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Studies on the Mechanism of Bile Pigment Formation in Vivo. II. On the Process of Intrahepatical Production of Bilirubin.

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Studies on the Mechanism of Bile Pigment Formation in Vivo. II. On the Process of Intrahepatical Production of Bilirubin.*

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Abstract

1. When hemolysed blood is administered orally to rabbits, in cases of healthy and those with blocked reticulo-endothelial systems, a transient increase in the verdohemoglobin (M. Engel) is seen in circulating blood, while in that of rabbits with impaired parenchymal liver cells, no such increase occurs. 2. On irrigation of hemolysed blood through rabbit livers, in healthy rabbits production of indirect bilirubin may be demonstrated while in that with blockage of the reticulo-endothelial system or with impaired liver parenchymal cells, this may not be seen. Moreover, in this case of blockade of the reticulo-endothelial system. production of verdohemoglobin may be demonstrated. while none whatsoever may be demonstrated in cases of impaired liver parenchmal. On the other hand on irrigation of verdohemoglobin and biliverdin solutions, in healthy and in impaired liver parenchymal cell cases, production of bilirubin may be observed while absolutely none was detected in cases of blocked reticulo-endothelial systems, 3. Concluding from the results stated above and those of clinical experiments stated elsewhere, the following process is assumed: when blood is imposed on the organism it is primarily phagocytosed by the reticulo-endothelial system, next dissolved to verdohemoglobin {M. Engel) in the parenchymal cells of the liver, and then dissolved into globin, iron, and biliverdin in the reticulo-endothelial system, of which biliverdin is further reduced to bilirubin. A portion of this remains in the circulating blood as indirect bilirubin, while the majority of it is esterized in the parenchymal cells of the liver, and proceeds to the bile ducts as direct bilirubin.

Studies on the Mechanism of Bile Pigment Formation in Vivo.
II. On the Process of Intrahepatical Production of Bilirubin.

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In the first chapter of this study, the authors assumed that not only the reticulo-endothelial system, but also the parenchymal cells of the liver participated in the production of bile pigments in vivo from hemoglobin. In order to further the certainty of this concept, we have proposed investigations concerning the metabolism of iron. Using rabbits, as in the former chapter, 15 cc. of hemolysed goat blood was administered orally to group of rabbits that were ; (a) healthy, (b) blockaded with India ink, and (c) poisoned with carbon tetrachloride. Thereupon, stimations were made on the total iron content of blood, serum iron amount, and *G. Barkan*'s[1)2)] so-called "easily split off iron." The result are to be seen in Table I – IV. Most noteworthy here is the transition of the easily split off iron ; in the case of a healthy rabbit with no imposition of hemolysed blood, it demonstrates a gradual decrease which is caused by the loss of blood itself, while in instances of imposition of hemolysed blood, it showed a transient increase, arriving at its maximum in three hours. When hemolysed blood is imposed on rabbits with blockage with India ink, although to only a slight degree, an increase may be seen. Contrary to this, in cases of hemolysed blood imposition after poisoning with carbon tetrachloride, there occurs a decrease which may be considered as caused merely be the loss of blood. Although it is difficult to imply deeply into the significance of these facts, and in spite of a few pros and cons concerning *G. Barkan*'s theory, this easily split off iron,[3)4)5)6)] as have been clarified by the authors in studies stated elsewhere[7)], the transition of intermediate products such as chole-

Alteration of total iron content of blood, serum iron amount, and G. Barkan's so-called "easily split off blood iron."

Table I. Administration of physiological saline.

(Healthy rabbits)

No.	Sex	Weight (kg)	Hemoglobin (g%)					Total blood iron (mg/dl)					Easily split off blood iron (mg/dl)					Serum iron (γ/dl)				
			(Before)	1°	3°	5°	7°	(Before)	1°	3°	5°	7°	(Before)	1°	3°	5°	7°	(Before)	1°	3°	5°	7°
I	♂	2.05	10.5	10.0	9.9	7.9	7.5	44.4	44.4	45.0	38.4	38.4	1.56	1.61	1.57	1.27	1.05	160	160	—	115	107
II	♀	1.80	9.5	9.5	9.7	6.6	5.8	49.0	49.6	44.0	40.0	34.0	1.20	0.82	0.75	0.52	0.37	165	157	175	67	57
III	♀	2.00	13.8	13.1	12.0	10.8	11.0	53.5	52.5	50.3	45.1	45.1	1.59	1.55	1.50	1.38	1.17	277	216	232	163	130

Table II. Administration of hemolysed blood.

