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Study of Mouse Hemoglobin by Starch-Gel Electrophoresis *

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The mouse hemoglobins were first demonstrated to exist in two distinct forms by Ranney and Gluechkson-Waelsch (1). Their observations were confirmed by Russell and Gerald (2), who classified mouse hemoglobins into single and diffuse types, using starch-block electrophoresis in 20 strains of inbred mice. Rosa, et al. (3) studied hemoglobins by starch-gel electrophoresis in 6 inbred strains of mice, and observed that the mice could be differentiated into 4 sub-groups on the basis of the hemoglobin pattern. Morton (4) reported 5 bands in a diffuse type of mouse hemoglobin, using starch-gel electrophoresis, and noted that the band 2 of the diffuse hemoglobin corresponded with the single hemoglobin type. More recently, Hutton, et al. (5) demonstrated that the mice with diffuse pattern have two distinct hemoglobins with different beta chains, while the mice with single pattern have only one kind of hemoglobin. They identified six chemically distinct hemoglobins in four inbred strains of mice. Studies on mouse fetal hemoglobin have shown that the electrophoretic pattern of early fetal hemoglobin differs from that of the adult hemoglobin, while late fetal hemoglobin and adult mouse hemoglobin have the same electrophoretic pattern (6, 7, 8).

Material and methods

Eighteen strains of inbred mice — C57BL/10, C57BL/6, C57BR/cd, C57L, C58, SWR, SEC/1Re, AKR, DBA/1, DBA/2, CBA, C3H/He, C3HeB/Fe, Fl/1Re, RF, 129, A and A/He, obtained from the Jackson Memoral Laboratories, Bar Harbor,

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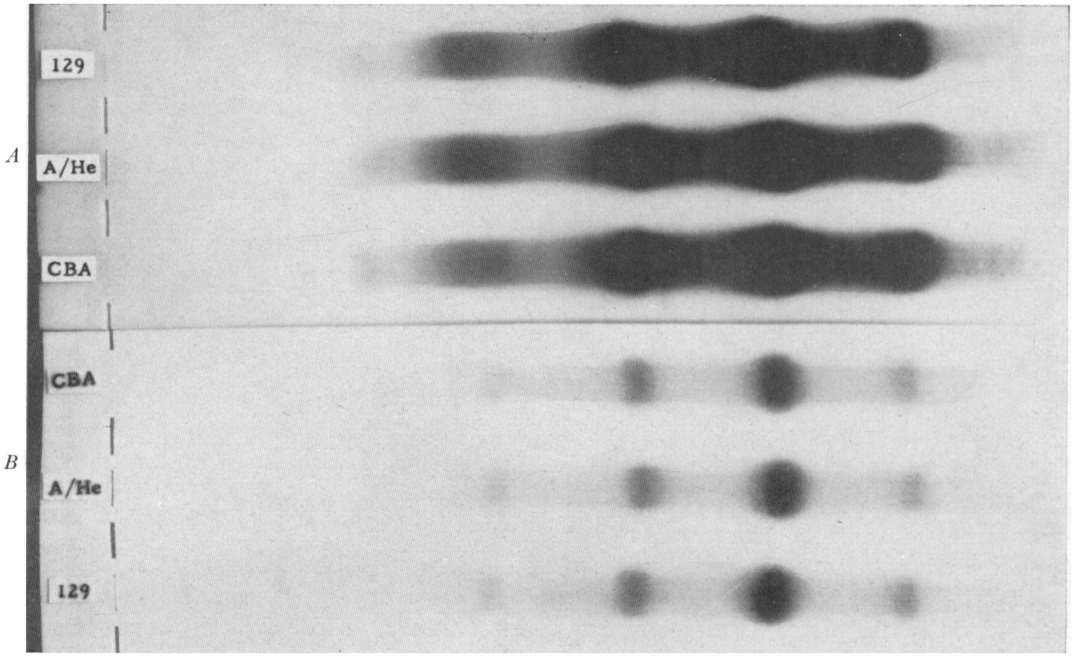

Fig. 1. The starch-gel electrophoretic pattern of hemoglobin of 129, A/He, and CBA strains. Two sections of the gel are shown. *Section A* was stained with benzidine and hydrogen peroxide and *Section B* was stained with amido-black 10B

