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Studies on the Me-chanism of Bile Pigment Formation in Vivo. Part Ⅰ. On the Cor-relation between the Production of Bile Pigments and Functions of the Parenchymal Cells of the Liver.

Kenji Yamaoka[*] Kiyowo Kosaka[†]

Shigeru Ariji[‡]

[*]Okayama University,

[†]Okayama University,

[‡]Okayama University,

Studies on the Me-chanism of Bile Pigment Formation in Vivo. Part Ⅰ. On the Cor-relation between the Production of Bile Pigments and Functions of the Parenchymal Cells of the Liver.*

Kenji Yamaoka, Kiyowo Kosaka, and Shigeru Ariji

Abstract

1. In normal adults and in patients of non-hepatic diseases a transient hyperbilirubinemia occurs after peroral administration of hemolysed blood. 2. In cases of severe anchylostomiasis the serum bilirubin displays a remarkable decrease, and on imposition of hemolysed blood, no hyperbilirubinemia occurs but a relative one may be seen. 3. In patients with highly impaired functions of the parenchymal cells of the liver, neither absolute nor relative hyperbilirubinemia occurs on similar imposition of hemolysed blood. 4. Imposed blood or hemoglobin seems primarily to be phagocytosed by the reticulo-endothelial system. 5. A similar transient hyperbilirubinemia is also seen in rabbits after peroral imposition of hemohsed blood. 6. When the functions of the reticulo-endothelial system are accelerated by administration of "Koha", even incases of nonimposition of blood a hyperbilirubinemia occurs, but when hemolysed blood is imposed an additional transient increase in the hyperbilirubinemia may be detected. 7. In cases of blockage of the reticulo- endothelial system, this degree in the occurrence of hyperbilirubinemia is somewhat lower. 8. In cases of impaired liver cells by carbon tetrachloride, this decline is especially remarkable, and only a tendency of occurence can be dected. Since it is very difficult to explain this fact only by the co-existing impairment in the reticulo-endothelial system, the decline in the functions of the parenchymal cells of the liver must be placed under consideration. 9. By absorption tests of the intestines and by serological procedures, it is apparent that the perorally administerred hemoglobin may be readily absorbed from the jejunum, under any of these conditions. 10. Consequently, as for the cause of the hyperbilirubinemia occurring after peroral administration of hemolysed blood, most naturaly the reticulo-endothelial system participates, but it is impossible to neglect the part payed by the parenchymal cells of the liver.

Studies on the Mechanism of Bile Pigment Formation in Vivo.

Part I. On the Correlation between the Production of Bile Pigments and Functions of the Parenchymal Cells of the Liver.

By

Kenji Yamaoka, Kiyowo Kosaka and Shigeru Ariji.

(The First Department of the Internal Medicine)

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The problem concerning the role played by the parenchymal cells of the liver in the production of bilirubin in vivo has been a subject of very prolonged debate. Primarily the liver was considered an excretive organ for bilirubin produced extra-hepatically. Since *O. Minkowsky & B. Naunyn*[1] advocated the principle of "without liver, no jaundice", which was based on their experiments with geese with extirpated livers, this concept was generally accepted. Neverthless, this was insufficient in concluding that the parenchymal cells of the liver were where bilirubin was produced. In 1913, *J. M. McNee*[2] under the auspices of *L. Aschoff* reviewed the experiments of *Minkowsky & Naunyn*, and asserted the location of bilirubin production to be in the reticulo-endothelial system. In 1919, *G. Lephene*[3] after blocking the stellar cells with collargol, poisoned birds with arsine (AsH_3), whereupon only slight jaundice could be seen. *H. Eppinger*[4] has demonstrated similar results with dogs. Subsequently various authors have reviewed this theory, among which the experiment of *F. C. Mann, J. L. Bollmann & T. B. Magath*[5] was most remarkable. At present, that bilirubin is produced in the reticulo-endothelial system, seems to be an accepted concept. Nerverthless, although the number is small, there are still authors who firmly assert that the locality of production is in the parenchymal cells of the liver (*R. Duesberg*[5], *Miura*[7]), furthermore, since the process of bilirubin production in vitro has been clarified, bilirubin production in the body's fluid has become a possibility. While on the other hand, much doubt remains in the question whether or not the whole process of bilirubin production from

hemoglobin can be fully explained by the functions of the reticulo-endothelial system alone. Therefore, a reexamination of the productive mechanisms of the reticulo-endothelial system seems to be necessary.

