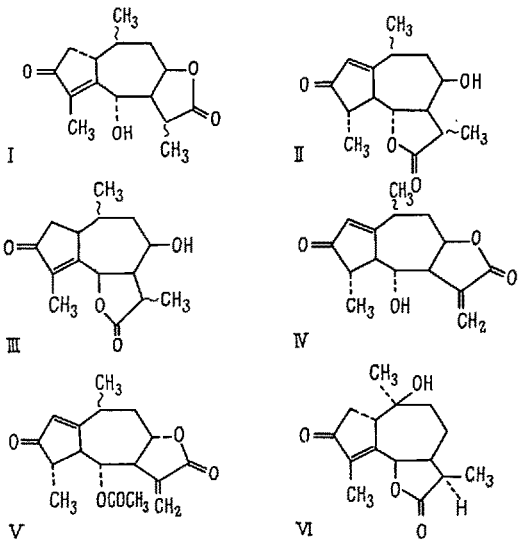
The conformation of I is fixed by the ring fusion, and demands that the carbon-carbon bond at C_1 be *quasi*-equatorial to the seven-membered ring. Consequently the substituents at C_6, C_7, and C_8 will be *e'*, *e'*, and *a'*, respectively.

The assignment of stereochemistry to the remaining substances, desacetylisotenulin (II), helenalin (IV), balduilin (V, or the equivalent structure with the lactone and acetoxyl interchanged), and isophotosantonic lactone (VI), rests on the following arguments. All of these substances have been shown to have the configuration indicated at C_1, C_4 and C_5 by rotatory dispersion studies[4]. In addition tenulin, helenalin, and balduilin have the same configuration at C_7 and C_{10}[5]. These compounds, as well as geigerin (I), and isophotosantonic lactone (VI) are all readily hydrogenated to saturated ketones under mild conditions[3,4]. On the other hand, desacetylneotenulin (III) which has unnatural stereochemistry at C_1 and is formed under conditions which should not disturb the configuration at C_7, is not readily hydrogenated[4,6]. During this interconversion, in order to maintain the *e'* conformation at the ring fusion it is necessary for the seven membered ring to flip to an alternate conformation, causing all the other substituent groups to undergo corresponding conformational changes. The differences in ease of hydrogenation may then be explained by the presence of a bulky *e'* group at C_7 in tenulin, being changed to *a'* in desacetylneotenulin. This group then shields the double bond from hydrogenation. On this basis the stereochemistry at C_7 is assigned as shown in desacetylisotenulin, helenalin, balduilin, and isophotosantonic lactone.



The assignment of configuration to the hydroxyl groups rests upon the following observations. Dihydroisotenulin on basic hydrolysis and subsequent reacidification affords desacetyldihydroisotenulin and an isomeric lactone resulting from lactonization at C_8. On this basis the oxygens at C_6 and C_8 must be equivalent (*cis*), and on the basis of our assumptions regarding the stereochemistry of these lactones, *vide supra*, they must also be *cis* to the carbon chain at C_7. This gives rise to the partial stereochemistry for desacetylisotenulin as illustrated (II).

[5] W. Herz, R. B. Mitra, and P. Jayaraman, Abstr. Papers 136th National Meeting of the American Chemical Society, Sept. 13-18 (1959), p. 50 P.

[6] B. H. Braun, W. Herz, and K. Rabindran, J. Amer. chem. Soc. *78*, 4423 (1956).

Helenalin (IV), and its derivatives, on hydrolysis and reacidification are recovered unchanged[7,8] indicating that the groups at C_7 and C_8 are *cis*, and that the C_6 hydroxyl is probably *trans* to the side chain. Herz has recently shown that balduilin (V) is epimeric with tenulin at C_6, and helenalin at C_8[5], giving balduilin the structure shown.

