Discrepant Phenomenon between BSP-retention and ICG-retention Tests

Tomiko OKA and Minoru MIZUTA

First Division, Department of Internal Medicine, Yamaguchi University School of Medicine, Ube, Japan

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SUMMARY

Binding ability of indocyanine green (ICG) to the serum proteins was examined in patients with hepatitis whose ICG tests were much more impaired in comparison with sulfobromophthalein sodium (BSP) tests (group H), patients with familial type of abnormal ICG retention (group F), and healthy adults (group N) as the control. The difference of binding ability of ICG to the serum proteins affected the abnormal retention of ICG in all groups, particularly in the group H. A qualitative difference in binding of ICG to the serum proteins was noted between both groups of H and F. The appearance of abnormal retention of ICG could be due to different etiology in between these two groups.

Notwithstanding a difference in excretory mechanism between indocyanine green (ICG) and sulfobromophthalein sodium (BSP)[1], a good correlation between both dye retention tests is noticed in liver disease.

Discrepant phenomenon between these dye retention tests, however, is sometimes found. Some cases whose BSP test is more impaired in comparison with ICG test are found among patients with hepatitis[2-4], and some cases whose ICG test is more impaired in comparison with BSP test are also detected in patients with or without liver disease[2,3,5-10]. The most interesting example is the familial type with abnormal retention of ICG without any liver disease[10,11].

We have examined the binding ability of ICG to serum proteins in patients with hepatitis whose ICG test is distinctly impaired compared with the BSP test and in patients who showed abnormal ICG retention of familial type, because this kind of study may be useful for clarification of the excretory mechanisms of the liver.

SUBJECTS AND METHODS

1) Subjects

Subjects with hepatitis diagnosed on liver biopsy specimens (group H) are described in Table 1. Seven sera from five patients were examined. It was called as the discrepant phenomenon, when the value of ICG retention test at 15 minutes (ICG-R) was over 20% higher than that of

Table. 1 Clinical data of the group H

	Clinical course	ICG retention test R-15min%	BSP retention test R-45min%	Serum bilirubin mg%	Serum GPT* U
Case 1 Acute hepatitis	0 day	100	—	26.7	59
	14 days	100	—	18.2	120
	39 days*	56	9	3.4	122
	2 months	33	14	1.2	140
	4.5 months	29	3	1.7	40
	12 months	10.5	—	0.5	8
Case 2 Chronic hepatitis	0 day	92	16	2.2	40
	26 days*	44	14	1.0	41
	54 days*	60	12	0.5	24
Case 3 Chronic hepatitis	0 day	100	—	0.8	30
	3 months	100	—	0.5	17
	8 months*	76	31	0.5	17
Case 4 Chronic hepatitis	0 day	—	—	0.5	29
	26 days	33	—	0.5	—
	67 days*	35	8	0.5	17
Case 5 Chronic hepatitis	0 day	—	—	0.5	21
	15 days*	28	3.5	0.5	18

*Estimation of ICG-bound protein
*Normal value: below 35U

Table. 2 Clinical data of the group F

Case	ICG retention R-15min%	BSP retention R-45min%	Serum bilirubin mg%	Serum GPT U
1-A	86.2	2.3	0.4	14
2-A	46.7	6.6	0.3	18
3-A	48.5	5.7	0.9	5
4-A	82.8	0	0.8	14
5-B	78.4	3.4	0.9	26
6-C	79.4	3.8	0.8	13
7-C	67.8	0	0.5	8

the BSP retention test at 45 minutes (BSP-R).

In case 1 with acute hepatitis, ICG-R was 100% both at the first day and the 14th day of admission and it was 56% at the 39th day though BSP-R was 9%. This discrepant phenomenon had been seen until 4.5 months after the admission and subsided at the 12th month when the liver function tests were normalized.

In the case 2 with chronic hepatitis, ICG-R and BSP-R at the first day of admission were 92% and 16% rospectively. At the 54th day of admission ICG-R retained at distinctly impaired level, while liver function tests including BSP retention test were virtually normalized.

The familial type of subjects with abnormal ICG retention (group F) are described in Table 2. Seven sera from seven subjects from three families (A, B and C) were examined. The serum was kindly offered from Drs. Kondo and Kuchiba and details of the patients were previously reported by them[10,11].

In addition, nine sera from nine normal subjects (group N) whose ICG-R and BSP-R were both 0% were examined.

