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EDITORIAL

EXPERIMENTAL LATHYRISM—A MOLECULAR DISEASE

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THE AIM of this review is to discuss current ideas and trends in research on experimental lathyrism. It is not concerned with an extensive documentation of the original literature on this topic.

Experimental lathyrism (odoratism) designates a diseased state which develops in laboratory animals fed on the seeds of sweet peas (*Lathyrus odoratus*). Experimental lathyrism may also be caused by extracts of Lathyrus seeds and by a number of synthetic substances called lathyrogens (surveyed in the accompanying table). Lathyrogens induce generalized changes in the mesenchymal tissues and provide an experimental model for studying the patho-physiology of connective tissues. For the history of lathyrism and for summaries of experimental data the reader is referred to the excellent reviews of Selye,[1] Kulonen,[2] Levene[3] and Tanzer.[4]

Experimental lathyrism is manifested not only by a general weakness of mesenchymal tissues but also by disorders in the growth of cartilage(s) and of bones, which are specifically referred to by the term *osteolathyrism.* Though enchondral ossification does occur in lathyric animals, irregular hyperplastic cartilage is formed in the epiphyseal regions with greatly enlarged epiphyseal discs. Malformations of long bones are caused by changes in the epiphyseal discs as well as by the formation of exostoses developing at the sites of attachments of big muscles exposed to continuous tension. The chest of lathyric animals is deformed, the spine shows kyphoscoliotic alterations and the intervertebral discs are loosened with consequent prolapse of the nucleus pulposus into the spinal canal.[5, 6] Manifestations of the effects of lathyrogens in the vascular system are usually called *angiolathyrism.* Histologically the underlying cause is an inhibition of elastic fibre formation in the vascular wall together with increased fibroblast proliferation and the formation of irregularly arranged collagenous fibres. The result of these changes is a lowered resistance of the vascular wall to stretching and the formation of aneurysms.[7] Some lathyrogens have a higher affinity for nervous tissues—e.g. iminodipropionitrile—causing manifestations of so-called *neurolathyrism.* After several days administration of iminodipropionitrile to growing rats or mice, the ECC syndrome (Excitement with Choreiform and Circling Movement) develops. Degenerative changes are found in the cells of the anterior cornus of the spinal chord.[8]

Lathyric animals show less reaction to inflammatory stimuli. The granuloma pouch (formed after subcutaneous injection of air and croton oil), contains less exudate in lathyric animals and the walls of the pouch are thinner.[9] Similarly the maturation of collagenous fibres in abscesses (provoked by irritation with turpentine oil) is delayed in lathyric rats, even though the proliferation of fibroblasts is not affected.[10] In healing wounds, the morphology of fibroblasts and of the ground substance is abnormal in lathyritic animals. Far fewer leukocytes are attracted to the site of injury in the burnt skin of lathyritic animals.[11,12] In lathyric rats the reactions of connective tissues are diminished in the course of experimental liver cirrhosis, and the production of carrageenin granulomae and experimental pulmonary silicosis.[13–15] The reactivity of connective tissues is also lowered in lathyritic rats in irritative periarthritis (induced by formaldehyde) and another form of experimental arthritis cannot be provoked in these (lathyritic) animals by injections of Freund's adjuvants.[16,17] Tumours caused by 7,12-di-methylbenzanthracene grow more quickly in lathyric rats and the total mass of the tumour is greater.[18] Walker's tumour 256 shows a higher invasiveness into lathyritic bone tissue.[19]

While there is no overall similarity between the pathology of lathyritic animals and the pathology of human connective tissues, nevertheless in certain details there are clear resemblances as seen in Marfan's syndrome (bone changes, dissecting aneurisms) and in infantile cortical hyperostosis (Caffey's syndrome).[20] Patients with dissecting aneurysm of the aorta have an unusually high incidence (35 per cent) of kyphoscoliosis and deformities of the chest.[21]

PROTEINS OF THE CONNECTIVE TISSUE AND LATHYRISM

It is generally accepted that lathyrogens act upon the collagenous component of connective tissue and not upon the mucopolysaccharide components (see next paragraph). The question of the mode and site of action of the lathyrogens upon the collagenous structure still remains unsolved, even though some authors assume that they have eliminated, or on the contrary confirmed, some of the supposed modes of action of lathyrogens, on the basis of their experimental work.

