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MECHANICAL PROPERTIES OF TISSUES OF LATHYRITIC ANIMALS

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Lathyrism (or odoratism) is a condition produced by ingestion of seeds of the sweet pea (*Lathyrus odoratus*), in which condition abnormalities in the mechanical properties of tissues are found, for example, aneurism of the aorta. Few quantitative observations on the mechanical properties of tissues of affected animals have been made (Levene & Gross, 1959; Bell & Sharma, 1960; Levene, 1961; J. Viljanto, T. Tuominen and E. Kulonen, personal communication). As we have used mechanical tests to measure certain properties of the collagenous framework of normal tissues it seemed of interest to apply the same tests to the tissues of lathyritic animals. We have applied them to the tissues of rats fed on sweet-pea meal, and to chick embryos after injection into the egg of β-aminopropionitrile, which appears to be the lathyrism-producing substance in the sweet pea. Aorta and skin from affected rats showed a drop of about 50 % in tensile strength, calculated per unit cross-sectional area of collagen found by chemical analysis, but intestine showed no significant change. Skin from chick embryos showed a greater diminution in tensile strength, to about a fifth, associated with approximately a fifteenfold increase in extensibility measured by the rate of extension under constant load. The concentration of collagen was the same in the tissues of normal and lathyritic animals.

METHODS

Animals

Rats. Virgin female albino rats of the local stock were used. The control animals received as much as they wanted of MRC diet No. 86, the experimental animals the same diet mixed with an equal weight of meal made from the seeds of the sweet pea (*Lathyrus odoratus*). The experimental diet was started when the rats were 6–7 weeks old and was continued for 3–4 weeks.

The animals were killed by stunning and breaking the neck and the following samples were removed for testing:

1. Skin. Cylinders 5 mm long cut from each rear leg round the lower end of the tibia.

2. Aorta. Adjacent lengths of 5 mm from the middle of the thoracic aorta cut from the vessel after it had been removed from the body.

3. Intestine. Adjacent lengths of 5 mm cut from the rectum, in the region between the kidneys and the bifurcation of the aorta, after it had been removed from the body.

Chick embryos. Eggs were incubated for 14 days at 37° C, after which 20 mg β-aminopropionitrile (Abbot Laboratories) in 0·2 ml. sterile distilled water was injected under the chorio-allantoic membrane through a hole made in the shell with a dental burr (Levene & Gross, 1959).

Mechanical tests were done on rings of skin approximately 5 mm long cut from the neck. The neck was cut completely through and the vertebral column then removed. The ring of tissue left was used for testing. After the test, tissues which played no part in it were removed, e.g. feathers, trachea, and the sample was blotted and weighed. This was done after the tests rather than before because the tissues of the lathyritic chicks were exceedingly fragile and difficult to handle at all without tearing.

Mechanical tests

The methods used have been described before (Harkness & Harkness, 1959; Cullen & Harkness, 1960), and it is sufficient to say that the rings of tissue, in oxygenated Locke's solution at 37° C, were placed as a belt round two parallel rods 0·6 mm in diameter, one fixed and the other moveable, and stretched by pulling the rods apart. The samples of tissue were in pairs and the following tests were made in sequence:

Tensile strength (*A*). The load on the tissue was increased rapidly until the tissue ruptured. Tension per unit cross-sectional area of collagen was calculated from the length of the belt at the time of rupture and the total collagen content, assuming this to be evenly distributed about the rods. In the course of these tests measurements of the size of the collagenous framework up to breaking tension were obtained; l_z, inner circumference of the belt of tissue at zero tension, obtained by linear extrapolation; and k, difference between l_z and the circumference obtained by extrapolating to breaking tension (Harkness & Harkness, 1959; Cullen & Harkness, 1960).

Extensibility (*B*). The tissue was subjected to a constant load equal to one fifth of that found necessary in test *A* to break it, and the rate of increase in the circumference of the belt of tissue was measured after it had become constant. Symbols: K = rate of increase in circumference (mm/min), l_0, circumference (mm) obtained by linear extrapolation to zero time. Extensibility, measured by K/l_0, is the fractional increase in circumference per unit time. Loads were left on for 2–3 hr, except in the case of skin from rats, when they were left on overnight. To compare treated and untreated animals the value was corrected to a constant tension per unit cross-sectional area of collagen calculated for length l_0, the method of correction being based on the relation between load and K/l_0 determined in a separate series of tests.