2) Assay of the ICG-binding to the serum proteins

A mixture of 1.0 ml of serum and 0.01 mg of ICG was stirred vigorously and kept for 30 minutes at room temperature. The mixture

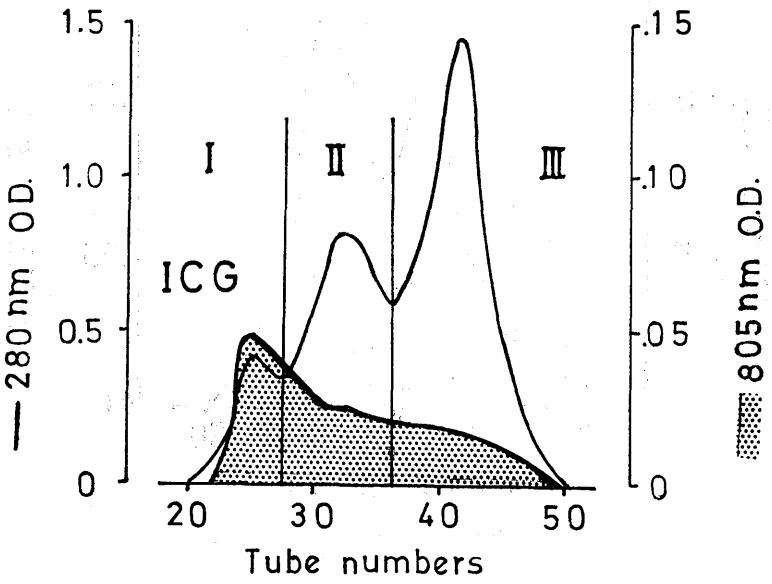

Fig. 1. Normal subject

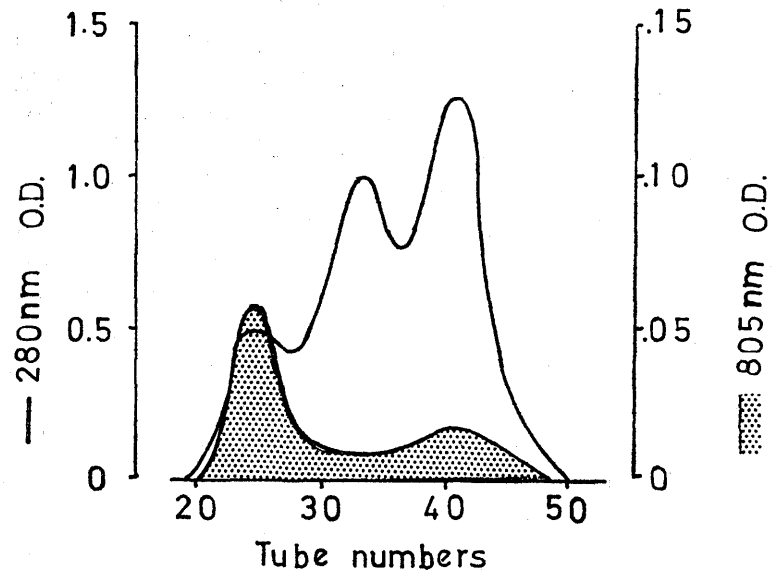

Fig. 2. Case 1 (acute hepatitis, ICG-R 56%)

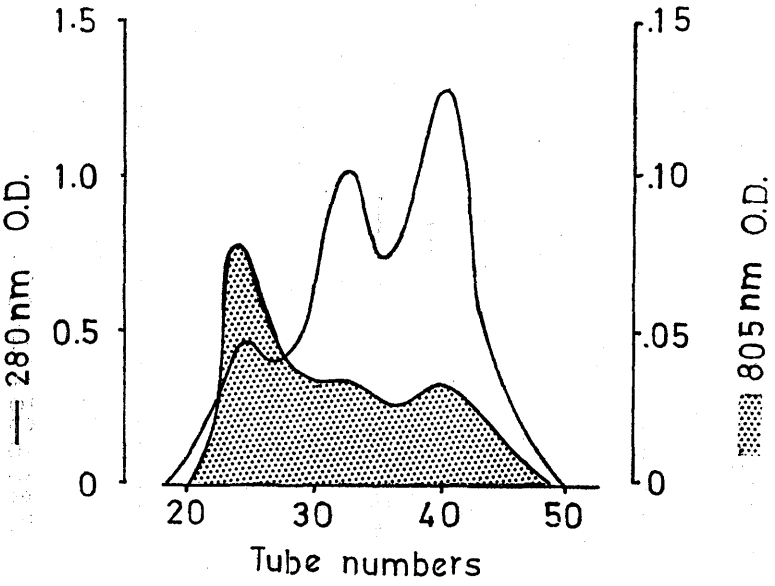

Fig. 3. Case 1(acute hepatitis, ICG-R 29%)

was applied on a Sephadex G-200 gel filtration column 2.6×51 cm, using 0.01 M Nacl as the eluting fluid. Flow rate was 12 ml/h and 3.8 ml/tube[12]. Protein or ICG concentration in the effluent solution was determinedd by 280 nm or 805 nm absorbance respectively.