In our opinion, lathyrogens may influence the collagenous component of connective tissues in the following phases of its development:

1. By influencing the cellular enzymes synthesizing collagen.
2. Influencing the enzymatic system of fibriliogenesis—insofar as this process is enzymatically controlled (hitherto only supposed by some authors[22]).
3. Specifically influencing the properties of the collagen macromolecule, by direct binding of the lathyrogen to this molecule at a place which is decisive for fibrillogenesis and for the formation of cross links.

It should be noted that this last effect (No. 3) involving a linkage of the lathyrogen to the collagen, differs from the two previous possible effects (Nos. 1 and 2) listed above which do not necessarily require direct interaction of the lathyrogen with collagen or its precursors.

In principle, there are two approaches to decide between these two types of mechanism:

(a) Through the pharmacological approach—that is by modifying the structure of the effective lathyrogens and by detecting the "active centre" in the collagen molecule which binds lathyrogens (assuming that such a centre really exists).

(b) Through studying the structural differences between lathyritic collagen and normal collagen.

A considerable amount of work has been devoted to this pharmacological approach, it may be concluded that in lathyrogens the effective group is a primary amino group* (see Table 1). Furthermore the amino group in the lathyrogen must be activated in a similar way as the amino group(s) capable of forming Schiff's bases.

TABLE 1. COMPOUNDS TESTED FOR LATHYROGENIC ACTIVITY*

Compounds proved to be lathyrogenic	Compounds proved to be inactive
aminoacetonitrile	dicyanamide
N-methyleneaminoacetonitrile	α-aminopropionitrile
β-aminopropionitrile	N-methylaminoacetonitrile
2-cyanopropylamine	γ-aminobutyronitrile
N-(γ-L-glutamylamino)-β-propionitrile	indolylacetonitrile
hydrazine	tris-(β-cyanoethyl)amine
N,N′-dimethylhydrazine	γ-trimethylamino-α-hydroxybutyronitrile
N,N-dimethylhydrazine	β-methoxypropionitrile
thiosemicarbazide	β-hydroxypropionitrile
acetone semicarbazone	potassium cyanide
glycine hydrazide	acetonitrile
glutamic acid hydrazide	acrylonitrile
cyanoacetic acid hydrazide	succinic acid dinitrile
benzoic acid hydrazide	phenylhydrazine
p-nitrobenzoic acid hydrazide	guanylurea
isonicotinic acid hydrazide	4,5-dioxovaleronitrile-5-semicarbazone
nicotinic acid hydrazide	sodium fumarate
cyanamide†	sodium propionate
β, β′-iminodipropionitrile†	acetamidine
carbohydrazide†	glycine amide
thiocarbohydrazide†	glycine hydroamic acid
β-mercapto-methylamine†	6-aminonicotinamide
	methylamine
	ethylamine
	n-propylamine
	hydroxylamine
	ethanolamine
	taurine
	cyanoacetic acid
	4-aminomethylpyridine

* Table according to Rosmus *et al.*[54]; the original table (ref. 54) includes details of the experimental conditions of testing for lathyrogenic activity, attempts to quantitate these tests and references to the original literature.

† These compounds do not produce all the typical lathyritic symptoms.

The study of the differences between lathyritic and normal collagen has so far not yet passed the bounds of phenomenology—differences have been noted in the content of soluble collagen in connective tissues,[23] in the composition of denaturation products of soluble collagens,[24, 25] and in reconstituting properties of soluble collagens.[26, 27] Conclusions drawn from these studies are at present less unanimous than those reached through the pharmacological type of studies, but it is clear that the formation of the intra- and intermolecular cross links is defective in lathyrism.

Another important question of course arises from the pharmacological studies, namely, whether the amino group is in fact the actual group blocking cross-link

* The only exception is N,N-dimethylhydrazine; some other compounds listed in table do not have a primary amino group, e.g. acetone semicarbazone and N-methyleneaminoacetonitrile, but they can be readily hydrolysed to a compound with a primary amino group.

formation. We mentioned the qualitative agreement between lathyrogenic activity and reactivity of these same lathyrogens with carbonyl compounds to form Schiff's bases. This suggests that the normal collagen cross linkages are of the type found in Schiff's bases (aldimines) and it is their formation which is being blocked by lathyrogens. Levene[28] has pointed out the difference between the linkage of 2,4-dinitrophenylhydrazine to lathyric and normal collagens and several other authors have discussed the possible existence of carbonyl groups in collagen.[29, 30] These carbonyl substances have been recently identified by Rosmus and Deyl[31] as keto-acids and by Rojkind *et al.*[32] as so-called "collagenalose" (an aldehyde of hitherto undefined structure).

