DNA can cross the brain barrier and reach the brain cells without losing its primary and secondary structures. Indeed after *A. tumefaciens* [$^3$H]DNA has been injected intraperitoneally, an important part of the radioactive molecules of the DNA extracted from the brains sediment at the level of the bacterial DNA after CsCl gradient centrifugation. Autoradiographs show that the radioactivity is located mainly in the nuclei. The foreign [$^3$H]RNA injected is probably completely broken down and reutilized for synthesis since after extraction of the [$^3$H]RNA in the brain no bacterial RNA can be traced.

Our results show that the bacterial RNA extracted comes from the brain cells and not from contaminating bacteria. The following observations support this argument. The brains extracted from the infected frogs are sterile as shown by plating. This is confirmed by autoradiography, which shows no labeled bacteria even though it is difficult by this technique to score bacteria when there are less than $3 \times 10^3$ bacteria per brain. If we take into consideration only the autoradiographs and, for the sake of the argument, admit that we have missed $2 \times 10^3$ bacteria per brain, this amount could not account for the bacterial RNA found in the animals. Indeed, if, before RNA extraction, $10^7$ bacteria labeled in vitro with [$^3$H]uridine (for 3 hours in the presence of brain tissues in Ringer solution) are added to five brains of control frogs labeled in vivo with [$^3$H]uridine, no bacterial RNA can be detected by our hybridization method (the total amount of RNA contained in $10^7$ bacteria is negligible compared to the bulk of the animal RNA). Apparently the bacterial RNA in the brain cells depends on the capacity of these cells to synthesize their own RNA. Indeed when the brains of the infected frogs are labeled in vitro, the amount of bacterial RNA recovered from the brain cells decreases while the synthesis of frog RNA is lowered in the same cells. This is also an indication that the bacterial RNA extracted does not come from bacteria.

The bacterial RNA is synthesized directly in the brain cells. Indeed brains of infected frogs labeled in vitro still synthesize bacterial RNA. Under these conditions the labeled bacterial RNA cannot be transferred from labeled bacteria in the circulatory blood system into the brain cells. Besides, the bacterial RNA does not seem to be able to reach the cells before being broken down. Moreover, bacterial RNA is synthesized in frog auricles that have been in direct contact with the bacteria-free supernatant of *B. subtilis* (*16*). It is difficult to conceive of the same experiment with brains which survive in vitro with difficulty.

Purified bacterial DNA is not transcribed once injected, even though it readily penetrates the brain cells without losing its primary and secondary structures. Bacterial DNA can be transcribed in plant or animal cells only if it is accompanied by its own DNA-dependent RNA polymerase (*2, 16*) as seems to be the case when it is spontaneously released by living bacteria.

These results suggest that, after a bacterial infection, even an organ which has a protective barrier against bacteria can be reached by their informative molecules. The pathological or neurophysiological implications of these results are still difficult to evaluate.

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17. We thank Dr. J. J. Dreifuss (Institut de Physiologie, Ecole de Médecine, Université de Genève) and Dr. M. Buscaglia (Laboratoire d'Endocrinologie, Université de Genève) for their advice concerning brain physiology and anatomy. We thank Miss J. Henri, Miss. A. Cattaneo, and Miss F. Devaud for technical assistance. Supported by a grant from the Fonds National Suisse de la Recherche Scientifique.

27 December 1971; revised 25 September 1972 ■

## Hemoglobin Lepore Trait: Globin Synthesis in Bone Marrow and Peripheral Blood

Abstract. *There was decreased synthesis of the β-globin chain in the peripheral blood, and equal synthesis of α- and non–α-chains in the bone marrow of three patients with hemoglobin Lepore trait, similar to the findings in patients with heterozygous β-thalassemia. There is a relative instability of the synthetic mechanism for normal β-chain in these patients.*

Hemoglobin Lepore (Hb Lepore) is a structurally abnormal hemoglobin composed of two normal α-chains, and two chains containing an $NH_2$-terminal portion with the amino acid sequence of δ-chains, and a COOH-terminal portion with the sequence of β-chains (*1, 2*). The Lepore globin chains presumably arose from nonhomologous pairing and subsequent crossing-over between the genes for δ- and β-chains. Three different types of Lepore globin have been described which differ in the region of crossing-over: Hb Lepore Hollandia (*2, 3*) Hb Lepore Baltimore (*4*), and Hb Lepore Boston (*1, 5*). With Hb Lepore trait there is mild anemia, hypochromia, and microcytosis, similar to that seen in heterozygous β-thalassemia, while with homozygous Hb Lepore there is a severe anemic disorder similar to that with homozygous β-thalassemia. These clinical observations indicate that the synthesis of the Lepore chain is markedly decreased, as compared to that of α-chain.

