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 β^S decreases more rapidly than that of α-chains as the red cell matures (*17*). The structurally abnormal β^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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References and Notes

1. C. Baglioni, *Proc. Nat. Acad. Sci. U.S.A.* **48**, 1880 (1962).
2. J. Barnabas and C. J. Muller, *Nature* **194**, 931 (1962).
3. C. C. Curtain, *Aust. J. Exp. Biol. Med. Sci.* **42**, 89 (1964).
4. W. Ostertag and E. W. Smith, *Eur. J. Biochem.* **10**, 371 (1969).
5. D. Labie, W. A. Schroeder, T. H. J. Huisman, *Biochim. Biophys. Acta* **127**, 428 (1966); C. Baglioni and V. Ventruto, *Eur. J. Biochem.* **5**, 29 (1968).
6. D. Heywood, M. Karon, S. Weissman, *Science* **146**, 520 (1964); D. J. Weatherall, J. B. Clegg, M. A. Naughton, *Nature* **208**, 1061 (1965); F. Conconi, A. Bargellesi, S. Pontremoli, V. Vigi, S. Volpato, D. Gaburro, *ibid.* **217**, 259 (1968).
7. A. Bank and P. A. Marks, *Nature* **212**, 1198 (1966).
8. A. S. Braverman and A. Bank, *J. Mol. Biol.* **42**, 57 (1969).
9. E. Schwartz, *Science* **167**, 1513 (1970).
10. V. M. Ingram, *Biochim. Biophys. Acta* **28**, 539 (1958); C. Baglioni, *ibid.* **48**, 392 (1961).
11. N. Quattrin, P. Bianchi, R. Cimino, L. DeRosa, E. Dini, V. Ventruto, *Acta Haematol.* **37**, 266 (1967).
12. J. B. Clegg, M. A. Naughton, D. J. Weatherall, *Nature* **207**, 945 (1967); Y. W. Kan, E. Schwartz, D. G. Nathan, *J. Clin. Invest.* **47**, 2515 (1968).
13. E. Schwartz, *N. Engl. J. Med.* **281**, 1327 (1969).
14. Y. W. Kan and D. G. Nathan, *J. Clin. Invest.* **49**, 635 (1970).
15. S. M. Weissman, I. Jeffries, M. Karon, *J. Lab. Clin. Med.* **69**, 183 (1967).
16. J. M. White, A. Long, P. A. Lorkin, H. Lehmann, J. Reeve, *Nature New Biol.* **235**, 208 (1972).
17. F. Gill and E. Schwartz, *Clin. Res.* **20**, 470 (1972).
18. H. H. Kazazian, Jr., and H. A. Itano, *J. Biol. Chem.* **243**, 2048 (1968).
19. R. M. Winslow and V. M. Ingram, *ibid.* **241**, 1144 1(966).
20. R. F. Rieder and D. J. Weatherall, *J. Clin. Invest.* **44**, 42 (1965); A. V. Roberts, D. J. Weatherall, J. B. Clegg, *Biochem. Biophys. Res. Commun.* **47**, 81 (1972).
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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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References and Notes

1. M. Rothschild, B. Ford, M. Hughes, *Trans. Zool. Soc. London* **122**, 417 (1970).
2. M. Rothschild and B. Ford, *ibid.*, in press.
3. M. Rothschild, *Sci. Amer.* **213**, 44 (Dec. 1965).
4. We thank N. Wilson for sending us unfed specimens of *C. simplex* bred from nest material of *Sylvilagus floridanus* obtained in northern Iowa.

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Choline: High-Affinity Uptake by Rat Brain Synaptosomes

Abstract. *Synaptosomes from rat brain accumulate choline by two kinetically distinct processes, a high-affinity uptake system [Michaelis constant* (K_m) $= 1 \times 10^{-6}$M], *and a low-affinity system* ($K_m = 9 \times 10^{-5}$M). *The high-affinity uptake system requires sodium, and is associated with considerable formation of acetylcholine. The low-affinity uptake system is less dependent on sodium, and does not appear to be associated with a marked degree of acetylcholine formation. The high-affinity choline uptake appears to represent selective choline accumulation by cholinergic neurons.*

As it lacks the capacity to synthesize choline, the nervous system depends on choline uptake for cholinergic function (*1*). Choline transport systems in the kidney (*2*), erythrocytes (*3*), brain slices (*4*), and brain synaptosomes (*5*) have a relatively low affinity for choline, and have little associated acetylcholine formation, so that it is not clear whether the choline is accumulated selectively into cholinergic neurons. Recent reports that brain synaptosomes form a significant amount of acetylcholine when low concentrations of choline are present suggest that there may be more than one uptake system for choline (*6*).

We have demonstrated two kinetically distinct transport systems for choline into synaptosomes in the rat brain. A high-affinity uptake system, highly dependent upon the presence of sodium, is associated with considerable acetylcholine formation, while the low-affinity choline uptake system is much less dependent on sodium, and results in little acetylcholine formation (*7*).