It may be assumed therefore that lathyrogens block some of the carbonyl groups in collagen, which are then not available for the formation of cross links. In disagreement with this mechanism are the experiments of Orloff and Gross,[33] who were not able to prove the linkage of radioactive β-aminopropionitrile to collagen soluble in 1 M NaCl. Stalder and Stegemann[34] found β-alanine (the hydrolytic product of β-aminopropionitrile) in the total hydrolysate of lathyric collagen, furnishing proof for the direct link of β-aminopropionitrile to collagen. Also, Wood[27] found that semicarbazide *in vitro* blocked collagen fibrillogenesis during the phase of nucleation centre formation, which also confirms the hypothesis that lathyrogen can interact directly with collagen.

We therefore consider the best-founded hypothesis concerning the mechanism of lathyrism to be the blocking of carbonyl groups, present in the tropocollagen molecule, by lathyrogens—in this way decreasing the possibility of forming intra- and intermolecular cross links. Tanzer[4] in his review favours another hypothesis: he considers the direct link of lathyrogens to collagen to be very unlikely and discusses the possibility that so-called telopeptides[22] participate in the formation of collagen cross linkages. He also emphasizes the possibility that lathyrogens attack the collagen molecule just in these telopeptide regions. Both these hypotheses are not in fact so far opposed to each other, as it would seem at first sight. Rosmus and Deyl[31] proved that practically all the keto-acids and a part of the "collagenalose" are to be found just in the region of the telopeptides (or that they are enzymatically split from collagen at the same time as the telopeptides without disturbing the basic structure of the tropocollagen molecule).

We therefore consider that further detailed studies of the telopeptide regions of the tropocollagen molecule may contribute a lot to our knowledge of the mechanism of action of lathyrogens upon connective tissues.

A recent paper[52] describes similar inhibition of crosslinking in elastin by lathyrogens. It is known that crosslinking in elastin involves two unusual amino acids, desmosine and isodesmosine. The biosynthesis of these two particular amino acids[53] is thought to proceed via an aldehyde intermediate. Therefore the action of lathyrogens in blocking the formation of these amino acid cross-links supports those theories which suppose the carbonyl-blocking activity of lathyrogens underlies their effect on connective tissues.

GROUND SUBSTANCE OF THE CONNECTIVE TISSUE AND LATHYRISM

In the original reports on lathyrism, the mechanism of action of lathyrogens was explained by their intervention in the biosynthesis, and consequent effect upon the

structure, of mucopolysaccharides in the ground substance. A whole series of reports has now accumulated describing changes in the mucopolysaccharides in lathyritic animals, which however, cannot yet be interpreted in terms of a common denominator, as in the case of changes in collagenous proteins in lathyrism. Quantitative analyses of the various components of mucopolysaccharides and glycoproteins (hexosamines, sialic acid etc.) in bone and cartilage tissue, in the skin and vascular wall showed either a decrease, unchanged levels, or even an increase in these components.[35–43] Lathyrogens *in vivo* and *in vitro* reduce the incorporation of radiosulphate into the epiphyseal cartilage mucopolysaccharides of rats, even if this decrease cannot be proved in the early phases of the development of lathyric changes.[44] Differences do exist between the effects of the various lathyrogens *in vitro*. Whereas β-aminopropionitrile and β-mercaptoethylamine inhibit the incorporation of radiosulphate into slices of epiphyseal tissue, semicarbazide—an otherwise effective lathyrogen—has no effect on this process. Parallel observations of the effects of lathyrogens on radiosulphate incorporation, on the extractibility of acid mucopolysaccharides and collagenous proteins have revealed that lathyrogens do not lead to changes of metabolism and extractibility of mucopolysaccharides, even though the extractibility of collagenous proteins in these particular experiments was considerably increased[41, 46] (due to diminished cross-linking).