The serum proteins which were bound to ICG were divided into three fractions by means of gel filtration as described in Figs. 1, 2 and

Table. 3 Correlation between the protein-bound ICG and the protein ratio, the ICG-R or minus BSP-R, and between the ICG-R minus BSP-R and the protein-bound ratio/the protein ratio

Correlation between	Fraction	Group	r	p<
protein-bound ICG ratio (y - axis) and protein ratio (x - axis)	I	N	0.61	0.05
		H	0.63	0.1
		F	0.82	0.025
	II	N	0.57	0.1
		H	0.69	0.05
		F	0.75	0.025
	III	N	0.11	not sig.
		H	0.18	not sig.
		F	0.83	0.025
ICG-R (y - axis) and protein-bound ICG ratio (x - axis)	I	H	0.84	0.01
		F	−0.13	not sig.
	II	H	−0.71	0.05
		F	−0.35	not sig.
	III	H	−0.34	not sig.
		F	0.48	not sig.
ICG-R minus BSP-R (y - axis) and protein-bound ICG ratio (x - axis)	I	H	0.86	0.01
		F	−0.04	not sig.
	II	H	−0.74	0.05
		F	−0.52	not sig.
	III	H	−0.32	not sig.
		F	0.59	0.1
ICG-R minus BSP-R (y - axis) and protein-bound ICG ratio / protein ratio (x - axis)	I	H	0.70	0.05
		F	0.15	not sig.
	II	H	−0.35	not sig.
		F	−0.45	not sig.
	III	H	−0.58	0.1
		F	0.66	0.1

Table. 4 Two-way classification

Relationship between	Subjected groups	Fraction	Fs	P<
protein ratio* and protein-bound ICG ratio*	N:H	I	7.26	0.01
		II	6.89	0.01
		III	2.11	not sig.
	N:F	I	1.75	not sig.
		II	9.14	0.01
		III	1.75	not sig.
	H:F	I	4.92	0.05
		II	1.19	not sig.
		III	0.88	not sig.
ICG-R and protein-bound ICG ratio*	H:F	I	11.05	0.01
		II	8.56	0.01
		III	3.70	not sig.
ICG-R minus BSP-R and protein-bound ICG ratio*	H:F	I	15.60	0.001
		II	17.55	0.001
		III	7.55	0.01
ICG-R minus BSP-R and protein-bound ICG ratio / protein ratio*	H:F	I	9.18	0.01
		II	11.01	0.01
		III	8.23	0.01

* Arcsine transformation
n: N(9), H(7), F(7)

3. These protein fractions were named I, II and III from the side of globulin, successively. Distribution ratios of both protein and dye in each fraction were called the protein ratio and the protein-bound ICG ratio respectively.

3) Statistical analysis

In each protein fration, a statistical analysis was applied to the relationship between the protein-bound ICG ratio and the protein ratio, between the protein-bound ICG ratio and the ICG-R, between the protein-bound ICG ratio and the ICG-R minus BSP-R (degree of discrepancy), and between the degree of discrepancy and the protein-bound ICG ratio/the protein ratio (ICG-binding ability of the protein fration).

The data were also analysed using two-way classification. When the numerical value was represented as percentage, it was changed by arcsine transformation.

The summarized data of the statistical analysis are described in Table 3 and 4.

RESULTS

1) Examples of gel filtration

In the serum of normal subjects, as shown in Fig. 1, ICG was distributed mainly in the protein fractions I and II, however, small amounts of the dye appeared in the protein fraction III.

In the serum of the patient with acute hepatitis (case 1), as shown in Fig. 2, ICG which was bound to the protein fraction I was distinctly increased, whereas the ratio of protein concentration in each fraction was unchanged. And, as shown in Fig. 3, the distribution of ICG in each protein fration returned to normal with improvement of liver function.