The authors while undergoing measurements of serum bilirubin in patients of various diseases, became aware of the fact that in patients with peptic or duodenal disorders, where even a slight internal hemorrhage occurs, causing but a very weakly positive occult blood reaction in the feces, a transient minimal hyperbilirubinemia was detected, the amount of which was between 1.25 and 1.9 mg%. While in cases of severe anchylostomiasis where the hemoglobin measurements read from between 15 and 30% (*Sahli*), and pronounced positive occult blood reactions in the feces were seen, no hyperbilirubinemia was demonstrated. In such cases, on investigation of the congo-red index, although not always parallel, a decline in the functions of the reticulo-endothelial system was noticed. (Tab. I.)

Table I.

Name	Disease	Occult blood in the stool	Serum bilirubin	Congo-red index
E. M	Peptic ulcer	+	1.22 mg%	60.9
A. K.	Duodenal ulcer	+	1.35	54.2
K. M.	Peptic ulcer	+	1.92	63.1
healthy adults :— average			0.49	50~70
M. K.	Anchylostomiasis (grave)	+	0.35	70.4
S. K.	,,	+	0.32	71.0
K. Y.	,,	+	0.27	75.0
I. S.	,,	±	0.25	69.2

Thereupon, 15 cc of hemolysed human blood was imposed perorally on these patients and a similar phenomenon was seen, as may be seen in Tab. II. While in patients of severe anchylostomiasis, after administration of blood, an increase in the serum bilirubin was seen within limits of normal measurements. In other words, although no absolute hyperdilirubinemia occurs, relative one is seen. On the other hand, in diseases where the functions of the parenchymal cells of the liver are markedly impaired, such as in cirrhosis of the liver, etc., it was clarified that neither absolute nor

Table II. Alteration of serum bilirubin after peroral imposition of hemolysed blood.

(Case 1. Adult persons)

Case	Age	Sex	Before	After				Quartity of increase
				1.5 hour	5 ,,	9 ,,	24 ,,	
			mg%	,,	,,	,,	,,	,,
1	29	♂	0.52	1.34	1.02	1.21	0.58	0.82
2	27	♀	0.35	1.12	0.77	0.21	0.25	0.77
3	27	♂	0.58	1.36	1.67	1.20	0.68	1.11
4	21	♂	0.58	1.05	0.58	0.42	0.55	0.47
5	21	♂	0.43	0.97	1.02	0.67	0.51	0.59
6	47	♀	0.62	1.73	1.05	0.93	0.57	1.11
7	67	♂	0.51	1.22	0.97	0.94	0.58	0.71

(Cass 2. Patients)

Disease	Before	After				Quantity of increase	Galactose. test	*Takta*-reaction	Phenothiazin-test
		1.5 hour	5 ,,	9 ,,	24 ,,				
	mg%	,,	,,	,,	,,	,,			
Anchylostomiasis (grave)	0.10	0.36	0.42	0.20	0.29	0.32			
,,	0.30	0.45	0.40	0.39	0.31	0.15			
,,	0.14	0.14	0.41	0.27	0.14	0.27			
,,	0.14	0.10	0.39	0.29	0.17	0.25			
,,	0.13	0.39	0.20	0.20	0.20	0.26			
,,	0.12	0.13	0.36	0.55	0.15	0.43			
Primary Carcinoma of the liver	0.77	0.71	0.83	0.73	0.58	0.06	+	++	++
Hepatitis	0.41	0.55	0.42	0.40	0.49	0.14	+	++	++
Catarrhalic Jaundice	0.51	0.59	0.49	0.47	0.54	0.08	+	++	++
Cirrhosis of the liver	0.44	0.52	0.42	0.39	0.43	0.08	+	++	++

relative hyperbilirubinemia appears. Of course, on considering the congo-red index in these cases of cirrhoses a decline in the function of the reticulo-endothelial system may be detected, but this alone is insufficient in explaining this phenomenon. Furthermore, because this imposition of blood or hemoglobin causes an incline in the congo-red index as shown in Tab. III, this verifies that the reticulo-endothelial system takes this hemoglobin as a foreign body quite similar as in the case of India ink.