(Healthy rabbits)

No.	Sex	Weight (kg)	Hemoglobin (g%)					Total blood iron (mg/dl)					Easily split off blood iron (mg/dl)					Serum iron (γ/dl)				
			(Before)	1°	3°	5°	7°	(Before)	1°	3°	5°	7°	(Before)	1°	3°	5°	7°	(Before)	1°	3°	5°	7°
I	♀	2.60	12.8	11.0	10.9	11.8	12.4	60.0	57.0	62.0	57.0	56.5	1.11	1.64	2.83	1.60	1.57	180	142	243	75	102
II	♀	1.95	12.7	12.8	11.5	9.2	9.8	65.2	56.8	56.0	57.6	56.8	1.08	1.32	1.03	0.26	0.60	169	109	99	42	42
III	♂	1.90	12.5	10.6	10.5	10.3	9.8	53.5	48.0	46.1	43.5	41.9	0.87	1.26	1.88	1.49	1.15	135	70	108	45	151

Table III. Administration of hemolysed blood.

(Rabbits blockaded with India ink)

No.	Sex	Weight (kg)	Hemoglobin (g%)					Total blood iron (mg/dl)					Easily split off blood iron (mg/dl)					Serum iron (γ/dl)				
			(Before)	1°	3°	5°	7°	(Before)	1°	3°	5°	7°	(Before)	1°	3°	5°	7°	(Before)	1°	3°	5°	7°
I	♂	1.90	5.0	5.5	5.5	3.0	—	30.0	24.8	25.7	14.2	—	0.70	0.67	0.83	0.47	—	288	382	420	205	—
II	♂	1.95	13.8	11.9	9.6	10.0	7.9	56.6	53.6	53.6	39.9	—	1.16	1.14	1.26	0.63	0.59	73	88	93	36	—
III	♀	1.85	8.5	8.2	9.2	7.0	7.2	50.1	40.6	39.1	30.6	28.8	0.60	0.69	0.99	0.77	0.99	205	292	97	184	132

Table IV. Administration of hemolysed blood.

(Rabbits poisoned with carbon tetrachloride)

No.	Sex	Weight (kg)	Hemoglobin (g%)					Total blood iron (mg/dl)					Easily split off blood iron (mg/dl)					Serum iron (γ/dl)				
			(Before)	1°	3°	5°	7°	(Before)	1°	3°	5°	7°	(Before)	1°	3°	5°	7°	(Before)	1°	3°	5°	7°
I	♂	2.05	13.0	12.6	12.1	10.5	12.2	57.0	52.0	—	48.0	43.0	1.76	1.05	0.85	0.68	1.06	270	274	342	288	219
II	♀	1.80	13.5	13.3	13.4	10.2	9.8	50.6	52.6	52.6	45.2	40.6	0.82	0.33	0.71	0.37	0.62	114	240	178	208	187
III	♀	2.00	15.8	14.7	12.8	13.5	14.4	57.0	61.0	55.0	54.0	47.0	1.87	1.77	1.78	1.53	1.03	—	—	192	—	103

globin (*Lemberg, R.*[8]), or verdohemoglobin (*Engel, M.*[9]) in the process of bile pigment production, may be presumed from the findings stated above. Therefore, we assume that the parenchymal cells of the liver are more intimately related than the reticulo-endothelial system in the production of this choleglobin (*Lemberg, R.*) or verdohemoglobin (*Engel, M.*). This fact has been clarified *in vitro* by *H. Fischer* and *F. Lindner*[10], *O. Warburg* and *E. Negelein*[11], *P. Karrer* and *H. V. Euler* and *H. Hellström*[12], *E. Edlbacher* and *A. Segesser*[13], *H. Fischer* and *H. Libowitzky*[14], *M. Engel*[9], *G. Barkan*[2] and especially by *R. Lemberg* and his collaborators[15]. As have been clarified by the forementioned authors, bilirubin is produced from hemoglobin, or to state in detail, hemoglobin undergoes oxidation and reduction caused by molecular oxygen, ascorbic acid and other related reducing substances, and transforms into choleglobin (*Lemberg, R.*) or verdohemoglobin (*Engel, M.*) which in turn dissolves into iron, globin and biliverdin, this latter being transformed into bilirubin after undergoing reduction. This complicated process may also be seen in the living organism, and furthermore, that the roles played by the parenchymal cells of the liver and the reticulo-endothelial system are of completely different nature, may be the reason for which such complicated and contrary reaction are to be seen in the organism.

