Acta Medica Okayama

Volume 8, *Issue* 2 1952 *Article* 2

JUNE 1952

Studies on the Mechanism of Bile Pigment Formation in Vivo. III. On the Transition of Biliverdin, and Bilirubin in the Bile of Rabbits.

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Studies on the Mechanism of Bile Pigment Formation in Vivo. III. On the Transition of Biliverdin, and Bilirubin in the Bile of Rabbits.*

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Abstract

1. In the bile of rabbits, the metabolisms of biliverdin and bilirubin are in a solucible state, and which have a ratio of 2: 1 in normal animals. 2. In the production of biliverdin, the liver, especially the parenchyma of the liver has a very important role, while that of the reticulo-endothelial system is rather minor. However, in the case of glucose administration, the reduction of bilirubin from biliverdin is performed in the reticulo-endothelial system, thus conferring an important part of this system. 3. The production of bilirubin is performed primarily extrahepatically, and the participation of the extrahepatical reticuloendothelial system is of a conservative nature, thus denying us any willingness to agree to the theory of bilirubin production in the reticulo-endothelial system. 4. On administration of hemolysed blood, bile pigments in bile demonstrate a remarkable increase, while as compared when injected into the auricle veins in cases of administration through the portal vein a decline in the functions of the liver reticulo-endothelial system is seen, causing a decrease in biliverdin amount. In the former modus of administration, an occasional stimulation of the liver reticulo- endothelial system is seen, causing reduction of biliverdin to bilirubin. 5. Concluding from these facts, biliverdin in rabbit bile occupies the role of an intermediate product in the production and metabolism of bilirubin.

Studies on the Mechanism of Bile Pigment Formation in Vivo.
III. On the Transition of Biliverdin, and Bilirubin in the Bile of Rabbits.

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Received for publication on October 26, 1951

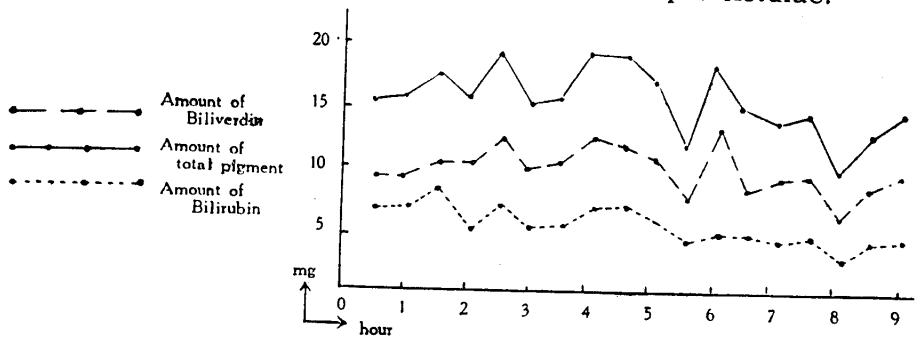
The concept that biliverdin is an oxide of bilirubin has been established for some time, but *R. Lemberg*[1)2)] during his pursuit of biliverdin pigments found throughout the natural world, noticed that their structures were intimately correlated to that of hemine IX, α, and since he had confirmed the fact that the so-called "green hemine" of *O. Warburg* and *E. Negelein*[3)] which is derived from hemine IX, α, was a ferrous complex salt of biliverdin, his collaborators[4)], *S. Edlbacher* and *A. V. Segesser*[5)], *H. Libowitzky*[5)7)8)], and *M. Engel*[9)] under the direction of *H. Fischer*, have all recognized biliverdin to be a foregoing substance in the production of bilirubin from hemoglobin.

Bile of healthy rabbits is colored green, and the majority of it is claimed by biliverdin, with a portion of bilirubin Whether this biliverdin foregoes bilirubin, or is an oxidated product of bilirubin is a point of much debate. *Kodama*[10)] considers it an oxidate, while *Amada*[11)] surmises it to be a substance foregoing bilirubin, although both are lack convincing evidence. One of the authors, *Yamamoto*[12)] has arrived at the conclusion that biliverdin in rabbit bile is for the most part a foregoing substance of bilirubin, from the following facts: Natural biliverdin may be divided into two substances, one easily soluble in ether or chloroform and the other non-soluble in these solvents. The former is a foregoing substance of bilirubin or, in other words, oxidated indirect bilirubin, while the later is an esterized form of it or an oxidate of direct bilirubin, in other words, this may be considered an oxidate of direct bilirubin.