There is a reduced synthesis of structurally normal β-chain in patients with β-thalassemia. In the peripheral blood of patients with heterozygous β-thalassemia, the amount of radioactive amino acid incorporated into the β-chain is approximately one-half that incorporated into the α-chain; in homozygotes, incorporation into β-chains is less than one-fourth that incorporated into α-chains (*6, 7*). A decrease in β-chain synthesis is also found in the bone marrow cells of patients with homozygous β-thalassemia (*8*). In con-

Table 1. Hematologic parameters and synthesis of globin chains in patients with Hb Lepore trait.

| Patient | Hemoglobin (g/100 ml) | Reticulocytes (%) | Hb Lepore (%) | Peripheral blood | | Bone marrow | |
|---|---|---|---|---|---|---|---|
| | | | | Radioactivity ($\beta$ + Lepore)/$\alpha$ | Specific activity $\beta/\alpha$ | Radioactivity ($\beta$ + Lepore)/$\alpha$ | Specific activity $\beta/\alpha$ |
| M.D. | 11.4 | 4.6 | 6.5 | 0.55 | 0.55 | 1.07 | 0.94 |
| C.C. | 12.6 | 1.9 | 4.3 | 0.64 | 0.57 | 0.94 | 0.84 |
| A.S. | 10.6 | 7.7 | 4.7 | 0.39 | 0.39 | 0.85 | 0.78 |

trast, the bone marrow of patients with heterozygous $\beta$-thalassemia synthesizes $\beta$-chains at a rate equal to that of $\alpha$-chains (*9*). This finding indicates that in heterozygous $\beta$-thalassemia balanced globin synthesis may occur in the nucleated red cells despite the presence of a $\beta$-thalassemia gene, but that there is unusually rapid decay of $\beta$-chain synthesis relative to that of $\alpha$-chain synthesis in the reticulocytes of these patients. We report here on studies of three patients with Hb Lepore trait, indicating that the phenomena of balanced globin synthesis in the marrow and instability of $\beta$-chain synthesis in the peripheral blood may occur in association with structurally abnormal hemoglobins as well as in patients with heterozygous $\beta$-thalassemia. In addition, the results show that the ability to synthesize the normal $\beta$-chain decreases more rapidly than that for $\alpha$-chain during red cell maturation in patients with Hb Lepore trait. The ratio of $\alpha$-chain to non-$\alpha$-chain synthesis is close to one in bone marrow cells of patients with impaired globin synthesis due to one of the non-$\alpha$-alleles. This compensation in the bone marrow is mainly achieved by increased synthesis of $\beta$-chains directed by the normal $\beta$-allele.

The three patients in the present study are from two southern Italian families. In one family, several patients in three generations had Hb Lepore, including a child heterozygous for $\beta$-thalassemia and Hb Lepore. A brother and sister from this family are included in the present study. In a second family, only the patient with Hb Lepore trait was studied. Each of the three patients had hypochromia, microcytosis, and decreased levels of Hb $A_2$. The abnormal hemoglobins from each of these patients were similar to each other in mobility on starch gel at *p*H 8.6, and the globin chains had identical mobilities in urea starch gel at *p*H 8.9. The tryptic peptide map of the abnormal globin chain in each family was similar to that of Hb Lepore Boston (*10*). Hemoglobin Lepore Boston is the only form of Hb Lepore that has been found in southern Italy (*11*)