Table III (a). Alteration of Congo-red index.

Case	Sex	Date	Congo-red index
F. T.	♀	6. 22	65.9
,,		6. 26	80.5
,,		6. 30	75.4
	80.5 — 65.9 = 14.6		
M. K.	♂	6. 28	65.8
,,		7. 1	73.8
,,		7. 5	75.4
	75.4 — 65.8 = 9.6		
N. M.	♀	5. 11	53.6
,,		5. 15	65.0
,,		5. 19	60.2
	65.0 — 53.6 = 11.4		
S. M.	♀	6. 23	66.3
,,		6. 27	60.2
,,		6. 30	56.4
	66.3 — 57.4 = 9.9		
I. A.	♂	4. 16	51.2
,,		4. 20	59.3
,,		4. 24	64.7
	64.7 — 51.2 = 13.5		

Table III (b). Alteration of Congo-red index after peroral imposition of human hemolysed blood.

Name	Sex	Date	Hemo-globin (cc)	Congo-red index	Date	Hemo-globin (cc)	Congo-red index	Date	Hemo-globin (cc)	Congo-red index
F. T.	♀	3.8	—	28.1	3.12	9	45.0	3.17	15	60.0
M. K.	♂	4.12	—	34.4	4.16	9	56.1	4.20	15	73.2
N. M.	♀	4.27	—	53.4	4.30	9	66.0	5.6	15	77.2
K. M.	♀	4.21	—	46.4	4.25	9	61.3	4.29	15	71.2

In the next stage, in order to confirm the above facts, experiments were performed on rabbits. Generally the quantity of bilirubin in rabbit serum is so slight that measurement of it is usually impossible, but peroral imposition of 5 cc of human hemolysed blood causes a hyperbilirubinemia beginning 1.5 hours and reaching a maximum 3 to 6 hours after administration as shown in Tab. IV. Disparity in the individual animals is rather great, ranging between 1.22 and 2.23, averaging 1.82 mg%. On administrations of large quantities of "Koha" (a photo-sensitized substance), this alone causes hyperbilirubinemia ranging between 0.07 to 0.79, averaging 0.47 mg%. Considering the congo-red, this may be considered as caused by a rise in the functions of the reticulo-endothelial system, which is a very interesting observation. On imposing "Koha"

Table IV. Alteration of serum bilirubin after peroral imposition of hemolysed blood.

(Healthy rabbits)

Rabbit No.	Weight (g)	Sex	Serum Bilirubin (mg%)						Congo-red index
			Before	1.5 hour	3 ,,	6 ,,	9 ,,	24 ,,	
1	1847	♂	0.00	1.54	2.23	0.45	0.38	0.07	
2	1924	♂	0.01	0.45	0.83	2.32		0.03	
3	2132	♂	0.02	0.75	1.01	1.46		0.01	42.1
4	1970	♂	0.04	1.47	2.03	1.28	1.59	0.02	39.5
5	2571	♀	0.00	0.45	1.09	0.71	1.85	0.00	48.3
6	2940	♀	0.00	1.67	1.71	2.09		0.03	21.5
7	2563	♂	0.04	1.32	1.25	0.91		0.00	38.1
8	1420	♂	0.00	1.95	2.02	1.63		0.00	31.8
9	2672	♀	0.00	0.10	1.12	1.66	1.24	0.00	46.7
10	2550	♂	0.00	0.81	1.22	0.26	0.19	0.00	56.4
11	1520	♂	0.00	0.45	1.09	0.71	1.85	0.03	

and hemolysed blood, as may be seen in Tab. V., when hemolysed blood is imposed and without administration of "Koha", the bilirubin value reaches 0.8 to 1.66, averaging 1.20 mg% while on the other hand, when "Koha" is administerred previous to the imposition of hemolysed blood, the bilirubin rises to 1.31 to 2.61, averaging 1.82 mg%. In animals that had demonstrated a hyperbilirubinemia caused by imposition of hemolysed blood, of 1.12 to 2.09, averaging 1.64 mg%, previous to any blocking with India ink, on execution of the latter, a decline in the hyperbilirubinemia to 0.99 to 1.71,

Table V (a). Alteration of serum bilirubin after administration of "Koha".

