus
C.L. = Cutaneous lesion

TABLE 2. *Functional lesions of liver cells (23 cases).*

With excretory dynamic disturbance				Without excretory dynamic disturbance			
12				11			
Hepatomegalia				Hepatomegalia			
With		Without		With		Without	
5		7		9		2	
Liver blood volume				Liver blood volume			
Reduction	Increase	Reduction	Increase	Reduction	Increase	Reduction	Increase
1	4	3	4	4	5	0	2
Evolutive phase				Evolutive phase			
La: 1	La: 4	La: 3	La: 4	La: 3 Li: 1	La: 5	0	La: 2
Response to treatment				Response to treatment			
II: 1	III: 2 II/III: 1 IV: 1	III: 2 I: 1	III: 2 I: 1	III: 2 I: 1 II: 1	III: 2 I: 2 II: 1	0	III: 1 I: 1

La = Advanced lepromatous
Li = Incipient
I = Good response to treatment

II = Regular response to treatment
III = Bad response to treatment
IV = New patients

TABLE 3. *No functional lesions of liver cells (6 cases).*

With excretory dynamic disturbance				Without excretory dynamic disturbance			
4				2			
Hepatomegalia				Hepatomegalia			
With		Without		With		Without	
3		1		0		2	
Liver blood volume				Liver blood volume			
Reduction	Increase	Reduction	Increase	Reduction	Increase	Reduction	Increase
2	1	1	0	0	0	2	0
Evolutive phase				Evolutive phase			
La. 2	Lm: 1	Lm: 1	0	0	0	La: 2	0
Response to treatment				Response to treatment			
I: 1 III: 1	II: 1	I: 1	0	0	0	II: 1 III: 1	0

La = Advanced lepromatous
Lm = Moderate lepromatous
I = Good response to treatment
II = Regular response to treatment
III = Bad response to treatment
IV = New patients

Considering those cases only that showed evidence of lesions or evident disturbances in cellular function or excretory dynamics, we made the following determinations: 58.6 per cent of the total cases showed functional deficit in polygonal cells; 45 per cent of the total cases showed a functional excretory deficit in the biliary ducts; 51.7 per cent of the total cases presented a liver blood volume increase; 44.8 per cent of the total cases presented a liver blood volume reduction.

The results set forth in Tables 2 and 3 indicate the absence of a direct relation between cases presenting functional derangement of the liver cells, excretory dynamic disturbances, hepatomegaly and a reduction or increase of the liver blood volume. There was also no evident direct relation between the disease's evolutive phase and its response to treatment with the parameters above enumerated.

DISCUSSION

The radioiodine labeled rose bengal test showed the presence of functional lesions of the liver polygonal cells in a large proportion of patients with lepromatous leprosy.

No correlation was noted between the hepatocellular functional deficit and the excretory dynamic disturbances, nor with liver blood increase or reduction, nor with the clinical stage of the disease and the response to treatment.

Hemodynamic factors may change the functional stage of polygonal cells and cannot be differentiated from those produced by cellular lesions unless the plasmatic flow rate is determined. Thus, so that the evaluation of the hepatocyte functional stage may be effective, it is also necessary to determine the minimum liver blood flow.

The conditions for the increase or reduction of the liver blood volume do not present any parallel with functional condition of the liver cells.

The use of I^{131} represents progress for hepatocyte functional study, because through the chromopexic and chromoexcretory hepatocyte capacity, it is possible to determine the hepato-cellular functional state.

It is necessary to perform the biopsy puncture and the histologic study concomitantly with the I^{131} rose bengal test in order to determine the existence of histo-functional correlation in patients with lepromatous leprosy.

SUMMARY

The authors studied the hepatocyte functional state in 29 lepromatous leprosy cases, through the radioiodine-labeled rose bengal test as described by Loewenstein. The procedure for quantitative analysis of the uptake-excretion curve is determined by three constants:

1. Uptake half time or interval of time in which a half part of the dye circulating in the blood is absorbed by the liver.
2. Excretion half time or interval of time in which a half part of the dye absorbed by the liver is excreted by the biliary ducts.
3. Liver blood volume in percentage relation with the total blood volume.

The 29 cases were subdivided into two groups: (1) those showing functional lesions of the liver cells, and (2) those without such lesions. Other subdivisions were made according to the presence or absence of excretory dynamic disturbances, to hepatomegaly, to reduction or increase of the liver blood volume, to the evolutive phase of the disease and to response to treatment.

Of those cases presenting evidence of lesions or disturbances of the cellular function or excretory dynamics, 58.6 per cent showed functional deficit of the polygonal cells; 45 per cent showed excretory functional deficit of the biliary ducts; 44.8 per cent showed reduction in the liver blood volume, and 51.7 per cent showed increase in the liver blood volume.

