吸光度法によるブリリアントグリーン染色Heinz小体の測定

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Attempt of Spectrophotometric Determination of Heinz Bodies Stained with Brilliant Green

Eri OGAWA, Chizuru ŌTA, Hiroshi FUJISE, and Kosaku KOBAYASHI[1)]

Department of Pathology II, and [1)]Internal Medicine II, School of Veterinary Medicine, Azabu University, Fuchinobe, Sagamihara 229, Japan

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The Heinz body (HzB) is defined as the aggregation of denatured hemoglobin, appearing in red blood cells during the process of hemolytic anemia caused by various oxidant drugs or chemicals [3, 5, 6, 7, 8]. Degree of HzB formation is generally estimated by HzB counts as % of red cells containing HzB in the blood films stained supravitally by several methods [2, 4, 9, 10, 11]. Brilliant green stain is specific to HzB or various forms of irreversively denatured hemoglobin, and the procedure is simple [10]. The value of HzB counts serves as a definite marker in diagnosis of oxidative hemolysis and its severity. However, it does not actually refer to the amount of denatured hemoglobin, as it simply shows the % of red cells carrying HzB, regardless of number or size of inclusion bodies. We have observed that dogs with onion poisoning had two or three large inclusion bodies, while dogs with phenylhydrazine poisoning showed numerous fine particles in each cell. In both cases, we have no idea to decide which agent causes more damage to hemoglobin. Asakura and co-workers have estimated denatured hemoglobin by measuring the absorbance of hemichrome at 280 and 414nm [1]. However, in our examination, these peaks were not specific to hemichrome, and interfered with stromal protein and the existence of remaining oxyhemoglobin in the system. The present work deals with our attempt to determine denatured hemoglobin by measuring the brilliant green-stained HzB with a spectrophotometer, and the results were compared with that of classical HzB counts.

Four dogs (either sex) weighing 8–10kg were used. Two were given p.o. the onion soup equivalent to 5g of raw onions per kg of body weight once a day for 3 successive days, while the other two received a single i.v. injection of phenylhydrazine hydrochloride (40 mg/kg of body weight). Venous blood was collected into heparinized tubes, and HzB counts were routinely performed. Separately, a 0.2m*l* of blood sample was allowed to hemolyze in 5m*l* of distilled water and centrifuged at 7,800×g for 10 min. The precipitate including stroma and HzB was mixed well with 50μ*l* of 0.05% brilliant green in 1% saline, followed by three washes in distilled water. The stained precipitate was dissolved thoroughly by adding 1m*l* 30% acetic acid, 1m*l* ethanol, and 2m*l* 1/15 M phosphate buffer (pH6.6), successively. The solution containing stained-HzB showed two strong peaks at 404nm and 625nm (Fig. 1). On the other hand, brilliant green alone had a single peak at 625nm, and the stroma not stained showed a Soret band of 404nm with the remaining hemoglobin and denatured hemoglobin attached to the membrane (not shown). A peak around 630nm expected from the presence of denatured hemoglobin or methemoglobin was not seen, probably due to its small extinction coefficient (not shown). Next, red cells showing 100% HzB counts from a phenylhydrazine-injected dog were diluted stepwise with normal red cells, and the brilliant green-stained hemoglobin was measured as described above. The decline of absorbance at 625nm was proportional to the dilution (Fig. 1), indicating that a peak at 625nm is characteristic of the brilliant green-stained HzB or denatured hemoglobin, and reflects its actual amount.

Dogs given the onion soup had a sudden increase of HzB counts, reaching the peak nearly

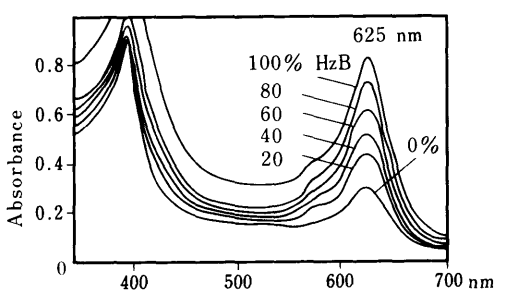

Fig. 1. Absorption spectra of solutions containing the brilliant green-stained HzB at various dilutions. The spectra were measured against water with a Hitachi Spectrophotometer model 220.

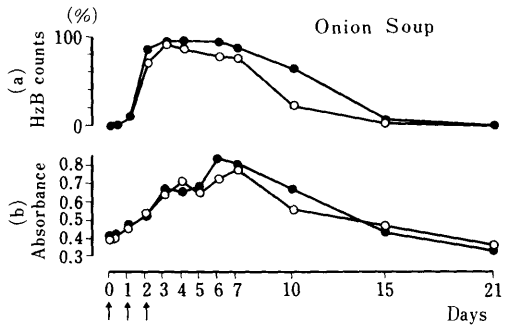

Fig. 2. Changes of (a) HzB counts and (b) absorbance of the brilliant green-stained HzB in two dogs (● and ○) given p.o. the onion soup.