In order to clarify this point more deeply, irrigation experiments were performed on rabbit livers with *Ohashi*'s irrigation apparatus[16]. In the first stage irrigation was performed with hemoglobin, *i.e.*, the irrigation fluid was concocted by adding 1 cc. of distilled water to 0.5 cc. of human blood and diluted with 80 cc. of *Ringer*'s solution and 20 cc. of rabbit blood, irrigation having a duration of one hour. Consequently, as may be seen in Table V – VIII, indirect bilirubin produced in healthy rabbit liver ranged from 0.33 to 0.42 mg%, averaging 0.37 mg%, while on the other hand in rabbit livers with blockaded reticulo-endothelial systems or with intoxication with carbon tetrachloride, the estimates proved to be within the limits of estimation differences or in other words no appreciable indirect bilirubin could be detected.

In the next stage irrigation was performed with 2 cc. of human blood corpuscles hemolysed with 2 cc. of distilled water. and diluted with 100 cc. of *Ringer*'s solution. In instances of blockade of the reticulo-endothelial system, verdohemoglobin (*Engel, M.*)

Table V. Irrigation with hemolysed blood.

Rabbits	indirect Bilirubin	direct Bilirubin
Healthy cases	0.33 mg%	0
	0.42	0
	0.36	0
Cases in reticulo-endothelial blocking	0.00	0
	0.02	0
	0.01	0
Cases in liver parenchymal cell disorders	0.00	0
	0.00	0
	0.01	0

Table VI. Irrigation with hemolysed blood.

Rabbits	Verdo-hemoglobin	indirect Bilirubin
Healthy cases	—	0.56 mg%
	—	0.48
	—	0.54
Case in reticulo-endothelial blocking	++	0.02
	+	0.00
	+	0.01
Case in liver parenchymal cell disorder	±	0.00
	—	0.01
	—	0.00

Table VII. Irrigation with „Verdohemochrome" solution.

Rabbits	indirect Bilirubin	direct Bilirubin
Healthy case	0.20 mg%	0
	0.39	0
	0.18	0
Case in reticulo-endothelial blocking	0.00	0
	0.00	0
	0.00	0
Case in liver parenchymal cell disorder	0.17	0
	0.14	0
	0.08	0

Table VIII. Irrigation with Bilirubin solution.

Rabbits	indirect Bilirubin	direct Bilirubin
Healthy case	0.49 mg%	0
	0.35	0
	0.27	0
Case in reticulo-endothelial blocking	0.00	0
	0.00	0
	0.00	0
Case in liver parenchymal cell disorder	0.28	0
	0.14	0
	0.14	0

was detected in degrees of (+) to (++) positive, while in cases of rabbits livers intoxicated with carbon tetrachloride, no verdohemoglobin (*Engel, M.*) could be detected. Consequently, as has been induced from the results of estimations of the fore-mentioned easily split off iron, the parenchymal cells of the liver have an important role in the production of verdohemoglobin (*Engel, M.*), while it may

be said that the reticulo-endothelial system of the liver has hardly no significant part in this function. If so, since no final production of bilirubin may be seen when hemolysed blood is irrigated in cases of blocked reticulo-endothelial systems. we assume that this is caused by the fact that the reticulo-endothelial system participates in the production of bilirubin from verdohemoglobin (*Engel, M.*). Therefore, the irrigation experiments were performed with the following solutions; Verdohemochrome made according to *R. Lemberg*'s method [15], dissolved in 10 cc. of a phosphate buffer solution (pH 7.2) to which 90 cc. of *Ringer*'s solution was added, on the other hand, 5 cc. of biliverdin solution made according to *M. Engel*'s method with diluting with *Ringer*'s solution to make the total 100 cc., after revising the pH to 7.2. In healthy rabbits and in rabbit livers intoxicated with carbon tetrachloride, indirect bilirubin could be perceived, although there was slight difference in the amount found, while in rabbit livers with blocked reticulo-endothelial systems indirect bilirubin could not be demonstrated. Consequently, in the liver of rabbits, production of bilirubin from verdohemochrome and biliverdin is ascribed to the reticulo-endothelial system. In other words, it is possible to conclude that the reticulo-endothelial system of the liver detaches iron from verdohemochrome to produce biliverdin which is in turn succeedingly reduced to bilirubin. However, as the prosthetic groups of verdohemoglobin (*Engel, M.*) may not always be identified as verdohemochrome (*Lemberg, R.*)[15], it may be somewhat hasty to conclude immediately that the reticulo-endothelial system produces bilirubin from verdohemoglobin (*Engel, M.*) via biliverdin. Nevertheless the authors[17)18)] have reported an unique form of bilirubin, with ferric ion as complex salt, which was found in urine of a salvarsan jaundice patient accompanied with a discussion of the mechanism of its production, and surmising the results, it may be considered that the reticulo-endothelial system conducts the process of biliverdin production from verdohemoglobin (*Engel, M.*), *i.e.* the segregation of iron and biliverdin. Therefore it was possible to affirm this productive mechanism. Moreover, in this irrigation experiment all bilirubin obtained was of the indirect type while that of the direct type was not to be seen.

