Table 1. Activity of L-dopa decarboxylase in regions of the brains of patients with and without Parkinson's disease. The controls are patients without any known neurological disease. Enzyme activity is expressed as the number of counts per minute (minus blanks) per milligram of protein. Blanks ranged from 15 to 30 count/min per milligram of protein. Results are the mean ± S.E.M. The numbers in parentheses are the number of patients analyzed.

| Brain area | L-Dopa decarboxylase activity | |
|---|---|---|
| | Control patients | Patients with Parkinson's disease |
| Putamen | 864 ± 271 (9) | 38 ± 10 (6) |
| Caudate nucleus | 641 ± 200 (10) | 55 ± 14 (6) |
| Hypothalamus | 238 ± 91 (4) | 72 ± 21 (3) |
| Temporal cortex | 39 ± 8 (9) | 20 ± 3 (5) |
| Cerebellar cortex | 33 ± 9 (7) | 38 ± 9 (6) |
| Subcortical white matter | | |
| Cerebral | 17 ± 4 (10) | < 5 (5) |
| Cerebellar | < 5 (5) | < 5 (5) |

material of the human brain (*7*). In brief, brain tissue was homogenized in ice-cold isotonic dextrose and then incubated for 20 minutes (37°C) in 0.1*M* phosphate buffer (*p*H 7.0) and 0.6 m*M* pyridoxal phosphate. Carboxyl-labeled D,L-dopa was then added (final concentration of $2.5 \times 10^{-3}M$), and after 2 hours the reaction was terminated by the addition of acid, causing the release of the evolved carbon dioxide. The latter was trapped in hyamine hydroxide and transferred to a scintillation vial for estimation of radioactivity. Blanks contained *p*-bromo-*m*-hydroxybenzyloxyamine (Brocresine), a potent inhibitor of L-dopa decarboxylase. In the present study we used this method to determine whether there are any abnormalities in L-dopa decarboxylase activity in the brains after death of patients who had suffered from Parkinson's disease (*8*).

The activity of L-dopa decarboxylase was greatly reduced in the caudate nucleus and putamen in the brains of patients with Parkinson's disease as compared with the values obtained from brains of patients not suffering from Parkinson's disease (Table 1). The decrease of the enzyme activity in the hypothalamus was less pronounced. No significant difference could be detected between the controls and patients with Parkinson's disease in respect to L-dopa decarboxylase in the cerebral and cerebellar cortex.

The decrease in L-dopa decarboxylase activity along with that of dopamine in the striatum from patients with parkinsonism suggests that a large proportion of this dopamine-forming enzyme is located in the nigro-striatal dopaminergic neurons that degenerate in parkinsonism. This conclusion is in agreement with the results obtained in animals with experimental brain lesions which interrupt the nigro-striatal dopaminergic neurons (*9*).

It is unknown whether the reduction in the striatal L-dopa decarboxylase activity in patients with parkinsonism occurs prior to, or as a consequence of, the degeneration of the substantia nigra and the nigro-striatal dopaminergic pathway. However, in relation to the beneficial action of L-dopa in Parkinson's disease, it should be noted that this enzyme is normally not rate limiting in the biosynthesis of dopamine (*10*). We found significant, although low, activity of L-dopa decarboxylase (5 to 10 percent normal) still present in the striatum from patients with Parkinson's disease. Therefore, sufficient amounts of dopamine may be formed from the administered L-dopa to functionally counteract the striatal dopamine deficiency and thus alleviate those symptoms of Parkinson's disease (akinesia, rigidity, and possibly tremor) which are assumed to be striatal in origin. This conclusion appears to be supported by our initial data (*11*) showing an elevation of homovanillic acid in the striatum of patients treated with L-dopa as compared with untreated patients. At present it is unknown whether the homovanillic acid was formed only in the surviving dopamine neurons or in other striatal structures—for example, the serotonin neurons; it should be noted, however, that the concentration of striatal serotonin was not significantly different between the two groups of patients (*12*). Since, however, the homovanillic acid concentration in the patients treated with L-dopa was also elevated in brain areas other than the striatum (*11*) (which is in agreement with the distribution pattern of the decarboxylase) the possibility exists that the striatum might not be the only site of the pharmacological effects or side effects, or both, of L-dopa.

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14 September 1970; revised 16 October 1970 ■

## Antimalarials: Effects on in vivo and in vitro Protein Synthesis

Abstract. *The antimalarials quinine, chloroquine, primaquine, and quinacrine inhibited the uptake and incorporation of amino acids in vivo, but these drugs had considerably less effect on cell-free protein synthesis. The results indicate that the primary effect of the four drugs on protein synthesis is blocking of amino acid uptake by the cells.*

Inhibition of protein synthesis in vivo by antimalarial drugs has been observed in bacteria (*1*), in *Plasmodium knowlesi* (*2*), and in the ciliate protozoan *Tetrahymena pyriformis* (*3*). In these reports, however, it was proposed that this effect was the result of an indirect action of the drugs. Ciak and Hahn (*1*) and Polet and Barr (*2*) suggested that the inhibition of protein synthesis could be ex-

Table 1. Inhibition of uptake and incorporation of $^{14}$C-labeled amino acids by antimalarial drugs and cycloheximide in vivo.

| Addition | Concentration (mole/liter) | Inhibition (mean ± S.E.) | |
|---|---|---|---|
| | | Uptake (%) | Incorporation (%) |
| Quinine | $2.5 \times 10^{-4}$ | 68.6 ± 2.12 | 73.0 ± 1.95* |
| Chloroquine | $7.0 \times 10^{-4}$ | 75.6 ± 2.62 | 76.0 ± 3.49* |
| Primaquine | $2.7 \times 10^{-4}$ | 57.8 ± 1.60 | 57.7 ± 4.35* |
| Quinacrine | $1.8 \times 10^{-5}$ | 27.8 ± 0.82 | 30.2 ± 0.95* |
| Cycloheximide | $5.0 \times 10^{-6}$ | 48.9 ± 0.87 | 95.5 ± 0.74 |

* $P$ for uptake versus incorporation $> .05$.