Chemical tests

Estimation of collagen content of samples. At the end of the mechanical tests samples were placed in 2 or 5 ml. of 6 N-HCl, according to weight, and hydrolysed for 4 hr at 40 lb./sq.in. (2·8 kg/cm²) pressure in an autoclave; the hydroxyproline content of the hydrolysate was estimated by the method of Neuman & Logan (1950) and the collagen content of the sample was obtained by multiplying the value by 7·46. Analyses of amino acids in the particular collagens in these tissues are not available. The factor 7·46 assumes a hydroxyproline content of 13·4 % in the collagen, about the middle of the fairly narrow range of hydroxyproline content found in mammalian tissues. The value found by Neuman & Logan (1950) for fowl tendon was 13·5 %. A more recent full analysis of amino acids in gelatin from fowl tendon gave a figure of 14 % (Leach, 1957). No figures are available for fowl skin but it is unlikely, in view of the fact that large differences between tissues in individual species have not been found, that the true factor is far from 7·46, which we have accordingly used for the chick skin also.

RESULTS

General effects of experimental treatment

Rats fed on the experimental diet grew more slowly than those on the normal diet (Table 1, lines 10 and 11). They showed obvious signs of deformity of bone structure, for example, angulation of the sternum. Bones were fragile and easily crushed. Chicks treated with β-aminopropionitrile showed the same sort of defect; though the most obvious deformities, unusual angulations and mobility in the leg joints, appeared to be due primarily to softening of the epiphysial cartilages.

TABLE 1. Weight and collagen content of samples of skin, intestine and aorta used in tests on normal and lathyritic rats

	Normal	Lathyritic
Skin		
1. Weight (mg)	24·2 ± 1·6	15·0 ± 0·8
2. Total collagen (mg)	2·43 ± 0·19	1·64 ± 0·14
3. Concentration of collagen (g/100 g)	10·6 ± 0·4	11·0 ± 0·6
Intestine		
4. Weight (mg)	33·0 ± 3·1	31·6 ± 3·6
5. Total collagen (mg)	0·53 ± 0·04	0·52 ± 0·06
6. Concentration of collagen (g/100 g)	1·63 ± 0·08	1·65 ± 0·08
Aorta		
7. Weight (mg)	3·6 ± 0·5	3·3 ± 0·5
8. Total collagen (mg)	0·33 ± 0·01	0·26 ± 0·03
9. Concentration of collagen (g/100 g)	10·5 ± 1·6	8·1 ± 0·5
10. Body weight at start (g)	78 ± 2	78 ± 2
11. Body weight at death (g)	152 ± 5·1	102 ± 3·4

The estimate of variation is the standard error of the mean. There were 6 rats in each group and 12 samples, except for normal aortas where there were 10, and normal intestine where there were 9. All samples used in tests on tissues of rats are bulked together in this table.

TABLE 2. Weight and collagen content of samples of skin used in tests on normal and lathyritic chick embryos

	Normal	Lathyritic
Number of samples	7	8
Weight (mg)	129 ± 9	107 ± 8
Total collagen (mg)	1·31 ± 0·06	1·06 ± 0·09
Concentration of collagen (g/100 g)	1·06 ± 0·07	1·00 ± 0·04

The estimate of variation is the standard error of the mean.

Weight and collagen content of samples

The samples from the lathyritic animals were in general a little lighter and contained less total collagen than samples from normal animals (Tables 1 and 2). These differences appear to reflect a difference in body size, and there was no significant difference between samples from the two groups in the concentration of collagen in the tissues.

