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The Role of the Pituitary in the Aging of Collagen*

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Hypophysectomized (hyd.) animals do not grow and they live a shorter life than the normals. There are similarities to early aging (*Smith*, 1926). As based on observations of human pathology, latterly the opinion has grown that premature aging may be caused by an involution of the pituitary.

In contrast to this *Goodman* (1951), *Beznák* (1955), *C. E. Hall* (1960), *M. C. Hall* (1961), *Jones* and *Krohn* (1961) and *Selye et al.* (1961) found signs of slower aging in hyd. animals: such are the lack of evolution of the gonads, or the delayed onset of proteinuria (*Everitt* and *Duval*, 1965).

Olsen and *Everitt* in 1965 also studied the aging of collagen. They compared tail tendon fibers of hyd. 200, 500 and 580-day-old rats with those of normals of similar age and found that after hypophysectomy the aging of collagen fibers was retarded. The biological age of collagen fibers was tested with the breaking time after immersion in 7 m Urea (after *Elden* and *Boucek*, 1962).

More than 2½ years ago we also started experiments on the aging of collagen fibers in hyd. animals, which will be referred to here.

Methods

We used the mechanical age test for collagen fibers, as introduced in 1956 for rat tail tendons, based on "*thermic denaturation tension*" which increases with age. Tension can be measured with an isotonic or isometric method. In the present paper the isometric method was used on rat tail tendon fibers 5 cm long and 2.5–3 mg weight.

For skin(corium-)-collagen our test for "*labile collagen*" was used. This shows at 10 min at 65° C a higher solubility for hydroxyproline (Hypro) in young and a lower

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solubility in old animals. The abdominal and the dorsal skin was used in double analyses. Hypro was assessed after *Stegemann* (1958).

Experiments

Rats of our own (Wistar) colony were used. 40 females were hyd. at the age of about 1½ months with a body weight of 110 to 130 g. Similar animals of the same age served as controls. Hypophysectomy was performed in ether narcosis, in the pharyngeal way by suction. The animals received 5% glucose-solution for drinking and the usual rat biscuits as food.

The weight of the animals was controlled every two weeks. It remained, with 2 exceptions, markedly constant. The influence of sugar solutions will be discussed in another paper.

The animals were lively and had good fur until the last decrease of body weight started. The oldest animals had a hump, which may be an expression of changed skeletal stability. With the exception of two animals – not used here – which showed growth and two with middle ear disease, all died naturally, the last after

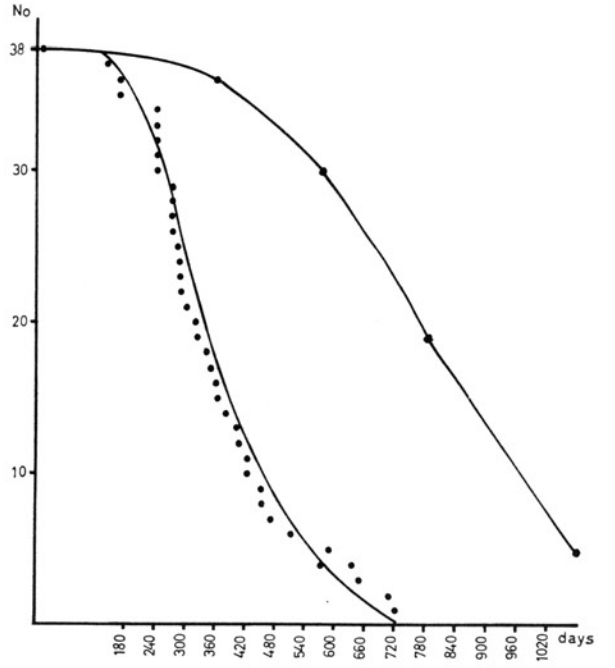

Fig. 1. Survival of 38 hypophysectomized animals, compared with that of normal rats.

two years. Dissection showed maximal atrophy of the adrenals and prepuberal gonads. No lung or other infections were seen.

The survival of these animals is shown in Fig. 1 and compared with our normal colonies survival curve as published over several years on a large number of animals (*Gsell*, 1963). 50% survival appears at 330 days, while in normal females it is 780 days. Maximal survival was also less; only 5% lived over 660 days and died with 720 days (24 months), while in normals 1100-day-old animals had been seen. This agrees with the data of *Everitt* (1965).

Collagen age tests were performed on the tail tendons and on ventral and dorsal skin as mentioned above.