Maine, were used in the present study. The hemolysates were prepared according to the technique of Smith (9), and were used in a concentration of 1-2 gm. per cent within 24 hours of blood collection. The horizontal and vertical starch-gel electrophoresis was performed using Tris-EDTA buffer, pH 8. 6., originated by Dr. O. Smithies and modified by Dr. S. H. Boyer (personal communication by S. H. Boyer). A stock buffer containing 109 gm 2-amino-2(hydroxy-methyl)-1,3-propanediol, 5.84 gm ethylenediamine tetracetic acid and 30.9 gm boric acid, was prepared. Dilutions 1:7, 1:5, and 1:20 of the above stock buffer were used in cathode tank, anode tank, and in preparation of starch-gel, respectively. The electrophoresis was carried out at 350V for 14-16 hours, at 4°C. After sectioning the gel, one-half was stained with benzidine and hydrogen peroxide (Smithies (10)), while the other half was stained with amido-black 10B. Hemoglobin of late fetuses (more than 2 weeks old), newborn, and young animals at different ages, was also studied, and compared with that of the adult mice.

Results and conclusions

The results were essentially the same with both horizontal and vertical gels, except for the wider separation of the bands in the latter. The hemoglobin pattern in 7 strains — C57BL/10, C57BL/6, C57BR/cd, C57L, C58, SWR, and SEC/1Re — was found to be single-band type. In earlier experiments (11), five components were observed in the remaining 11 strains — AKR, DBA/1, DBA/2, CBA, C3H/He, C3HeB/Fe, Fl/1Re, RF, 129, A, and A/He. When electrophoretic run was carried

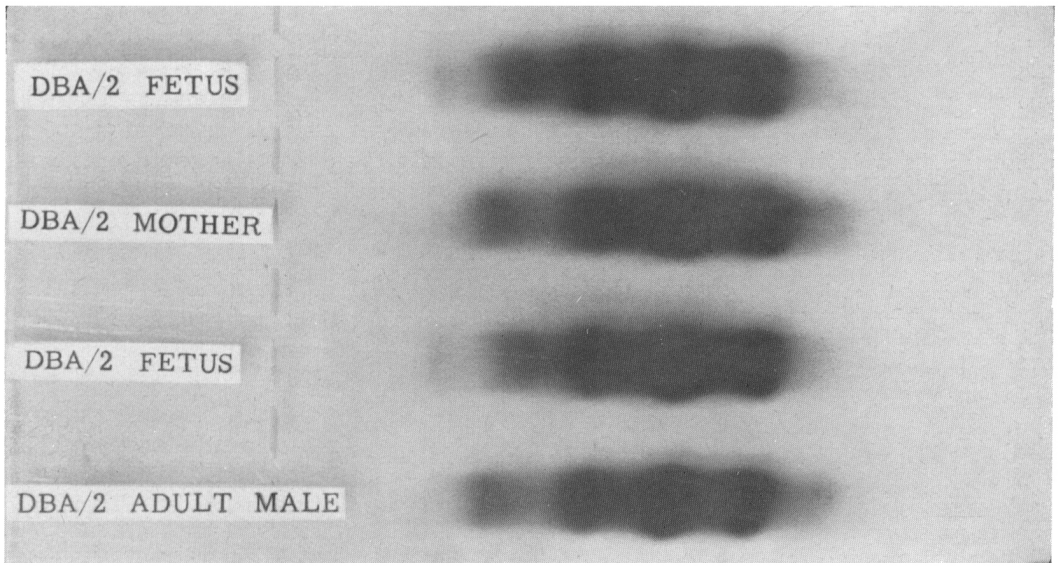

Fig. 2. The starch-gel electrophoretic pattern of hemoglobin of DBA/2 foetus, mother, and adult male. The hemoglobin concentration in the hemolysates was the same (1 gm per cent)

out for 20 hours, the fourth band was actually found to be composed of two bands. Under these conditions, 6 components were consistently obtained in the above 11 strains (Fig. 1). No difference in the electrophoretic mobility of the hemoglobin band in the strain with single band and of the different bands in the strains with 6 components could be detected. The findings support the classification of Russell and Gerald (2), as opposed to that of Rosa et al (3). In the strains with 6 components, band 2 was the strongest and corresponded with the hemoglobin band of " single " type strains. Bands 4 and 5 were weak, the sixth being the weakest one in contrast to the observations of Morton (4), who found that the fourth band was the weakest.