Therefore, biliverdin in rabbit bile is assumed to be a substance preceding bilirubin.

The authors in pursuing the transition of biliverdin, and bilirubin in the bile of rabbits, have endeavored to clarify the position occupied by biliverdin in bile in the process of bilirubin production from hemoglobin, and through consideration of the correlation between biliverdin metabolism and the liver, we have attempted to clarify a fraction of the bile pigment metabolism in the living organism.

Fig. 1, Case of simple fistulae.



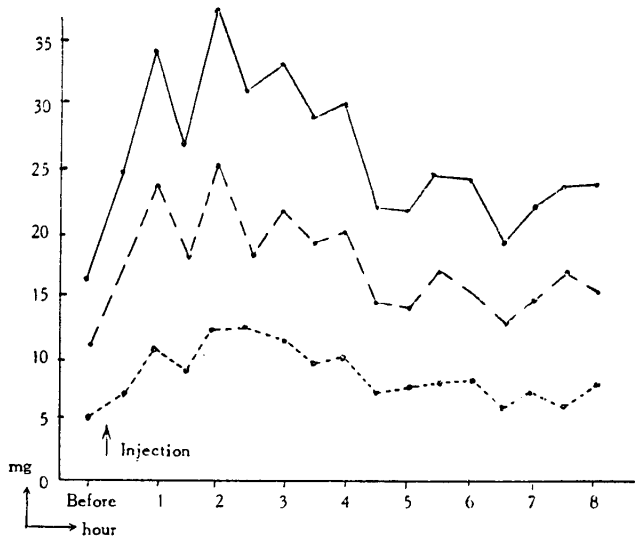
In the first stage, on healthy rabbits and rabbits receiving various procedures, bile duct fistulae were constructed surgically, and the following determinations made: Amount of bile, total pigment concentration, biliverdin concentration and comparative analysis of various pigments were determined on bile collected every 30 minutes, and their transitions observed. (Amounts of pigments were expressed with by the hundred times of the actual readings.)

In bile from cases simple fistulae, the biliverdin amount was approximately double that of bilirubin, as may be seen in Fig. 1, or the former ranged between 8 to 12 mg. while the latter from 4 to 6 mg. Maintaining this ratio of 2 : 1, both were seen to display a parallel transition.

Immediately after beginning of bile excretion, physiological saline, warmed to body temperature, was injected into the auricle veins in doses of 5 ml. per body weight, and as seen in Fig. 2, it resulted in a remarkable cholagogic reaction whereas the amount excreted doubled that compared with non-administered cases, although the biliverdin-bilirubin ratio still remained 2 : 1, with a

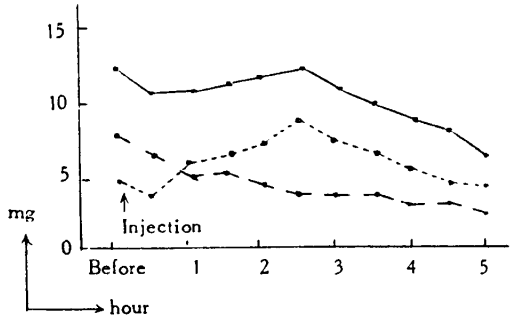
parallel transition. Whether this cholagogic action merely signifies a washing out of bile or rather an acceleration of bile production is as yet unknown.

Fig. 2. Healthy case injected physiological saline.



In the next stage, a 20% glucose solution in doses of 5.0 ml. per kilogram body weight was injected likewisely, into the auricle veins of rabbits before onset of bile excretion. Hardly no cholagogic action was seen, as may be observed in Fig. 3. The total bile pigment amount was close to that of non-administered cases. From the early stages of the procedure, the concentrations of biliverdin and bilirubin, or the concentration curves of the pigments display an intersection; in other words the bilirubin displaying an increase while the biliverdin a decrease, and at the same time the total pigment amount remaining unchanged. From this phenome-

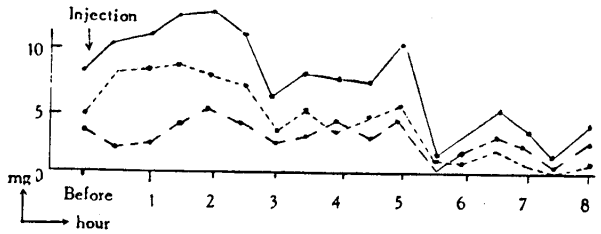
Fig. 3. Healthy case injected a 20% glucose solution.



non, therefore, it may be considered that a reduction of biliverdin to bilirubin has occurred.