No.	Weight (g)	Sex	Date	Congo-red index	Serum Bilirubin (mg%)	No.	Weight (g)	Sex	Date	Congo-red index	Serum Bilirubin (mg%)
1	2743	♂	7. 17	36.8	0.00	3	2510	♀	7. 19	53.1	0.01
			7. 20	21.0	0.24				7. 22	30.4	0.12
			7. 23	18.7	0.79				7. 25	13.7	0.53
2	2373	♂	7. 17	55.0	0.00	4	2337	♀	7. 19	47.6	0.00
			7. 20	23.4	0.10				7. 22	26.2	0.29
			7. 23	25.3	0.17				7. 25	20.4	0.49

Table V (b). Alteration of serum bilirubin after administration of "Koha" and hemolysed blood.

(Rabbits)

No.	Weight (g)	Sex	Congo-red index	Serum Bilirubin (mg%)					
				Before	1.5 hour	3 ,,	6 ,,	9 ,,	24 ,,
1	2880	♀	B. 46.7	0.00	0.10	1.12	1.66	1.24	0.00
			A. 18.7	0.09	1.72	1.72	2.16	1.37	0.11
2	2730	♂	B. 56.4	0.00	0.80	0.72	0.26	0.19	0.00
			A. 15.3	0.18	2.01	1.28	0.86	1.53	0.16
3	2990	♀	B. 64.8	0.00	0.70	0.50	0.50		0.00
			A. 40.7	0.05	0.12	1.21	1.81		0.09
4	2840	♂	B. 43.1	0.00	0.20	0.70	1.11		0.00
			A. 41.1	0.01	0.90	1.31	0.30		0.08

averaging 1.28 mg%, was observed. (Tab. VI.) Therefore, it is obvious that the reticulo-endothelial system has a definite correlation with the hyperbilirubinemia caused by peroral imposition of hemolysed blood, which, however, does not seem so very poignant. In the next stage carbon tetra-chloride was administerred perorally in doses of 0.4 to 0.5 cc In animals demonstrating a hyperbilirubinemia of 2.04 to 2.23, averaging 2.12 mg% caused by imposition of hemolysed blood previous to administration, at 30 hours after administration, when hemolysed blood was repeatedly imposed, the hyperbilirubinemia that has ranged between 0.85 to 4.5 mg%, increased from 0.07 to 0.47, averaging 0.28 mg%. (Tab. VII.) This

Table VI. Alteration of serum bilirubin after peroral imposition of hemolysed blood.

(Rabbits with blocked reticulo-endothelial system)

Rabbit No.	Weight (g)	Sex	Blocking	Congo-red index	Serum Bilirubin				
					Before	1.5 hour	3 ,,	6 ,,	24 ,,
1	2870	♀	Before	41.5	0.00	1.67	1.72	2.09	0.03
			After	54.1	0.00	1.21	0.23	0.21	0.07
2	2740	♂	Before	38.3	0.04	1.32	1.25	0.91	0.00
			After	49.2	0.00	1.23	0.29	0.25	0.09
3	1790	♀	Before	49.2	0.00	1.12	1.03	0.82	0.00
			After	62.4	0.00	0.99	0.72	0.77	0.00
4	1740	♂	Before	31.8	0.00	1.93	2.02	1.63	0.00
			After	46.2	0.00	1.71	1.42	0.63	0.00

Table VII. Alteration of serum bilirubin after peroral imposition of hemolysed blood.

