



(86) Date de dépôt PCT/PCT Filing Date: 1998/01/05
(87) Date publication PCT/PCT Publication Date: 1998/07/16
(45) Date de délivrance/Issue Date: 2007/10/30
(85) Entrée phase nationale/National Entry: 1999/06/28
(86) N° demande PCT/PCT Application No.: DK 1998/000001
(87) N° publication PCT/PCT Publication No.: 1998/030900
(30) Priorités/Priorities: 1997/01/06 (DK0009/97);
1997/07/07 (DK0823/97); 1997/12/01 (DK1383/97)

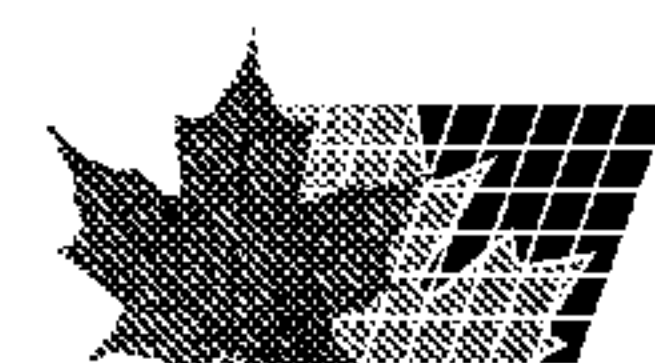
(51) Cl.Int./Int.Cl. *A61K 31/522* (2006.01),
A61K 31/00 (2006.01), *A61K 31/52* (2006.01),
A61P 27/02 (2006.01)
(72) Inventeur/Inventor:
TRIER, KLAUS, DK
(73) Propriétaire/Owner:
KLAUS TRIER APS, DK
(74) Agent: GOWLING LAFLEUR HENDERSON LLP

(54) Titre : PROCEDE DE SELECTION

(54) Title: SCREENING METHOD FOR COMPOUNDS ACTIVE IN TREATING MYOPIA AND HYPERMETROPIA

(57) **Abrégé/Abstract:**

The invention relates to a method for identification of substances which are applicable for treatment or prevention of an insufficient longitudinal growth of the eye (hypermetropia) or for treatment or prevention of an excessive longitudinal growth of the eye (myopia); substances identified by the method for treating or preventing conditions related to the longitudinal growth of the eye; substances and mixtures of substances for the preparation of a pharmaceutical composition for the treatment or prevention of abnormal growth of the axial length of the eye. The identification involves measuring the effect of the substances on the retinal pigment epithelium of the eye, e.g. by detecting the metabolic effect of the substance on the retinal epithelium, the effect on the standing potential or the effect on the proteoglycans of the scleral tissue of the eye, by way of EOG examination, by way on the size of the so-called c-wave in ERG-recordings, or by the state of the Ca²⁺-channels or on the [³H]-ryanodine receptors of the retinal pigment epithelium.



WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

(51) International Patent Classification ⁶ : A61K 31/52		A3	(11) International Publication Number: WO 98/30900
			(43) International Publication Date: 16 July 1998 (16.07.98)
(21) International Application Number: PCT/DK98/00001		(81) Designated States: AL, AM, AT, AT (Utility model), AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, CZ (Utility model), DE, DE (Utility model), DK, DK (Utility model), EE, EE (Utility model), ES, FI, FI (Utility model), GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK (Utility model), SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).	
(22) International Filing Date: 5 January 1998 (05.01.98)			
(30) Priority Data:			
0009/97	6 January 1997 (06.01.97)	DK	
0823/97	7 July 1997 (07.07.97)	DK	
1383/97	- 1 December 1997 (01.12.97)	DK	
(71) Applicant (for all designated States except US): KLAUS TRIER APS [DK/DK]; Tingskiftevej 6, DK-2900 Hellerup (DK).			
(72) Inventor; and			
(75) Inventor/Applicant (for US only): TRIER, Klaus [DK/DK]; Tingskiftevej 6, DK-2900 Hellerup (DK).		Published With international search report.	
(74) Agent: PLOUGMANN, VINGTOFT & PARTNERS; Sankt Annæ Plads 11, P.O. Box 3007, DK-1021 Copenhagen K (DK).		(88) Date of publication of the international search report: 10 December 1998 (10.12.98)	

(57) Abstract

The invention relates to a method for identification of substances which are applicable for treatment or prevention of an insufficient longitudinal growth of the eye (hypermetropia) or for treatment or prevention of an excessive longitudinal growth of the eye (myopia); substances identified by the method for treating or preventing conditions related to the longitudinal growth of the eye; substances and mixtures of substances for the preparation of a pharmaceutical composition for the treatment or prevention of abnormal growth of the axial length of the eye. The identification involves measuring the effect of the substances on the retinal pigment epithelium of the eye, e.g. by detecting the metabolic effect of the substance on the retinal epithelium, the effect on the standing potential or the effect on the proteoglycans of the scleral tissue of the eye, by way of EOG examination, by way on the size of the so-called c-wave in ERG-recordings, or by the state of the Ca²⁺-channels or on the [³H]-ryanodine receptors of the retinal pigment epithelium.

Screening Method

The invention relates to a method for identification of substances which are applicable for treatment or prevention of an insufficient longitudinal growth of the eye
5 (hypermetropia) or for treatment or prevention of an excessive longitudinal growth of the eye (myopia); substances identified by the method for treating or preventing conditions related to the longitudinal growth of the eye; substances and mixtures of substances for the preparation of a
10 pharmaceutical composition for the treatment or prevention of abnormal growth of the axial length of the eye.

Background of the invention

Myopia is caused by the length of the eye being too big in relation to the optical strength of the cornea and the lens
15 so that the picture of a distant object is focused in a point in front of the retina, whereas the picture produced on the retina will be blurred. In other words, myopia is caused by an anomaly between the length of the eye (the axial length) and the refraction in the cornea and the lens.

20 The longitudinal growth of the eye (from approximately 17 mm at birth to approximately 24 mm) during the childhood is caused by expansion of the eye content and thus stretching the immature connective tissue in the sclera of the eye which adjusts to the new size of the eye. Normally the eye will
25 reach its permanent length at the age of 12, at which time the connective tissue in the sclera reaches an appropriate degree of maturity and the longitudinal growth of the eye will stop.

In myopic persons the longitudinal growth of the eye is too
30 high and the longitudinal growth of the eye continues for a longer period of time than in normal individuals.

WO 98/30900

PCT/DK98/00001

2

Hypermetropia is caused by the length of the eye being too short in relation to the optical strength of the cornea and the lens. Hypermetropia usually prevails at birth and is normally recorded at the age of 3-5 years. Subsequently it will reduce in proportion with the growth of the eye until the age of 12, from which age it will remain constant for the rest of the persons life.