However, *Miyake*[13] of this laboratory, has brought notice to the fact that glucose possesses the ability to inhibit or delay the production of verdohemoglobin (*Engel, M.*) from hemoglobin. Therefore, the above mentioned phenomenon may be interpreted as a function of glucose. *Lemberg, R.* and *R. A. Wyndham*[14] have demonstrated that biliverdin is easily reduced into bilirubin in various living organs, especially the liver, and these authors have interpreted this reduction as being caused by the acceptor biliverdin receiving reductive action from activitated hydrogen, which in turn is produced through various carbohydrates as substrate under the influence of dehydrogenase, which is found in the organs. The above mentioned reduction of biliverdin to bilirubin through glucose may be interpreted precisely as being caused by this mechanism.

Fig. 4. Case with impaired liver caused by carbon tetrachloride, injected physiological saline.



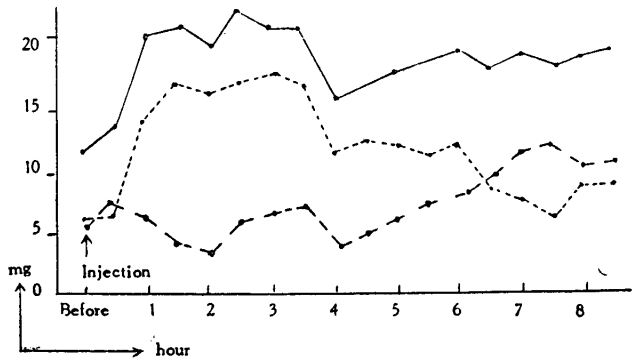
In the next experiment, bile fistulae were constructed in rabbits with impaired livers caused by carbon tetrachloride. On administration of physiological saline, as may be seen in Fig. 4, notice should be placed on the remarkable decrease of biliverdin concentration and the pigment amount. Since carbon tetrachloride is considered primarily as toxic toward the parenchymal cells of the liver, this remarkable decrease of biliverdin is considered as based on impairment of the liver parenchyma, consequently, rendering it inevitable to conclude that the functions of the liver parenchyma are greatly concerned in the production of biliverdin. When compared with biliverdin, bilirubin also displays a decrease, but on consideration of the accumulation of this pigment in blood caused by impairment of liver parenchyma, we must conclude that its pro-

duction should be considered as being of little difference compared with normal instances.

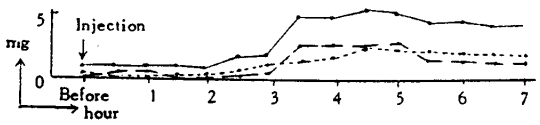
On establishment of bile fistulae after intoxication with carbon tetrachloride, when glucose is administered, and when the degree of intoxication is slight, a partial cholagogic action is seen, as may be noticed in Fig. 5, (case 1), with pronounced transition of biliverdin to bilirubin. In the latter half of this procedure, a tendency toward increase in the excretion of pigments was observed, which was interpreted as being brought on by recovery of the functions of the liver caused by influences of glucose. This phenomenon was not observed in normal cases (Fig. 5, case 2).

Fig 5. Case with impaired liver caused by carbon tetrachloride, injected 0.20% glucose solution.

(Case 1)



(Case 2)



In the next stage, bile fistulae were constructed in rabbits with blocked reticulo-endothelial systems. On administration of physiological saline, when the degree of blockage was rather slight as may be seen in Fig. 6, a cholagogic action was seen, but with the progress of its severity, this action was altogether prohibited. When the degree of blockage was far advanced, as in Fig. 7, both the concentrations of biliverdin and bilirubin, as with the amount of

pigments, displayed a decrease, of which that of biliverdin was the most remarkable.