Tail tendon fibers have, in all cases, a lower denaturation tension during thermic denaturation than those of their normal sister animals. The relation of denaturation tension of hyd. animals (TH) to normals (TN) is always less than 1. $Q = TH/TN < 1$. This is shown in Fig. 2 where the straight line is the average of $Q = 0.73$ in 32 hyd. animals. About half of the animals has

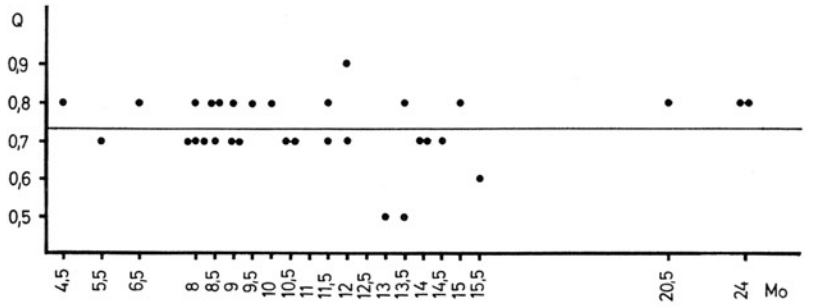

Fig. 2. Denaturation tension of tail tendon fibers after hypophysectomy in relation to normal tension of control animals.

$Q = 0.8$ and the other half $Q = 0.7$. Thus the aging of the collagen fibers is retarded by about $^1/_5$ to $^1/_3$. But it should be stressed, that the aging-process itself is present in the collagen fibers, in spite of the complete inhibition of the animals growth.

More complicated is the analysis of the aging of the collagen of the skin. We first found that there is a distinct difference of labile collagen in normal animals between ventral and dorsal skin. Labile collagen in normals is always more abundant in the ventral skin (NV) than in the dorsal (ND) skin. $Q = NV/ND > 1$; it becomes larger than 1 after the ninth month and up to 2–3 times

larger in 24-month-old animals. This is shown in Fig. 3. This has already been observed formerly (*Verzár*, 1964). Thus it is important that the comparison of skin from hyd. and normal animals is taken from the same body regions.

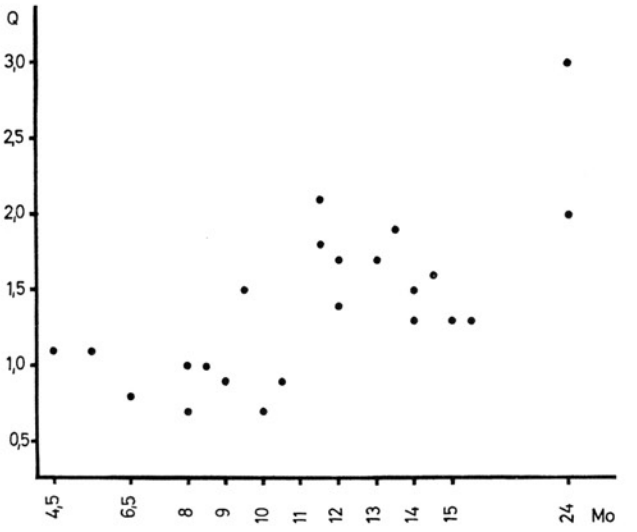

Fig. 3. Relation (Q) of labile collagen in normal animal's ventral to dorsal skin.

The relation of hypophysectomized animal's dorsal skin (HD) to normal dorsal skin (ND) is shown in Fig. 4. $Q = HD/ND$ shows that, up to about 9 months, the hyd. animal's dorsal skins "labile collagen" is not very different from normal, $Q = \pm 1$. In later months, the hyd. animal's skin contains much more labile collagen, and at 24 months of age more than twice as much. $Q = HD/ND =$ 2 to 3. This shows that, while in normals collagen ages, that of hyd. animals does not, or much less.

The same correlation has been made with the ventral skin (HV) of the hyd. animals and the ventral skin of normals (NV) in Fig. 5. This shows that up to about the 11th months the hyd. animal's skin has more labile collagen, i.e. its collagen does not age. But after 11 months, in contrast to this, in normal animal's ventral skin labile collagen increases rapidly, while in the hyd. it does not. Therefore up to 11 months it is more than 1, $Q = HV/NV > 1$ and then falls to values of less than 1, $Q = HV/NV = 0.5$.

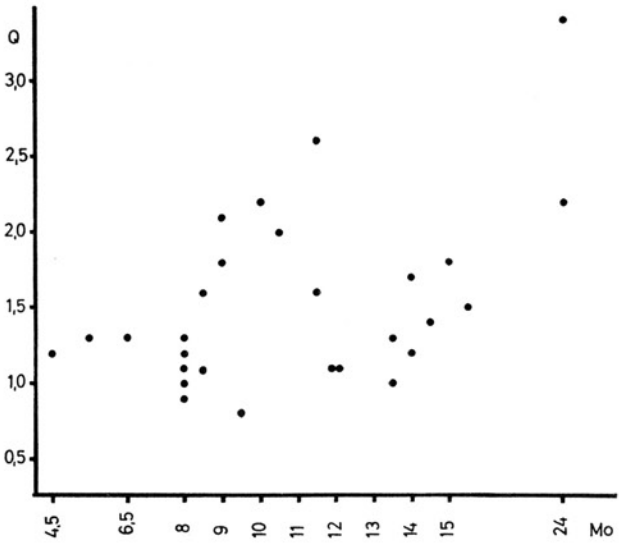

Fig. 4. Relation (Q) of labile collagen of dorsal skin of hypophysectomized animals to normal ones.