The corpus striatum of male Sprague-Dawley rats (body weight, 150 to 200 g) was dissected (*8*), and homogenized in 30 volumes of ice-cold 0.32*M* sucrose containing $10^{-7}M$ Soman (pinacolyl-methylphosphorofluoridate) in a Potter-Elvehjem glass homogenizer fitted with a Teflon pestle. At the concentration employed, Soman completely inhibited acetylcholinesterase without influencing choline uptake. The homogenate mixture was centrifuged at 1,000*g* for 10 minutes, the pellet discarded, and the supernatant fluid centrifuged at 17,000*g* for 60 minutes. The resultant pellet was reconstituted to the original volume with 0.32*M* sucrose, and 0.1 ml of the tissue suspension was added to 1.9 ml of a Krebs-phosphate solution, *p*H 7.4, containing 11.1 m*M* glucose and varying concentrations of [^{3}H]choline (17 curie/mmole; Amersham/Searle). Under these incubation conditions, even if all the endogenous choline had leaked out of the particles and choline levels increased during incubation (*9*), the resultant concentration of choline in the medium would be only $1 \times 10^{-7}M$, and hence contribute much less than the exogenous [^{3}H]choline to the final concentration of choline in the medium. The mixture was incubated at 30°C for 4 minutes, after which 50 μl each of choline (0.4*M*) and neostigmine (0.04*M*) were added, and the mixture was centrifuged at 27,000*g* for 15 minutes. The large amount of nonradioactive choline terminated the reaction by diluting the [^{3}H]choline. Neostigmine was added to inactivate any cholinesterase that may have regenerated. After the pellets were washed with ice-cold 0.9 percent NaCl containing 1.0 m*M* neostigmine, they were centrifuged at 48,000*g* for 10 minutes and the accumulated radioactivity was extracted into Triton X-100 : toluene scintillation fluid, and assayed by liquid scintillation spectrometry.

The relative amounts of labeled choline, acetylcholine, betaine, and phosphorylcholine were determined by high-voltage paper electrophoresis (*10*). The amount of radioactivity increased linearly with time for at least 4 minutes at all concentrations of [^{3}H]choline employed, and with varying concentrations of brain tissue. When pellets obtained from these incubations were layered on continuous sucrose density gradients (0.32 to 1.5*M*) and centrifuged for 90 minutes (*11*), the radioactivity was localized to an area of the gradient rich in synaptosomes, and separable from the bulk of activity of monoamine oxidase, a mitochondrial marker. Hypotonic shock completely liberated the radioactivity found in particulates. These observations indicate that radioactivity accumulated after incubation with [^{3}H]choline was localized predominantly in the synaptosomal fraction. The homogenates can be prepared rapidly, and therefore presumably undergo less deterioration than do purified synaptosomes. As essentially all radioactivity accumulated by homogenates is localized in the synaptosomes, all kinetic studies, unless otherwise indicated, were performed with homogenates. The kinetics of uptake were determined by double-reciprocal plots (*12*), and by least-squares fitting of a substrate velocity curve to the data by computer programs; this provided the Michaelis constants (K_m) and their standard errors (*13*) (Fig. 1).

Crude synaptosomal preparations of the corpus striatum were incubated with concentrations of [^{3}H]choline varying from $5 \times 10^{-7}M$ to $2 \times 10^{-2}M$. Double-reciprocal plots of the accumulated radioactivity always resulted in curves (Fig. 2) that could be resolved into two components graphically, as well as by computer analysis, with a calculated K_m of $1.2 \times 10^{-6}M$ and about $9.4 \times 10^{-5}M$, for the high- and low-affinity uptake systems, respectively. We found a similar dual affinity uptake system for [^{3}H]choline in synaptosomal preparations of the rat cerebral cortex. After separating the crude synaptosomal preparation into synaptosomal and mitochondrial fractions (*14*), we found the high-affinity choline transport system localized in the synaptosomal fraction.

Electrophoretic analysis of the radioactivity accumulated in pellets of homogenates showed that at a [^{3}H]choline concentration of $5 \times 10^{-7}M$, 70 percent of accumulated radioactivity was present as acetylcholine. This proportion decreased progressively to 61 percent, 47 percent, 23 percent, and 16 percent at [^{3}H]choline concentrations of $1 \times 10^{-6}M$, $1 \times 10^{-5}M$, $5 \times 10^{-5}M$, and $1 \times 10^{-4}M$, respectively. Negligible amounts of [^{3}H]phosphorylcholine were present until concentrations of [^{3}H]choline in the medium reached $5 \times 10^{-5}M$ and $1 \times 10^{-4}M$. At these concentrations, phosphorylcholine accounted for 8 percent and 10 percent, respectively, of the accumulated radioactivity. No [^{3}H]betaine was detected