(Rabbits with impaired liver)

No.	weight (g)	Sex	CCl_4 (cc)	Liver dis-order.	Congo-red index	Hippuric acid test	Phenothiazin test	Serum Bilirubin (mg%)					
								Before	1.5 hour	3 ,,	6 ,,	9 ,,	24 ,,
1	1920	♂	0.5 once	before	39.5	—	—	0.04	1.47	2.04	1.28	1.59	0.02
				after	48.3	++	++	4.50	4.51	4.51	4.57		
2	2110	♀	0.2 twice	before	44.3	—	—	0.00	1.54	2.23	0.45	0.38	0.07
				after	51.7	++	++	0.85	0.90	1.34	1.02	1.00	1.12

is a very remarkable decrease as compared with the case in reticulo-endothelial blocking. In this case, the function of the reticulo-endothelial system shows a decline, but on consideration of the congo-red coefficient, it is very slight as compared with blocking with India ink. Therefore, this remarkable decrease in the production of bilirubin cannot be fully explained by dysfunction of the reticulo-endothelial system alone. On consideration of (a) the properties of carbon tetrachloride that was used in this experiment, (b) the results of simultaneously performed examinations on the functions of the liver parenchyma and (c) hyperbilirubinemia caused by impairment of excretion of bilirubin by the parenchymal cells of the liver; these phenomena should be imputed to dysfunctions of the parenchymal cells of the liver.

During the course of these previous experiments, naturally, the absorption of hemoglobin from the intestines should be considered. On this point, investigations were performed through observation of the transition of intestinal contents, and the possibility of this absorption was demonstrated. Furthermore, through serological procedures, this was reaffirmed. Namely, on the following four groups of rabbits; (a) healthy, (b) "Koha" administerred, (c) blocked with India ink, (d) poisoned with carbon tetrachloride; precipitation were performed after peroral administration of 5 cc of hemolysed human blood. In this test, rabbit serum immunized with human hemoglobin, having a precipitation titer of over 20,000× dilution was used. Results of the test are seen in Tab. VIII. The chief factor participating in this precipitation is globin and even when disregarding the question of to what extent the substituting molecular group of hemoglobin undergoes changes, and whether or not it has seceded itself from the carrier-globin, rabbit

Table VIII. "Glodin" of peroral imposed human hemolysed blood in peripheral blood-stream of rabbits.

(This shows 2 Cases in 4 Cases)

a) Healthy rabbits.

Case 1. Weight 2140 g ♀

		2 hour					4 ,,					6 ,,				
	Antigen titer / Antiserum titer	100	200	500	1000	2000	100	200	500	1000	2000	100	200	500	1000	2000
Globin sedimentation titer	0	++	++	++	++	+	++	++	++	++	±	++	+	+	+	±
	2	++	++	++	++	+	++	+	+	+	±	+	±	±	±	−
	4	++	+	+	+	+	+	+	+	±	−	+	±	±	−	−
Bilirubin (mg%)	Before 0.02	0.36					1.40					0.45				

Case 2. Weight 2550 g ♀

		2 hour						4 ,,						6 ,,					
	Antigen titer / Antiserum titer	50	100	200	500	1000	2000	50	100	200	500	1000	2000	50	100	200	500	1000	2000
Globin sedimentation titer	0	++	++	++	++	++	+	++	++	++	++	++	+	+	+	+	±	±	−
	2	+	+	+	+	+	±	+	+	+	±	±	−	±	±	±	−	−	−
	4	+	+	+	+	+	±	+	+	+	±	±	−	±	±	±	−	−	−
Bilirubin (mg%)	Before 0.00	0.83						1.05						0.80					

b) ,,Koha" administered rabbits.

Case 1. Weight 1950 g ♀

		2 hour						4 ,,						6 ,,					
	Antigen titer / Antiserum titer	50	100	200	500	1000	2000	50	100	200	500	1000	2000	50	100	200	500	1000	2000
Globin sedimentation titer	0	++	++	++	++	++	++	++	+	+	+	+	+	++	+	+	+	+	±
	2	+	+	+	+	+	+	+	+	±	±	±	±	+	+	±	±	±	−
	4	+	+	+	±	±	±	+	±	−	−	−	−	+	±	−	−	−	−

Bili-rubin (mg%)	Before 0.04	1.34						1.72						1.09			
Case 2. Weight 2000 g ♀																	
		2 hour						4 ,						6 ,,			
	Antigen titer / Antiserum titer	50	100	200	500	1000	2000	50	100	200	500	1000	2000	50	100	200	500	1000	2000
Globin sedimentation titer	0	++	++	++	++	++	++	++	+	+	+	+	±	++	+	+	±	±	−
	2	++	+	+	+	+	+	+	+	+	±	±	−	+	+	±	−	−	−
	4	+	+	+	+	+	±	±	±	±	±	−	−	±	±	−	−	−	−
Bili-rubin (mg%)	Before 0.04	1.53						1.97						0.83			

c) Rabbits blocked with india ink.