This shows that in normals the collagen in the ventral skin of grown-up animals is partly newly formed, while in hyd. animals it

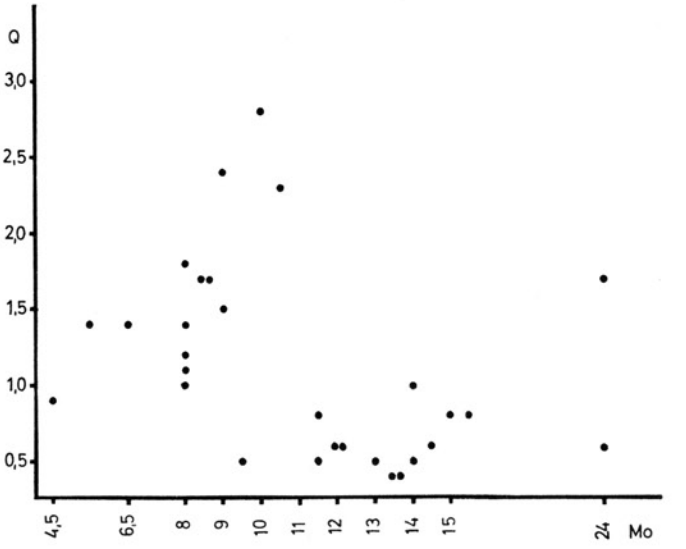

Fig. 5. Relation (Q) of labile collagen of ventral skin of hypophysectomized animals to normal ones.

remains in the less aged state and no new collagen is produced. The collagen production in the normal ventral skin is connected with the growth of these animals and the increase of the abdominal volume.

Fig. 6 shows that in hyd. animals the labile collagen in the ventral and dorsal skin, Q = HV/HD is first not very different, but tends to fall after the 11th months. But after 24 months, it was again about 1.

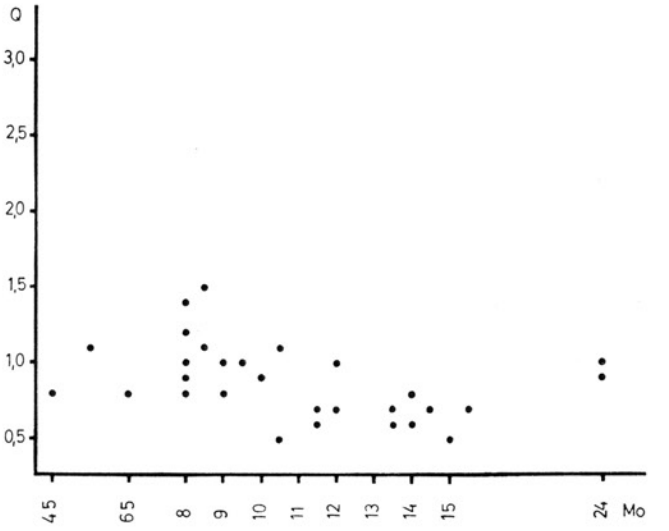

Fig. 6. Relation (Q) of labile collagen of ventral skin to dorsal skin in hypophysectomized animals.

This Fig. 6 should be compared with Fig. 3 in which the same correlation was made for normal ventral and dorsal skin, Q = NV/ND. In the normal animal formation of young collagen is obvious, while no new formation occurs in the hyd. rat.

It follows that in the hyd. animals the collagen of the skin remains relatively young compared with normal skin and there is also no production of new (young) collagen. On the contrary is in the normal animals ventral skin in later life new collagen is produced.

In Fig. 7 we compare medium values, in three age groups of hyd. and normal animals of labile collagen of dorsal and ventral skin. Since the number of cases for each month was too small, three groups were formed with the medium values of 6- (4.5–7.5), 9- (7.5–10.5), and 13- (10.5–15.5) month-old animals. The mean