Fig. 6. Case with blocked reticulo-endothelial system, injected physiological saline.
(Case 1)

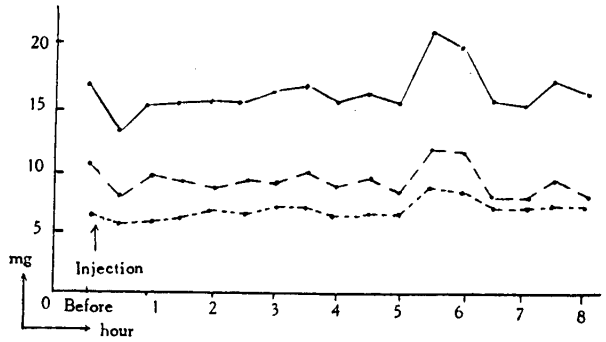
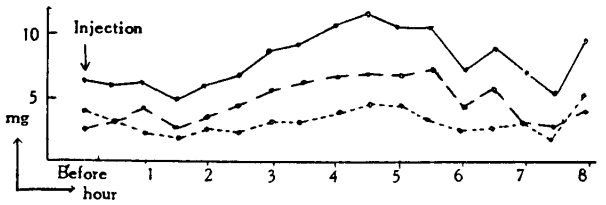


Fig. 7. Case with blocked reticulo-endothelial system, injected physiological saline.
(Case 2)



Furthermore, when glucose was administered intravenously to these cases of bile fistulae combined with blocked reticulo-endothelial systems, as may be seen in Fig. 8, a more superior cholagogic reaction may be demonstrated as compared with cases of injections of physiological saline under identical conditions, and is especially remarkable in the easily stages of the procedure. Transition of biliverdin to bilirubin may be seen to some extent when the degree of blockage is slight, but when it is pronounced, as may be seen in Fig. 9, this transition may not be detected. This reduction of biliverdin, differing from the case of carbon tetrachloride intoxication, may be ascribed entirely to the impairment of the reticulo-endothelial system, therefore this indicates that the reduction of biliverdin is performed in the reticulo-endothelial system.

In order to supplement the above stated experiments, further experiments of impositions of hemolysed blood were performed.

Fig. 8. Case with blocked reticulo-endothelial system, injected a 20% glucose solution.
(Case 1)

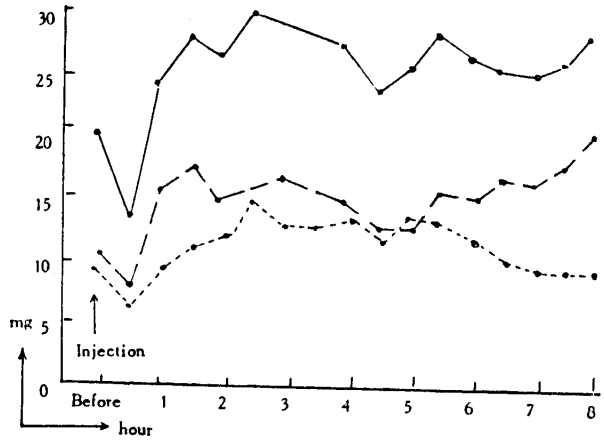
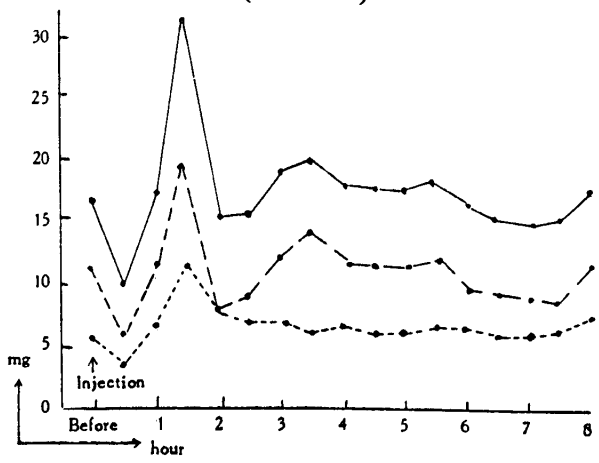


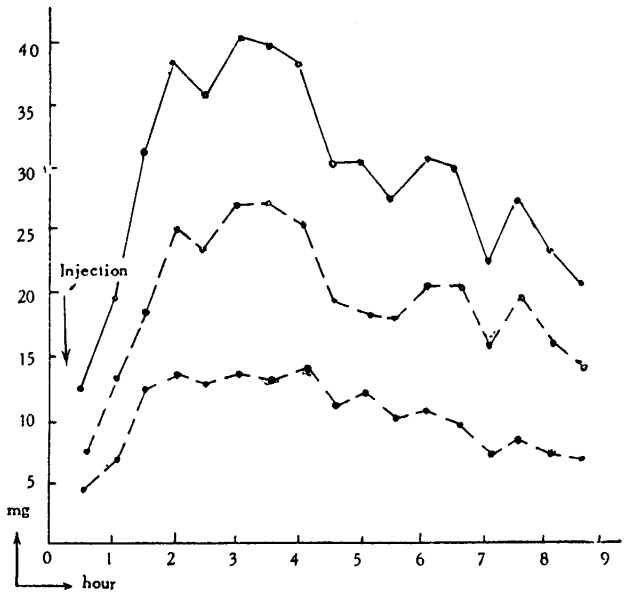
Fig. 9. Case with blocked reticulo-endothelial system, injected a 20% glucose solution.
(Case 2)