Case 1. Weight 1700 g ♂																	
		2 hour						4 ,,						6 ,,			
	Antigen titer / Antiserum titer	50	100	200	500	1000	2000	50	100	200	500	1000	2000	50	100	200	500	1000	2000
Globin sedimentation titer	0	++	++	++	++	++	++	++	++	++	++	++	++	++	++	+	+	+	+
	2	++	++	++	++	++	+	++	+	+	+	+	+	+	+	+	+	+	+
	4	+	+	+	±	±	±	+	+	+	±	±	±	+	+	+	±	±	−
Bili-rubin (mg%)	Before 0.00	0.45						0.58						0.71			
Case 2. Weight 1650 g ♀																	
		2 hour						4 ,,						6 ,,			
	Antigen titer / Antiserum titer	50	100	200	500	1000	2000	50	100	200	500	1000	2000	50	100	200	500	1000	2000
Globin sedimentation titer	0	++	++	++	++	++	+	++	++	++	++	++	+	++	++	+	+	+	±
	2	+	+	+	+	+	+	+	+	+	+	+	±	+	+	+	±	+	±
	4	+	+	+	±	±	−	+	+	+	±	±	−	+	+	+	±	−	−
Bili-rubin (mg%)	Before 0.01	0.46						0.96						0.36			

P) Rabbits poisoned with carbon tetrachloride.

Case 1. Weight 2210 g ♂

		2 hour						4 ,,						6 ,,					
	Antigen titer / Antiserum titer	50	100	200	500	1000	2000	50	100	200	500	1000	2000	50	100	200	500	1000	2000
Globin sedimentation titer	0	++	++	+	+	+	−	++	++	++	+	+	+	++	++	++	+	+	+
	2	+	+	±	±	±	−	++	+	+	+	+	±	++	+	+	+	+	±
	4	+	+	±	±	±	−	+	+	+	+	±	±	+	+	+	+	+	±
Bilirubin (mg%)	Before 0.63 (0.18)	0.58 (0.14)						0.57 (0.15)						0.64 (0.17) total (direct)					

Cese 2. Weight 2250 g ♂

		2 hour						4 ,,						6 ,,					
	Antigen titer / Antiserum titer	50	100	200	500	1000	2000	50	100	200	500	1000	2000	50	100	200	500	1000	2000
Globin sedimentation titer	0	++	++	+	+	+	±	++	++	++	+	+	+	++	+	+	+	+	±
	2	+	+	+	+	+	−	++	+	+	+	+	±	+	+	±	±	±	−
	4	+	+	+	±	±	−	+	+	+	+	+	±	+	+	±	±	−	−
Bilirubin (mg%)	Before 1.37 (0.60)	1.43 (0.58)						1.21 (0.58)						0.96 (0.45) total (direct)					

serum demonstrates a very high precipitation titer under the above stated conditions, which can still be detected after several hours. Consequently, this verifies that sufficient absorption of hemoglobin prevails in the intestines. Thus, since there may be detected hardly no remarkable difference in the intestinal absorption of hemoglobin under the various conditions stated above, it is not too much in stating that the cause of hyperbilirubinemia not occurring in carbon tetrachloride poisoning, as compared with the case of blocking of the reticulo-endothelial system, is due to impairment of the liver parenchymal cells. Furthermore, to conclude that the parenchymal cells of the liver have a far more important part in the production of bilirubin than the reticulo-endothelial system may be considered no exaggeration.

Experimental Procedures.

I. Clinical Experiments.

i) Peroral administration of hemolysed blood.

