DNP and EDTA, transformed the active center of myosin A ATPase to one similar to that which functioned with ITP as the substrate(3,4,5). With the solvents employed, Ca^{++}-ITPase indeed behaved very similarly to the PCMB modified Ca^{++}-ATPase. However, in urea, dioxane and to some extent in ethylene glycol, its behavior was quantitatively different from the DNA moderated Ca^{++}-ATPase and in urea and ethylene glycol, it was entirely different from the EDTA-activated ATPase (Fig. 1 to 3).

The previously described experiments demonstrated that compounds capable of interacting with hydrogen and hydrophobic bonds may alter the enzymatic function of the active center of myosin A in the concentration range where their effects on the secondary and/or tertiary structure of the protein were very small(6,7,8,9). The quantitatively different sensitivities of the DNP and PCMB modified enzyme site toward the various solvents investigated could serve as further proof that these modifiers which differ chemically act through different groups of the protein (4,10). These results also suggest that minor structral changes, even at some distance from the locus where ATP is being split, may change the "flexibility of the active site." In the case of this enzyme, the space occupied by the substrate on the protein molecule is probably much smaller than the "conceptual" active site.

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Effect of Ascorbic Acid on Tissue Deposition of Chloroquine in the Guinea Pig. (30288)

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The problem of the so-called chloroquine* (CQ) retinopathy, originally pointed out by Hobbs, Sorsby, and Freedman(1), has become increasingly important in recent years as a result of the widespread use of this drug at high daily dose levels for alleviation of such conditions as rheumatoid arthritis(2) and systemic lupus erythematosus(3). The incidence of retinopathy is not especially high (4,5), but it is a very serious complication for those so afflicted. The condition appears to be reversible if detected in time, and medication is discontinued(6,7); nevertheless it is obvious that effective methods for accelerating the elimination of CQ would be desirable. The use of such agents as ammonium chloride has been suggested(5); this compound has long been known to produce such an effect(8). It is also known that CQ has a marked affinity for the eyes of pigmented rats, undoubtedly due to their relatively high content of melanin(5,9,10). Recently Perez *et al*(11) demonstrated that the binding of CQ to melanin is decreased by ascorbic acid. This suggested 2 possibilities: 1) retinopathy may develop more quickly or be less readily reversed in subjects customarily on a low ascorbic acid intake, and 2) administration of high doses of ascorbic acid might be advantageous in subjects who have been tak-

* Available from Winthrop Laboratories, New York, under the trade name of Aralen®.

TABLE I. Effect of Ascorbic Acid on Tissue Distribution of Chloroquine in the Guinea Pig.*

Tissue analyzed	Ascorbic acid intake		
	Deficient	Adequate	Excessive
Plasma	.42 ± .03	.41 ± .05	.27 ± .04
Muscle	35 ± 7	20 ± 4	7.4 ± .7
Heart	45 ± 3	35 ± 4	24 ± 3
Liver	132 ± 23	135 ± 33	74 ± 9
Spleen	167 ± 25	139 ± 16	88 ± 9
Eye	169 ± 16	190 ± 34	183 ± 20
Lung	315 ± 35	278 ± 48	177 ± 25
Kidney	818 ± 50	732 ± 42	658 ± 38

* Values given as μg/g; mean ± s.e. There were 8 pigs in each group, chosen at random from the survivors, except in the deficient group, in which only 8 animals survived.

ing CQ and in whom early retinopathy has been diagnosed. It was felt that this general hypothesis should be tested in an ascorbic acid-dependent species, and the English guinea pig (*Cavia porcellus*) was chosen.

Experimental. Three groups of 10 male pigs weighing initially 300-375 g were maintained on an ascorbic acid-deficient diet,† and the following medications were given, subcutaneously, 6 days per week:

Group A: CQ, 20 mg/kg (as sulfate).

Group B: CQ, 20 mg/kg, plus 1.8 mg/kg of ascorbic acid.

Group C: CQ, 20 mg/kg, plus 18 mg/kg of ascorbic acid.

