of duplicate tubes. The parasitemia was about 4%, and the reticulocyte count was less than 0.5%. The washing procedure removes over 90% of the leukocytes.

Figure 2 shows the effects of pepstatin on the incorporation of precursors into protein and nucleic acids. In the latter case less than 10% of the incorporation is into DNA, as shown by selective removal of RNA, from the filter paper discs[12]. A dose dependent response was obtained. Protein synthesis was inhibited by about 40% and nucleic acid synthesis by 80% at the highest pepstatin concentration tested. Some variability in the sensitivity to the inhibitors was encountered in different experiments. We have not yet been able to account for this.

Cells were prepared as described for Figure 1. However, the adenosine content of the medium was reduced by 50% (to 5 μg/ml), the parasitemia was 11%, the volume of the incubation medium was reduced to 0.225 ml, and 0.075 μCi of isoleucine-^{14}C were added. For incorporation into nucleic acids, cells were incubated with 0.125 μCi of orotic acid-6-^{14}C (specific activity 25.2 mCi/mmole). In this experiment, counts in infected cells have been corrected for counts into an equivalent number of red cells.

If these inhibitors are indeed acting to prevent hemoglobin breakdown by the parasite, it might be possible to reverse the inhibition by supplying the cells with amino acids. Figure 2 (insert) shows the results of such an experiment. The addition of a mixture of amino acids partially reversed the inhibition due to pepstatin, at least during the first two hours. With leupeptin (not shown), a solution of either amino acids or proteose peptone partially reversed the inhibition.

Our data suggest that protease inhibitors may be considered as potential antimalarial agents. Thus, 4 compounds that inhibit the parasite protease also inhibited macromolecular synthesis in infected red cells. These findings are consistent with our working hypothesis[1,2] that agents that inhibit hemoglobin breakdown could inhibit parasite growth through deprivation of amino acids. The finding that this inhibition was partially overcome in the presence of amino acids or proteose peptone is consistent with this hypothesis. The marked inhibition of pepstatin on precursor incorporation into nucleic acid remains to be explained, as it is not clear if this is a direct effect, or if it is secondary to the inhibition of protein synthesis.

Our data are also consistent with results from experiments in which 'growth' of *P. knowlesi* was measured by following the increase in parasitemia in infected red cells over a 24 h period[7]. Pepstatin and chymostatin concentrations as low as 0.01 μg/ml markedly inhibited the increase in parasitemia. Leupeptin actually caused a decrease in the parasitemia, but much higher concentrations were required. It is not clear if the high concentrations needed for inhibition in the present study are due to a species difference or to a difference in the method for measuring 'growth'. However, the present findings indicate that it may be of value to test other inhibitors of proteolytic enzymes on growth of the malarial parasite.

Zusammenfassung. Nachweis, dass diverse Hemmstoffe der proteolytischen Enzyme den Einbau ihrer Vorgänger in Protein und RNS von *Plasmodium berghei* bei in vitro-Kultur in Mäuse-Erythrozyten verhindern, eine Hemmung, die teilweise durch Aminosäuren rückgängig gemacht wird. Diese Daten sprechen für einen Aminosäure-Entzug durch die Proteinase-Hemmstoffe im Parasiten, wobei der Hämoglobinabbau blockiert wird.

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Structure Dependent Catalytic Effect of Cupric Ion on the Hydrolysis of Cephalosporins

All natural and semisynthetic penicillins are catalytically hydrolyzed by cupric ion to the corresponding microbiologically inactive penicilloic acids or a complex of penicilloyl-copper ion[1-4]. We have found that cephalosporins, the structural relatives of penicillins, have a structure-dependent sensitivity to the hydrolysing action of cupric ion.