The stereochemistry at C_{10} in isophotosantonic lactone (VI) rests upon the following argument. Dehydration of (VI) with thionyl chloride in pyridine affords an exocyclic, non-conjugated olefin[3], while acid catalyzed dehydration gives the doubly unsaturated, conjugated ketone. On this basis we propose that the C_{10} hydroxyl group is *cis* to the hydrogen at C_1, otherwise dehydration with thionyl chloride should have afforded the conjugated isomer *via* a *trans* elimination involving the activated hydrogen at C_1. The configuration at C_6 is assigned as shown, with inversion taking place during the formation of VI from santonin by analogy with the santonin-desmotroposantonin conversion[9]. The stereochemistry of the methyl group in the side chain is considered to be unchanged from that in santonin[10], in view of the fact that the conditions for the formation of VI from santonin do not appear vigorous enough to cause epimerization of a group adjacent to a saturated carbonyl. A similar argument may be used in support of the proposed configuration at C_7, which is the same as that in santonin.

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Zusammenfassung

Die Stereochemie der Sesquiterpene Tenulin, Balduilin und Helanilin sowie isophotosantonisches Lakton wird behandelt und provisorische Strukturformeln für diese Substanzen vorgeschlagen.

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[9] Huang-Minlon, J. Amer. chem. Soc. *70*, 611 (1948).

[10] R. B. Woodward and P. Yates, Chem. & Ind. *1954*, 1319. – E. J. Corey, J. Amer. chem. Soc. *77*, 1044 (1955).

The Effect of Insulin on Production of Granulation Tissue in Rats[1]

The effect of hormones on connective tissue has been extensively studied. The formation of granulation tissue – one of the basic reactions of the mesenchyme – is influenced by several hormones. The stimulation of this process by STH, thyroxine, and aldosterone and the inhibition by cortisone and sexual steroids is well known[2–9].

[1] Presented at the 25th meeting of the Hungarian Physiological Society, Szeged, July 1959.

[2] M. Taubenhaus and G. D. Amromin, J. Lab. clin. Med. *36*, 7 (1950).

[3] M. Taubenhaus and G. D. Amromin, Endocrinol. *44*, 359 (1949).

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In recent years several data became known which point to a parallelism of some metabolic effects of STH and insulin. According to LUKENS, the protein anabolic action of STH is dependent on insulin[10]. Growth hormone seems to stimulate the secretion of insulin[11]. In view of the stimulating effect of growth hormone on granulation tissue production, it seemed of interest to examine a possible effect also of insulin. From the field of experiments dealing with hormonal influences on granulation tissue, primarily those seemed convincing wherein direct (local) hormonal effects could be demonstrated[12–18].

For the quantitative examination of granulation tissue formation, we employed essentially the method of MEIER, SCHULER, and DESAULLES[12]. Albino rats weighing 100 to 150 g were used. Cotton pellets weighing 50 mg of the same shape and size were prepared and sterilized. The pellets were impregnated with insulin dissolved in 0·2 ml 0·0033 *N* HCl. Control pellets were impregnated with 0·2 ml of the solvent alone. A glucagon-free crystalline zinc insulin preparation with an activity of 25·3 U/mg was used. To each animal one pellet containing insulin and one control pellet were implanted subcutaneously under the abdominal skin, 2 cm from the midline on both sides. The animals were killed 5 days after the operation and the granulomas formed were dissected out. Care was taken not to include any pre-existing structure. The granulomas were dried to constant weight at 110°C and weighed. The insulin effect represented the difference between the dry weight of granulomas from the control and trated side. Similarly the effect of growth hormone and the joint effect of growth hormone and insulin was examined. In the latter group a pellett impregnated with STH was implanted on one side, and another one containing the same amount of STH besides insulin on the other side.

[10] F. D. W. LUKENS and S. M. MCCANN, in *The Hypophyseal Growth Hormone Nature and Actions* (Blakiston, New York 1955), p. 225.

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[17] B. L. BAKER and W. L. WHITAKER, Endocrinol. *46*, 544 (1950).