Approximately 25% of the population are myopic. In some myopic persons the axial length is normal (physiological myopia, of <-4 dioptre), in other persons, the axial length grows from the age of 8-10 unproportionally much until the approximate age of 20, and subsequently the axial length and thus the myopia are stable (intermediary myopia, glass strength of from approximately -4 to -8 dioptres).

Finally, in rare cases a continuously growing axial length throughout the entire life can be seen, often connected with bulges in weak areas of the eye wall (scleraectasies). Here the myopia can reach extreme levels for glass strengths of up to approximately -40 dioptres (Excessive/pathological myopia).

The intermediary form, and the excessive one in particular, is connected to a high risk of severe sight threatening complications such as e.g. retinal detachment, degenerative changes in the yellow spot of the eye (macula degeneration) and glaucoma.

In the Western part of the world, severe myopia is among the most important causes of blindness.

The group of myopic persons with a glass strength of more than -6, which comprises parts of the intermediary group and the entire excessive group, comprises approximately 2% of the population, e.g. in Denmark approximately 100 000 persons (Curtin, B.J.: The myopys: Basic science and clinical management, Harper and Row, Philadelphia, (1985)).

The cause of axial length conditional myopia is unknown.

It is however known that the longitudinal growth of the eye can be increased by disturbance of the image formation on the retina, eg. experimentally by sewing together the eye lids of
5 test animals (visual deprivation) (Yinon, U., Current Eye Research, vol. 3, 4, 677-690, 1984).

Administration of dopaminergic substances (apomorphine) in test animals exposed to visual deprivation inhibits the development of myopia. (Iuvone, P.M., Invest. Ophthalmol.
10 Vis. Sci., 32, 1674-77 (1991)).

US-patent No. 5,055,302, Laties and Stone, shows a method for control of abnormal postnatal growth of the eye of an animal with the application of vasoactive intestinal peptide (VIP), PH1 or analogues of such peptides. Such peptides were found
15 to restrain the axial longitudinal growth of a myopic eye.

US-patent No. 5,122,522, Laties and Stone, shows a method for control of abnormal postnatal growth of the eye of an animal with the application of pirenzepine, an anticholinergic substance (M1 Muscarine antagonist). The axial longitudinal
20 growth was inhibited by administration of pirenzepine.

PCT-patent application publication No. WO 94/25034, Laties and Stone, shows a method for control of abnormal postnatal growth of the eye of an animal with the application of tricyclic substances (antidepressiva). The axial longitudinal
25 growth was inhibited by administration of tricyclic substances.

However, in most cases myopia and hypermetropia are benign conditions which can easily be corrected by means of glasses. In order to justify a medical treatment of these conditions,
30 such treatment must be effective at relatively low dosages and roughly without any side effects, accordingly, as appli-

cation of VIP, dopaminergic, anticholinergic or tricyclic substances is connected to risk of side effects as, simultaneously, the substances have considerable psychochemical effects these prior art substances are not suitable for such
5 treatment.

It is also a theory that the growth of the eye can be caused by passive stretching of the scleral connective tissue (Norton, T.T., Invest. Ophthalmol. Vis. Sci., 37(3), S324 (1996), Siegwart Jr., J.T., Invest. Ophthalmol. Vis. Sci., 37(3),
10 S324 (1996)). Thus it is been shown possible to trigger irreversible stretching of the sclera in young rabbits by increasing the intraocular pressure but it is not possible to stretch the sclera in mature rabbits (Greene, P.R., ARVO Abstracts, 1978, p. 297). However, tests with reduction of
15 the intraocular pressure by means of beta-blocking eye drops in humans developing myopia have no effect (Jensen, H., Acta Ophthalmol., Suppl. 200, 69 (1991)).

There is no model for animal experiments which precisely corresponds to the human conditions. As mentioned above, it
20 is possible to provoke myopia in some animals, e.g. cats and chicken, by sewing together the eye lids of newborn animals, but partly this experimental myopia develops much more rapidly than in humans, and partly the biological age of the animal (newborn) does not correspond to the same age when the
25 myopia typically occurs in human (8-12 years). Furthermore, in chicken the sclera is considerably anomalous as it partly consists of cartilage.

As the conditions of the eye related to the refractory system is extremely common and preventive treatment is to be applied
30 to children, probably during years of treatment, effective substances should be very safe. Accordingly, it would be a considerable improvement if a method for identification of a number of substances having an effect on the longitudinal growth of the eye was available. Among substances such ident-
35 ified, it would subsequently be possible to select appropri-

WO 98/30900

PCT/DK98/00001

5

ate substances characterized by high efficiency and few side effects.

The present invention is related to methods for identification of substances or groups of substances being candidates
5 for the treatment or prevention of disease of the eye related to the longitudinal growth of the eye.

One of the methods for identification of effective substances according to the present invention is related to the fact that, developmentwise, the retinal pigment epithelium is a
10 part of the retina and forms an electrochemically active cell layer which is located between the choroid membrane of the eye and the neuronal part of the retina (neuroretina). It forms an electrically tight barrier and due to active ion transport (based on the $\text{Na}^+\text{-K}^+$ pump) it creates a difference
15 in potential (the standing potential), the cornea-fundal potential, between the inner and the outer part of the eye of approximately 5 Mv.

Due to the anatomical conditions of the eye, this electrical field is in the nature of a dipole with + at the cornea and -
20 at the back pole of the eye. The size and changes of the standing potential can thus be estimated with electrodes placed on each side of the eye by means of sideways movements of the eye. (EOG examination (electrooculography which is well known in the art).

25 The standing potential is furthermore positively correlated with the size of the so-called c-wave in ERG-recordings (electroretinography, which is well known in the art).

According to one aspect of the present invention, the growth of the eye is related to the size and changes of the standing
30 potential of the retinal pigment epithelium of the eye.

The transepithelial potential of the retinal pigment epithelium is maintained by a difference in ionic concentration between the cytoplasm of the pigment epithelial cell and

the internal and external surface. The state of the Ca^{2+} -channels determines intracellular Ca^{2+} . According to one aspect of the present invention, it is believed that substances affecting the state of Ca^{2+} simultaneously affect the standing potential created by the retinal pigment epithelium. The state of the Ca^{2+} -channels may be determined by [^3H] ryanodine binding as the plant alkaloid ryanodine has been extensively used to study the functional interaction of the Ca^{2+} -release channel (Meissner G: Ryanodine receptor/ Ca^{2+} release channels and their regulation by endogenous effectors, Annu Rev Physiol 1994;56:485-508).

Accordingly, in a further aspect of the invention, the effect of different drugs on [^3H]-ryanodine binding can be used to screen for drugs that either strengthens the scleral connective tissue (increases the content of proteoglycans in sclera), or weakens it (decreases the content of proteoglycans in sclera).