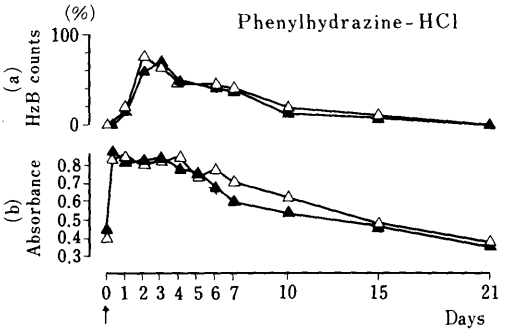

Fig. 3. Changes of (a) HzB counts and (b) absorbance of the brilliant green-stained HzB in two dogs (▲ and △) injected i.v. with phenylhydrazine-HCl.

100% on day 2 or 3 after administration, but only a few fine particles in each cell (Fig. 2a). During the following 4 days, these inclusion bodies developed larger in size and more in number. The absorbance of the solution containing stained-HzB, however, increased gradually from day 1 towards the peak of inclusion body development on day 6 or 7 (Fig. 2b). The absorbance appeared to reflect the amount of denatured hemoglobin, and its relatively slow increase was probably associated with the gradual and continuous effect of the onion soup. It was clearly demonstrated that the hemoglobin denaturation was progressive, in spite that the HzB counts were plateau and onion feeding had been stopped.

Injection of phenylhydrazine produced a different pattern of HzB formation from onion soup administration. HzB was not seen in the blood films until 24 h, while the absorbance of the solution containing stained-HzB demonstrated a sharp increase reaching the peak at 4 h after the injection (Fig. 3). Nearly 80% of each cell had numerous fine particles on day 2 or 3 which gradually developed larger in size during the following 5 days, but the absorbance of the stained-HzB remained fairly constant. It is contrasted to the HzB formation in onion poisoning which showed the parallel relationship between absorbance and actual amount of denatured hemoglobin. The feature of HzB formation in phenylhydrazine poisoning can be accounted for by the acute and direct effect of phenylhydrazine on the red cells, poducing a lot of fine particles which aggregate and grow larger without further denaturation of hemoglobin. Although the fine inclusion bodies at very early stage were too small to be seen for the counting, strong denaturation of hemoglobin was clearly demonstrated by a marked increase of the absorbance at 625nm.

Our present work has shown that the absorbance of stained-HzB reflected adequately the denaturation of hemoglobin. Our method is not satisfactory for a routine use, and also requires to exhibit the extinction coefficient of the stained pigment. However, the fluctuation in absorbance of the stained-HzB may display the practical change of denatured hemoglobin amount during HzB anemias. Moreover, this method provides us a parameter for the estimation and comparison of morphologically different HzBs resulting from various causes.

REFERENCES

1. Asakura, T., Minakata, K., Adachi, K., Russell, M. O., and Schwartz, E. 1977. *J. Clin. Invest.* 59: 633–640.
2. Beutler, E. 1983. pp. 1603. In: *Hematology*, 3rd ed., (William, W. J., Beutler, E., Erslev, A. J., and Lichtman, M. A. eds.), McGraw Hill Book Co. New York.
3. Beutler, E. 1969. *Pharmacological Reviews* 21: 73–103.
4. Dacie, L. V., and Lewis, S. M. 1975. pp. 140–141. In:*Practical Haematology*, 5th ed. Churchill Livingstone, Edinburgh London and New York.
5. Harley, J. D., and Mauer, M. 1960. *Blood* 16: 1722–1735.
6. Harley, J. D., and Mauer, M. 1961. *Blood* 17: 418–433.
7. Jandl, J. H. Engle, L. K., and Allen, D. W. 1960. *J. Clin. Invest.* 39: 1818–1936.

8. Rifkind R. A., and Danon, D. 1965. *Blood* 25: 885–896.
9. Schalm, O. W. 1964. *J. Am. Vet. Med. Assoc.* 145: 1184–1188.
10. Schwab, M. L., and Lewis, A. E. 1969. *Am. J. Clin. Path.* 51: 673–675.
11. Tanabe, T. 1978. *J. Tokyo Med. Coll.* 36: 603–617 (in Japanese).

要　　約

吸光度法によるブリリアントグリーン染色 Heinz 小体の測定(短報)：小川絵里・太田ちづる・藤瀬　浩・小林好作[1](麻布大学獣医学部病理学第2講座，[1]内科学第2講座)―― 溶血後の遠心沈渣をブリリアントグリーンで超生体染色し，可溶化後625nm で吸光度を測定する方法により，タマネギ煮汁の経口投与またはフェニルヒドラジンの静脈注射により惹起した溶血性貧血犬の赤血球を経時的に観察したところ，従来の方法に比べて，ヘモグロビン変性量をより実質的に反映する成績が得られた.