procaine hydrochloride[5] which prevented the spindle acceleration to repetitive stimulation of the gastrocnemius nerves (at 200-300/sec, 0.05 msec, 6–10 times α-threshold), abolished in most units the afferent discharge to stimulation of Deiters' nucleus. The spindles tested, therefore, were activated through γ-fibres. In some receptors, however, excitation from Deiters' nucleus persisted in spite of procaine blockage and gallamine administered at the height of γ-paralysis (Figure 2). The persistence of spindle activation in the presence of γ-nerve blockage and extrafusal muscle paralysis shows that in some gastrocnemius spindles the effect from Deiters' nucleus is also mediated by fusimotor fibres larger than γ-efferents.

Riassunto. La stimolazione ripetitiva del nucleo di Deiters produce la contrazione del muscolo gastrocnemio, che si accompagna ad una scarica afferente fusale. La risposta dei recettori fusali dipende non soltanto dall'attività delle fibre γ, ma anche da quella di fibre fusimotorie di più grande diametro, probabilmente di natura α.

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Two Forms of the Effect of α,α'-Dipyridyl and 1,10-Phenanthroline on Synthesized Hydroxyproline-Deficient Collagen

We have shown in our previous work[1,2] that some chelating agents inhibit the hydroxylation of proline-C^{14} and thus modify the biosynthesis of collagenous proteins in skin slices of chick embryos. By application of α,α'-dipyridyl (1 m*M*) collagen was formed which did not contain hydroxyproline, but its proline-C^{14} activity was doubled when compared with normal collagen of control samples. By the effect of 1,10-phenanthroline of the same concentration, however, a complete inhibition of proline hydroxylation as well as a partial decrease of proline-C^{14} incorporation into collagen occurred. Due to a certain structural similarity of both chelating agents, this contradictory effect was surprising because it pointed to a different mechanism of interference into the sequence of processes involved in the synthesis of the collagen molecule. Therefore, we were interested to find out how the above-mentioned different effect on the hydroxylation of proline and on its incorporation into collagen depends on the concentration of the chelating agent used.

Chick embryo skin slices were incubated for 2 h at 37 °C in a Krebs-Ringer bicarbonate buffer with 5 μC proline-C^{14} and with appropriate concentration of chelating agents. After isolation, purification and acid hydrolysis of collagen and non-collagenous proteins (purity checked by proline/hydroxyproline ratio – see Table), the specific activity of proline-C^{14} and hydroxyproline-C^{14} was determined according to PETERKOFSKY and PROCKOP[3]. As non-collagenous proteins are considered all proteins which precipitate in hot TCA. The collagenous proteins remain in the supernatant. For further methodical details see [1]. The results (Table) proved unequivocally that α,α'-dipyridyl within the concentration range of 0.1–1 m*M* inhibits completely the hydroxylation of proline, whose activity in the collagen molecule was, however, the double

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Effect of different concentrations of α,α'-dipyridyl and 1,10-phenanthroline on hydroxylation and incorporation of proline-C^{14} into collagenous and non-collagenous proteins

Substance	Collagenous proteins				Non-collagenous proteins		Proline/hydroxyproline (amount)
	Hydroxyproline-C^{14}		Proline-C^{14}		Proline-C^{14}		
	cpm/μ*M*	Residual activity	cpm/μ*M*	Residual activity	cpm/μ*M*	Residual activity	
Control	14,000	100%	16,400	100%	60,000	100%	1.18
α,α'-Dipyridyl							
1 m*M*	143	1	31,800	194	61,800	101	1.17
0.5 m*M*	630	4.5	27,900	170	69,800	117	1.15
0.1 m*M*	1,980	14	29,500	180	71,100	118	1.15
1,10-Phenanthroline							
1 m*M*	35	0.25	5,900	37	15,900	26	1.28
0.5 m*M*	360	3	11,000	70	35,700	59	1.10
0.1 m*M*	734	5	15,000	94	60,300	100	1.08
0.05 m*M*	494	4	19,400	118	45,500	76	1.20