The hemoglobin pattern of C57BL/6 foetus and newborn was found to be identical to that of the adult animal, while in two strains with multiple components

— AKR and DBA/2 — the sixth band was found to stain more intensely in the foetus and newborn (Fig. 2), as compared with the corresponding band in the mother or other adult animals of the same strain.

Summary

The hemoglobin pattern of 18 inbred strains of mice was studied by starch-gel electrophoresis. In 7 strains (C57BL/10, C57BL/6, C57BR/cd, C57L, C58, SWR, and SEC/1Re) the electrophoretic pattern was found to be of single-band type: in the remaining 11 strains (AKR, DBA/1, DBA/2, CBA, C3H/He, C3HeB/Fe, Fl/1Re, RF, 129, A, and A/He), 6 components were constantly observed when the electrophoretic run was carried out for 20 hours. Hemoglobin from late C57BL/6 fetuses showed an electrophoretic pattern identical to that of the adult animal. Hemoglobin from AKR and DBA/2 late fetuses and newborns showed an electrophoretic pattern similar to that of the adult animal, but the slowest band was more intensely stained as compared with the corresponding band of the adult animal.

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RIASSUNTO

L'emoglobina di 18 ceppi puri di topi è stata studiata mediante elettroforesi su gel d'amido. In 7 ceppi (C57BL/10, C57BL/6, C57BR/cd, C57L, C58, SWR e SEC/1Re) il tracciato elettroforetico era costituito da un'unica banda; nei rimanenti 11 ceppi (AKR, DBA/1, DBA/2, CBA, C3H/He, C3HeB/Fe, Fl/1Re, RF, 129, A, e A/He) il tracciato, ottenuto dopo 20 ore di separazione elettroforetica, presentava sei bande.

Il tracciato elettroforetico dell'emoglobina di feti C57BL/6, prossimi al termine della vita fetale, era identico a quello degli animali adulti. Nei ceppi AKR e DBA/2 il tracciato dell'emoglobina dei feti prossimi al termine e dei neonati era simile a quello degli adulti, con la sola eccezione della banda più lenta, che appariva colorata più intensamente nei tracciati dei feti e dei neonati rispetto a quelli degli adulti.

RÉSUMÉ

L'hémoglobine de 18 lignées pures de souris a été étudiée moyennant l'électrophorèse sur gel d'amidon. Chez 7 lignées (C57BL/10, C57BL/6, C57BR/Cd, C57L, C58, SWR e SEC/1Re) le tracé électrophorétique était composé par une seule bande; tandis que chez les 11 lignées restantes (AKR, DBA/1, DBA/2, CBA, C3H/He, C3HeB/Fe, F1/IRe, RF, 129, A e A/He) le tracé, obtenu après 20 heures de séparation électrophorétique, présentait 6 bandes.

Le tracé électrophorétique de l'hémoglobine de fétus C57BC/6, approchant le terme de la vie fétale, était identique à celui des animaux adultes. Chez les lignées AKR et DBA/2 le tracé de l'hémoglobine des fétus approchant le terme et des nouveaux-nés se rapprochait de celui des adultes, à l'exception de la bande la plus lente qui paraissait colorée plus intensivement dans les tracés des fétus et des nouveaux-nés que dans ceux des adultes.

ZUSAMMENFASSUNG

Es wurde das Hb von 18 reinen Mäusestämmen durch Elektrophorese auf Stärkegel untersucht. Bei 7 Stämmen (C57BL/10, C57BL/6, C57BR/Cd, C57L, C58, SWR und SEC/1Re) bestand die elektrophoretische Kurve aus nur einem Streifen; bei den übrigen 11 Stämmen (AKR, DBA/1, DBA/2, CBA, C3H/He, C3HeB/Fe, Fl/1RE, RF, 129, A und A/He) bastand die nach 20—stündiger elektrophoretischer Trennung erhaltene Kurve aus sechs Streifen.

Die elektrophoretische Kurve des Hb der Föten C57BL/6, die bald am Ende ihres Fötaldaseins angelangt waren, verlief genau wie die der erwachsenen Tiere. Bei den Stämmen AKR und DBA/2 war die Kurve des Hb der fast wurfreifen Föten und der neugeborenen Tiere ähnlich der Kurve der Erwachsenen, mit Ausnahme des langsameren Streifens, der in den Kurven der Föten und der Neugeborenen deutlicher gefärbt erschien als bei den erwachsenen Tieren.

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