Biochemical Pharmacology, 1961, Vol. 6, pp. 56–60. Pergamon Press Ltd., Printed in Great Britain.

BIOCHEMISTRY OF EXPERIMENTAL LATHYRISM

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Abstract—Experimental lathyrism may be of importance in an understanding of diseases of connective tissue, since symptoms of lathyrism occur as a weakness of fibrous tissue and disordered growth of cartilage and bone. Changes observed in peripheral tissue and general metabolic disturbances are discussed. The collagen content of skin may be altered. Experimental evidence showed that the alkali-soluble hydroxyproline was increased five-fold; but good analytical data were not obtainable on the constituents of alkali-soluble collagen. The deposition of collagen was studied. The metabolic defect can be approached by analysis of blood and urine, and by study of the metabolism of β-aminopropionitrile. It is suggested that in lathyrism the general metabolism of nitrogenous components is disturbed.

FOR practical reasons lathyrism would hardly deserve much attention for its own sake. The interest is due to the similarity between its symptoms and those of many changes adherent to diseases of the connective tissue.[1] When the mechanism of experimental lathyrism is understood, it will appreciably contribute to our understanding of the formation and degeneration of the connective tissue. Also means can be developed for the prevention of undesirable fibrous scars and adhesions.

The essential features of lathyrism caused by sweet peas and specifically by their content of β-aminopropionitrile have been reviewed frequently.[2, 3] The symptoms manifest themselves as a weakness of the fibrous tissue and as a disordered growth of cartilage and bone. Also the nervous system is affected, but here I limit myself to the connective tissue. As the starting point we may recall the histological description of healing wounds in lathyritic rats.[4, 5] "The granulation tissue was thinner, and showed numerous extravasated red blood cells and foci of an eosinophilic material containing fine, disoriented fibrils. The number of fibroblasts per unit area was decreased and throughout the experimental period these cells were more spindle-shaped. The number of collagen fibrils was also decreased. In the cut edge of panniculus carnosus the fibroblastic proliferation was more intense in experimental than in control animals".[4]

All the connective tissue changes may be explained by the defect in the deposition of collagen fibres: the weakness of healing scars, the aneurysmas of aorta and hernias, the retardation in weight gain, disordered growth of cartilage cells and the malformations. I suggest that the breaking of the regular columns of cartilage cells[6] and the formation of teratomas[7] in lathyrism are related, and both are due to the lack of a guiding fibrous matrix.

The deviations from the normal in connective tissue can be discussed at two levels: (a) the changes observed in the peripheral tissue, and (b) the general metabolic disturbance.

PERIPHERAL TISSUE

Some investigators claim that the *collagen* content of the skin is not altered,[2] that its electron-microscopic appearance[1] and the thermal shrinking point[8] are regular. However, in a healing wound the number of the fibrils[4, 9] and the tensile strength[8] are decreased, as also the formation of hydroxyproline in the granuloma tissue.[10] The fragility of chick embryos is increased in lathyrism.[11] Some of the conflicting results depend on the experimental conditions, and the defect is revealed only in circumstances where the collagen fibre formation is put to a special test and not complicated by reactive fibroblastic proliferation in sites where the tissue is dislocated by the mechanical stress.

In view of these statements we wished to obtain information on the *precursor fractions of collagen.* In scorbut or adrenocortical hyperfunction the precursor of the fibrous collagen, the alkali- or neutral salt-soluble collagen, is decreased indicating that the defect in the fibre formation lies, at least partly, in protein synthesis.[12, 13] Quite the contrary seems to be the case in lathyrism. The alkali-soluble hydroxyproline is increased fivefold.[14] This is in agreement with those authors[15] who have shown that in lathyrism there is a defect in the precipitation of the collagen fibres in the cartilage but that the total content of collagen is not changed. Other recent reports also indicate an increased solubility of collagen in lathyritic rats[5] and chick embryos.[11]