TABLE 3. Tensile strength and circumference of the collagenous framework of rings of tissue, found by loading at constant rate to the point of rupture

	Rat skin		Rat aorta		Rat intestine		Chick skin	
	Normal	Lathyritic	Normal	Lathyritic	Normal	Lathyritic	Normal	Lathyritic
1. Number of samples	6	6	6	6	6	6	7	8
2. Breaking load (g)	320 ± 32	100 ± 10	157 ± 13	71 ± 4	91 ± 8	70 ± 2	59 ± 7	8 ± 1
3. Breaking tension (kg/mm² collagen)	3·65 ± 0·36	1·68 ± 0·28	2·92 ± 0·45	1·42 ± 0·10	3·75 ± 0·41	2·81 ± 0·30	1·41 ± 0·23	0·24 ± 0·08
4. Circumference at break (mm)	38·9 ± 1·1	35·4 ± 0·9	8·6 ± 0·1	7·7 ± 0·2	33·2 ± 0·8	31·8 ± 0·6	42·7 ± 2·1	44·6 ± 2·0
5. l_z (mm)	31·1 ± 1·1	28·0 ± 0·7	7·2 ± 0·1	6·3 ± 0·1	25·7 ± 0·6	24·3 ± 0·3	33·4 ± 0·8	29·9 ± 0·7
6. k/l_z	0·23 ± 0·02	0·25 ± 0·02	0·18 ± 0·01	0·20 ± 0·01	0·27 ± 0·02	0·31 ± 0·04	0·25 ± 0·03	0·45 ± 0·08

The estimate of variation is the standard error of the mean. Rates of loading were 40 g/min for rat skin, 20 g/min for rat aorta and intestine, 20 g/min for normal chick skin and 4 g/min for skin of lathyritic chicks. l_z is the circumference found by linear extrapolation of the tension-length curve to zero load; k/l_z is the fractional increase in circumference between zero and breaking tension.

Size of collagenous framework

The natural size of the collagenous framework estimated by linear extrapolation of the tension–length curve to zero load (Table 3, l_z, line 5) was a little smaller in the affected than in the normal animals.

Tensile strength of tissues

In aorta and skin from lathyritic rats the value of tensile strength calculated per unit cross-sectional area of collagen was about half that found in normal animals (Table 3, line 3). In chick skin the difference was greater, tensile strength in the lathyritic animals being about one fifth of that in the normal. A difference of the same sort was found in the rat's intestine, but it was not significant (at the 5 % level).

Extensibility of tissues

Relation of load to rate of extension. In order to compare tissues it was necessary to correct the rate of extension under constant load to a standard load per unit cross-sectional area of collagen. The relation between rate of extension and load was examined in samples of skin and intestine (rectum) taken from normal rats. First a sample was loaded to the point of rupture; then another (skin of other side), or others (rectum) were subjected to prolonged tension with loads ranging from 5 to 40 % of the breaking load.

TABLE 4. Relation between load and rate of extension in rings of skin and intestine from rats

		A. Skin		
Tension at l_0 (g/mm² collagen)	76 ± 9	177 ± 13	304 ± 11	439 ± 33
Rate of extension (K/l_0, $(10^{-3}$ min$)^{-1}$)	0·92 ± 0·24	2·78 ± 0·32	4·85 ± 0·38	7·15 ± 0·69
Circumference (l_0, mm)	30·3 ± 0·9	32·7 ± 0·7	32·6 ± 0·7	33·8 ± 0·8
Body weight of rats (g)	101 ± 3	107 ± 4	105 ± 1	105 ± 4
		B. Rectum		
Tension at l_0 (g/mm² collagen)	380 ± 50	600 ± 20	850 ± 40	1440 ± 130
Rate of extension (K/l_0, $(10^{-3}$ min$)^{-1}$)	0·21 ± 0·05	0·37 ± 0·03	0·46 ± 0·07	0·86 ± 0·16
Circumference (l_0, mm)	23·2 ± 0·7	22·1 ± 0·5	24·8 ± 0·3	26·0 ± 0·8
Body weight of rats (g)	118 ± 7	101 ± 2	109 ± 5	104 ± 6

The estimate of variation is the standard error of the mean. K (mm/min) is the rate of extension of inner circumference of rings of skin; l_0 is the value of circumference obtained by extrapolating the linear part of the time circumference curve to zero time. There were six samples in each group, except the highest tension group for skin which contained five.