Samples of peripheral blood and bone marrow from each patient were incubated with L-[$^{14}$C]leucine for 2 hours. Red cells were washed and lyzed, and the $\alpha$- and $\beta$-chains were separated by chromatography on carboxymethyl cellulose in 8*M* urea (*12, 13*). The absorbance of each fraction was determined at 280 nm; radioactivity was measured in a liquid scintillation spectrometer. The radioactivity of each chain was determined by totaling the radioactivities of the tubes containing that chain. The specific activities for the $\beta$- and $\alpha$-chains were determined from the tubes containing the material that produced the peaks in the graphs of absorbance and radioactivity (Fig. 1). The activities were calculated by dividing the number of counts per minute by the absorbance, after correcting for the difference in extinction coefficients of the two chains (*12, 13*). We did not determine the specific activity of Lepore globin because the small volume of the bone marrow samples did not allow separation of sufficient quantities of this protein for accurate comparisons.

The separation of globin chains from peripheral blood and bone marrow in one patient is shown in Fig. 1. The results of the studies in the three patients are summarized in Table 1. The amount of radioactivity in the marrow samples of the patients was 6 to 12 times that found in the peripheral blood samples prepared at the same time. In each patient there was a decrease in the amount of radioactive amino acid incorporated into $\beta$-chains relative to that incorporated into $\alpha$-chains in the peripheral blood, as indicated by ($\beta$ + Lepore)/$\alpha$ radioactivity ratios. These ratios are similar to those which have been found in Italian patients with $\beta$-thalassemia trait by us [0.57 ± 0.08 (1 standard deviation, S.D.)] and by others (*7, 13–15*). In control patients, the $\beta/\alpha$ chain ratio in the peripheral blood was 0.99 ± 0.05 (1 S.D.). In contrast, in each of the three bone marrow samples, the ($\beta$ + Lepore)/$\alpha$ ratio was in the range of the $\beta/\alpha$ ratio found in normal persons and in the bone marrows of patients with $\beta$-thalassemia trait (*9*). The ratios of specific activities of $\beta/\alpha$ chains are in agreement with these findings. Thus, the results described in the three patients with Hb Lepore trait are identical to those seen in patients with heterozygous $\beta$-thalassemia.

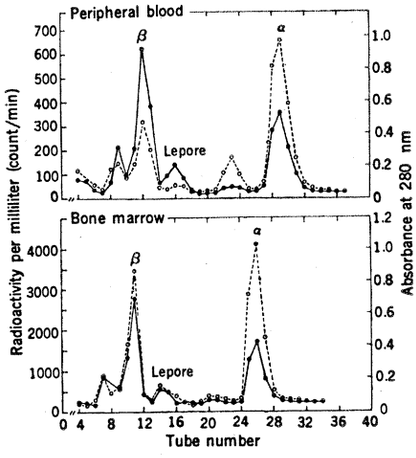


Fig. 1. Separation of globin chains from peripheral blood and bone marrow of patient A.S. by chromatography on carboxymethyl cellulose in 8*M* urea at *p*H 6.7. The point of elution of each chain was confirmed by conductivity measurements. Absorbancy is uncorrected. Solid line, absorbance; dotted line, radioactivity.

In one previous study of purified hemoglobin from two patients with Hb Lepore trait, no imbalance of globin synthesis was found in the peripheral blood (*15*). However, a recent study of one Turkish-Cypriot patient with Hb Lepore Boston showed that the ratio of radioactivity incorporation of ($\beta^A$ + Lepore)/$\alpha$ in the peripheral blood was approximately 0.5 (*16*).

The findings in the three patients described here indicate that the discrepancy between balanced globin synthesis in bone marrow red cells and unbalanced globin synthesis in reticulocytes in patients with $\beta$-thalassemia trait is a phenomenon that is not unique to patients with classical thalassemia. This phenomenon depends only on the presence of a gene causing marked underproduction of $\beta$-chains. The presence of moderately decreased mean cell hemoglobin (MCH) of 20.3, 20.9, and