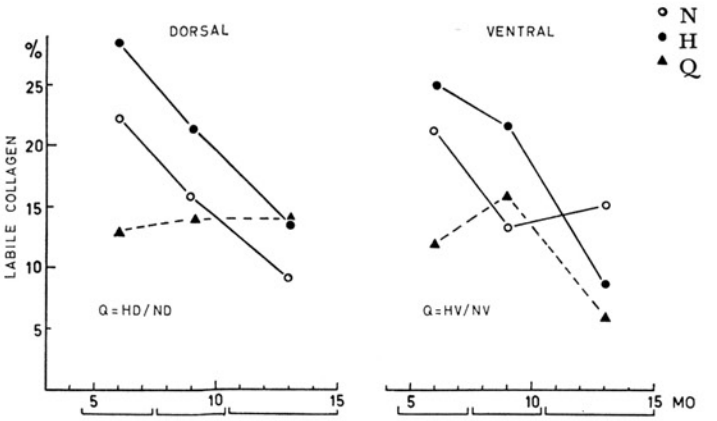

Fig. 7. Labile collagen (Hydroxyproline) in dorsal and ventral skin of groups of animals of different ages. H ● hypophysectomized animals; N ○ normal animals; Q = HD/ND and Q = HV/NV▲; Left dorsal skin. Right ventral skin.

values of labile collagen (labile Hypro) for each group show a decrease with age, thus the collagen ages both in normals and hyd., but in normals it ages more quickly than in hyd.

In Fig. 7, also the values of Q for HD/ND and for HV/NV are given. They show a difference between ventral and dorsal skin. In the dorsal skin it remains constant (1.3–1.4)* while in ventral skin it increases until 9 months and falls at 13 months, because new collagen is produced.

Discussion

Experiments on collagen aging were followed in hyd. rats up to 24 months of survival without growth.

The tail tendon collagen fibers show aging, but this remains $^1/_5$–$^1/_3$ below the tension values of normal growing sister animals. The inhibition of growth slows the increase of aging but does not abolish it. The same is shown by the analysis of skin (corium). This also ages in spite of the fact that the animals do not grow; but it is considerably slower than in normals. There is a special situation in the ventral skin, which in the normal adult animals after the 11 months grows and new collagen is formed, while this is not the case in the non-growing hyd. animals. The growth of the normal animals induces new collagen formation in the ventral corium, together with the growth of the abdominal content.

* Scale of Q is $^1/_{10}$ of left scale.

The main result is that aging of collagen is present in non-growing animals after hypophysectomy; however, it is slowed down by about $^1/_5$–$^1/_3$. We conclude that if metabolic intrinsic factors are the cause of collagen aging, they are also present after hypophysectomy, but are slowed down.

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Summary

1. The survival curve of 38 female rats after hypophysectomy at the age of 1 ½ month confirms observations of others. 50% survival is 330 days and maximal survival 720 days for about 5%. This is about half of the life duration of normal rats.

2. Collagen fibers of tail tendons after hypophysectomy, tested with heat denaturation tension show at every age $^1/_5$–$^1/_3$ lower values than normals. Aging still occurs, but slower than in normals.

3. Collagen of the skin (corium) similarly shows aging but this is also considerably diminished in velocity.

4. Differences between dorsal and ventral skin were noted and explained by growth of ventral skin in the normal grown-up rat.

Zusammenfassung

1. Die Überlebenskurve von 38 weiblichen im Alter von 1 ½ Monaten hypophysektomierten Ratten bestätigt die Beobachtungen von andern. 50% überlebten 330 Tage und das maximale Alter war 720 Tage bei etwa 5%. Das ist etwa die halbe Lebensdauer von normalen Ratten.

2. Die Collagenfasern des Rattenschwanzes, geprüft mit der Spannungsmessung bei Hitzedenaturierung, zeigen nach Hypophysektomie stets $^1/_5$–$^1/_3$ niedrigere Werte als bei normalen.

3. Collagen der Haut (Corium) altert auch, aber ebenfalls mit wesentlich geringerer Geschwindigkeit als bei normalen.

4. Unterschiede zwischen Rücken- und Bauchhaut werden mit dem Wachstum der Bauchhaut bei den normalen Ratten erklärt.

Résumé

1. La courbe de survie de 38 rats femelles hypophysectomisés à l'âge d'un mois et demi confirme les observations faites par d'autres auteurs: 50% des animaux atteignent l'âge de 330 jours et 5% celui de 720 jours. Il y a donc une réduction de moitié environ de la durée de vie.

2. Si l'on étudie la contraction thermique des fibres collagènes des tendons de la queue des rats hypophysectomisés, on constate, à tous les âges, que les tensions nécessaires pour inhiber la contraction thermique sont inférieures à la normale du $^1/_3$ ou du

1/5. Il y a donc un vieillissement de la fibre collagène, mais il est plus lent que chez les animaux normaux.

3. Le même phénomène s'observe au niveau de la peau (chorion) dont le vieillissement est également ralenti considérablement.

4. Les auteurs ont observé une différence entre la peau du dos et celle du ventre; ils l'expliquent par la croissance de la peau ventrale chez le rat adulte.

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