According to a still further theory behind the present invention, the biomechanical strength of the tissue is believed to be decisive for the stretching of the sclera rather than a change of the intraocular pressure as described in the prior art above. Substances increasing or decreasing the strength of the scleral tissue may be used according to the present invention for the treatment or prevention of disease relating to the longitudinal growth of the eye. In a further aspect, substances having a substantially selective effect on the posterior part of the sclera is preferred.

In a still further aspect of the present invention, substances having an effect on the content of proteoglycans in the scleral tissue may be used for the treatment or prevention of disease relating to the longitudinal growth of the eye.

Connective tissue such as eg. sclera mainly consists of scattered cells in a base substance of proteoglycans (pro-

WO 98/30900

PCT/DK98/00001

7

teins added to glycosaminoglycanes (branched polysaccharides)) and collagen fibres. The biomechanical properties of the tissue are determined by the content of these components and their organisation (Scott, J.E., Dermatan Sulphate Proteoglycans, Portland Press, 1993).

Furthermore, it has been demonstrated that fibroblasts in cell cultures may react to physiological electrical fields by taking on an oblong shape and orienting itself with the longitudinal axis orthogonally with the direction of the field (Erickson, C.A, et al., Cell Biol, Vol. 98, January 1984, 296-307). It has also been demonstrated that the connective tissue substance which is produced by fibroblasts may be determined by the shape of the cell (Evangelisti, R. et al, Eur. J. Histochem., 37, 161-172, 1993).

15 Brief description of the present invention

The present invention relates to a method for identification of substances which have an effect, either inhibiting or increasing, on the longitudinal growth of the human eye.

In another aspect, the invention relates to a method for treating and/or preventing myopia and hypermetropia and to pharmaceutical compositions for such treatment, as well as for use of substances for the preparation of medicaments useful for such treatment or prevention.

According to the present invention it is presumed that some of these substances act mainly on the retinal pigment epithelium and in addition on the receptors of the neuroretina.

In accordance with the present invention it is however believed, that the substances in the last end exerts the treating or preventing effect through an effect on the ion transport in and out of the cells in the retinal pigment

WO 98/30900

PCT/DK98/00001

8

epithelium and thereby exerts its effect on the longitudinal growth of the eye.

In one aspect the invention comprises screening of an optional number of substances for the effect on the metabolic activity in the retinal pigment epithelium of the eye.

This effect may be measured by different methods. One of these methods relates to the activation of the Ca^{2+} -release channel. This Ca^{2+} -release channel may be influenced by different effectors on receptors on the cell membrane.

10 Examples of such receptors are the ryanodine receptor (RyR) and the inositol triphosphate (IP_3) receptor.

In another aspect, the present invention relates to the surprising finding that substances affecting the size of the standing potential are useful for the treatment or prevention of abnormal growth of the axial length of the eye.

Accordingly, by measuring of effect of the substances on the electrochemical potential over the retinal pigment epithelium, the so-called "standing potential", or the effect on the so-called "c-wave" by electro retinography (ERG),

20 suitable substances for treating or preventing conditions related to the longitudinal growth of the eye are easily identified.

Substances increasing the standing potential or c-wave will inhibit the longitudinal growth of the eye and will thus be applicable for treatment of myopia. Substances reducing the standing potential or c-wave will increase the longitudinal growth of the eye and will thus be applicable for treatment of hypermetropia.

In a further aspect of the invention it is believed that physiological electrical fields are important in relation to the extracellular matrix of the scleral tissue and thereby to the biomechanical properties of sclera. An increase of the

electrical field is believed to influence the connective tissue cells in the sclera to take on an oblong form and deposit orthogonally on the field, i.e. in the longitudinal direction of the sclera, making the tissue organise more
5 appropriately as concerns content of base substance and packing of collagen fibres. After long time treatment the sclera will be more resistant to draft and thus less likely to give in to the intraocular pressure, thus avoiding development of myopia.

10 In a further aspect of the invention, substances affecting the composition of the proteoglycanes may be used for the treatment of abnormalities of the longitudinal growth of the eye.

In a further aspect, the invention comprises a method involving
15 analysis of sclera tests from test animals after treatment (0-6 months) with substances influencing the sclera. Accordingly, the method comprises identifying one or more of the following elements: the effect of the substance on the proteoglycanes; the distribution between various glycosaminoglycane types; and the content of collagen specific amino
20 acids. The analysis may additionally comprise identifying the density of collagen fibrils and the distribution between various fibrils diameters by means of electron microscopy.

According to the present invention, it is possible that the
25 effect on the composition of the proteoglycanes and/or the collagen specific amino acid present in the connective tissue of the sclera of the eye is due to an influence on the metabolical activity in the retinal pigment epithelium of the substance of the eye.

30 Substances increasing the content of proteoglycanes and collagen specific amino acids will strengthen the connective tissue in the sclera, reduce the longitudinal growth of the eye and thus work against myopia. Substances increasing the content of dermatane sulphate in proportion to the other

WO 98/30900

PCT/DK98/00001

10

glycosaminoglycanes will furthermore strengthen the connective tissue in the sclera and work against myopia. Substances increasing the density of collagen fibrils and increasing the diameter of the fibrils will furthermore strengthen the
5 connective tissue in the sclera and work against myopia.

Substances reducing the content of proteoglycanes and collagen specific amino acids will weaken the connective tissue in the sclera, increase the longitudinal growth of the eye and thus work against hypermetropia. Substances reducing
10 the content of dermatane sulphate in proportion to the other glycosaminoglycanes will weaken the connective tissue in the sclera and work against hypermetropia. Substances reducing the density of collagen fibrils and reducing the diameter of the fibrils will furthermore weaken the connective tissue in
15 the sclera and work against hypermetropia.

In a further aspect, the invention relates to a method for identifying substances affecting the composition of the proteoglycanes of the sclera of the eye, and thereby exert an effect of the strength of the connective tissue.

20 Brief description of the figures

Fig. 1 shows the inhibiting effect of a combination of caffeine and L-cystine on the longitudinal growth of the human eye.

Fig. 2 shows an example of an analysis curve for amino acids
25 by the application of HPLC.

Fig. 3 shows the general formula of xanthine and of three derivatives thereof, caffeine, theophylline and theobromine, respectively. As appears from the figure, caffeine and theobromine are substituted in position 7 by a methyl group.

Fig. 4 shows the content of hydroxyproline in anterior and posterior sclera after treatment with the substances described in Example 2.

Fig. 5 shows the content of hydroxylysine in anterior and posterior sclera after treatment with the substances described in Example 2.

Fig. 6 shows the content of proline in anterior and posterior sclera after treatment with the substances described in Example 2.

Fig. 7 shows the content of proteoglycanes in anterior and posterior sclera after treatment with the substances described in Example 2.

Fig. 8 shows the content of hydroxyproline in anterior and posterior sclera after treatment with the substances described in Example 4.

Fig. 9 shows the content of hydroxylysine in anterior and posterior sclera after treatment with the substances described in Example 4.