Some of the discrepancies between different reports of the effects of lathyrism on the mucopolysaccharide component of connective tissue may be explained by differences in the analytical methods and the choice of different time intervals over which the examinations had been carried out. The incorporation of ^{35}S-radiosulphate by tissue cultures of L-fibroblasts was actually increased by lathyrogen.[47] The results of some perfusion experiments are especially interesting. In these experiments the livers of lathyritic animals incorporated less sulphate than the livers of normal animals but even when β-aminopropionitrile was added to the perfusate in healthy animals, sulphate incorporation did not decrease.[48] These findings indicate that different results may be obtained when using the whole animal, as opposed to only isolated tissues. Furthermore the time interval, from beginning the administration of lathyrogens will certainly be of importance as it is known that quantitative biochemical changes occurring in tissues after the administration of toxic substances may be compensated at various intervals of time.[49] Tanzer[4] has pointed out that far larger doses of lathyrogens are needed to bring about changes in the mucopolysaccharide components of connective tissues than are needed to cause changes in the structure of collagenous proteins. Changes in the mucopolysaccharide components could then be an absolutely unspecific manifestation. Greater attention should be paid to the study of the effect of lathyrogens on the mucopolysaccharide link to the protein carrier in the proteinpolysaccharide of cartilage. It is known that this link is sensitive to proteolytic enzymes and recently the possibility that these enzymatic systems may be activated by lathyrogens has been pointed out.[50, 51]

REFERENCES

1. H. Selye, *Revue Can. Biol.* **16**, 1 (1957).
2. E. Kulonen, K. Juva, L. Mikkonen, T. Nikkari, A. Salmi and T. Tuominen, *Biochem. Pharmac.* **6**, 56 (1961).
3. C. I. Levene, *Fedn. Proc.* **22**, 1386 (1963).
4. M. L. Tanzer, *Int. Rev. Connective Tissue Research*, vol. 3, p. 91, Academic Press, New York (1965).