2) Relationship between the protein-bound ICG ratio and the protein ratio

Relationship between the protein-bound ICG ratio and the protein ratio is shown in Fig. 4. In each of the groups, a good correlation was

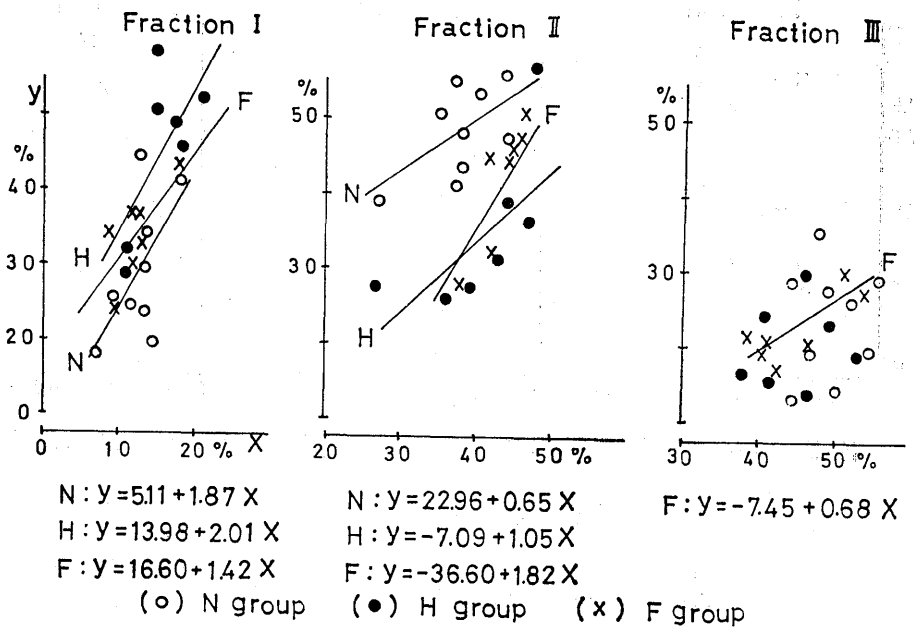

Fig. 4. Relationship between the protein-bound ICG ratio (Y-axis) and the protein ratio (X-axis)

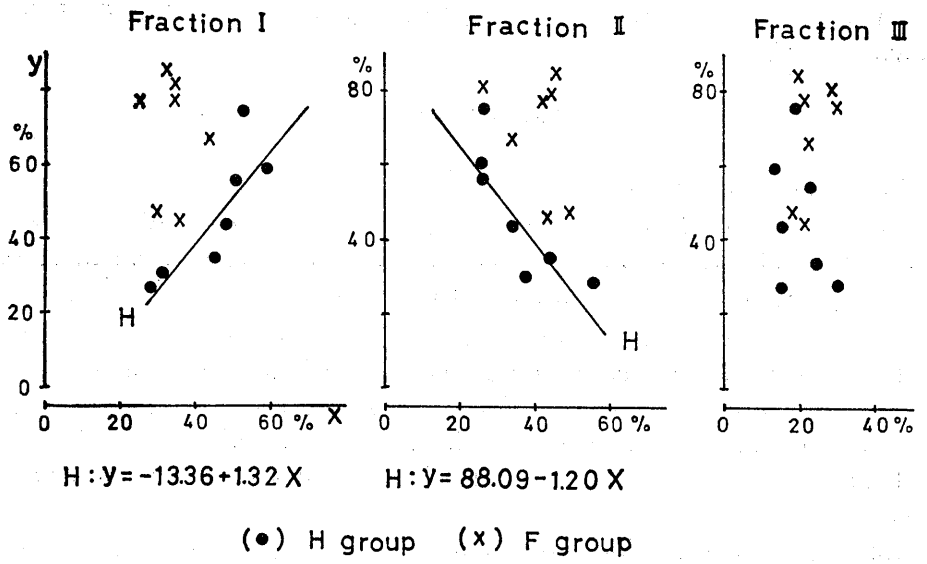

Fig. 5. Relationship between the protein-bound ICG ratio (X-axis) and the ICG-R (Y-axis)