Fig. 10 shows the content of proline in anterior and posterior sclera after treatment with the substances described in Example 4.

Fig. 11 shows the content of proteoglycanes in anterior and posterior sclera after treatment with the substances described in Example 4.

Fig. 12 shows the correlation between the effect of different methylxanthines on the content of proteoglycanes in posterior sclera and the effect of the same methylxanthines on [³H] ryanodine binding capacity compared to that of the control, xanthine. The values appears from Table 13. Caffeine is represented by 1,7-dimethylxanthine since this is the most

dominating metabolite in serum of rabbits after feeding with caffeine. As appears from the figure, a ³H ryanodine binding exceeding 130% compared to xanthine seems to be needed to produce a positive effect on the content of proteoglycanes in sclera.

Interesting embodiments of the aspects of the invention appear from the appended claims.

Detailed description of the invention

The present invention applies the surprising finding that substances which have an increasing effect on the standing potential or c-wave of the eye by ERG will increase the scleral connective tissues ability to resist abnormal growth or stretching, and thus work against development of myopia. The increased resistant may according to the present invention be due to increase in the content of proteoglycanes and/or collagen specific amino acids such as hydroxyproline, hydroxylysine and proline. Furthermore, an increase in the diameter of the collagen fibrils may also contribute to the effect according to the invention.

It is furthermore believed that the specific glycosaminoglycanes of the proteoglycanes may have an effect on the structure so that an increased proportion of e.g the content of dermatane sulphate in percentages is related to increased mechanical strength of the sclera and thus also work against development of myopia. Similarly, a decrease works against development of hypermetropia

In a further embodiment, the invention applies the surprising finding that substances which reduce the standing potential or c-wave of the eye by ERG will, in similarity, reduce the content of proteoglycanes and/or collagen specific amino acids, reduce the content of dermatane sulphate in percentages, reduce the diameter of the collagen fibrils, and thus

WO 98/30900

PCT/DK98/00001

13

reduce the scleral connective tissues ability to resist stretching, and thus reduce the level of hypermetropia.

In order to examine whether a substance is able to produce a change in the connective tissue substance of the sclera, it is normally necessary to treat test animals for a long time, presumably several months. Therefore it would be very resource consuming to test a large number of substances by means of this method alone. By confining oneself to testing substances which influence the standing potential or c-wave of the eye by ERG, it becomes much more likely to identify substances which with few or no side effects are able to influence the scleral connective tissue and thus be applicable for treatment of myopia or hypermetropia.

As will be described in the following, the invention further relates to the surprising finding, that substances effecting the metabolic state of the cells of the retinal pigment epithelium are also able to induce the effects described above. This effect may be detected in different ways, such as by the state of the Ca^{2+} -channels. In addition, the state of the Ca^{2+} -channels may be determined by [^3H] ryanodine binding, or in another preferred embodiment of the present invention, by the inositol triphosphate (IP_3) receptor. Inositol triphosphate increase intracellular calcium.

Accordingly, substances binding to the receptors and thereby results in an increase in the intracellular calcium ions may be utilized for the treatment of myopia, and substances which inhibit the receptors may be used in the treatment of hypermetropia in accordance with the teaching of the present invention.

The method according to the invention for screening substances effective of treating disorders of the eye related to the axial length of the eye comprises identifying substances having an effect on the retinal pigment epithelium. It is believed that substances having an effect on the more primi-

tive pigment epithelium and a rather limited effect on neural tissue will result in a treatment having less side effects compared to the use of substances having a substantial effect on the central nervous system.

- 5 Accordingly, the present invention in a still other aspect relates to the use of substances wherein the effect on the pigment epithelium is primarily on the ion exchange over the cell membrane. This effect may be regarded as a metabolic effect. Accordingly, in a further aspect of the invention,
- 10 the method is for screening substances effective of treating disorders of the eye related to the axial length of the eye and comprises identifying substances having an effect substantially on the pigment epithelium compared to the effect on the neuroretina.
- 15 The present invention relates in its broad definition to a method for screening substances effective of treating disorders of the eye related to the axial length of the eye. The method essentially comprises the identification of substances having an effect on the retinal pigment epithelium of the eye
- 20 as this effect on the retinal pigment epithelium has a clear connection to the function of the control of the axial growth of the eye.

As the function of the retinal pigment epithelium is possibly also affected by a direct or receptor effect on neuroretina,

25 the effect on the retinal pigment epithelium may be through an effect of the substance on the neuroretina. In a further aspect of the invention, the substance may have an effect directly on the neuroretina together with a direct effect on the retinal pigment epithelium. In a further aspect the

30 effect on the neuroretina also induces an effect on the retinal pigment epithelium.

The ion exchange pumping on the cell membrane is driven by cAMP. An effect of a substance to be screened may exert an inhibiting or increasing effect on the pump and thereby on

WO 98/30900

PCT/DK98/00001

15

the function of the retinal epithelium. In other words it could be expressed by the fact that the substances to be screened are selected by the metabolic effect of the substance on the pigment epithelium.

- 5 One preferred method according to the invention is to identify the effect on the pigment epithelium by means of the standing potential and/or on the c-wave by electro retinography (ERG). In a further aspect, also the effect on the a-wave and/or on the b-wave in the neuroretina is measured whereby
10 it is possible to identify substances having an effect substantially on the retinal pigment epithelium compared to an effect on the neuroretina where the latter is shown as an effect on the a-wave and/or on the b-wave.

In order to identify substances effective of inhibiting the
15 longitudinal growth of the eye, substances increasing the standing potential and/or the c-wave are selected. These substances may be used for the treatment or prevention of myopia.

In order to identify substances effective of increasing the
20 longitudinal growth of the eye, substances decreasing the standing potential and/or the c-wave are identified. These substances may be used for the treatment or prevention of hypermetropia.

In a further embodiment of the invention a method for screening
25 substances effective of treating disorders of the eye related to the axial length of the eye is disclosed. The method comprises identifying substances having an effect on the composition of the proteoglycans and/or the collagen specific amino acid present in the connective tissue of the
30 sclera of the eye.

This method may in one embodiment be by treating an animal with the substance and measuring the effect on the composition of the connective tissue of the sclera.

In another embodiment the method comprises adding the substance to a tissue culture comprising retinal pigment epithelium and fibroblasts and identify the impact of the substance on the production and composition of the proteoglycans and/or the collagen specific amino acid produced by the fibroblasts in the tissue culture.

The method according to the invention also comprises identifying substances effective of inhibiting the longitudinal growth of the eye. These substances are such substances which increase the content of proteoglycans of the sclera, and may be used for the treatment or prevention of myopia.

Accordingly, substances effective of increasing the longitudinal growth of the eye are the substances decreasing the content of proteoglycans of the sclera. These substances may be used for the treatment or prevention of hypermetropia.

