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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not more than 6 h. For determination of catalatic activity FEINSTEIN's[9] perborate method with slight modifications was used.

The screening procedure was carried out by adding 0.003 ml blood to 5 ml 2.5% perborate solution. Those cases, unable to decompose this amount of perborate within 10 min, were further evaluated by using decreasing amounts (4, 3, 2, and 1 ml) of the same solution. This procedure permits the detection of activity levels corresponding to intervals of 0–20; 20–40; 40–60; and 60–80% of the normal level. No better accuracy was attempted for the purpose of this study. A group of 20 normal subjects was studied by this method. Their average catalase activity was 0.526 ± 0.018 ($\overline{X} \pm 5$) expressed as mM of decomposed perborate.

Results. Among the 10,009 blood samples of different individuals, 26 were found to have an enzymatic activity below the normal range. They were distributed as follows: 20–40% interval 4 samples; 40–60% interval 20 samples and in the 60–80 interval 2 samples. The percentage of hypocatalasemia is therefore 0.26%, which means one per 384 individuals. If we take all the cases together, not considering the posibility of the existence of several alleles, the frequency would be 0.0013, with a confidence limit of 0.008 to 0.0019. We have not found cases of acatalasemia (term coined by TAKAHARA and MIYAMOTO[7]), and only one of the individuals was affected with pyorrhea, a woman whose catalatic activity turned out to be lowest in this screening. A daughter of this woman was also affected with the same illness, but her catalatic activity was normal.

Discussion. The data available do not allow one to decide to which type of AEBI's[5] classification the hypocatalasemia cases found in Spain may be attributed. The variability of catalase activity found in this population may be of genetic origin, such as the presence of several alleles, or it may be due to experimental variation since the number of cells employed for this analysis was not exactly determined. In cases of ferropenic and haemolytic anaemia, normal values are found when catalatic activity is related to haemoglobin content (PAUL and ENGSTED[10]). It is for this reason that, when measuring absolute values, these subjects may appear as hypocatalatic. It was assumed that the haemoglobin concentration would be approximately normal in all the samples analyzed.

It may also be that hypocatalatic subjects have been overlooked. If there is an overlap between the activities of hypocatalatics and normals, those individuals with an activity of over 80% may have been scored as normals. Due to this overlapping, it is possible that our figures underestimate the real number of hypocatalatics (i.e. in cases of group IIIb deficiency, AEBI[5]). On the other hand, the presence of enzyme inhibitors in blood serum, as demonstrated by PAUL and ENGSTED[10], may contribute to an overstimation.

The figure for the frequency of the acatalasemia gene (0.0013) probably represents a maximal value. Nevertheless our values are in agreement with the assumption that – although the average gene frequency seems to vary from country to country (TAKAHARA 1967 in AEBI[5]), the acatalasemia gene is of worldwide distribution.

Resumen. Se ha estudiado la actividad catalásica en la sangre de 10.009 españoles residentes en Madrid, por el método de FEINSTEIN[9]. Se encontraron 26 individuos cuya actividad catalásica oscila entre el 20% y el 80% de la normal. Estos individuos se consideraron como hipocatalasémicos. Se comparan estos datos con los de otros autores y se discute su posible significado.

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Difference in Mechanical Properties of Adjacent Sarcomeres in Single Striated Muscle Fibres of the Horseshoe Crab (*Tachypleus gigas*)

Striated muscle fibres contain both contractile and visco-elastic elements. Such visco-elastic elements could be made to reveal themselves when the muscle is overloaded i.e. under so-called isometric conditions. HILL[1] suggested that during isometric contraction the actively contractile elements would shorten at the expense of the passive elastic elements. Since then, several methods have been designed to test his suggestion[2–6]. However all the methods used to measure the properties of the elastic elements were based on estimations from the force-velocity relation and the relation between tension and time derivative of isometric tension during tetanus[7]. In this paper we have used the method of simultaneous measurements of adjacent sarcomere length changes to study the behaviours of adjacent sarcomeres in single striated muscle fibres during isometric twitch[8a]. The observed difference in the average velocities of adjacent Z lines in some special cases is considered to be an index of the difference in the visco-elastic elements of adjacent sarcomeres.

Materials and methods. The experiments were performed on the short accessory muscle (SAM), a receptor muscle[8b], in the walking leg of the Asiatic horseshoe crab, *Tachypleus gigas*. These muscle fibres were selected for study because of their broad striations (2–12 μm) and small diameters

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