The rate of extension was found to be linearly related to load within the limits of variation, which were large (Table 4; Figs. 1 and 2). This relation

was later used to adjust values of rate of extension to constant load. The value of l_0 increased slightly with load, as would be expected if there were a simple elastic element in the tissue. The regression of l_0 (mm) on tension

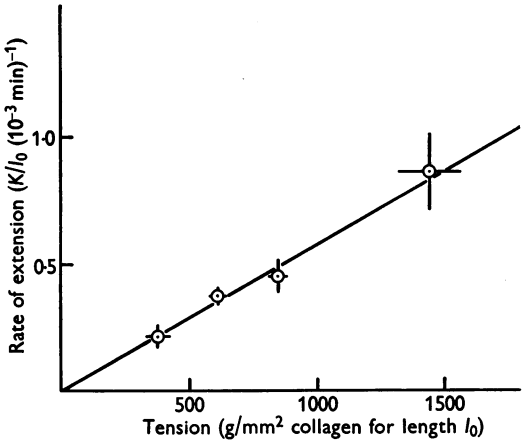

Fig. 1. Effect of load on rate of extension of intestinal wall (rectum). The length of the lines through each point is twice the standard error of the mean.

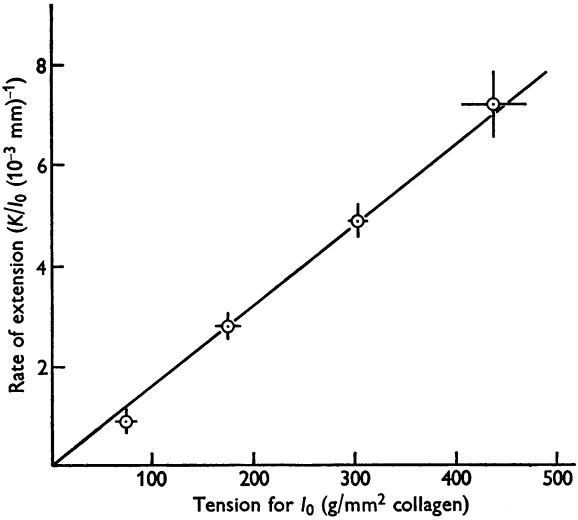

Fig. 2. Effect of load on rate of extension of skin. The length of the lines through each point is twice the standard error of the mean.

f(g/mm^2 collagen) was calculated. In the equation $l_0 = a + bf$, for skin and intestine, respectively, the values of a were 29·7 and 21·3 mm; of b $10{\cdot}9 \times 10^{-3}$ and $3{\cdot}3 \times 10^{-3}$ mm^3/g. The value of a can be regarded as a measure of the circumference of the collagenous framework under no

tension, and it is of interest that in both skin and intestine it was close to the value of l_z obtained by extrapolating the linear part of the length–tension curve to zero. Values of the latter were 29·2 and 21·3 mm, respectively.

Comparison between extensibility of normal and lathyritic tissues. The results are given in Table 5. The mean values of K/l_0 ($(10^{-3}$ min$)^{-1}$) for skin and intestine of lathyritic rats were greater than for the controls, but the difference was not significant. For chick's skin, however, values were significantly (fifteen times) greater in lathyritic animals. An example is shown in Fig. 3. In correcting the figures to constant tension per unit cross-sectional area of collagen, the same linear relation between load and rate of extension as found for rat's skin was assumed.

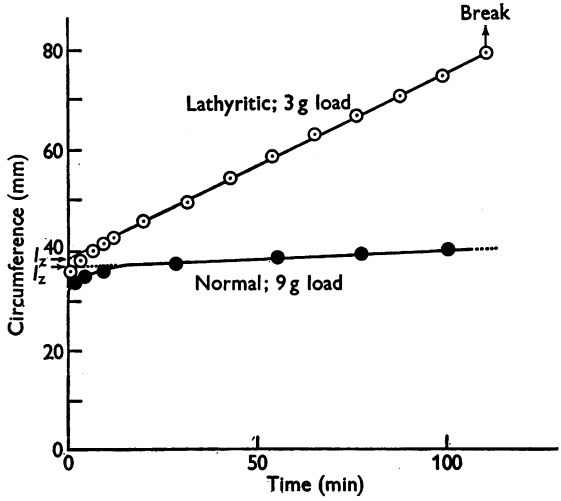

Fig. 3. Effect of constant load on inner circumference of rings of skin from neck of normal and lathyritic chicks. Loading for normal chick 197 g/mm² collagen, for lathyritic chick 77 g/mm² collagen, calculated for length l_0.