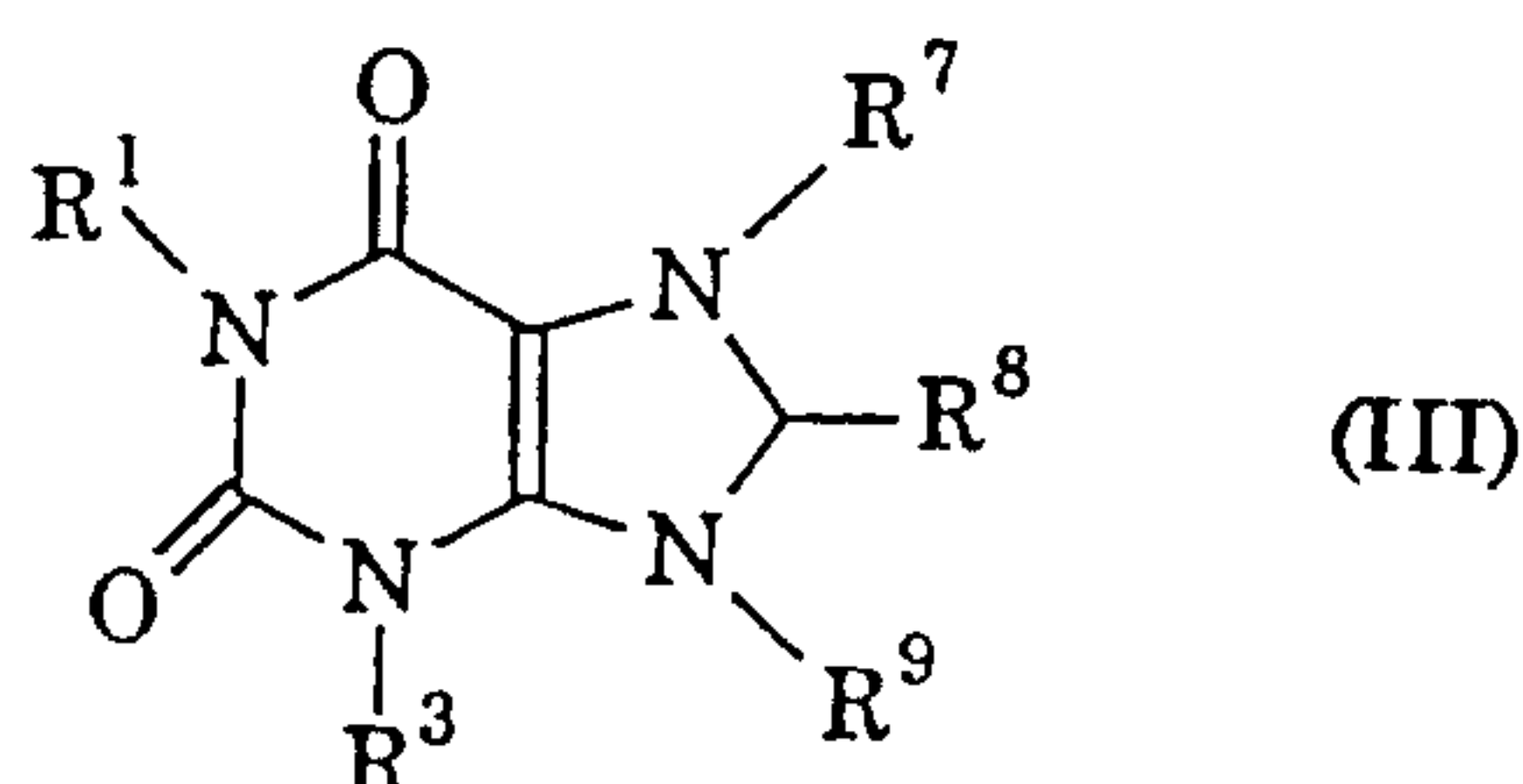
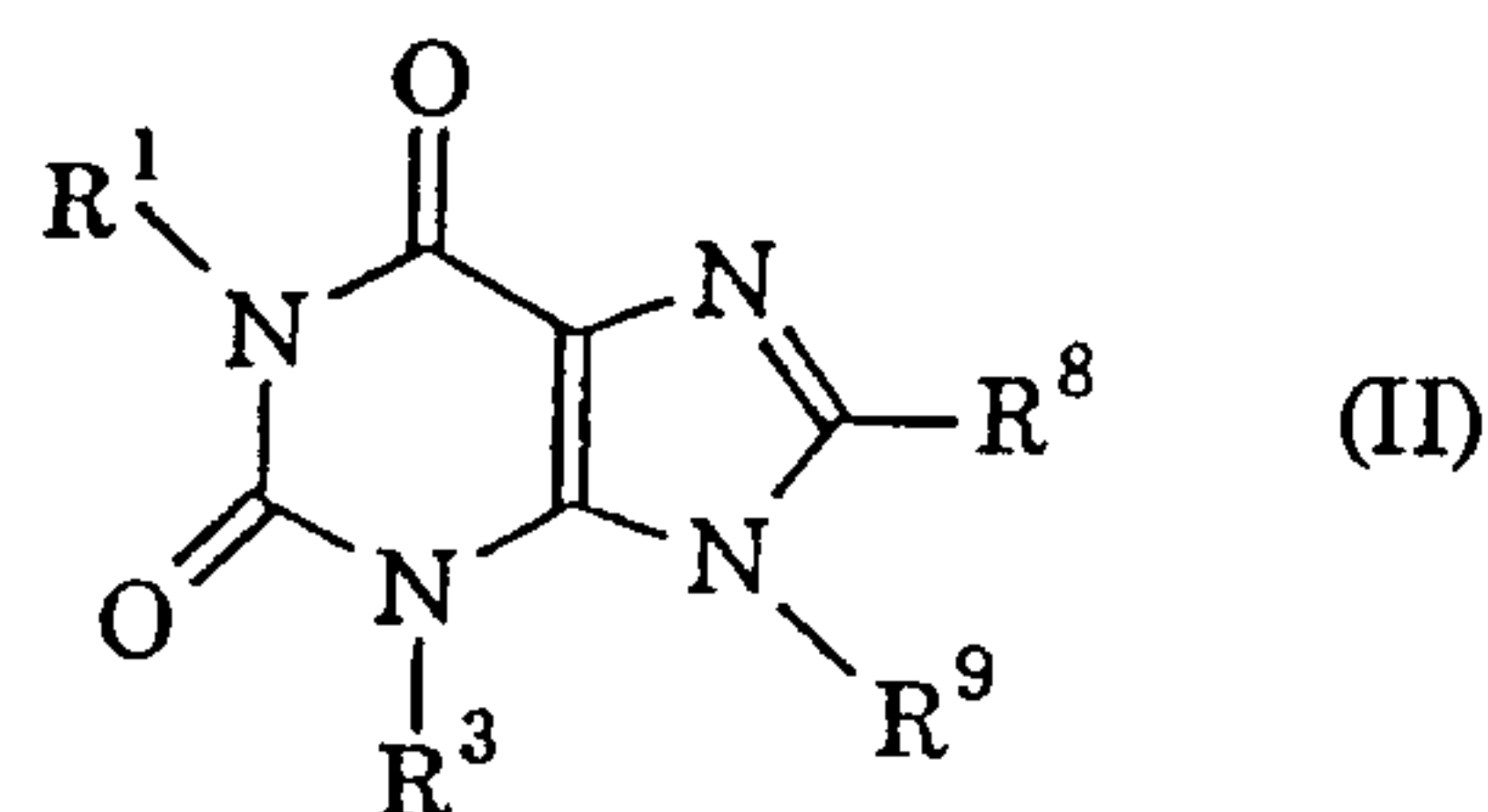
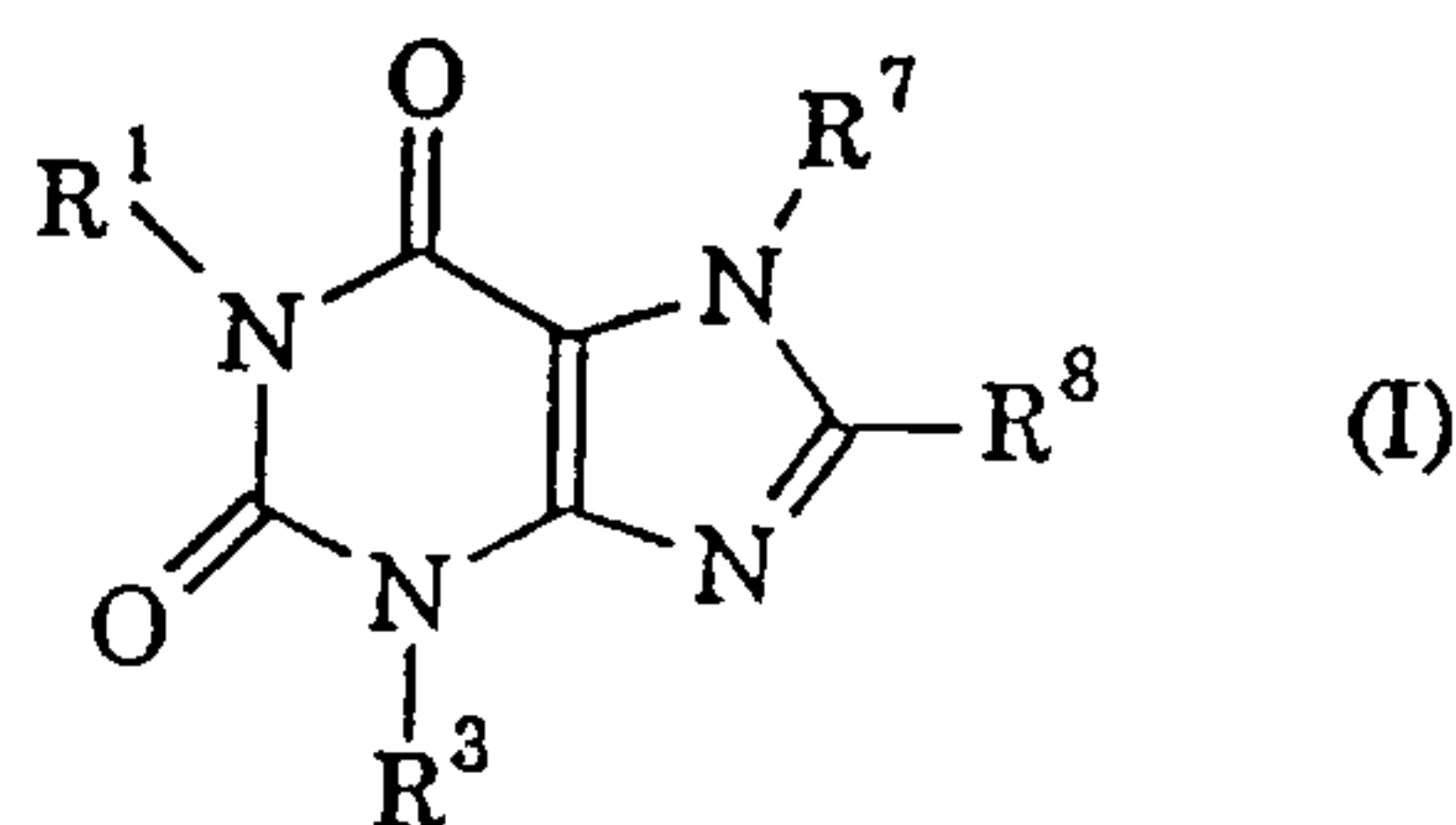
The effect of the substance on the proteoglycans can generally be identified within a period from as early as 1 day to about 12 weeks from the start of the treatment. However, normally at least one week of treatment is necessary for having an effect which can be measured with the existing analytical methods.