5. I. V. PONSETI and W. A. BAIRD, *Am. J. Pathol.* **28**, 1059 (1952).
6. I. V. PONSETI and R. S. SHEPARD, *J. Bone Jt Surg.* **36A**, 1031 (1954).
7. J. J. LALICH, H. W. WIRKA and C. D. ANGEVINE, *Pathologia. Microbiol.* **24**, 140 (1961).
8. G. ULLE, *Z. Zellforsch. mikrosk. Anat.* **56**, 130 (1962).
9. J. E. MIELKE, J. J. LALICH and D. M. ANGEVINE, *Proc. Soc. exp. Biol. Med.* **94**, 673 (1957).
10. J. V. HURLEY, E. STOREY and K. N. HAM, *Br J. exp. Pathol.* **39**, 119 (1958).
11. G. A. KRIKOS and J. L. ORBISON, *Archs Pathol.* **70**, 188 (1960).
12. R. R. BRUNS, G. A. KRIKOS and J. L. ORBISON, *Archs Pathol.* **72**, 512 (1961).
13. L. FIUME and G. FAVILLI, *Nature, Lond.* **189**, 71 (1961).
14. J. V. HURLEY and K. N. HAM, *Br. J. exp. Pathol.* **40**, 216 (1959).
15. C. I. LEVENE and I. BYE, *Fedn. Proc.* **23**, No. 2 (1964).
16. K. TRNAVSKÝ and Z. TRNAVSKÁ, *Med. exp.* **11**, 7 (1964).
17. C. STEFFEN, K. FORMANEK and R. TIMPL, *Z. Immunitäts-Allergieforsch.* **128**, 451 (1965).
18. J. S. SCHWEPPE, R. BAJERGA, L. HARRIS and R. A. JUNGMANN, *Nature, Lond.* **207**, 310 (1965).
19. C. MCCRARY, Y. AKAMTSU and J. L. ORBISON, *Archs Pathol.* **76**, 95 (1963).
20. R. J. G. SINCLAIR, *Bull. rheum. Dis.* **8**, 153 (1958).
21. W. B. BEAN and I. V. PONSETI, *Circulation* **12**, 185 (1955).
22. F. O. SCHMITT, in *The Biology of Connective Tissue Cells*, p. 40, The Arthritis and Rheumatism Foundation, New York (1962).
23. C. I. LEVENE and J. GROSS, *J. exp. Med.* **110**, 771 (1959).
24. G. R. MARTIN, J. GROSS, K. A. PIEZ and M. S. LEWIS, *Biochim. biophys. Acta* **53**, 599 (1961).
25. G. R. MARTIN, K. A. PIEZ and M. S. LEWIS, *Biochim. biophys. Acta* **69**, 472 (1963).
26. J. GROSS, *Biochim. Biophys. Acta* **71**, 250 (1963).
27. G. C. WOOD, *J. Pharm. Pharmac.* **15**, 134 T (1963).
28. C. I. LEVENE, *J. exp. Med.* **116**, 119 (1962).
29. J. M. LANDUCCI, J. POURADIER and M. DURANTE in *Recent Advances in Gelatine and Glue Research* (Ed. G. STAINSBY), p. 62, Pergamon Press, London (1958).
30. P. M. GALLOP, *Biophys. J.* **4**, 79 (1964).
31. J. ROSMUS and Z. DEYL, *Biochim. biophys. Acta*, in press.
32. M. ROJKIND, O. O. BLUMENFELD and P. M. GALLOP, *Biochim. Biophys. Res. Commun.* **17**, 320 (1964).
33. S. D. ORLOFF and J. GROSS, *J. exp. Med.* **117**, 1009 (1963).
34. K. STALDER and H. STEGEMANN, *Naturwissenschaften* **49**, 398 (1962).
35. A. A. CASTELLANI and C. CASTELLANI-BISI, *Proc. Soc. exp. Biol. Med.* **98**, 318 (1958).
36. A. PEDRINI-MILLE and V. PEDRINI, *Proc. Soc. exp. Biol. Med.* **119**, 322 (1965).
37. V. PEDRINI and A. PEDRINI-MILLE, *Proc. Soc. exp. Biol. Med.* **101**, 358 (1959).
38. S. OKUYAMA, *Fukushima J. med. Sci.* **11**, 77 (1961).
39. L. BOLOGNANI and A. V. LANERI, *Proc. Soc. exp. Biol. Med.* **108**, 111 (1961).
40. M. J. KARNOVSKY and M. L. KARNOVSKY, *J. exp. Med.* **113**, 381 (1961).
41. C. I. LEVENE, *Symposium Int. sur la Biochim. Physiol. Tissu Conjonctif, Lyon* (1965).
42. S. GABAY, F. VIVIANCO, F. RAMOS and C. J. DIAZ, *Archs Biochem. Biophys.* **92**, 86 (1961).
43. D. J. SMITH, *J. dent. Res.* **38**, 740 (1959).
44. B. ENGELFELDT, B. TEGNER and E. BERQUIST, *Acta path. microbiol. scand.* **49**, 39 (1960).
45. Y. K. SHINTANI and H. E. TAYLOR, *Lab. Invest.* **11**, 697 (1962).
46. W. A. PECK, I. MARIZ and W. H. DAUGHADAY, *J. Lab. clin. Med.* **62**, 407 (1963).
47. H. C. BICKLEY and J. L. ORBISON, *Lab. Invest.* **13**, 172 (1964).
48. D. W. JOHN, R. J. SOKOL and J. L. ORBISON, *Lab. Invest.* **13**, 346 (1964).
49. T. R. NIEDERLAND, *The Action of Salicylates in Organisms.* SAV, Bratislava 1963.
50. M. L. TANZER and J. GROSS, *J. exp. Med.* **119**, 275 (1964).
51. K. KÜHN, M. DURRUTI, P. IVANGOFF, F. HAMMERSTEIN, K. STECHER, H. HOLZMANN and G. W. KORTING, *Hoppe-Seyler's Z. physiol. Chem.* **336**, 4 (1964).
52. B. L. O'DELL, D. F. ELSDEN, J. THOMAS, S. M. PARTRIDGE, R. H. SMITH and R. PALMER, *Nature, Lond.* **209**, 401 (1966).
53. S. M. M. PARTRIDGE, *J. Soc. Leath. Trades Chem.* **49**, 41 (1965).
54. J. ROSMUS, Z. DEYL, K. TRNAVSKÝ and Z. TRNAVSKÁ, *Chekh. Fiziol.* **14**, 14 (1965).