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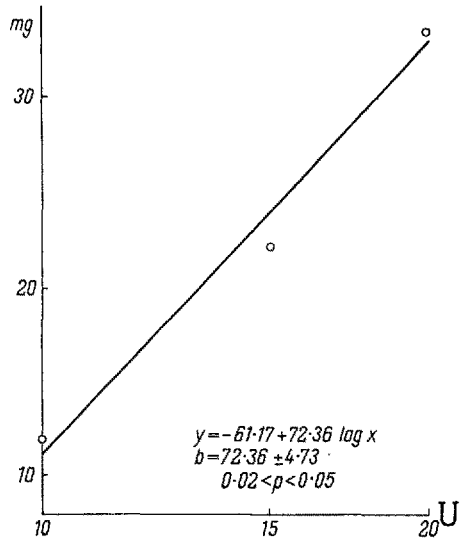
The results were analyzed statistically by the 'DD' method[19].

The results are summarized in the Table. It can be seen that significantly more granulation tissue formed around pellets impregnated with insulin. Our results confirm the stimulating effect of STH on granulation tissue and show that insulin also exerts its effect in the presence of STH.

There was a linear relationship between the log dose of insulin and the difference of dry weight of granulomas from treated and control side (Fig.).



Relationship between the dose of insulin and its effect on granulation tissue production. Ordinate: difference between dry weight of granulomas from treated and control side. Abscisse: dose of insulin in units

In histological sections, a cell-rich granulation tissue could be seen containing many capillaries, fibroblasts, plasma cells, and giant foreign body type cells. No qualitative difference was revealed in the histological appearance of insulin-treated and control tissue.

It seemed possible that this effect of insulin is a non-specific one and is due to the irritating effect of the insulin protein on the tissues. Therefore the effect of inactivated

[19] I. JUVANCZ, in *Klinikai Laboratoriumi Diagnosztika* (Müvelt Nép., Budapest 1955), p. 989.

Dose of hormone	Number of animals	Granuloma weight in mg		Difference in mg	*P*
		Treated side	Control side		
Insulin 10 units	14	148·7 ± 4·3	136·9 ± 2·9	11·8	< 0·01
Insulin 15 units	11	153·7 ± 4·8	130·5 ± 4·2	23·2	< 0·01
Insulin 20 units	18	161·6 ± 5·5	128·3 ± 3·1	33·3	< 0·001
STH 3 USP units	15	149·0 ± 8,7	132·2 ± 2·4	16·8	< 0·01
STH 2 USP units + Insulin 15 units	12	151,3 ± 3·2	140·0 ± 3·4	11·3	< 0·01
Insulin 15 unites (inactivated)	11	132,5 ± 1·0			
Insulin 20 units + adrenalectomy	8	156·5 ± 2·4	133·4 ± 1·8 133·5 ± 4·6	−0·9 23·0	> 0·1 < 0·01

Numbers preceeded by ± are standard errors of the mean

insulin was also examined. The inactivation of insulin was carried out according to the method of DU VIGNEAUD *et al.*[20]. The inactivated insulin which lost its blood-sugar-lowering effect was also ineffective in stimulating granulation tissue formation (Table). In this experiment the control pellets were impregnated with the cysteine solution used in the inactivation procedure. To avoid any possible loss of insulin, no attempt was made to separate insulin and cysteine after the reduction of insulin was accomplished.

The granulation tissue-stimulating effect of insulin is also apparent in the adrenalectomized animal (Table). The implantation of the pellets was carried out 4 days after adrenalectomy. The animals received 1 mg cortisol intramuscularly 24 and 48 h after adrenalectomy. The presence of the granulation tissue-stimulating hormone of the adrenal cortex, aldosterone[5] is therefore not necessary for the insulin effect.

Preliminary experiments carried out on hypophysectomized animals suggest that the presence of the hypophysis is also not necessary for the insulin effect. In accordance with the data in the literature, we found a diminished production of granulation tissue in hypophysectomized rats.

The mechanism and significance of the granulation tissue-stimulating effect of insulin is not known. In our experiments, we succeeded in demonstrating a local effect of a highly purified insulin. This would suggest that the effect is specific. Decreased production of granulation tissue in the diabetic organism, among other factors[21,22], may play a role in the diminished resistance to infection and delayed wound healing.