To apply an animal model to a human effect, it is generally preferred that the test animal is a mammal. However other animals such as birds and reptiles may employed in the present method.

As is evident for the above explanation, and due to the surprising finding that an effect on the retinal pigment epithelium may be utilized for treatment of disorders of the axial length of the eye, any substance may be subjected to the method according to the invention.

In one embodiment the invention relates to the screening of a substance selected from the group consisting of prostaglan-

dine and analogues thereof; and compounds of the general formula I, II or III



wherein R^1 , R^3 , R^7 , and R^9 are independently selected from the group consisting of hydrogen, optionally substituted C_{1-20} -alkyl, optionally substituted C_{2-20} -alkenyl, optionally substituted C_{4-20} -alkadienyl, optionally substituted C_{6-20} -alkatrienyl, optionally substituted C_{2-20} -alkynyl, optionally substituted C_{1-20} -alkoxycarbonyl, optionally substituted C_{1-20} -alkylcarbonyl, formyl, optionally substituted aryl, optionally substituted aryloxycarbonyl, optionally substituted arylcarbonyl, optionally substituted heteroaryl, optionally substituted heteroaryloxycarbonyl, optionally substituted heteroarylcarbonyl, carbamoyl, mono- and di(C_{1-20} -alkyl)aminocarbonyl, mono- and di(C_{1-20} -alkyl)amino- C_{1-20} -alkyl-aminocarbonyl, and halogen such as fluoro, chloro, bromo or iodo,

and

R^8 is selected from the group consisting of hydrogen, optionally substituted C_{1-20} -alkyl, optionally substituted C_{2-20} -alkenyl, optionally substituted C_{4-20} -alkadienyl, optionally substituted C_{6-20} -alkatrienyl, optionally substituted C_{2-20} -alkynyl, optionally substituted C_{1-20} -alkoxy, optionally