We have examined a large number of semisynthetic cephalosporins of diverse chemical composition for their sensitivity to the hydrolysing effect of cupric ion in aqueous solution. For convenience of assay a disc-agar diffusion method with *B. subtilis* ATCC 6633 as the test organism and a fixed concentration of cephalosporin (4 μg/ml) was used. For compounds with poorer intrinsic activities a test concentration of 8 μg/ml was employed with the same general pattern of results. The activity of the compounds was determined: a) in deionized water; b) in the presence of 1 μg/ml $CuSO_4$ (the average amount in human serum); and c) in a mixture containing 1 μg/ml $CuSO_4$ together with 5 μg/ml of D-penicillamine (Cuprimine). These solutions were kept at 37°C and samples were assayed microbiologically at 0, 3, 5 and 24 h. The results obtained with several known or commercially available cephalosporins are presented in Table I. It can be seen from this table that the activity of cephalosporins containing a phenylglycine moiety (cephaloglycin and to a lesser degree cephalexin and cephradine) is progressively lost in the presence of cupric ion. This degrading effect of cupric ion can be inhibited by D-penicillamine (Cuprimine), a highly specific copper chelating agent.

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Table I. The effect of cupric ion (Cu^{++}) and penicillamine (Pem) on the degradation of commercially available cephalosporins

Cephalosporin	Sampling Time (h)	4 µg/ml	4 µg/ml + 1 µg/ml Cu^{++}	4 µg/ml + 1 µg/ml Cu^{++} + 5 µg/ml Pem	Cephalosporin	Sampling Time (h)	4 µg/ml	4 µg/ml + 1 µg/ml Cu^{++}	4 µg/ml + 1 µg/ml Cu^{++} + 5 µg/ml Pem
Cephaloglycin	0	26[a]	25	27	Cefazolin	0	23	22	23
	3	24	16	26		3	23	22	23
	5	23	0	25		5	23	20	23
	24	9	0	15		24	20	20	23
Cephalexin	0	16	16	17	Cephalothin	0	30	30	30
	3	16	12	17		3	29	28	28
	5	16	10	17		5	28	28	28
	24	15	0	16		24	26	24	26
Cephradine	0	16	16	17	Cephalosporin C	0	15	14	15
	3	16	10	17	(40 µg/ml)	3	13	13	13
	5	16	10	17		5	12	12	12
	24	15	0	15		24	8	8	9
Cephaloridine	0	29	28	28	Cephacetrile	0	24	23	23
	3	28	28	28		3	23	22	23
	5	27	27	27		5	23	22	23
	24	26	25	26		24	18	17	20

[a] The numbers are diameters of inhibition zones in mm; incubation temperature 30 °C.

Table II. Copper degrading effect on cephaloglycin and SK & F 44065 and its protection with metal chelating agents

Compound	Sampling Time (h)	$CuSO_4$ (µg/ml) 0	10	5	1	Compound	Sampling Time (h)	$CuSO_4$ (µg/ml) 0	10	5	1
Cephaloglycin	0	27[a]	27	26	26	SK & F 44065	0	25	22	23	24
(4 µg/ml)	1	27	9	15	22	(4 µg/ml)	1	25	11	16	21
	2	27	0	0	20		2	25	0	0	21
	3	26	0	0	18		3	24	0	0	20
	5	23	0	0	0		5	23	0	0	0
	24	20	0	0	0		24	16	0	0	0
Cephaloglycin	0	27	26	27	26	SK & F 44065	0	25	24	25	25
(4 µg/ml +	1	26	26	27	27	(4 µg/ml +	1	25	23	25	25
cuprimine	2	27	27	27	27	cuprimine	2	25	24	24	25
10 µg/ml)	3	28	28	27	28	10 µg/ml)	3	25	25	25	25
	5	24	24	24	25		5	22	23	23	24
	24	21	20	24	21		24	19	22	23	20
Cephaloglycin	0	27	26	27	27	SK & F 44065	0	25	24	25	25
(4 µg/ml +	1	26	26	27	27	(4 µg/ml +	1	25	24	25	25
oxine	2	27	28	28	27	oxine	2	25	25	25	25
10 µg/ml)	3	27	27	28	27	10 µg/ml	3	25	26	26	26
	5	24	24	24	24		5	23	22	23	24
	24	20	0	23	20		24	16	17	23	18
Cephaloglycin	0	27	25	26	26	SK & F 44065	0	25	24	25	25
(4 µg/ml +	1	27	26	27	27	(4 µg/ml +	1	25	24	25	25
EDTA	2	27	27	28	28	EDTA	2	25	25	25	25
10 µg/ml)	3	27	27	28	28	10 µg/ml)	3	26	26	26	26
	5	26	26	26	27		5	26	25	26	26
	24	21	0	23	22		24	20	22	23	19

[a] The numbers represent zones of inhibition in mm; incubation temperature 30 °C.