First, hemolysed blood was administered via the auricle vein, and as may be seen in Fig. 10, the total pigment amount, bilirubin and biliverdin amounts all displayed an increase, reaching 4 times

that of non-administration cases. However, the biliverdin, bilirubin ratio still remained 2 : 1, and displayed a parallel transition as was seen in non-administered cases. In a single case, as to be seen in Fig. 11, reductive process of biliverdin to bilirubin was demonstrated, as was seen on administration of glucose, Consequently, administration of hemolysed blood in large quantities seems to stimulate the reticulo-endothelial system, activitating the production of bilirubin, and here we find evidence that biliverdin precedes bilirubin in its production.

Fig. 10. Healthy case, administered hemolysed blood via the auricle vein.
(Case 1)



When administrations were undertaken through the portal vein, as may be seen in Fig. 12, the total pigment amount shows a decrease on comparison with those of the auricle vein, and the points when biliverdin and bilirubin reaches their maximum displays an obvious difference, that of biliverdin preceding. This phenomenon is caused by the difference in the localization of administration, *i.e.*, contrary to the case of intra-auricle vein administration, in this case a sudden administration of hemoglobin of lower osmotic pressure than that in existing the portal vein may be

considered as influencing the liver to bring on this above mentioned phenomenon. Although the decrease of biliverdin is especially remarkable, the bilirubin amount displays hardly no significant change.

Fig. 11. Healthy case, administered hemolysed blood via the auricle vein. (Cace 2)

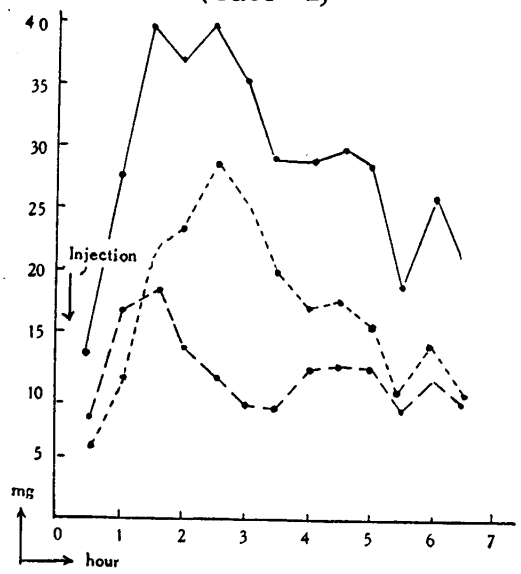
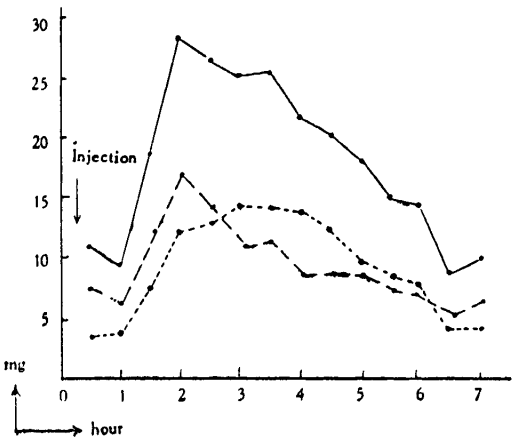


Fig. 12. Healthy case, administered hemolysed blood through the portal vein.

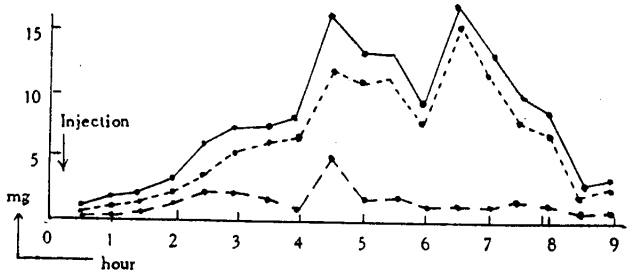


On administration of hemolysed blood after impairment of the liver has been established by carbon tetrachloride, as may be seen in Fig. 13, the bile amount, the concentration of biliverdin and the pigment amount all demonstrate a remarkable decrease, giving evidence to the fact that the parenchymal cells of the liver are intimately correlated in the production of biliverdin. On the other hand, in view of the impairment of the excretive functions of the liver, an increase of the bilirubin amount may be surmised.