As yet we have not obtained qualitative data on the alkali-soluble collagen in lathyrism, but it is observed that its precipitation *in vitro* during dialysis or storage is appreciably retarded for non-explained reasons. The proportion of the "directly reacting" (without hydrolysis) hydroxyproline was also clearly lower in lathyrism. We have observed an increase in the apparent tyrosine content of the gelatine derived from lathyritic bones,[8] but the possibility of impurities in the gelatine is not excluded and may be due to the relatively increased amount of blood vessels. Levene and Gross[11] found that the specific optical rotation, intrinsic viscosity and sedimentation constant of the collagen extracted from the treated chick embryos were essentially normal. They also concluded that the soluble collagen originates from fibrils formed prior to treatment with lathyrogenic agent. The extracted collagen could be reconstituted to typical striated fibrils.[11] Its molecular weight and axial ratio are normal.[22] Electron microscope analysis[16] shows that extraction of lathyritic tissue with cold 1 M saline causes a dissolution of the fibrils to fine filaments.

The available knowledge on the composition of other elements in the connective tissue is not quite unanimous. The incorporation of radioactive sulphate is increased in the femurs of the rats,[17] but this can be interpreted rather as a reaction similar the fibroblastic proliferation at the edges of the wound. We have some of our own data[14] indicating that the salt-soluble uronic acid was increased, especially when determined with carbazole reaction, indicating in the first place glucuronic acid and hence chondroitin sulphates A and/or C. Also it has been claimed that mucopolysaccharides are increased in the aorta,[18] but not in the skin. This is in some disagreement with the finding that galactosamine is decreased in growing epiphysial cartilage.[19, 20] This is confirmed but the mechanism is obscure.[21] Also it is worth mentioning that the neutral salt-soluble non-collagenous protein of the skin is increased.[14, 15]

METABOLISM

In studies of the peripheral tissue we have a concrete approach, i.e. the deposition collagen. The inability of the soluble collagen molecules to form stabilizing linkages

must have its original cause in the intermediary metabolism. Two lines of research are obvious to begin with: (a) the routine analyses on blood and urine, and (b) the metabolism of the β-aminopropionitrile itself.

The analyses of blood and urine have not contributed very much. Blood sugar and non-protein nitrogen are within normal limits;[22] on blood calcium, phosphorus and phosphatase the reports are so conflicting that no clear picture emerges.[3] It may be concluded that the mineral metabolism is not primarily affected. The renal readsorption of some substances (including tyrosine and histidine) is impaired.[23] The injurious substance, β-aminopropionitrile, has been detected in the urine[24] as also its detoxication product, cyanoacetic acid.[25] The stimulated formation of the red cells and haemoglobin[26] indicates an increased need of oxygen, and is discussed below.

Considering the occurrence of cyanoacetic acid in urine and the fact that the amino group must not be substituted but the cyano group can be replaced by SH group, it may be concluded that the amino group is the essential radical and that the detoxication involves a deamination. This approach was developed further by studying the effect of the inhibition of monoamino-oxidase (by iproniazid) on the development of lathyrism,[28, 28a] which was found to be aggravated. Corroborating evidence was sought with other hydrazines which do not inhibit the monoamino-oxidase. *Iso*niazid also has a similar effect on lathyrism[22] but is a weak inhibitor of monoamino-oxidase.[29] Carbazides and other hydrazines alone have been reported to cause symptoms in bone[30] similar to those caused by β-aminopropionitrile, and other mechanisms for the effect of iproniazid should not be overlooked in addition to the inhibition of monoamino-oxidase.

The deamination requires oxygen and it can be shown that in the presence of β-aminopropionitrile the oxygen consumption of the liver slices and epiphysial cartilage is increased by 50 per cent[27] which is in keeping with the increased hematopoiesis in the intact animal. However, the increase exceeds by far the calculated requirement for the detoxication of all the β-aminopropionitrile, and about one-tenth of the β-aminopropionitrile disappears. In liver slices from lathyritic rats the oxygen consumption was markedly decreased but again stimulated by β-aminopropionitrile. On dehydrogenation no change was observed (studied with Thunberg-technique with various substrates)[22].