In the tests on rat skin the constant load was left on overnight and the tissues were ruptured. An incidental finding which emerged was that the circumference at rupture was nearly the same whether the tissues were ruptured in this way or by rapidly increasing the load. For normal skin, circumferences at rupture were $39{\cdot}8 \pm 1{\cdot}2$ mm and $38{\cdot}9 \pm 1{\cdot}1$ mm for constant load and increasing load, respectively. For lathyritic skin the corresponding figures were $37{\cdot}9 \pm 1{\cdot}1$ mm and $35{\cdot}4 \pm 0{\cdot}9$ mm, respectively.

TABLE 5. Extensibility of tissues of normal and lathyritic animals

	Rat skin		Rat intestine		Chick skin	
	Normal	Lathyritic	Normal	Lathyritic	Normal	Lathyritic
1. Extensibility (K/l_0 (10^{-3} min)$^{-1}$) corrected to tension of 500 g/mm^2 collagen at l_0	$1{\cdot}09 \pm 0{\cdot}34$	$1{\cdot}34 \pm 0{\cdot}25$	$0{\cdot}37 \pm 0{\cdot}12$	$0{\cdot}44 \pm 0{\cdot}12$	$6{\cdot}4 \pm 2{\cdot}3$	102 ± 15
2. Circumference (l_0 mm)	$33{\cdot}3 \pm 1{\cdot}4$	$29{\cdot}9 \pm 0{\cdot}9$	$27{\cdot}9 \pm 0{\cdot}5$	$25{\cdot}9 \pm 0{\cdot}3$	$33{\cdot}9 \pm 1{\cdot}8$	$35{\cdot}9 \pm 0{\cdot}8$
3. Tension (g/mm^2 collagen calculated for l_0)	268 ± 24	149 ± 6	364 ± 23	390 ± 24	227 ± 30	55 ± 5

The estimate of variation is the standard error of the mean. The number of rats in each group is the same as in Table 3. Extensibility (K/l_0) was corrected to tension 500 g/mm^2 collagen at l_0. Loads were applied to rat intestine and chick skin for 2–3 hr, to rat skin overnight.

DISCUSSION

Properties of normal tissues

Tensile strength in normal tissue falls in the range between 1·5 and 4 kg/mm^2 collagen. Values for the cervix uteri of the rat previously determined (Harkness & Harkness, 1959; Cullen & Harkness, 1960) were in the range 1–2 kg/mm^2 collagen.

Extensibility. The extensibility of normal tissues shows a wider range of variation than tensile strength. Though values of K/l_0 for aorta were too low to measure accurately enough to make it worth while to record them, they were measured approximately and found to be below 0·1 $(10^{-3}\,\text{min})^{-1}$. At corresponding values of tension the figure for the cervix uteri at the end of pregnancy, the most extensible normal tissue found so far, would be 20–30 $(10^{-3}\,\text{min})^{-1}$ (Harkness & Harkness, 1959). Values for intestine and skin lay between these extremes (intestine 0·3 $(10^{-3}\,\text{min})^{-1}$; rat skin 1 $(10^{-3}\,\text{min})^{-1}$; chick skin 6 $(10^{-3}\,\text{min})^{-1}$). It is to be noted, however, that these wide variations in extensibility cannot be attributed with certainty entirely to differences in the linking of the collagenous framework. In skin, intestine and cervix collagen forms the major continuous structural framework in the tissue and must be involved, since the extension continues in time to the point of rupture. In the wall of the aorta, however, the elastin content is relatively high and may be higher than the collagen content (see Harkness, Harkness & MacDonald, 1957). In this case the low extensibility could be a property of the elastin rather than of the collagenous framework.