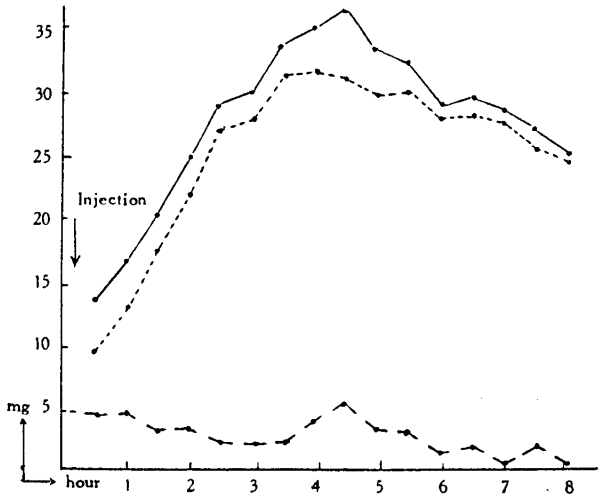
In cases of blockage of the reticulo-endothelial systems, when hemolysed blood was administered, a delay in biliverdin amount reaching its maximum was observed, giving the impression that a decline in the phagocytic functions of hemoglobin in the reticulo-endothelial system of the liver had occurred (Fig. 14). However, when the

degree of blockage progressed the maximum was hardly reached and contrarily, a decline was observed. On the other hand, the increase in the bilirubin pigment amount was very pronounced.

Fig. 13. Case with impaired liver caused by carbon tetrachloride, administered hemolysed blood.



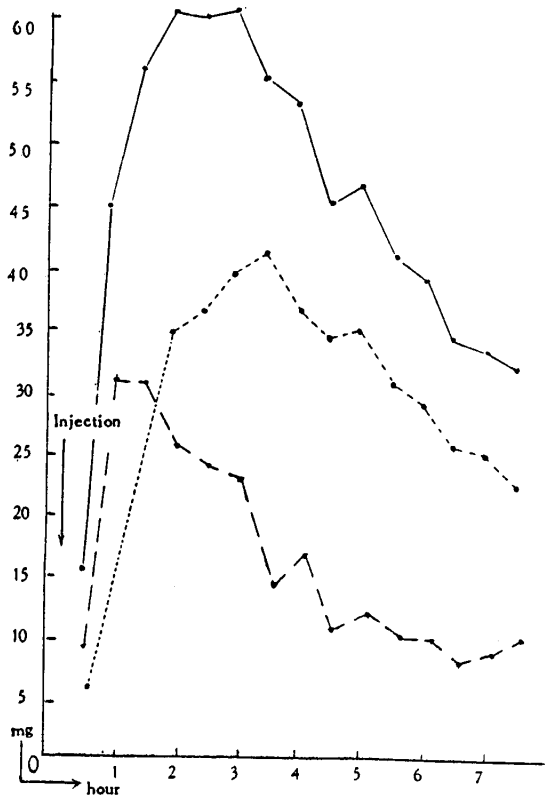
Fig, 14. Case with blocked reticulo-endothelial system, administered hemolysed blood.



In the next stage, the above experiments were repeated with simultaneous administrations of glucose. In the cases of normal rabbits, as may be seen in Fig. 15, the bile amount, pigment concentration, pigment amount all display a remarkable increase, especially the total pigment amount doubling that of non-administered cases. This may be caused by protection against the influence of the forementioned hypotonic heterogeneous hemoglobin administration, and furthermore, it causes an acceleration in produc-

tion. Other observations such as in cases of hepatic impairment caused by carbon tetrachloride, and blockage of the reticulo-endothelial system, as to be seen in Figs. 16, and 17 respectively, the findings were roughly parallel to those of individual glucose administrations.

Fig. 15. Healthy case, administered hemolysed blood and glucose.