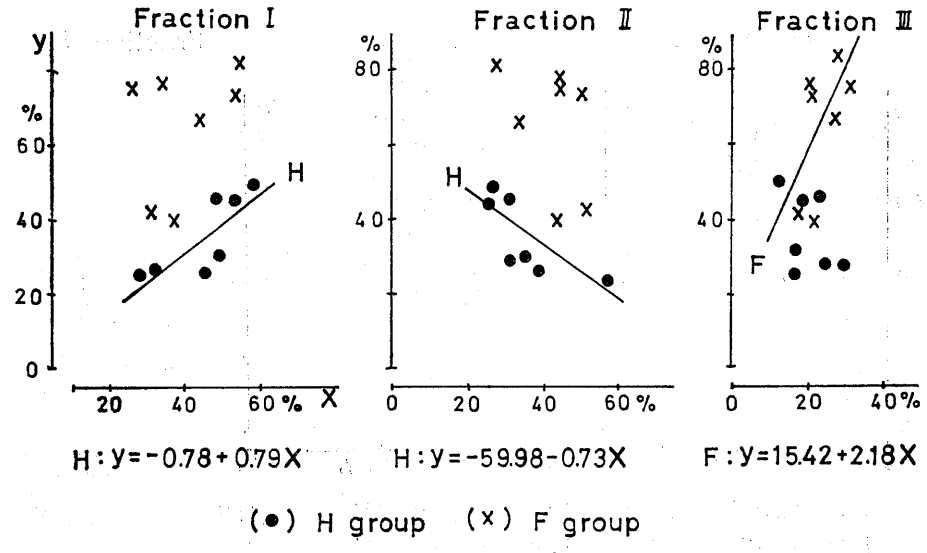

Fig. 6. Relationship between the protein-bound ICG ratio (X-axis) and the ICG-R minus BSP-R (Y-axis)

shown for fractions I and II. In fration III, however, this was shown only in the group F.

On two-way classification of the data from fraction I, a significant variance was found in all these groups, except for the relationship between both groups of N and F. The same result was also obtained on the fraction II. On the fraction III, no significant variance was shown in one another among these groups.

3) Relationship between the protein-bound ICG ratio and the ICG-R

Relationship between the protein-bound ICG ratio and the ICG-R is shown in Fig. 5. In group H, a positive correlation was shown in fraction I, a negative one was present in fraction II, and no significance was found in fraction III. In group F, no correlation was indicated in all fractions.

Regarding this relationship in fractions I and II, a significant variance was shown between both groups of H and F using two-way classification. However, in fraction III, no significant variance was indicated between both groups.

4) Relationship between the ICG-R minus BSP-R and the protein-bound ICG ratio

Relationship between the ICG-R minus BSP-R and the protein-bound

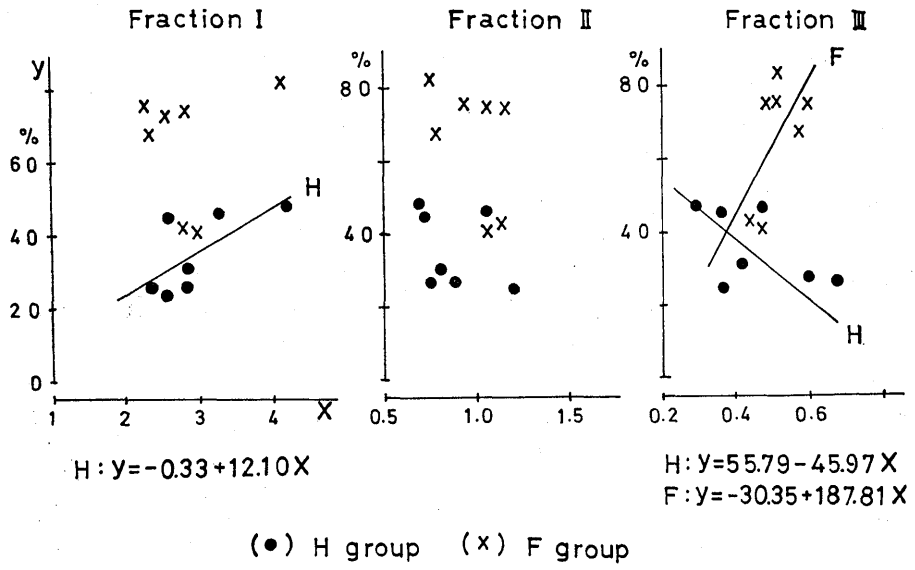

Fig. 7. Relationship between the ICG-R minus BSP-R (Y-axis) and the protein-bound ICG ratio/the protein ratio (X-axis)

ICG ratio was shown in Fig. 6. In the group H, concerning this relationship, a positive correlation was shown on the fraction I, a negative one was present on the fraction II and any relationship was never indicated on the fraction III. In the group F, a positive correlation was present merely on the fraction III.

