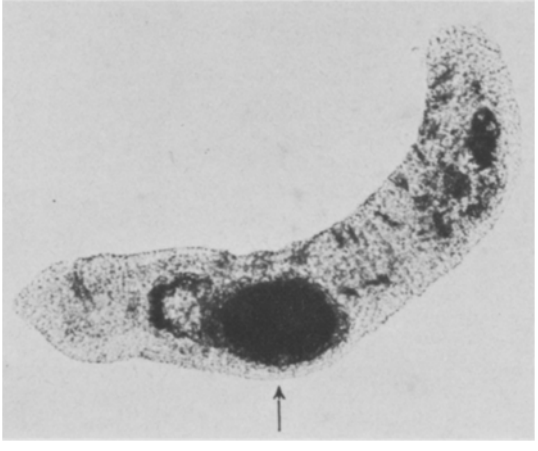
stages of *F. hepatica,* and on the 68th day, only 4 snails remained alive and all released cercariae. When cercariae were emerging, 10–15% of the *C. limnaei* present on snails caught and ingested some of them (at the rate of 1/2 cercariae/chaetogaster). Most cercariae were ingested tail-end first and became lodged in the gut (figure). Encysted metacercariae were never seen inside *C. limnaei* and these annelids did not even ingest any of the encysted metacercariae which were present in the environment. Those chaetogaster which were dislodged from the body of snails, and came to lie in the immediate environment, became less active and were unable to catch cercariae, whereas those in contact with snails remained active and capable of catching and ingesting cercariae.

Discussion. In this situation one would normally expect a control group of infected snails without *C. limnaei.* Strictly speaking, such a control group is not required as it has been clearly understood that infected snails release cercariae[10]. Alternatively, it is very difficult to carry out any observation on predation by chaetogaster which are dislodged from snails because the former lose activity soon after their separation from the latter. Hence no such control observations were carried out. However, it reveals the importance of commensalistic relationship of snails and chaetogaster to maintain the predatory habits. The present observation of the devouring of *F. hepatica* cercariae by *C. limnaei* provides additional information on their feeding behaviour[4].



Chaetogaster limnaei: after ingestion of *Fasciola hepatica* cercariae (indicated by arrow).

It is common to note that snails free from *C. limnaei* successfully 'take' infection and release cercariae of *F. hepatica,* whereas snails infested with this annelid do not readily 'take' infection of *F. hepatica* in the laboratory[10]. This clearly indicates that *C. limnaei* inhibits the invasion of miracidia into snails, and devours cercariae as they emerge. Previous workers considered the association of *C. limnaei* with snails as either commensalistic[4], predacious[6], or mutualistic[2]. Nevertheless, whatever the nature of the relationship, it would seem that *C. limnaei* may control snail populations as well as limit trematode infections in snails. The latter may occur directly, by the ingestion of miracidia, and indirectly, by the ingestion of cercariae. The ingestion of cercariae is also of direct importance as far as limiting *F. hepatica* infection in the definitive hosts. Although a number of control measures are being suggested and implemented to eradicate fascioliasis, successful control has not been achieved.

Recently Samson and Wilson[11] have shown ducks to be an effective biological control agents for *F. hepatica* in USA; other biological methods need to be explored. Hence, it would seem possible that the presence of *C. limnaei* on *L. tomentosa* could be exploited as a multi-facet control measure for fascioliasis. However, much more information on the ecology and physiology of this annelid seem warranted before such a possibility could be realized.

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Collagen synthesis of cultured fibroblast from Werner's syndromes of premature aging[1]

T. Tajima, K. Iijima and T. Watanabe

Department of Pathology and Division of Cellar biology, Tokai University School of Medicine, Boseidai Isehara, Kanagawa 259-11 (Japan), 17 April 1978

Summary. The difference of collagen producibility between 2 groups of skin fibroblasts from patients with Werner's syndrome with skin change and with normal skin, and the difference of collagen accumulation to cell layer between skin fibroblast from Werner's syndrome and controls were studied.

In recent years, some reports indicate that the cultured skin fibroblasts from patients with Werner's syndrome, a typical inherited premature aging disease, have shorter life span in culture[2,3] increased portion of heat-labile enzymes[4,5] and retarded rate of DNA replication[6]. It is assumed that collagen synthesis or fibre formation disorder may exist in the skin of the patients, because dermal atrophy on extremities and face is a typical clinical sign of this syndrome[2].

We now report the difference of collagen synthesis, differentiated function of skin fibroblasts from 2 cases of Werner's syndromes compared with that of normal skin firboblast and human diploid fibroblast.