23.0 pg/cell in the patients with Hb Lepore trait, and the presence of similar amounts in patients with β-thalassemia trait indicates that increased synthesis of β-chains and decreased synthesis of α-chains are both important in achieving balanced globin synthesis in the bone marrow. The observation of a decreased ratio of β/α chains in the peripheral blood and equal synthesis of β- and α-chains in the marrow is compatible with a lack of stability of normal β-chain production in these patients, perhaps due to an unstable messenger RNA (mRNA) produced in excess by the normal β gene. These findings are similar to those in patients with sickle–β-thalassemia where the rate of production of $\beta^S$ decreases more rapidly than that of α-chains as the red cell matures (*17*). The structurally abnormal $\beta^S$ chain serves as a marker for the production of globin chain by the "normal" β-chain gene in these patients. The total globin synthesis in the cells of patients with Hb Lepore trait, β-thalassemia trait, and sickle-thalassemia is not restored to normal, as hypochromia and microcytosis were evident in all patients we studied in these groups.

Decreased synthesis of Lepore globin chains may be related to the fact that the initial sequence is derived from the δ-chain gene. Delta chains are present in normal individuals in amounts that are about 1/40 those of β-chains. Each chain is coded at only one locus on the DNA molecule, but δ-chain synthesis may be slower than that of the β-chain once the chains have been initiated (*18, 19*). In addition, the synthesis of the δ-chain decreases markedly between the time the cell is present in bone marrow, and the time the cell becomes a reticulocyte, possibly due to an unstable mRNA for δ-chain (*19, 20*). In the three patients studied here, there was 4.3 to 6.5 percent Hb Lepore in the peripheral blood, despite the fact that in two patients synthesis of Hb Lepore could not be detected by incorporation of radioactivity into Lepore globin in the peripheral blood, and in the third patient only minimal synthesis was detected. In contrast, synthesis of Lepore chains was detected in the bone marrow in each patient. The synthesis of Lepore globin appears to be similar in this respect to that of the δ-chain.

The mechanisms for achieving balanced globin chain synthesis in the nucleated red cell suggested by the experiments described here may be representative of control mechanisms for protein synthesis in other cells of the body. The direct measurement of instability of mRNA in human cells must await the development of an accurate assay of mRNA.

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21. This work was supported in part by grants AM 12896, AM 05212, and H 6374 from the U.S. Public Health Service and a grant from the Cooley's Anemia Foundation. We thank V. Watson for technical assistance.
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12 June 1972; revised 21 August 1972

## Breeding Cycle of the Flea Cediopsylla simplex Is Controlled by Breeding Cycle of Host

Abstract. *Maturation of female* Cediopsylla simplex *takes place on the pregnant rabbit and nestlings but not on the estrous doe. If matured fleas are transferred to the estrous doe their ovaries are resorbed.*

Unfed male and female specimens of *Cediopsylla simplex* Baker, obtained as larvae from the nest of cottontail rabbits (*Silvilagus floridanus*), were released onto domestic rabbits (New Zealand Whites). We found that this species of flea undergoes maturation on the pregnant doe but not on the estrous doe. Female fleas that are moved onto a rabbit during the last week of her pregnancy mature and develop chorionated eggs within 72 hours; if they are returned to an estrous doe their ovaries regress rapidly and the developing oocytes are resorbed. If fleas are again transferred directly to newborn nestlings when regression is complete, the ovaries mature again and chorionated eggs develop. Copulation occurs on the body of newborn young 16 hours after transfer and egg-laying follows.

*Cediopsylla simplex* therefore appears to have an essentially similar life cycle to that of the European rabbit flea *Spilopsyllus cuniculi* Dale, which is also linked to and dependent upon the sex cycle of the host. The unmatured flea also produces yolk-laden oocytes if cortisone is sprayed on externally while it is feeding on the estrous doe. The unfed female responds faster to the concentration of hormones in the blood of the pregnant doe than does *S. cuniculi* (*1*), but the time lag between transfer to the day-old rabbits and copulation (*2*) may be slightly longer. The unfed *C. simplex* did not fix to the ears when first introduced to the host but ran freely in the fur on the head, face, and neck of the adult rabbit; on the newborn young it dispersed all over the body and did not concentrate for feeding in the sacral region. It is more active and a better jumper than *S. cuniculi*.

It was previously suggested (*3*) that other "hormone-bound" fleas might occur among related genera parasitizing rabbits and hares in the New World. That this is now shown to be the case indicates that the specialization in question must be of considerable antiquity.

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