The conditions for reduction or increase of liver blood volume did not parallel the functional conditions of the liver cells. Likewise, the presence of hepatomegaly, the disease's evolutive phase, and the response to treatment, did not correspond to the hepatocyte functional state.

As hemodynamic factors and the functional state of the liver cells may change independently, it is necessary to perform the test simultaneously with the measurement of minimum liver blood flow in order to achieve an effective result.

RESUMEN

Los autores estudiaron el estado funcional de las células hepáticas en 29 casos de lepra lepromatosa, mediante la prueba del rosa de bengala marcado con radioyodo en la forma descrita por Loewenstein. El procedimiento para el análisis cuantitativo de la curva de absorción-eliminación es determinado por tres constantes:

1. Mitad del tiempo de absorción o intervalo de tiempo en el cual una mitad del colorante circulante en la sangre es absorbido por el hígado.
2. Mitad del tiempo de excresión o intervalo de tiempo en el cual una mitad del colorante absorbido por el hígado es excretado por los conductos biliares.
3. Volumen de sangre en el hígado en una relación de porcentaje con el volumen total de sangre.

Los 29 casos se subdividieron en dos grupos: (1) aquellos que mostraban lesiones funcionales de las células del hígado, y (2) aquellos que no tenían este tipo de lesiones. Se hicieron otras subdivisiones de acuerdo con la presencia o ausencia de alteraciones dinámicas de excresión, hepatomegalia, disminución o aumento del volumen de sangre en el hígado en la fase evolutiva de la enfermedad y a la respuesta al tratamiento.

De aquellos casos que presentaron evidencia de lesiones o trastornos de la función celular o de la dinámica de excresión, 58.6% mostraron déficit funcional de las células polygonales; 45% mostraron un déficit funcional excretorio de los conductos biliares; 44.8% mostró disminución en el volumen sanguíneo en el hígado, y 51.7% mostró aumento en el volumen sanguíneo del hígado.

Las condiciones para le disminución o aumento de volumen sanguíneo del hígado no fueron paralelas a las condiciones funcionales de las células del hígado. De igual manera, la presencia de hepatomegalia, la fase evolutiva de la enfermedad, y la respuesta al tratamiento, no correspondieron al estado funcional de las células hepaticas.

Como factores hemodynámicos y el estado funcional de las células del hígado pueden cambiar independientemente, es necesario efectuar el exámen simultaneamente con la medida de un mínimun de corriente sanguíneo del hígado para conseguir un resultado efectivo.

RÉSUMÉ

Utilisant l'épreuve du rose bengale marqué au radioiode, telle qu'elle a été décrite par Loewenstein, les auteurs ont étudié l'état fonctionnel hépatocytique chez 29 malades at-

teints de lèpre lépromateuse. Le procédé employé pour analyser de façon quantitative la courbe reliant la charge du produit à son excrétion, est déterminé par 3 constantes:

1. La demi-période de charge, ou intervalle de temps durant lequel la moitié du colorant circulant dans le sang est absorbé par le foie;
2. La demi-période d'excrétion, ou intervalle de temps durant lequel la moitié du colorant absorbé par le foie est excrété par les canaux biliaires;
3. Le volume sanguin du foie pris comme pourcentage du volume sanguin total.

Les 29 cas ont été divisés en deux groupes: (1) ceux qui montraient des lésions fonctionnelles des cellules hépatiques, et (2) ceux qui ne montraient pas ces lésions. D'autres subdivisions ont été faites, en se basant sur la présence ou l'absence de troubles dynamiques de l'excrétion, sur l'hépatomégalies, sur la diminution ou l'augmentation du volume sanguin hépatique, ainsi que sur le degré évolutif de la maladie et sur la réponse au traitement.

Parmi les cas montrant des signes de lésions ou des troubles de la fonction cellulaire ou de la dynamique d'excrétion, 58.6% ont témoigné d'une déficience fonctionnelle des cellules polygonales, 45.0% ont montré une déficience dans la fonction excrétoire des canaux biliaires, 44.8% ont montré une diminution du volume sanguin hépatique, et 51.7% ont montré une augmentation dans le volume sanguin du foie.

Les conditions nécessaires pour la diminution ou l'augmentation du volume sanguin hépatique n'ont pas pu être mises en parallèle avec les conditions fonctionnelles des cellules hépatiques. De même, l'état fonctionnel hépatocytique ne correspondait pas à la présence d'hépatomégalie, au degré d'evolution de la maladie, et à la réponse au traitement.

Du fait que les facteurs hémodynamiques et l'état fonctionnel des cellules du foie peuvent changer de façon indépendante, il est nécessaire, si l'on veut obtenir un résultat utile, de pratiquer en même temps que cette épreuve une mesure du débit sanguin minimum dans le foie.

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