substituted C₂₋₂₀-alkenyloxy, carboxy, hydroxy, optionally substituted C₁₋₂₀-alkoxycarbonyl, optionally substituted C₁₋₂₀-alkylcarbonyl, formyl, optionally substituted aryl, optionally substituted aryloxycarbonyl, optionally substituted aryloxy, optionally substituted arylcarbonyl, optionally substituted heteroaryl, optionally substituted heteroaryloxycarbonyl, optionally substituted heteroaryloxy, optionally substituted heteroarylcarbonyl, amino, mono- and di(C₁₋₂₀-alkyl)amino, carbamoyl, mono- and di(C₁₋₂₀-alkyl)aminocarbonyl, amino-C₁₋₂₀-alkylaminocarbonyl, mono- and di(C₁₋₂₀-alkyl)amino-C₁₋₂₀-alkylaminocarbonyl, optionally substituted C₁₋₂₀-alkylcarbonylamino, guanidino, carbamido, optionally substituted C₁₋₂₀-alkanyloxy, sulphono, optionally substituted C₁₋₂₀-alkylsulphonyloxy, nitro, sulphanyl, optionally substituted C₁₋₂₀-alkylthio, and halogen such as fluoro, chloro, bromo or iodo.

Preferably, R¹, R³, R⁷, and R⁹ are independently selected from the group consisting of hydrogen, optionally substituted C₁₋₆-alkyl, optionally substituted C₂₋₆-alkenyl, optionally substituted C₂₋₆-alkynyl, optionally substituted C₁₋₆-alkylcarbonyl, formyl, optionally substituted aryl, optionally substituted arylcarbonyl, optionally substituted heteroaryl, and optionally substituted heteroarylcarbonyl,

and

R⁸ is selected from the group consisting of hydrogen, optionally substituted C₁₋₆-alkyl, optionally substituted C₂₋₆-alkenyl, optionally substituted C₂₋₆-alkynyl, optionally substituted C₁₋₆-alkoxy, optionally substituted C₂₋₆-alkenyloxy, carboxy, hydroxy, optionally substituted C₁₋₆-alkoxycarbonyl, optionally substituted C₁₋₆-alkylcarbonyl, formyl, optionally substituted aryl, optionally substituted aryloxycarbonyl, optionally substituted aryloxy, optionally substituted arylcarbonyl, optionally substituted heteroaryl, optionally substituted

heteroaryloxycarbonyl, optionally substituted heteroaryloxy, optionally substituted heteroarylcarbonyl, amino, mono- and di(C₁₋₆-alkyl)amino, carbamoyl, mono- and di(C₁₋₆-alkyl)aminocarbonyl, optionally substituted C₁₋₆-alkylcarbo-
5 nylamino, guanidino, carbamido, optionally substituted C₁₋₆-alkanoyloxy, sulphono, optionally substituted C₁₋₆-alkylsulphonyloxy, nitro, sulphonyl, optionally substituted C₁₋₆-alkylthio, and halogen such as fluoro, chloro, bromo or iodo.

In a further embodiment, R¹, R³, R⁷ and R⁹ are independently
10 selected from the group consisting of hydrogen, optionally substituted C₁₋₆-alkyl, optionally substituted C₂₋₆-alkenyl, optionally substituted C₂₋₆-alkynyl, optionally substituted aryl,

and

15 R⁸ is selected from the group consisting of hydrogen, optionally substituted C₁₋₆-alkyl, optionally substituted C₂₋₆-alkenyl, optionally substituted C₂₋₆-alkynyl, optionally substituted C₁₋₆-alkoxy, optionally substituted C₂₋₆-alkenyl-oxy, carboxy, hydroxy, optionally substituted aryl, optional-
20 ly substituted aryloxy, optionally substituted heteroaryl, optionally substituted heteroaryloxy, optionally substituted heteroarylcarbonyl, amino, nitro, sulphonyl, and halogen such as fluoro, chloro, bromo or iodo.

In a further embodiment the invention relates to the scree-
25 ning of a substance selected from the following group of substances selected based on different criteria with respect to inhibiting or stimulating effects known from the literature. The group comprises prostaglandine and analogues thereof such as F2 alpha analogues including PhXA41, prostag-
30 landin receptor agonists including PGF 2 alpha, 17-phenyl trinor PGE2, and U46619; adenosine A2-receptor agonists such as 5' (N-ethyl)-carboxamido adenosine, alpha-2D adrenergic receptor agonists and antagonists; alpha-2-adrenergic agonist such as UK-14,304; prostaglandin agonists such as 16-phenoxy-

WO 98/30900

PCT/DK98/00001

20

PGF2 alpha, cloprostenol, 17-phenyl-PGF2 alpha, fluprostenol, and PhXA85; insulin; bumetanide, peptides such as bradykinin, arginine vasopressin, bombesin, substance P, pituitary adenylate cyclase activating polypeptide, atrial natriuretic peptide, somatostatin analogues such as Tyr11-somatostatin-14, Leu8,D-Trp22, and Tyr25-somatostatin-28; muscarine receptor agonists and antagonists such as the Carbachol (stimulates production of inositolphosphate by way of M3 muscarinic receptors), 4-diphenylacetoxy-N-methylpiperadine methiodide, atropin and pirenzepine (blocking the effect); calcitriol receptor agonist and antagonists, acetylcholin (increases intracellular calcium by inositol triphosphate receptors), nonsteroidal antiinflammatory drugs such as niflumic acid; Thapsigargin; A23187; phosphodiesterase inhibitors such as rolipram and zaprinast, 4-chloro-3-ethyl-phenol; Bastidin; Veratridine; estrogens and derivatives thereof; Tamoxifen; and Bay K 8644 (1,4-Dihydro-2,6-dimethyl-5-nitor-4-(2(triflouromethyl)-phenyl)-3-pyridinencarboxylic acid methyl ester.

20 Upon screening, substances selected due their ability of treating myopia or hypermetropia based on the criteria defined in the present text may be used for the preparation of a medicament for treating or preventing disorders of the eye related to the axial length of the eye for a method for treating or preventing the disorder by administration of a pharmaceutically effective amount of the substance to an individual in need thereof.