Regarding on this relationship of each fraction, no significant variance was indicated between both groups when the data were analysed using two-way classification.

5) Rerationship between the ICG-R minus BSP-R and the protein-bound ICG ratio/the protein ratio

Relationships between the ICG-R minus BSP-R and the protein-bound ICG ratio/the protein ratio is shown in Fig. 7. In group H, a posititive correlation was shown in fraction I, a negative one was present in fraction III, and no relationship was indicated in fraction II. In group F, a positive correlation was present only in fraction III. No significant variance was indicated between these groups on two-way classification.

DISCUSSION

In case 1 with acute hepatitis, the discrepant phenomenon between ICG and BSP retentions subsided when his liver function was normalized. It is, therefore, quite clear that the discrepancy was based on the acute hepatic impairment. In the other cases with chronic hepatitis, it is uncertain whether the discrepancy was actually based on the hepatic lesion, because the result of dye retention tests prior to the onset of illness was unknown. In this report, however, we have regarded the discrepant phenomenon demonstrated in the patient with hepatitis as based on the hepatic lesion.

In the fractions I and II, in each of the groups N, H and F, a positive correlation was shown in the relationship between the protein ratio and the protein-bound ICG ratio, and, in fraction III, it was shown merely in the group F. At the first inspection of the data, the retention of ICG seems to be affected mostly by the dye-binding ability of the protein fractions I and II. When the data were analysed using two-way classification with arcsine transformation, however, there was no significant variance in the above-mentioned relationship between groups H and between groups H and F. Therefore, the serum proteins of each group may have a qualitatively specific property in the binding of ICG.

As shown in case 1, at the early stage of acute hepatitis, ICG was distributed abundantly in the protein fraction I, but, in the convalescent stage, the dye distributed in nearly normal wise through all of the three

fractions. The ratio of protein concentration of each fraction was unchanged all the time. The phenomenon suggests that the value of ICG retention test n the patient with hepatitis may be largely dependent upon the amounts of ICG in protein fraction I.

The same conclusion is suggested from the facts that both values of the ICG-R and the ICG-R minus BSP-R in the group H were positively correlated with the amounts of dye in the protein fraction I and negatively correlated with that in the protein fraction II.

In the group F, on the other hand, the retention of ICG was suggestively dependent upon the amounts of dye in protein fraction III, because the value of ICG-R minus BSP-R was positively correlated with the protein-bound ICG ratio only in protein fraction III.

It may be considered that the ICG-binding ability of the protein can be caluculated as the protein-bound ICG ratio/the protein ratio in each fraction though the concentration of protein in each fraction was not analysed quantitatively.

When the relationship between the ICG-binding ability of the pro -tein and the ICG-R minus BSP-R is examined, a positive correlation was demonstrated both in the protein fraction I of the group H and in the protein fraction III of the group H. Furthermore, a significant variance about this relationship was shown between the groups H and F. It is suggested, also, that the serum proteins of each of the groups may have a qualitatively different property in the binding of ICG.

In most of patients with chronic hepatitis the concentration of serum albumin is decreased and the lowered concentration of serum albumin may bring about an increased clearance of bilirubin or BSP[12,13]. On the contrary, the clearance of ICG may be scarcely influenced by the concentration of serum albumin because the dye is abundantly bound to the globulin fraction and is slightly bound to the albumin fraction.

Accordingly, it can be assumed that the discrepant phenomenon between both dye retention tests in the patient with hepatitis is attributable to differences of character in the dye-binding to the serum protein. Naturally, many more important problems are unsolved on the process of dye excretion such as hepatic uptake through the liver cell membrane, hepatic intracellular transport and biliary excretion[13,14,15].

In the cases with the familial type of abnormal ICG retention, the disturbance of hepatic intracellular transport is suggested by abnormal intracellular deposition of pigments in the liver on electron microscopy [8,10].

In the cases with familial type of abnormal ICG retention, the value of the ICG retention test was appreciably affected by the dye-binding ability of the protein fraction III which contains albumin. In this protein fraction, ICG may be bound preferably to the serum lipoprotein, because the dye is bound more strongly to lipoprotein than to albumin[12,16,17]. The concentration of the serum β-lipoprotein was examined in four cases of group F, but the result was not conclusive.

In spite of many unsolved problems, it was concluded that a qualitative difference in binding of ICG to the serum proteins was noted between the both groups of H and F.

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