Table III. Effect of cupric ions on experimental oral therapy with cephaloglycin and SK & F 44065 in mouse infected with *E. coli*

Compound	Dissolved in (and administered orally)	ED_{50} (mg/kg) Exp. 1	Exp. 2
Cephaloglycin	Saline	6.2	15.5
	$CuSO_4$ solution (1 μg/ml)	48	42
	D-Penicillamine (cuprimine) (5 μg/ml)	22	—
	$CuSO_4$ (1 μg/ml) + cuprimine (5 μg/ml)	12	—
SK & F 44065	Saline	7.5	9.5
	$CuSO_4$ solution (1 μg/ml)	35	25
	D-Penicillamine (cuprimine) (5 μg/ml)	9	—
	$CuSO_4$ (1 μg/ml) + cuprimine (5 μg/ml)	9.2	—

The data in Table II demonstrate that cephaloglycin and SK & F 44065 [3-(5-methyl-1, 3, 4-thiadiazol-2-ylthiomethyl)-7-(phenylglycine acetamido)-3-cephem-4-carboxylic acid], another phenylglycine-type cephalosporin behave similarly. The degrading effect of increasing concentrations of cupric ion and the neutralization of this effect by different chelating agents is shown for both cephalosporins.

The rate of hydrolyzing effect of cupric ion on various phenylglycine-type cephalosporins was found to be a function of the substituent at the 3-position of the cephalosporin nucleus and also was influenced by substitution on the phenylglycine moiety. The results obtained with a larger number of phenylglycine-type cephalosporins will be published in detail elsewhere. The degrading effect of $CuSO_4$ on the antimicrobial activity of cephaloglycin and SK & F 44065 was found also in mouse in an infection-protection assay with *E. coli* (Table III). The ED_{50} values of the 2 compounds are significantly increased (loss of potency) when they were dissolved in $CuSO_4$ solution (1 μg/ml) and administered orally to mice. The simultaneous addition of D-penicillamine is able to counteract this effect of cupric ion.

These experiments clearly demonstrate that the orally active phenylglycine-type semisynthetic cephalosporins (but not the other types) are inactivated by cupric ion as are the penicillins. The implications of these findings touch several aspects of cephalosporin research including bioassay, stability, formulation and experimental and clinical trials.

Résumé. Toutes les pénicillines sont dégradées par les ions de cuivre avec la formation d'acide pénicilloïque. Parmi les céphalosporines demisynthétiques seuls les dérivés contenant du phénylglycine sont sensitives à l'effet dégradant du cuivre. La D-pénicillamine est capable de le contrecarrer.

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Frequency of Hypocatalasemia in a Sample of Spanish Population

According to NISHIMURA et al.[1], individuals whose catalatic activity in blood is below the normal range must be considered as hypocatalatics. AEBI et al.[2] have detected in a screening study activities varying from 16 to 85% of the normal levels. Investigations carried out by TAKAHARA et al.[3]; HAMILTON et al.[4]; AEBI et al.[5] and BAUR[6], suggest that one or more autosomic codominant genes with variable expressivity including 0 (recessive) are responsible for this defect (TAKAHARA et al.[3]). So far, data on the frequency of this deficiency in different human groups are very scarce. There are available data only on populations from Asia (TAKAHARA[7] in AEBI[5]) Switzerland (AEBI[2]) and a Jewish family from Iran (SZEINBERG et al.[8]).

The object of this study is the determination of hypocatalasemia frequency in a sample of the spanish population.

A total of 10,009 specimens have been sampled from subjects visiting the Ambulatorio 'Pedro Gonzalez Bueno', all of them living in the Eastern areas of Madrid, most of them immigrants from other spanish provinces (77% from Southern and 17% from the Northern Castilian plateau and 6% from the surrounding provinces or even of mixed origins).

Methods. A sample of 2 ml of blood from every subject was mixed with 0.5 ml of a 3.8% citrate solution. Before analysis the blood was keept at room temperature for

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