without competing amino acids or albumin, the uptake index is presumably unaffected by plasma NAA competitors or by the TRY binding capacity of albumin. Recent studies of the physiological regulation of brain TRY have focused on determining the relative importance of the latter two factors. Our data suggest that not only brain TRY but also brain methionine, tyrosine, phenylalanine, and histidine may be influenced by the activity of the blood-brain transport system.

It has been proposed that the NAA transport system of the blood-brain barrier transports the NAA's by facilitated diffusion into an extracellular fluid compartment (ECF), from which they are actively transported into brain cells (*5*). After PCA, increased capillary NAA transport would permit a greater influx of NAA's into the ECF, thus making the NAA's more available to the concentrative, active transport systems of the brain cells. The increased brain/plasma ratios of the NAA's after PCA thus probably do not reflect concentrative transport across the blood-brain barrier itself, but rather a decrease in the normal restrictions on blood-ECF transport. The relatively low brain/plasma ratios of leucine, isoleucine, and valine in both PCA and control animals may be explained if these amino acids are readily catabolized by brain. These results thus strikingly confirm the hypothesis that the rate-limiting factor in the availability of NAA's to the brain is the activity of carrier-mediated blood-brain (blood-ECF) transport.

These results also strongly support the postulated role of the NAA's in the pathogenesis of hepatic encephalopathy and demonstrate a previously unsuspected involvement of the liver in the regulation of blood-brain transport activity. Fischer *et al.* (*14*) described the use of a specially formulated intravenous feeding solution in the treatment of hepatic encephalopathy. This solution, which is low in aromatic amino acids and high in branched-chain amino acids compared to conventional parenteral nutrition formulas, was designed to permit increased protein tolerance in a group of patients traditionally incapable of ingesting adequate amounts of dietary protein without encephalopathy. If patients with cirrhosis and portal-systemic shunting of the circulation also have increased blood-brain NAA transport, then increasing their plasma levels of leucine, isoleucine, and valine should result in decreased brain uptake of the aromatic amino acids, which may interfere with normal neurotransmitter metabolism. Further study is required to determine whether increased NAA transport is a unique pathological response to impaired liver function or whether the normal physiological regulation of brain NAA content is achieved in some part by this mechanism.

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$$\frac{(\text{dpm } ^{14}\text{C}/\text{dpm } ^{3}\text{H})_{\text{brain}}}{(\text{dpm } ^{14}\text{C}/\text{dpm } ^{3}\text{H})_{\text{injectate}}} \times 100$$
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Chloroquine Resistance Produced in vitro in an African Strain of Human Malaria

Abstract. *After continuous cultivation in the presence of chloroquine, an African strain of the malaria parasite,* Plasmodium falciparum, *acquired resistance to the drug. The resistance was stable and comparable in vitro to that occurring naturally in a strain from Southeast Asia. This suggests that chloroquine resistance, absent until now in Africa, might arise in the future.*

Chloroquine-resistant strains of *Plasmodium falciparum* have occurred thus far only in Asia and Latin America (*1*). Since chloroquine is the antimalarial drug of choice, and since *P. falciparum* remains a major cause of morbidity and mortality in Africa (*2*), the emergence of such strains in this continent could have very serious consequences. We have now demonstrated in vitro the potential for chloroquine resistance in an African strain of *P. falciparum*.

Strain FCR-3 (FMG) was isolated in September 1976 from a patient in the Gambia, West Africa, and since then has been kept in continuous culture (*3*) in our laboratory. Using a petri dish test described by Trager *et al.* (*4*), together with a multiwells test, we determined its response in vitro to chloroquine (Fig. 1, A and B). Repeated experiments over an 8-month period showed a constant pattern of sensitivity to the drug. The parasite growth was completely inhibited by a 48-hour exposure to a concentration of 0.1 μg of chloroquine base per milliliter of medium. This was consistent with results reported from the Gambia by Smalley (*5*), who used a different in vitro method described by Rieckmann *et al.* (*6*).

To produce resistance, we maintained a line of FCR-3 in continuous culture by the petri dish–candle jar method of Jensen and Trager (*7*), in a medium containing progressively higher concentrations of chloroquine. We used 35-mm petri dishes with 1.5 ml of 8 percent red cell suspension, changing the medium daily and subculturing to fresh blood cells every 4 days. Beginning with a barely inhibitory concentration of 0.01 μg/ml, we increased the chloroquine level stepwise as the growth of the parasites permitted. After 1 month, or 15 cycles of asexual multiplication, the experimental line had been adapted to a medium containing 0.1 μg of chloroquine base per milliliter of medium, three times the effective mean plasma concentration for a drug-sensitive strain (*8*).

The experiment illustrated in Fig. 2 confirmed at that time the emergence of a new strain, unaffected by the same concentration of chloroquine (0.1 μg/ml) that proves lethal to the original African strain. Continuing the stepwise increase, we are now growing the new strain, R-FCR-3, in the presence of 0.13 μg/ml, and have maintained some lines derived from it for up to 8 weeks at 0.16 μg/ml. Multiplication of the parasite

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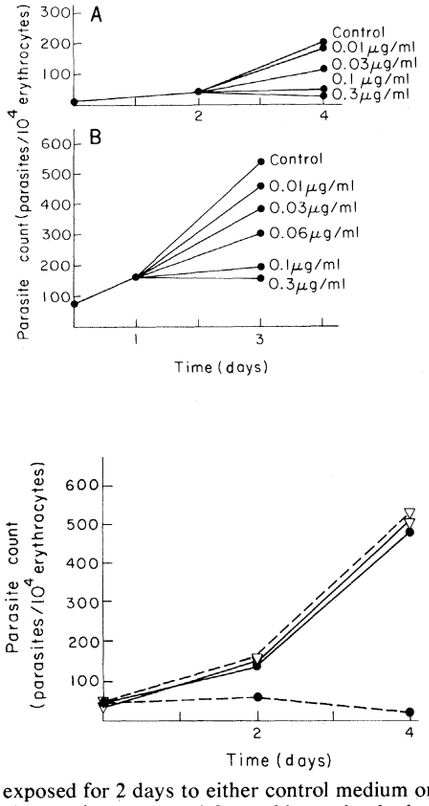
in the presence of 0.2 μg/ml has not been obtained yet, after 6 months of continuous exposure to chloroquine.

