Shrinkage Temperature of Eye Collagen

DURING a study of the shrinkage temperature of skin collagen, normal human eye collagen was included in the control material. A measure of difference was found between the sclera, which behaved as ordinary mammalian collagen[1-5], and the cornea, which showed a lower hydrothermal stability, in the sense that it shortened at a lower temperature than sclera or skin collagen.

Although the structure of the transparent cornea is well documented[6,7], and descriptions exist of the analytical differences between it and the sclera[8], there seems to be no ready reference to a lower shrinkage temperature.

Using a modified Theis apparatus[9] the results were as shown in Table 1.

Table 1

Speci-men	Human eye	Sex	Age	55	56	57	58	59	60	61	62	63	64	65	
314	Normal cornea	F	66				1								
314	Normal sclera	F	66								1				
318	Normal cornea	M	84		1										
318	Normal sclera	M	84								1				
321	Normal cornea	F	81		1										
321	Normal sclera	F	81								1				
323	Normal cornea	M	62	1											
323	Normal sclera	M	62								1				
324	Normal cornea	M		1											
324	Normal sclera	M									1				
325	Normal cornea	F	60	1											
325	Normal sclera	F	60									1			
330	Normal cornea	M	18								1⎫ Astro-				
330	Normal sclera	M	18								1⎬ cytoma				
333	Normal cornea	M		1											
333	Normal sclera	M										1			
291	Normal cornea	M					1								
272	Normal cornea	F	86	1								1			
STB1	Normal cornea	M	69		1										
STB2	Normal cornea	M	58				1								
STB3	Normal cornea	F	82		1										
STB3	Normal sclera	F	82						1						
STB4	Normal cornea	M	65				1								
STB4	Normal sclera	M	65						1						

These findings relate only to the translucent area of normal eyes; investigation of pathological specimens is pending.

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Biochemical Chlorination of Streptomyces aureofaciens

EARLIER work on the chlorination mechanism of Streptomyces aureofaciens established that chlorine of chlorinated fatty acids was incorporated into the molecule of 7-chlorotetracycline (CTC)[1,2].

Recently, Sekizawa has found that the chlorine of chlorpropanediols was also incorporated into CTC and, moreover, this compound was able to overcome the effect of known chlorination inhibitors[3-5]. Sekizawa also reported that after the addition of chlorpropanediols to the media of oxytetracycline-producing strains, CTC could be detected[6]. He therefore supposed chlorpropanediols to enter directly into the tetracycline (TC) molecule during the course of biosynthesis preceding the chlorination reaction.

The conditions of chlorine incorporation were investigated by the application of $Na^{36}Cl$, using 2,5-dimercaptothiadiazole[1,3,4] as chlorination inhibitor. Regarding organic chloro-compounds, the incorporation of chlorine from 1-^{14}C-acetate (MCA), 2-^{14}C-MCA and 3-chlorpropanediol[1,2] was investigated. Incorporation of the carbon of monochloracetates into TC was also detected.

Strains of CTC-producing Str. aureofaciens 'B-28' (ref. 7) and the tetracycline producer Str. aureofaciens 'CDSD-314' (ref. 8) were used for the present experiments. The synthetic medium and experimental conditions were the same as reported earlier[7,9].

Using isotopically labelled NaCl it was shown that TDA exerts its inhibitory action in the first oxidative stage of chlorination. $Na^{36}Cl$ could be detected in the broth of chlortetracycline fermentations inhibited by TDA. Similarly, chloride could be detected about the end of fermentations of TC-producing 'CDSD-314' strain. These facts support the assumption that inhibition of chlorination was effected in the first phase of the biosynthetic chain, genetically manifest during the course of strain production[8]. But, in contrast to the inhibition of TDA, in the case of strain 'CDSD-314' copper did not suspend the inhibition of CTC production; the genetic block has been found to be irreversible (Table 1).

Table 1 shows that, in the case of Streptomyces aureofaciens 'CDSD-314', the 'residual reaction' supposed by Sekizawa[6] to be general in chlorination reactions could not be found. In our opinion the appearance of a 'residual reaction' in the case of predominantly TC-producing strains depends on the localization of the genetic block. We suppose, however, that at the very first stage of chlorination there is no alternative pathway in the biogenesis of CTC; later, there may be a partial separation of the reaction routes.

By the use of 1-^{14}C-MCA and 2-^{14}C-MCA it could be shown that, in the case of strain 'B-28', the chlorine of MCA. decomposed enzymatically, was incorporated into the CTC molecule. It was found that TDA did not inhibit the incorporation of carbon from MCA 1-C and 2-C into the tetracycline molecule. Similarly, in the case of strain 'CDSD-314', independently from the genetic block, carbon atoms of MCA were incorporated into TC (Table 2). We suppose that the first step of chlorine utilization is an enzymatic cleavage of the chlorine of MCA to inorganic chloride during the metabolism of both strains. Afterwards, evidently, chloride will be incorporated into CTC only in the case of the CTC-producing strain 'B-28'.

The possibility reported by Sekizawa that propanediole may be a direct precursor for CTC would have been confirmed if the production of CTC could have been detected at the end of the fermentation, after giving chlorpropanediole to the culture of the exclusively TC-producing strain 'CDSD-314'.

Experimental results obtained in our laboratory did not support the assumption of Sekizawa. CTC could not be detected in the fermentation broth of strain 'CDSD-314', thus excluding the possibility that chlorpropanediol acts as a direct precursor of CTC, if it is assumed that, in general, the biosynthesis is similar in TC- and CTC-producing

Table 1

Strain	Materials*	Total antib. activity mcg/ml.	Breaking per mg of antibiotics	CTC (%)	Chloride det. at the end of fermentation
B-28	—	1,080	1,512	100	32
B-28	0·2 mM TDA	1,040	396	26	80
B-28	0·2 mM TDA +0·3 mM CuSO₄	825	1,180	74	38
CDSD-314	—	1,370	38	0	98
CDSD-314	0·2 mM TDA	1,280	32	0	96
CDSD-314	0·2 mM TDA +0·2 mM CuSO₄	1,010	36	0	99
—	—	—	40†	—	96

* As an isotope source 0·4 μc./90 ml., $Na^{36}Cl$ was added at 0 h for each experiment.
† Activity was related to an equal quantity of butanolic extract. The small activity observed in the control flask may be due to a small amount of ^{36}Cl dissolved in butanol.