On summarizing these observations on bile collected from bile duct fistulae, several odd points are noticeable: *i.e.*, on injections into the portal vein, influences bestowed on the liver were always pronounced, especially on injections of hypotonic hemoglobin as foreign substance, influences on liver especially the reticulo-endothelial system was remarkable, consequently incurring decrease in the biliverdin amount, and furthermore, in cases of blockage of the reticulo-endothelial system, this influence was doubled, therefore causing a very remarkable decrease in biliverdin amount.

On summarizing these observations on bile collected from bile duct fistulae, several odd points are noticeable; *i.e.*, when physiological saline is administered, bilirubin appearing in instances of liver impairment demonstrates contrarily, an increase, while with blockage of the reticulo-endothelial system, a decrease in biliverdin is seen, and the bilirubin amount does not accompany the total bile amount in its decrease. Furthermore, in experiments with impositions with hemoglobin, in healthy animals, the decrease in biliverdin was remarkable, while little change was seen in the bilirubin amount.

in liver impairment cases with carbon tetrachloride, compared with the influences on biliverdin, the bilirubin amount displayed tendency toward increase; and finally in cases of reticulo-endothelial system blockage, as the severity of the blockage increases, the decrease in biliverdin and the increase in bilirubin were very remarkable. These facts seem to contradict the mechanism of bilirubin production clarified in Part II, but as mentioned before, the reticulo-endothelial system of the liver not only participates in this reductive process, but also in the phagocytosis of hemoglobin. Moreover, on observation of the excretions of biliverdin and bilirubin in the bile of untreated rabbits, they are independent, of one another, thus giving the impression that their locations of production are varied. Consequently, on consideration of the possibility that biliverdin is produced intrahepatically while bilirubin extrahepatically, the contradiction mentioned above easily solves itself. When

Fig. 16. Case with impaired liver caused by carbon tetrachloride, administered hemolysed blood and glucose.

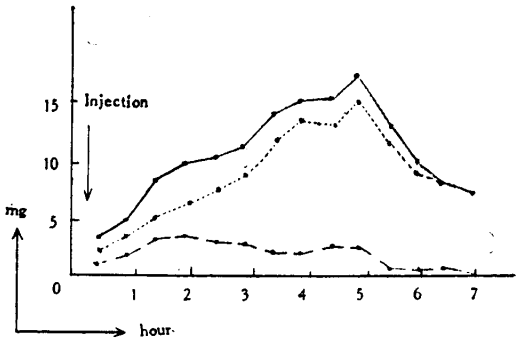
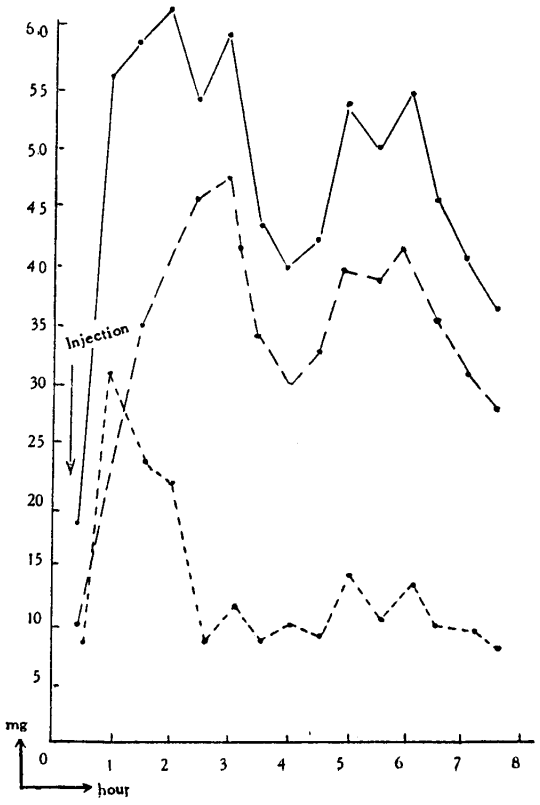


Fig. 17. Case with blocked reticulo-endothelial system, administered hemolysed blood and glucose.



injections are administered to the portal vein, the influences on the liver are great, especially on injections of hypotonic hemoglobin as foreign substance, effects on the liver notably on the reticulo-endothelial system are remarkable, resulting in a decreases in the biliverdin amount, moreover, in cases of blockage of the reticulo-endothelial system, the influence is doubled, causing a remarkable decrease in the biliverdin amount. On the other hand, any hemoglobin that is not taken care of in the liver, is humorally transformed into bilirubin extrahepatically, therefore, the lower the production of biliverdin, the higher is the tendency of increase in bilirubin production. Here we find another answer to the contradiction stated above.