The chloroquine resistance produced after 4 months was a stable characteristic, persisting after removal of the drug from the medium. Resistant lines were allowed to grow in normal medium for up to 4 weeks, then returned to the previous concentration of chloroquine, 0.13 μg/ml, where they continued to multiply readily.

The degree of resistance obtained in vitro after 5 months was comparable to that encountered in nature. A chloroquine-resistant isolate from Vietnam has been maintained in our laboratory as the FCR-1 (FVO) strain (*9*). Previous studies demonstrated its high level of resistance, RIII (*1*), in vivo (*10*) and in vitro (*11*). Repeated tests have detected no fluctuation in resistance during 1 year of continuous culture and after storage in liquid nitrogen. We compared the Vietnamese (FCR-1) and the newly adapted (R-FCR-3) strains by the petri dish test, exposing both for 48 hours to chloroquine, and found a similar response. Neither of the two strains was affected by 0.1 μg/ml, and inhibition occurred at 0.3 μg/ml for both strains, the inhibition being slightly more marked for the Vietnamese (FCR-1) than for the adapted (R-FCR-3) strain (Fig. 3, A and B). In the same experiment, a control culture of the original African strain (FCR-3) showed, as expected, complete inhibition at 0.1 μg/ml (Fig. 3C).



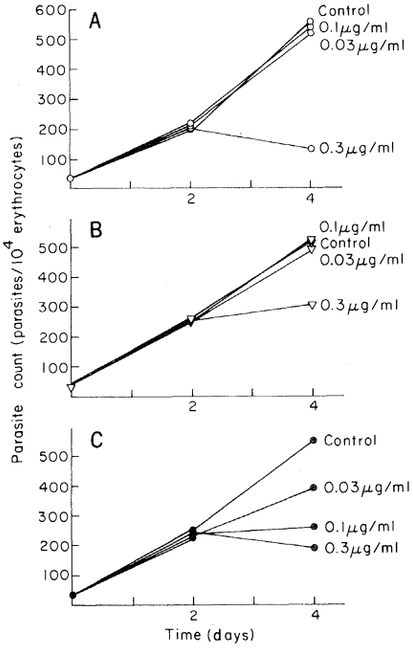


Fig. 1 (top left). Effect of chloroquine on the original African (FCR-3) strain. (A) Petri dish test: Parasites were grown in 35-mm petri dishes in normal medium for 2 days, then exposed for 2 days to either control medium or medium containing different concentrations of chloroquine (prepared from chloroquine hydrochloride, Aralen, Winthrop, 40 mg of base per milliliter): 0.01, 0.03, 0.06, 0.1, and 0.3 μg of chloroquine base per milliliter of medium. Parasite counts were made at the beginning of the experiment, and at the beginning and the end of exposure. Each point represents the average count for four dishes, with negligible variation within each group. (B) Multiwells test: Parasites were grown for 1 day in normal medium in a single 50-mm petri dish, then distributed into individual 16-mm wells containing control medium or different concentrations of chloroquine as above. Parasite counts were made as above, each point representing the average of duplicate wells. Fig. 2 (bottom left). Comparison of the original African strain (FCR-3) and its derived resistant strain (R-FCR-3) after 1 month of continuous exposure to chloroquine. Both strains were cultivated for 4 days, under identical conditions, in either normal medium or medium contining 0.1 μg of chloroquine base per milliliter of medium. Symbols: ●——●, FCR-3 in normal medium; ●----●, FCR-3 in 0.1 μg of chloroquine per milliliter; ▽——▽, R-FCR-3 in normal medium; ▽----▽, R-FCR-3 in 0.1 μg of chloroquine per milliliter. Each point represents the average of triplicate dishes. Fig. 3 (right). Response to chloroquine of the Vietnamese (FCR-1), African (FCR-3), and adapted (R-FCR-3) strains, by the petri dish test. Parasites from the three strains were grown for 2 days in normal medium, under identical conditions, and then exposed for 2 days to media containing no chloroquine, or 0.3, 0.1, or 0.03 μg of chloroquine base per milliliter of medium. Each point represents the average of duplicate dishes. (A) FCR-1 (Vietnamese) strain. (B) Adapted (R-FCR-3) strain. (C) Original African (FCR-3) strain.

This study in vitro indicates that an African strain of *P. falciparum* has the genetic capability to develop chloroquine resistance. Epidemiological factors unfavorable to the emergence of resistant strains might account for their absence from Africa so far. This situation is not necessarily stable, and chloroquine resistance represents a real threat to that continent. Recent reports of decreased clinical response to the drug in some African localities, although unconfirmed by strict World Health Organization standards (*12*), hint that a delicate balance is perhaps on the verge of being upset.

The continuous cultivation method made possible this first experimental production of chloroquine resistance in *P. falciparum*. Investigations into the mechanisms of drug resistance in malaria will be facilitated by direct biochemical comparison between resistant strains and the sensitive strains from which they were derived.

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