The variations between the measured values of extensibility in different tissues are in general such as might be expected from their different functions; for example, the high extensibility of the cervix at the end of pregnancy, and the low extensibility of the aortic wall which is permanently under tension during life. Extensibility of skin was, however, unexpectedly high. Tension in the walls of the aorta (dog) during life can be calculated to be about 100 g/mm^2 of collagen. If the collagenous framework were as extensible as in skin this tension would produce an increase in circumference of about 3 %/hr.

Extension of skin during normal growth

Alteration in the size of the collagenous framework during normal growth may involve absorption and formation anew at a larger size, for example, in bone. Growth by such a process, involving the continuous destruction of collagen as it proceeds, must result in a high 'turnover' rate for this substance. But in soft tissues which show continuous extension under load, such as skin, growth could occur without destruction of

collagen, by the molecules or fibrils slowly moving relatively to one another into new positions. No 'turnover' of collagen would be necessary. All the information needed to establish whether the growth of the collagenous framework of skin involves destruction and replacement or extension is not available. But such information as there is, from rates of incorporation of isotopically labelled amino acids, indicates that 'turnover' is too slow for growth to be entirely by the former method without extension of the framework. The figures available come from experiments in which a single dose of labelled amino acid was given initially. Animals were killed subsequently and the activity in this amino acid or another derived from it was measured. In the earlier work (rats, Neuberger & Slack, 1953) α-^{14}C-glycine was used. In later work ^{14}C-labelled lysine has been used with isolation of the derived hydroxylysine (Kao, Hilker & McGavack, 1960, 1961). In such experiments on growing animals a rapid rise in activity in the amino acid isolated from collagen is found, to a maximum in a matter of days, followed by a much slower fall with a half-time measured in weeks or months.

The course of this change in the isotopic content of the collagen can be explained, in general terms, on the assumption that the initial rise is caused by the new formation of collagen from an amino-acid pool of high isotopic concentration, and the subsequent fall is caused, partly by its destruction later ('turnover'), but mainly by the addition of collagen with low isotopic content formed in growth.

Turnover can be assessed from the change in isotope content of the collagen with time by subtracting the effect of growth dilution. To do this it is necessary to know the rate of growth of collagen and the time course of the isotopic concentration in the pool of amino acid used by the synthesizing cells. If one assumes that this time course is the same as that of free α-^{14}C-glycine in plasma measured by Henriques, Henriques & Neuberger (1955) up to 18 hr after a single injection of labelled material, and if one extrapolates exponentially, one finds that over 90 % of the total isotopic incorporation will take place in the first day. Measurement of isotopic activity in plasma-free glycine of rats by Smith & Armstrong (1961) from 3 days onwards after administration provides confirmatory evidence that extrapolation is reasonable. If as an approximation one assumes that all the isotope is incorporated in the first day, and if one computes from the change in body weight the effect of growth dilution allowing for the fact that skin collagen increases faster than body weight (see Harkness, 1961), one finds that the fall in isotopic activity is completely (data of Kao *et al.* 1961) or nearly completely (data of Neuberger & Slack, 1953) accounted for; so that little or no turnover is needed to explain the results. The weakness in the argument is the assumption that the time course of

isotopic activity in the free amino acid used to synthesize the collagen is the same as in the plasma. Henriques *et al.* (1955) found that, after a single injection of labelled glycine, the activity of free amino acid in liver after the first half hour followed a time course not greatly different from that in plasma; but in skeletal muscle equilibration of plasma and tissue was slower. In such a case the low activities in the pool after the first day may not be negligible. However, Harkness, Marko, Muir & Neuberger (1954) found that the activity in the most soluble form of collagen in the skin varied with time similarly to that in plasma protein, which means that the free amino-acid pool from which it is formed must be like that of liver rather than skeletal muscle. Though it is in theory possible that collagen molecules are broken down and the parts immediately used to form new collagen, a process which would not be detectable by this type of isotopic investigation, the most reasonable conclusion is that the turnover of collagen in skin is low. As the collagen macromolecule is by nature virtually inextensible, extension of the whole tissue must involve movement of the parts of the framework relatively to one another. It is therefore interesting to compute from our measurements the tension which would be required to extend the tissues at the rates found in normal growth. If we take a rate of growth in body weight of 2 %/day, which is of the order which might be found in rats of the size used, and assume a rate of linear extension in skin proportional to the cube root of this, a tension of about 5 g/mm^2 of collagen would be needed, or 0·5 g/mm^2 of whole tissue. This is approximately the tension in the wall of a capillary, calculated on the assumption that the diameter is 10 μ, the wall thickness 2 μ, and the pressure of the fluid contents 20 mm Hg. Much higher tensions can be exerted by cells, for example, those of skeletal muscle which can produce a tetanic tension of the order of 30 g/mm^2.