Seven normal adults (5 male and 2 female) and patients of various diseases, particularly anchylostomiasis, and hepatic disorders were selected for this experiment. From 2 or 3 days before the experiment diets of meat and physical exertion were prohibited. Fifteen cc of hemolysed human blood diluted to about 45 cc with destilled water was introduced directly into the stomach by means of a duodenal tube before breakfast. Meals were allowed only after 5 hours following this procedure, and furthermore, diets containing meats, stimulants, and alcoholic beverages were prohibited for the following 24 hours.

ii) Transition of the congo-red index after peroral imposition of hemoglobin.

On 4 normal adults hemolysed blood was administerred with the same procedures as above. Five hours after administration the congo-red index was determined, and as may be seen in Tab. III (b) the index demonstrates an incline. On comparing this incline with the results of tests performed on normal adult (Tab. III (a)) the congo-red coefficient in cases of hemoglobin imposition may be seen to display a very remarkable incline. This also demonstrates that the imposed hemoglobin, as in the case of imposed India ink, has been phagocytosed by the reticulo-endothelial system.

II. Animal Experiments.

i) Peroral administration of hemolysed blood.

Five cc of freshly obtained normal human blood diluted with destilled water was administerred to rabbits weighing about 2 kg directly into the stomach by means of a Nelaton tube (No. 7) before breakfast.

ii) Blocking of the reticulo-endothelial system.

By standard methods, India ink was injected into the auricular veins of normal rabbits, in doses of 2 cc per body weight daily for 4 days. Before and after each injection. the congo-red index was seen to ascertain whether blockage was prevalent.

iii) Acceleration of the functions of the reticulo-endothelial system with "Koha".

On thin strips of potatoes, 3 tablets of "Koha" (each con-

taining 0.1 mg of Illuminal U II.) were attached and administerred to rabbits daily for three days. When insufficient acceleration of the reticulo-endothelial system functions were detected, through congo-red coefficient, three more days of administration were added to assure acceleration.

iv) Impairment of the parenchymal cells of the liver.

Standard methods of peroral administration of carbon tetrachloride to obtain the desired impairment, were performed on rabbits. Impairment of the parenchymal cells of the liver was verified by histo-pathological examinations.

v) Examinations on the intestinal absorption of hemoglobin.

Using standard surgical procedures, the intestines of rabbits were exposed, and at the upper most part of the jejunum a portion, —measuring 10 cm was ligatured at both ends, taking precautions not to impair the mesenterial blood vessels. By the same method a 10 cm portion of the descending colon was stopped off by ligature. Then thrusting a syringe into the central part of each, the contents of the intestines were rinsed out 3 times with normal saline that had been warmed to 37°C. Thereupon, 5 cc of human blood diluted with 5 cc of destilled water were injected. After ligature of the injection point, the abdomen was closed temporarily. One hour later, the abdomen was reopened, the contents of the ligatured intestinal portions taken out, diluted to its original volume and the hemoglobin amount determined by *Sahli*'s method. Results are as in Tab. IX (a). As for control, the degree of segregation of hemoglobin in intestines was determined in the following way; in re-

Table IX. Examinations on the intestinal absorption of hemoglobin.

(a)

No.	Before						After 1 hour					
	Jejunum		Colon descend.		Serum Bilir. (mg%)		Jejunum		Colon descend		Serum Bilir. (mg%)	
	Imposed blood	Hemo-globin	Imposed blood	Hemo-globin	Pfortar vein	Intra-cor.	Residual blood	Hemo-globin	Residual blood	Hemo-globin	Pfortar vein	Intra-cor.
	(cc)	(%)	(cc)	(%)								
1	5	56	5	56	0.00	0.00	3	25	5	54	0.34	0.71
2	5	56	5	56	0.00	0.01	3.8	28	4.5	57	0.42	0.56
3	5	51	5	51	0.00	0.00	2.75	39	4.8	47	0.32	0.41

(b) For control.