We are indebted to Dr. W. R. KIRTLEY of Eli Lilly and Company, for glucagon-free crystalline zinc insulin, Lot No. 499667 and to Dr. F. PAULSEN of Ferring AB, Malmö, for growth hormone, supplied as 'Somacton'. Our thanks are due to Dr. L. KISS for performance of the hypophysectomies.

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Institute of Pathophysiology, Medical University of Szeged (Hungary), July 21, 1959.

Zusammenfassung

Mit dem Fremdkörpergranulomtest wurde festgestellt, dass lokal appliziertes Insulin die Bildung des Granulationsgewebes bei der Ratte fördert. Inaktiviertes Insulin bleibt ohne Effekt. Die Insulinwirkung findet auch in Anwesenheit des Wachstumshormons statt.

[20] V. DU VIGNEAUD, A. FITCH, E. PEKAREK, and W. W. LOCKWOOD, J. biol. Chem. *94*, 233 (1931/32).
[21] L. JUHLIN, Acta physiol. scand. *45*, 369 (1959).
[22] B. K. FORSCHER and H. C. CECIL, J. appl. Physiol. *13*, 278 (1958).

Spinal Afferent pathway of the Tactile Placing Reaction

In most textbooks of physiology, the view is held that tactile discrimination depends on dorsal column pathways and to some extent also on the crossed spino-thalamic tract. The assumption has been that the most highly discriminative tactile functions are subserved by the dorsal column pathway. However, it has been demonstrated that in cat evoked potentials in the somatic sensory areas remain after section of the dorsal column, but disappear after a lesion in the dorsal part of the lateral funicle[1].

Tactile placing is a cortical reflex requiring a high degree of spatial sensory discrimination. In the present experiments we have tested tactile placing in the cat's hindlimb, after lesions in the spinal cord had been made to interrupt different ascending pathways. Complete section of the dorsal column does not interfer with tactile placing but ipsilateral tactile placing was entirely abolished after a small superficial lesion in the medio-dorsal part of the lateral funicle. It is possible that this lesion in the lateral funicle may have interrupted a small fraction of pyramidal fibres, but several findings indicate that the loss of placing is not due to a motor deficiency; the cats did not show any defects in movements and visual placing could be performed accurately. There was no loss of the placing reaction in either hindlimb after a large lesion in the ventral quadrant interrupting pathways with the classical location of the spino-thalamic tract.

It is therefore concluded that the afferent link of the placing reaction is an uncrossed pathway in the medio-dorsal part of the lateral funicle.

Electrophysiological experiments have demonstrated the existance of eight pathways in the dorsal part of the lateral funicle, five of which are functionally independent subdivisions of the dorsal spino-cerebellar tract[2]. One of these non spino-cerebellar pathways is activated exclusively by low threshold cutaneous afferents, the discharge being adequately elicited by very light touch from an extremely restricted receptive field. The axons of this pathway are located medially to those of the dorsal spino-cerebellar tract[2]. Disappearance of tactile placing was found after a medial lesion interrupting the axons of this pathway but leaving the majority of the dorsal spino-cerebellar axons. On the other hand, there was no loss of tactile placing after a more laterally placed lesion, which interrupted the majority of the dorsal spino-cerebellar axons sparing the more medially located exteroceptive pathway.

It is assumed that this ascending pathway is the afferent link of the tactile placing reaction.

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Zusammenfassung

Die exterozeptive Aufsetzreaktion der Hinterpfote der Katze nach verschiedenartigen Rückenmarksverletzungen ist untersucht worden. Nach Entfernung des Hinterstranges bleibt dieselbe erhalten, sie verschwindet nach oberflächlicher Verletzung im medialen dorsalen Teil des Seitenstranges. Es wird angenommen, dass eine in diesem Rückenmarksteil verlaufende exterozeptive Bahn der afferente Schenkel der Aufsetzreaktion ist.

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