Properties of tissues of lathyritic animals

Composition of tissue. The absence of any difference in the concentration of collagen in the tissues of lathyritic and normal animals confirms the findings of Levene & Gross (1959) and Levene (1961). Kalliomaki, Yli-Pohja & Kulonen (1957) found no difference in hydroxyproline content of normal and lathyritic rat skin per unit dry weight; they found the hydroxyproline content of bone significantly higher (about 25 %) than normal.

Mechanical properties. Levene & Gross (1959) applied a constant load to the heads of chick embryos to stretch the neck longitudinally and found that the time until the tissues ruptured was much less in the lathyritic animals. Levene (1961) obtained similar results on the aorta of chicks. Our results are in general agreement with these findings, though a direct quantitative comparison is not possible.

J. Viljanto, T. Tuominen and E. Kulonen (personal communication) found tensile strength per unit cross-sectional area of tendon, calculated from weight, reduced in lathyritic rats to about half the normal value, which, on the assumption that the tissues had the same concentration of collagen, is a drop of tensile strength of the same order as we found. Reduction in tensile strength of wounds in lathyritic animals has been reported (Kalliomaki *et al.* 1957) but in this case there is not enough evidence to justify the assumption that the same quantity of collagen is present as in normal wounds. In contrast to the above findings Bell & Sharma (1960) have reported no change in the strength of bone in lathyritic rats.

Nature of change in the tissues in lathyrism

The change from normal could theoretically be produced by reorientation of the fibre bundles so that they no longer lay in the direction of the mechanical stress applied. That breaking strength and extensibility do not change in the same ratio argues against this improbable explanation and the fact that our own mechanical tests and those of Levene & Gross (1959) on the chick were applied in directions at right angles (across and along the neck respectively), but gave essentially similar results effectively excludes it. The abnormality must therefore be in the nature of the ultimate linkage in the collagenous framework. Levene & Gross (1959) have shown that the collagen is more readily extractable from the affected tissues by solutions of sodium chloride but in solution behaves as normal collagen, and have suggested that there is a defect in inter-molecular cross-linkage. The present experiments do not throw any further light directly on the nature of the defect, though they define it in a more precise quantitative manner. They also show that in the chick the gross abnormality which develops in the mechanical properties of the collagenous framework does not lead to abnormality in the size of this framework, which suggests that the extensibility of the framework is not normally a limiting factor in its growth.

SUMMARY

1. Measurements have been made of the collagen content and some mechanical properties of tissues of lathyritic animals; skin, intestine and aorta from rats fed on a diet containing sweet-pea meal, and skin from chicks after injection of β-amino propionitrile into the eggs.

2. The concentration of collagen was found not to differ significantly in the normal and lathyritic tissues.

3. Tensile strength, calculated per unit cross-sectional area of collagen, in skin and aorta of lathyritic rats was about half that in normal animals. In skin of lathyritic chicks it was about one fifth that in normal chicks.

In intestine of rats no difference was found between normal and lathyritic animals.

4. The 'extensibility' of the tissues was measured by the rate of extension, after this had become constant under a constant load, corrected to standard tension per unit cross-sectional area of collagen. No differences between normal and lathyritic tissues were found in skin and intestine of rats, but extensibility was about fifteen times greater in the skin from lathyritic chicks than from normal ones. Evidence was obtained that this difference in extensibility was associated with no significant difference in the size of the collagenous framework, which suggests that extensibility is not a limiting factor in the growth of the latter.

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