It was intended that group A should receive no ascorbic acid, that group B should receive an amount equal to the normal daily requirement (0.5 mg), and that group C should receive an amount equal to 10 times the daily requirement. When one group (A) had been reduced by deaths to 8 pigs, all of the animals were sacrificed under chloroform anesthesia, by exsanguination. This situation developed in 14 days, during which time each pig had received 11 doses of CQ (220 mg/kg; the most recent dose was given 24 hours before sacrifice). Representative tissues were removed from the animals promptly and were kept frozen until they could be analyzed. The analyses were performed essentially by the Alving-Brodie method(12), except that the final estimation was spectrophotofluorometric(13). The recovery of CQ by this method was 85 ± 4%, and the desethyl metabolite was about 80% removed in the preliminary processing. The data are presented in Table I. (Note: At necropsy one eye from each animal was fixed in formalin for histopathological examination.)

Discussion. It is evident from the data that ascorbic acid administration profoundly affected the overall metabolism of CQ, altering its retention in all tissues *except the eye.* The order of increasing tissue concentrations in all groups was: plasma, muscle, heart, liver, spleen, eye,‡ lung, and kidney. This is the same general order as found in the rat (10) except for the reversal in position of spleen and kidney. Note, however, that in the albino rat the concentration in the eye exceeds only that in muscle and plasma, while in the pigmented rat the ocular concentration exceeds that in all other tissues. The tissue concentrations in the deficient group average about double those in the group receiving the excessive ascorbic acid intake, but when account is taken of the bulk of the muscle as compared to the other tissues, it becomes evident that the total body content of CQ in the deficient group must be about 4 times that in the "excessive" C intake group. The absolute concentrations of CQ in the "adequate" group are very similar to those apparently existing in the albino rat after a similar period of medication, but at twice the daily dose level(10).

It is of particular interest that the ratio of tissue concentrations in deficient *vs* excessive C intake groups is constant at 1.8-2.0 for heart, liver, spleen and lung. For the eye and kidney the ratio is practically unity, while for muscle it is 4.7. The ratio of plasma concentrations in these 2 groups is of the same order as in the tissues (excepting muscle): 1.55 ± 0.35. This means, in effect, that the tissue to plasma concentration ratios are not appreciably affected by ascorbic acid intake, except in muscle. These ratios are essentially as follows: heart, 85; liver, 300; spleen, 350; eye, 500; lung, 700; kidney, 2000. In some of the tissues there may

† Nutritional Biochemicals Co., Cleveland, Ohio; ascorbic acid deficient diet (guinea pig), supplied *ad lib.*

‡ Except perhaps in the excessive and deficient intake groups.

TABLE II. Effect of Ascorbic Acid on Tissue to Plasma Concentration Ratios* of Chloroquine in the Guinea Pig.

(Values given as means ± s.e.)

Tissue	Ascorbic acid intake		
	Deficient	Adequate	Excessive
Muscle	86 ± 20	47 ± 10	25 ± 3
Heart	111 ± 9	85 ± 9	90 ± 12
Liver	315 ± 15	311 ± 34	347 ± 68
Spleen	420 ± 20	347 ± 14	397 ± 22
Eye	413 ± 42	498 ± 55	930 ± 270
Lung	755 ± 66	730 ± 50	712 ± 84
Kidney	2030 ± 198	1710 ± 145	2420 ± 272

* Calculated for each tissue independently.

appear to be an upward or downward trend in the ratios with increasing ascorbic acid intake, but when the ratios are calculated for each individual tissue and the means ± s.e. of these values are determined (Table II), it becomes apparent that these trends are not real.

The overall effect of ascorbic acid on CQ metabolism observed herein recalls its effect on the metabolism of zoxazolamine in the guinea pig, as reported by Conney *et al,* and earlier observations concerning its effect on the metabolism of procaine, pentobarbital, and acetanilid(14).

Histopathological examination of the eyes of the pigs at necropsy failed to reveal any lesions. This result was to be expected, since it has not been possible thus far to produce ocular pathology of a type resembling human retinopathy in any experimental animal, by means of long-term high-dosage CQ medication(10,15).§

Summary. After 2 weeks of daily subcutaneous medication with chloroquine the order of increasing tissue concentrations in the English guinea pig was generally: plasma, muscle, heart, liver, spleen, eye, lung, and kidney. The level of ascorbic acid intake did not significantly affect the ocular concentration of the drug, nor did it significantly alter the tissue to plasma concentration ratios, except in the muscle. The apparent overall effect of ascorbic acid in significantly decreasing the tissue retention of this drug might prove to be a valuable property.

§ Except perhaps as noted by François and Maudgal (16).

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