Thus, the following conclusions may be derived ; in the production of biliverdin in rabbit bile, the parenchymal cells of the liver appear to possess the principal part while that of bilirubin belongs to extrahepatical productive system, and furthermore, the ratio of biliverdin and bilirubin production is 2 : 1. Therefore, the production of bile pigments in the rabbit is performed chiefly in the liver especially in the parenchymal cells of the liver, and moreover, physiologically, the reticulo-endothelial system has only a secondary or passive part in this production.

Experimental.

1. Procedures of Bile Duct Fistulae Construction in Rabbits.

Adult rabbits were fed on a standard diet, till 24 hours before operation when all feeding was prohibited. Next morning on laparotomy, a glass canule was inserted into the common bile duct (ductus choledochus), to which a thin rubber tube measuring 20 cm. in length was attached. Therefore, bile flowing from this tube was collected every 30 minutes. On consideration of the intestinohepatical circulation, the duration of the estimations were limited to 6 to 8 hours. Because there was anticipation of harm being done to the liver, ligatures of the ductus cysticus were not performed ; nevertheless, no flow of bile from the gall bladder was seen throughout the experiments. On bile collected in this manner, the amount of bile excretion, concentration of bile pigments, amounts of bile pigments (total bile, biliverdin. bilirubin) were determined. For the determination of pigment concentration, the method de-

scribed by one of the authors, *Yamamoto*[15] was utilized, represented by 1/100 of the product of the concentration and the bile amount. In this experiment, emphasis was lain mainly on the transition of the pigment amount, other points merely referred to.

2. *Experimental Animals.*

For the experiments, adult rabbits of around 2 kilograms were chosen. For cases of liver impairment, carbon tetrachloride was administered in doses of 0.3 ml. per kg. body weight, perorally, and offered for experiments 30 minutes after. Histopathological investigations followed the experiments to verify and criticize the results obtained. For cases of reticulo-endothelial system blockage, a 10% emulsion of India ink or a 1.0% solution of collargol was administered parenterally in doses of 4.0 ml. per kg, body weight, continuously for seven days. Thereafter, by utilizatian of ヨ. *Adler* and *Reimanns*' congo-red index, the blockage was verified.

3. *Preparation of hemolysed blood.*

Five ml. of rabbit blood was collected, immediately centrifuged; the plasma segregated and discarded, and after several washings with physiological saline to completely remove serum, to this blood cell mixture 3.0 ml. of distilled water was added, resulting in complete hemolysis, and to which additional distilled water was added to amount to 8.0 ml. This solution was administered in doses of 3.0 ml. per kg. body weight.

4. *Experiments of Combined Administration of Hemolysed Blood and Glucose.*

Immediately after flow of bile from bile duct fistulae that had been established in the method mentioned above, had commenced, 5.0 ml. per kg. body weight of a 20% solution of glucose were injected into the auricle veins, and 10 minutes later, hemoglobin prepared as above was injected in doses of 3.0 cc. per kg. body weight, into the mesenterial vein, and thereafter every 30 minutes bile was collected for 6 to 8 hours.

Conclusions.

1. In the bile of rabbits, the metabolisms of biliverdin and bilirubin are in a solucible state, and which have a ratio of 2 : 1 in normal animals.

2. In the production of biliverdin, the liver, especially the

parenchyma of the liver has a very important role, while that of the reticulo-endothelial system is rather minor. However, in the case of glucose administration, the reduction of bilirubin from biliverdin is performed in the reticulo-endothelial system, thus conferring an important part of this system.

3. The production of bilirubin is performed primarily extrahepatically, and the participation of the extrahepatical reticulo-endothelial system is of a conservative nature, thus denying us any willingness to agree to the theory of bilirubin production in the reticulo-endothelial system.

4. On administration of hemolysed blood, bile pigments in bile demonstrate a remarkable increase, while as compared when injected into the auricle veins in cases of administration through the portal vein a decline in the functions of the liver reticulo-endothelial system is seen, causing a decrease in biliverdin amount. In the former modus of administration, an occasional stimulation of the liver reticulo-endothelial system is seen, causing reduction of biliverdin to bilirubin.

5. Concluding from these facts, biliverdin in rabbit bile occupies the role of an intermediate product in the production and metabolism of bilirubin.

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