Since these findings implicate the energy metabolism, the content on energy-rich nucelotide phosphates was determined in the muscles and in chick embryos. In chick embryos the nucleotides did not change in any consistent manner.[31] In muscle there are differences in the nucleotide phosphates, but only at the later stages, and they may be due to secondary reasons.[22]

When the experimental diets were supplemented with sucrose, the signs of osteolathyrism were not very much affected, but the growth was definitely delayed in animals on the lathyritic diets.[22] On its face value, this result means that in lathyrism the utilization of sucrose is impaired and this is indicated by the retardation of the growth. All this presumes that the growth retardation can be an independent sign of lathyrism.

Many feeding experiments[2, 3] have been carried out to elucidate the mechanism of lathyrism. The working hypothesis has been that β-aminopropionitrile causes an unknown deficiency of some metabolic factors, and this deficiency would be compensated by suitable additions in the diet. This belief has a sound basis. In animals on

good diets the lathyrism develops slowly and can and be almost prevented when the diet is supplemented with protein. Several claims have been staked on the values of individual amino acids but the evidence has always remained in conclusive.[3] Neither has it been possible to find defects in the metabolism of individual amino acids or in their concentrations in the cartilage,[20] or chick embryo,[31, 32] except a reversal in the ratios of proline and hydroxyproline.[32]

The significance of proteins in the feeding experiments and the essential role of the amino group in the β-aminopropionitrile suggest the importance of studying *nitrogen metabolism*. Two lines seemed especially attractive, glutamine metabolism and transamination. Since the β-aminopropionitrile appears in the peas as an amide-like conjugate with glutamic acid, it was necessary to discover whether it interferes in the synthesis of glutamine. However, thus far all our attempts with brain glutamine synthetase have been negative or inconclusive. The experiments with heart transaminase were also quite negative. It is still too early, though, to conclude that these functions were not disturbed *in vivo*, where numerous other reactions and energy metabolism contribute to the final result.[33]

We have also some data[22] on the synthesis of nitrogenous components from ammonia marked with ^{15}N. In chick embryos the incorporation of ammonia nitrogen into purines was, after 4 days, exactly the same in normal and β-aminopropionitrile-treated eggs which otherwise showed good evidence of lathyrism. This seems to eliminate the nuclear metabolism from further consideration. Nor was there any difference in the incorporation of ammonia nitrogen into lipids or into trichloroacetic acid-soluble fraction (measured 3 days after injection of ammonium chloride into the yolk sac).

In chick embryos the incorporation of the β-nitrogen of β-aminopropionitrile was so low that the nitrile could not be used in concentrations high enough. Its detoxication by deamination was already discussed. It is rapidly removed from the body.[25] Some data are known on the metabolism of aminoacetonitrile, which is equally toxic, but its own metabolism is obviously different.[34]

CONCLUDING COMMENTS

In lathyrism the fibrous collagen is decreased, in spite of the abundance of its precursor. Secondary proliferation of the connective tissue is common, where the mechanical stress dislocates the originally weakened tissue.

The β-amino group is an essential radical of the aminopropionitrile. The symptoms of lathyrism are aggravated by hydrazines, including a monoamino-oxidase inhibitor, iproniazid. The detoxication involves an oxidative deamination, which partly explains the increased oxygen consumption by the tissue slices in the presence of β-aminopropionitrile and the stimulated haematopoiesis *in vivo*.

Brain glutamine synthetase or heart transaminase were not inhibited *in vitro* by β-aminopropionitrile. The nuclear metabolism does not appear to be disturbed.

It is suggested that in lathyrism the general metabolism of nitrogenous components is disturbed.

Acknowledgement—Our work presented above was supported by Sigrid Jusélius Foundation and the State Board of Science.

Note added in proof—The incorporation of $^{15}NH_4Cl$ into the collagen fractions in chick embryos was higher in lathyritic embryos than controls 2–3 days after the

simultaneous injection of β-aminopropionitrile and $^{15}NH_4Cl$. When the β-aminopropionitrile was administered 4 days later than $^{15}NH_4Cl$, it caused the replacement of the labelled nitrogen by non-labelled. It is not known whether the synthesis of the individual amino acids was affected, but there seems to be no disturbance in the conversion of proline to hydroxyproline.[35]

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