Thus on combining the results of this chapter and that of the 1st, we may establish the following concept: In the liver, the

reticulo-endothelial system assimilates hemoglobin as would a foreign body, and after entrusting it to the parenchmal cells of the liver, this latter transforms it into verdohemoglobin through oxidation and reduction. This verdohemoglobin (*Engel, M.*) is dissolved into globin, iron, and biliverdin in the reticulo-endothelial system. This biliverdin which after undergoing reduction, transforms so-called indirect bilirubin, of which one portion enters the blood stream, while the greater part of which is esterized in the parenchmal cells of the liver to become so-called direct bilirubin and enters the bile ducts. Therefore, the functions of the reticulo-endothelial system in the liver at least, proves to relatively simple, while complicated processes such as oxidation and reduction of hemoglobin may be ascribed to the parenchymal cells of the liver.

Experimental

1. Experiments concerning the transition of the total hemoglobin amount, total blood iron amount, serum iron amount, *G. Barkan*'s easily split off iron amount, after peroral administration of hemolysed blood to rabbits.

Fifteen cc. of hemolysed goat blood which was prepared according to the method described in Chapter I, was administered orally to adult rabbits weighing about 2 kilograms. Thereafter, hourly blood collections were performed by puncture of the jugular vein, and the total hemoglobin amount, total blood iron amount, easily split off iron amount, and the serum iron amount were determined. Hemoglobin determination was performed with *Hiraide et al.*'s method[19], while the total blood iron and serum iron amounts were determined by *Shinohara*'s[20] method modified by one of the authors, *Shimamura*[21], and the easily split off iron amount determined by *G. Barkan*'s[22) 23)] method. Moreover, the imposed hemolysed blood solution was prepared by washing 15 cc. of goat blood with normal saline several times, and thereupon adding distilled water to the sedimented blood corpuscles to the original volume.

2. Experiments of rabbit liver irrigation.

i) *Ohashi*'s rabbit liver irrigation apparatus was utilized.

ii) Methods for preparing the rabbits, extirpation of the livers, and the procedures of irrigation were according to those described by *Ohashi*[16].

iii) Determination of verdohemoglobin amount.

For determination of that in irrigating fluid, *M. Engel*'s[9] method was used.

iv) Estimation of bilirubin.

That of irrigation fluid was performed with the method described by *L. Jendrassik* and *Cleghorn*[24].

v) Preparation of verdohemochrome.

This was prepared from hemine according to *R. Lemberg*'s[15] method.

vi) Preparation of biliverdin solution.

According to *M. Engel*'s[9] method, ascorbic acid and molecular oxygen were applied to hemoglobin, thus producing verdohemoglobin, and from this biliverdin was segregated.

Conclusions.

1. When hemolysed blood is administered orally to rabbits, in cases of healthy and those with blocked reticulo-endothelial systems, a transient increase in the verdohemoglobin (*M. Engel*) is seen in circulating blood, while in that of rabbits with impaired parenchymal liver cells, no such increase occurs.

2. On irrigation of hemolysed blood through rabbit livers, in healthy rabbits production of indirect bilirubin may be demonstrated while in that with blockage of the reticulo-endothelial system or with impaired liver parenchymal cells, this may not be seen. Moreover, in this case of blockade of the reticulo-endothelial system, production of verdohemoglobin may be demonstrated, while none whatsoever may be demonstrated in cases of impaired liver parenchmal. On the other hand on irrigation of verdohemoglobin and biliverdin solutions, in healthy and in impaired liver parenchymal cell cases, production of bilirubin may be observed while absolutely none was detected in cases of blocked reticulo-endothelial systems,

3. Concluding from the results stated above and those of clinical experiments stated elsewhere, the following process is assumed: when blood is imposed on the organism it is primarily phagocytosed by the reticulo-endothelial system, next dissolved to verdohemoglobin (*M. Engel*) in the parenchymal cells of the liver, and then dissolved into globin, iron, and biliverdin in the reticulo-endothelial system, of which biliverdin is further reduced to bilirubin.

A portion of this remains in the circulating blood as indirect bilirubin, while the majority of it is esterized in the parenchymal cells of the liver, and proceeds to the bile ducts as direct bilirubin.

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