No.	Location	Before		After 1 hour	
		Blood	Hemoglobin	Blood	Hemoglobin
		(cc)	(%)	(cc)	(%)
1	Jejunum	5	56	4.8	55
	Colon des.	5	56	4.9	57
2	Jejunum	5	56	5.1	55
	Colon des.	5	56	5.2	56
3	Jejunum	5	51	4.8	53
	Colon des.	5	51	5.2	48

opening if the abdomen in the foregoing experiment, adjoining portions of intestine measuring 10 cm each were extirpated, and after rinsing out their contents, similar solutions of hemolysed human blood were injected into them and left for one hour, thereupon, the volume and hemoglobin amount was analysed on the remaining solution. The results obtained are to be seen in Tab. IX (b). Thus it is apparent that hemoglobin administerred perorally is absorbed in the intestines, especially in the jejunum although hardly no absorption is seen in the descending colon.

vi) Production of anti-human-blood-corpuscle serum.

On adult rabbits of about 2 kg body weight, *Kaji*'s[8] method of blood corpuscle unguentum percutaneous immunization was adapted. Seven days after 6 days of application, serum was segregated from blood taken from the auricular veins and its precipitation titer (antigen titer × antiserum titer) was determined. When it exceeded 20,000×8, total collection of blood was performed by incision of the jugular vein, and after segregation of the serum, this was placed in a regriferator for subsequent use.

vii) Demonstration of corpuscle globin in circulating blood.

Using the above mentioned anti-human-blood-corpuscle serum and utilizing precipitation tests, the precipitation titer were determined with hourly collected blood corpuscles as antigens. Decisions were made with consideration on field. However, according to *M. Ueno*[7], although this anti-human-blood-corpuscle serum is hogeneous specific, this reaction contains to some extent, side effects caused by heme. Therefore, to criticize the influences of this side effect, before the main experiment was performed, a

precipitation test using normal rabbit serum as antigen was carried out with the results shown in Tab. X, and as may be seen, the side effects in question may be considered negligible.

Table X.

(Case 1)

Antigen titer / Antiserum titer	50	100	500	1000	2000
0	+	−	−	−	−
2	±	−	−	−	−
4	−	−	−	−	−

(Case 2)

Antigen titer / Antiserum titer	50	100	500	1000	2000
0	+	±	−	−	−
2	+	−	−	−	−
4	−	−	−	−	−

III. Determination of Serum Bilirubin.

The method originated by *L. Jendrassik & R. A. Cleghorn*[10] was used.

IV. Determination of the Congo-red Index.

E. Adler & T. Reimanns'[11] method was applied. However, for calculation of the congo-red amount, *L. Heilmeyer's*[12] method of determining the amount of circulatory blood utilizing a photometer was used.

Conclusions.

1. In normal adults and in patients of non-hepatic diseases a transient hyperbilirubinemia occurs after peroral administration of hemolysed blood.
2. In cases of severe anchylostomiasis the serum bilirubin displays a remarkable decrease, and on imposition of hemolysed blood, no hyperbilirubinemia occurs but a relative one may be seen.
3. In patients with highly impaired functions of the parenchymal cells of the liver, neither absolute nor relative hyperbilirubinemia occurs on similar imposition of hemolysed blood.
4. Imposed blood or hemoglobin seems primarily to be phagocytosed by the reticulo-endothelial system.
5. A similar transient hyperbilirubinemia is also seen in rabbits after peroral imposition of hemolysed blood.

6. When the functions of the reticulo-endothelial system are accelerated by administration of "Koha", even in cases of non-imposition of blood a hyperbilirubinemia occurs, but when hemolysed blood is imposed an additional transient increase in the hyperbilirubinemia may be detected.

7. In cases of blockage of the reticulo-endothelial system, this degree in the occurrence of hyperbilirubinemia is somewhat lower.

8. In cases of impaired liver cells by carbon tetrachloride, this decline is especially remarkable, and only a tendency of occurence can be dected. Since it is very difficult to explain this fact only by the co-existing impairment in the reticulo-endothelial system, the decline in the functions of the parenchymal cells of the liver must be placed under consideration.

9. By absorption tests of the intestines and by serological procedures, it is apparent that the perorally administerred hemoglobin may be readily absorbed from the jejunum, under any of these conditions.

10. Consequently, as for the cause of the hyperbilirubinemia occurring after peroral administration of hemolysed blood, most naturaly the reticulo-endothelial system participates, but it is impossible to neglect the part payed by the parenchymal cells of the liver.

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