Table 2. The effect of antimalarial drugs and cycloheximide on cell-free protein synthesis.

| Addition | Concentration (mole/liter) | Inhibition* (%) |
|---|---|---|
| Quinine | $2.5 \times 10^{-4}$ | 3 |
| Chloroquine | $7.0 \times 10^{-4}$ | 15 |
| Primaquine | $2.7 \times 10^{-4}$ | 4 |
| Quinacrine | $1.8 \times 10^{-5}$ | 3 |
| Cycloheximide | $5.0 \times 10^{-6}$ | 79 |

* Each value is the mean of two determinations.

plained by an inhibition of RNA synthesis; and Ciak and Hahn supported their hypothesis by showing a decrease in the ribosome content of *Bacillus megaterium* upon exposure to chloroquine.

From our previous results with *T. pyriformis* (*3*), we suggested that this effect could be accounted for by an inhibition of energetics because quinine and quinacrine appeared to block the synthesis of all major cellular macromolecules. Although these hypotheses could adequately explain the in vivo inhibition of protein synthesis, our recent results, reported here, suggest another indirect mechanism which would account for the actions of the antimalarials. From an investigation of the drugs' effects on uptake as well as incorporation of amino acids in vivo, and determination of the direct effects of the drugs on cell-free protein synthesis, the primary effect of the antimalarials appears to be the inhibition of uptake of amino acids by the cells. Inhibition of precursor uptake has also been offered as a partial explanation of the effects of antimalarial drugs on nucleic acid synthesis (*4*).

Cultures of *T. pyriformis*, strain GL, were grown and exposed to the cyclic heat treatment for synchrony induction (*3*). At the end of the heat treatment, portions (10 ml) of the cells were pipetted into flasks containing 1.5 $\mu$c of a $^{14}$C-labeled amino acid mixture (algal profile) and an antimalarial drug, and a control flask containing only the radioactive precursors. At 0 and 80 minutes after the end of the heat treatment, 1.0-ml samples were removed, and the cells were sedimented by centrifugation at 400$g$ for 30 seconds. To determine the amount of amino acid uptake by the cells, 0.1-ml samples of the supernatants were placed on 2.3-cm filter paper disks, and dried at 60°C; the radioactivity was counted by liquid scintillation. Samples were also taken at 0 and 80 minutes and processed as described (*3*) to determine the amount of $^{14}$C-labeled amino acids incorporated into protein. In order to determine the direct effect of the antimalarials on protein synthesis, a cell-free protein synthesizing system was used. The preparation of this system and the method of assay have been described (*5*).

Quinine, chloroquine, primaquine, and quinacrine were used at concentrations that inhibit synchronized cell division in *T. pyriformis*. The effects of these antimalarials and of cycloheximide, a known protein synthesis inhibitor, on uptake and incorporation of amino acids were compared (Table 1). Both uptake and incorporation were markedly inhibited by quinine, chloroquine, and primaquine, but quinacrine had less effect. If the percentage of inhibition of incorporation is compared to that of uptake, no significant difference is observed for any of these antimalarials. Cycloheximide, however, produced markedly different values, almost completely inhibiting incorporation, but having considerably less effect on uptake. Since this antibiotic directly inhibits protein synthesis (*6*), it appears that a drug with this as the primary action will inhibit uptake considerably less than incorporation of amino acids. Since each of the antimalarials produced comparable effects on uptake and incorporation, this suggests that their primary action is not a direct inhibition of protein synthesis.

We next examined the direct effect of the drugs on protein synthesis in a cell-free system, and the results presented in Table 2 substantiate our conclusion stated above. At the concentrations used in vivo, the antimalarials had little effect on cell-free protein synthesis. Cycloheximide, however, inhibited this reaction by 79 percent at the concentration which produced 95 percent inhibition in vivo. Therefore, a direct inhibition does not appear to be the primary effect of the antimalarial drugs on protein synthesis.

One means of explaining our results is that the antimalarial drugs act by blocking the uptake of $^{14}$C-labeled amino acids by the cells. This action would produce an apparent inhibition of protein synthesis since the labeled precursors are not available to the intracellular protein synthesis machinery. This apparent inhibition would be observed in spite of the fact that the antimalarials have little direct effect on protein synthesis. The suggestion of others that the effects on protein synthesis is due to an inhibition of RNA synthesis cannot explain our results. If messenger RNA synthesis is inhibited, this would be observed as a direct inhibition of protein synthesis since the encoded message for amino acid incorporation contained in the messenger RNA would not be available to the protein synthesis machinery. Also, inhibition of RNA synthesis in *T. pyriformis* has only a slight effect on protein synthesis during the 80 minutes after the end of the cyclic heat treatment (*7*). Therefore, it appears that the effects of the antimalarials on in vivo protein synthesis may be explained by an inhibition of amino acid uptake.

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8. Supported by a Fried Foundation research grant and predoctoral fellowship to K.A.C. from the National Institutes of Health.

21 September 1970