The cultures were derived and propagated from the thigh skin of dermal atrophy (WF52-1), and from a trunk skin in where no obvious pathological change was detected (WF52-3) of a 52-year-old male patient, and from normal

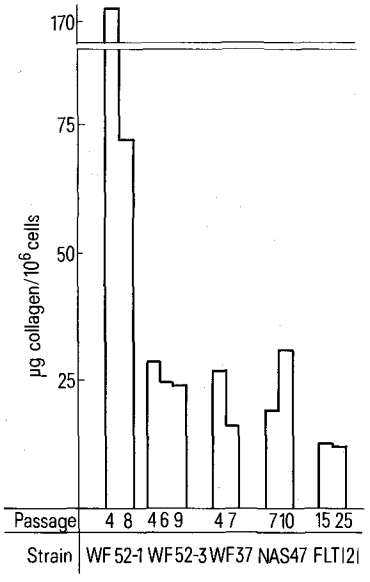

Fig. 1. Total content of hydroxyproline produced during 8 days of culture. Abscissa: cell strain and passage generation number. Ordinate: total hydroxyproline content of medium and cell layer showed as μg collagen/10^6 cells. Bovine tendon collagen was used as authenticity.

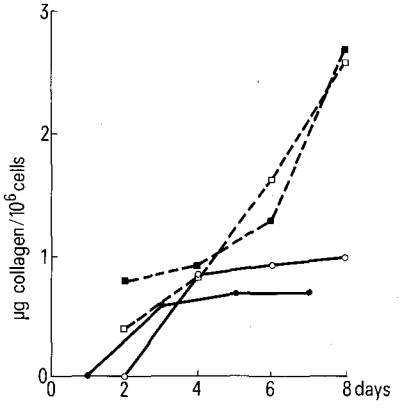

Fig. 2. The change of hydroxyproline accumulation in cell layer during culture period. Abscissa: days of culture. Ordinate: hydroxyproline content in cell layer showed as μg collagen/10^6 cells. Bovine tendon collagen was used as authenticity. FLT121 15th passage generation (□—□), NAS47 7th passage generation (■—■), WF52-3 4th passage generation (○—○), WF37 4th passage generation (●—●).

trunk skin (WF37) of a 37-year-old male patient with the syndrome. The controls were derived and propagated from trunk skin of a 47-year-old normal male and human diploid fibroblast strain FLT-121 originated from 21th week fetal lung. Collagen, produced as procollagen in culture[7-9] contained in cultured medium and cell layer, is measured as hydroxyproline content using the methods of Juva and Prockop[10]. Figure 1 showed the total hydroxyproline produced during 8 days of culture. WF52-1 cells showed considerable high concentration among these 5 cell strains. On the contrary, WF37 and WF52-3 showed nearly equal amount of hydroxyproline as in controls, during several successive passages. Figure 2 showed that 4 passages of WF37 and WF52-3 did not increase the accumulation of hydroxyproline in cell layer after 4th culture days; whereas in controls the accumulation increased with the elongations of culture days. On the contrary, 4 passages of WF52-1 cells

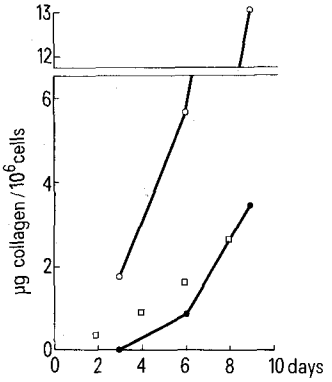

Fig. 3. The change of hydroxyproline accumulation in cell layer during culture period. Abscissa: days of culture, Ordinate: hydroxyproline content in cell layer showed as μg collagen/10^6 cells. Bovine tendon collagen was used as authenticity. NAS47 7th passage generation (□), WF52-1 4th passage generation (○—○), 8th passage generation (●—●).

The difference of collagen synthesis and accumulation in cell layer between 2 groups of fibroblasts from skin with skin change and from normal areas of patients with Werner's syndrome

Skin change	Collagen synthesis	Collagen accumulation in cell layer
+	Increase	Increase
−	No change	Decrease

increased the accumulation of hydroxyproline in cell layer considerably more than in controls during the culture period (figure 3).

The table summarizes the results of early passage of these cell strains which reflected the precise condition to host tissue in vitro. From the results shown in the table, we can conclude that disorder of collagen metabolism is in the systemic skin fibroblasts of the patient with this syndrome. Fleischmajer reported that increased portion of soluble collagen was detected in the skin of scleroderma-like area of this syndrome[11], which suggests relative decrease of the efficiency of fibrillogenesis is in the skin with skin change. Similarly our results, that skin fibroblasts from dermal atrophic area (WF52-1) showed increased collagen producibility, indicate the disorder of fibrillogenesis in the skin with skin change of Werner's syndromes.

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