of the activity of the controls. On the contrary, 0.1-0.05 mM concentrations of 1,10-phenanthroline inhibit almost completely the proline hydroxylation, but here the activity of collagenous proline-C^{14} is the same as in control collagen. This means that α,α'-dipyridyl blocks the formation of collagen hydroxyproline without influencing the sum of total imino acids in collagen. By the effect of 1,10-phenanthroline hydroxyproline-deficient collagen is formed, containing approximately half the amount of imino acids compared with normal collagen. Therefore, it can be presumed that the first substance inhibits the hydroxylation of proline at the final collagenous polypeptide stage, whereas 1,10-phenanthroline prevents either only the incorporation of proline to be hydroxylated or proportionally decreases the incorporation of all amino acids into the collagenous molecule so that both normal proline and proline to be hydroxylated participate even in this case in the resulting activity of proline. This latter hypothesis proved to be correct[4]. It is worth mentioning that the same results with both substances studied were achieved for the couple lysine/hydroxylysine by HURYCH and NORDWIG[5]. The interference of both chelates under investigation in proline hydroxylation is in favour of Fe^{2+} (or possibly Cu^{2+}) containing enzyme responsible for this reaction, since only Fe^{2+}, Cu^{+} or Cr^{3+} stimulated proline hydroxylation in vitro[6].

Zusammenfassung. Während der Inkubation von Hautschnitten der Hühnerembryonen mit C^{14}-Prolin entstehen bei Anwesenheit von 0,1-1 mM Dipyridyl und 0,05–1 mM 1,10-Phenanthrolin anomale Kollagene, die kein Hydroxyprolin enthalten. Mit Dipyridyl wird die normale Menge, mit Phenanthrolin nur die Hälfte der Iminosäuren eingebaut. Beide Stoffe beeinflussen in den angeführten Konzentrationen die Biosynthese (Inkorporation von C^{14}-Prolin) in nicht-kollagene Eiweißstoffe nicht.

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Ultrastruttura della calcificazione distrofica renale da sublimato

Nel quadro delle ricerche che si vanno da tempo compiendo nel nostro Istituto sul fenomeno della calcificazione animale, uno di noi ha in precedenti lavori (PALLADINI et al.[1,2]) sottolineato il valore che hanno i dati deducibili dallo studio delle calcificazioni tessutali abnormali ai fini della precisazione del ruolo svolto nel fenomeno fisiologico di precipitazione calcarea dalle varie sostanze e strutture che si riscontrano nel focolaio calcifico. In questo campo di ricerca si inquadrano i precedenti lavori (PALLADINI[3]; PALLADINI et al.[4]) sulla istochimica della calcificazione distrofica da sublimato nel rene; riportiamo in questa nota i dati ricavati dallo studio ultrastrutturale di questo tipo di calcificazione rivolto ad indagare il valore di elementi submicroscopici nella genesi del fenomeno indicato.

Frammenti di rene provenienti da ratti Wistar adulti trattati con 0.5 mg/100 g peso di $HgCl_2$ e sacrificati dopo 72 h sono stati fissati in gluteraldeide 3% in tampone fosfato pH 8.0; alcuni di essi sono stati rifissati in Osmio 2% in tampone veronal-acetato pH 7.4-7.6 con $CaCl_2$ 0.005 M. Opportune colorazioni di controllo (alizarina, v. Kossa) hanno dimostrato l'assenza di fenomeni di decalcificazione in seguito alle tecniche usate. Inclusione in vestopal W (KURTZ[5]), colorazione al Pb (KARNOVSKY[6]). Osservazione al M.E. HITACHI HU 11. L'osservazione è stata particolarmente diretta sui tubuli completamente necrotizzati, riconoscibili per la spessa membrana basale che li circonda, mentre tutto lo spazio una volta occupato dalle pareti tubulari appare riempito da detriti cellulari di varia opacità; tra questi detriti appaiono caratteristicamente due tipi di elementi: (a) numerosissimi mitocondri, rigonfi e tondeggianti, di varia opacità, in cui è ancora ben riconoscibile la doppia membrana e alcune cristae mentre la matrice è occupata da masse opache, flocculente (Figura 1) e da caratteristici agglomerati di piccole particelle intensamente osmiofile disposte ad anello, già osservate da MOLBERT et al.[7], in stretta relazione con le membrane delle criste, ma non comprese tra le due membrane delle criste stesse, bensì spesso tra le superfici affacciate di due criste vicine (Figura 1). Queste formazioni si sono dimostrate resistenti alla decalcificazione (EDTA 10% pH 7.3, HNO_3 5%) anche protratta fino a 2 h. (b) ammassi di varia dimensione di cristalli aghiformi (circa 600-800 Å × 30 Å) morfologicamente sovrapponibili a quelli descritti come cristalli di apatite in varie calcificazioni ectopiche (POLICARD et al.[8]; GIACOMELLI et al.[9]; BAUD et al.[10]). Gli agglomerati si presentano per lo più di forma tondeggiante o ovale e dell'ordine di grandezza dei mitocondri. Alcuni cristalli

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