In a further embodiment, the present invention relates to a method for treating and/or preventing myopia of a human eye comprising administering to an individual in need thereof a therapeutically effective amount of one or more substances selected from caffeine; 1,7-dimethylxanthine (paraxanthine), 7-methylxanthine (heteroxanthine), isobutylmethylxanthine (IBMX) and derivatives; 3-methylxanthine, 1-methylxanthine, 1-Hexyl-3,7-dimethylxanthine (pentifylline); 1,7-Dimethyl-xanthine; 1,3-Dipropyl-7-methyl-xanthine;

WO 98/30900

PCT/DK98/00001

21

- 7-Propylxanthine; 7 β -Chloroethyl-1,3-dimethylxanthine;
 3,7-Dimethyl-1-propargyl-xanthine; 3-Propylxanthine;
 1-(5-Oxohexyl)-3,7-dimethylxanthine (pentoxifylline);
 3-Isobutyl-1-methylxanthine; 3,9-Dimethylxanthine
 5 8-Cyclopentyl-1,3-dimethylxanthine;
 1,3-Bis(3-methylbut-2-butenyl-7-methylxanthine;
 3,7-Dihydro-7-methyl-1,3-dipropyl-1H-purine-2,6-dione;
 7-Methyl-1,3-diprenylxanthine (7-methyl-1,3-dipropyl-xanthine;
 7-Ethoxymethyl-1-(5-hydroxy-5-methylhex-methylxanthine (Tor-
 10 bafylline, "HWA 448");
 1-(5 hydroxy-5-methylhexyl)-3-methyl-7-propylxanthine (A
 80.2715);
 3,7-Dimethyl-1-(5-oxyhexyl)-xanthine (Pentoxifylline, "Tren-
 tal"); 3,7-Dimethyl-1-(5-hydroxyhexyl)-xanthine (Hydroxypen-
 15 toxifylline);
 1-Hexyl-3,7-dimethylxanthine (Pentifylline, "Cosaldon");
 3,7-Dimethyl-1-propargylxanthine (DMPX);
 (E)-8-(3,4-Dimethoxystyryl)-1,3-dipropyl-7-methylxanthine
 (KF 17837) (Lisofylline);
 20 1-(5-Hydroxy-5-methylhexyl)-3-methylxanthine
 (Albifylline, "HWA 138"); 3-Methyl-1-(5'-oxohexyl)-7-propyl-
 xanthine (Propentofylline, "HWA 285"); 1-(5-Hydroxyhexyl)-
 3,7-dimethylxanthine (BL 194);
 (E)-1,3-dipropyl-8-(3,4-dimethoxystyryl)-7-methylxanthine
 25 (KF 17.837); 1,3-di-n-butyl-7-(2'oxopropyl)-xanthine (Denbu-
 fylline); 1-n-butyl-3-n-propylxanthine (XT-044);
 7-(2,3-dihydroxypropyl)-theophylline (Dyphylline);
 7-Methyl-8-(2-hydroxy-N-methylethylamino)-theophylline (Cafa-
 minol); 7-(1,3-Dioxolan 2-ylmethyl)-theophylline (Doxo-
 30 fylline); 7-(2-Hydroxyethyl)-1,3-dimethylxanthine (Eto-
 fylline); 7-(2-Hydroxypropyl)-1,3-dimethylxanthine (Proxy-
 phylline); Pyridoxine-O-(theophyllin-7-ylethyl)sulphate
 (Pyridofylline); 7-(2-(3-diethylcarbamoxypropionyloxy)ethyl)-
 theophylline (Suxamidofylline); Piperazine bis (theophyllin-
 35 7-ylacetate) (Acepifylline); 8-benzyl-7-(2-(N-ethyl-N-2-hy-
 droxyethylamino)ethyl)theophylline (Bamifylline);
 2-amino-2-methylpropan-1-ol theophyllinate (Bufylline);
 7-(2,3-Dihydroxypropyl)-1,3-dimethylxanthine (Diprophylline);

*Trademark

7-(2-diethylaminoethyl)-1,3-dimethylxanthine camphor 10
sulphonate (Etamiphylline Camsylate); 3-Propylxanthine (En-
profylline); 4-amino-8-chloro-1-phenyl-(1,2,4)-triazolo (4,3-
a)quinoxaline (CP 66713); cysteine/cystine; glycine, forsko-
5 line; alpha-2-adrenergic agonist such as brimonidine (UK-
14,304), clonidine, apraclonidine, dapiprazole, moxonidine
(4-chloro-N-(4,5 dihydro-1H-imidasol-2yl)-6-methoxy-2-methyl-
5-pyridinamine), medetomidine, oxymetazoline, or derivatives
thereof; peptides such as bradykinin, arginine vasopressin
10 including V2 agonists, bombesin, substance P, pituitary
adenylate cyclase activating polypeptide; somatostatin ana-
logues such as Tyr11-somatostatin-14, Leu8,D-Trp22, and
Tyr25-somatostatin-28 including agonists of somatostatin sst2
receptors, neuropeptide Y including agonists of Y2 receptors,
15 and analogues of these peptides; calcitriol or analogues of
calcitriol or Vitamin D; muscarine receptor agonists such as
the Carbachol, acetylcholine or analogues thereof; nonste-
roidal antiinflammatory drugs such as niflumic acid;
prostaglandin and analogues thereof such as F2 alpha ana-
20 logues including PhXA41 (latanoprost), prostaglandin receptor
agonists including PGF 2 alpha, 17-phenyl trinor PGE2, and
U46619 FP, EP1, and TP receptor agonists), UF 021, 16-pheno-
xy-PGF2 alpha, cloprostenol, 17-phenyl-PGF2 alpha, fluproste-
nol, and PhXA85; Thapsigargin, A23187, Phosphodiesterase
25 inhibitors including rolipram and Zaprinas, 4-chloro-3-
ethylphenol and Bastidin, veratridine, esterogens including
analogues thereof; Bay K 8644 (1,4-Dihydro-2,6-dimethyl-5-
nitor-4-(2(trifluoromethyl)-phenyl)-3-pyridinencarboxylic
acid methyl ester; angiotensin converting enzyme inhibitors,
30 in particular captopril (SQ 14225); adenosine A2-receptor
agonists such as 5'-(N-ethyl)-carboxamido adenosine and 8-
phenylaminoadenosine (CV-1808); Candoxatril (neutral
endopeptidase 24.11 (NEP) inhibitor); Met-enkephalin, alpha-
endorphin or derivatives; and mixtures thereof.

35 L-cystine is a sulphurous amino acid which is a component of
proteins, e.g. collagen, where the above sulphurous cross-

links derive from it. The L-cystine is thus contained in ordinary food.

In a preferred embodiment, the method comprises use of a mixture of two or more substances having an additive effect
 5 on the myopia. In a preferred embodiment, the mixture has a synergistic effect. Both the additive effect and the synergistic effect may be measured by means of the methods described herein, such as by ERG.

In a still further embodiment, the present invention relates
 10 to methods for treating and/or preventing hypermetropia of a human eye comprising treatment by means of therapeutically effective amounts of one or more substances selected from theophylline, xanthine, 1,9-dimethylxanthine;
 1,3-Dipropyl-8-(2-(5,6-epoxynorbonyl)-xanthine;
 15 8-Cyclopentyl-1,3-dipropylxanthine (CPDPX);
 8-Sulphophenyltheophylline; 1,3-Dipropyl-8-(4-acrylate)phenylxanthine (BW-A1433); (1-Propyl-11C)8-dicyclopropylmethyl-1,3-dipropylxanthine
 (11C)KF15372 and 11C-ethyl and 11C-methyl derivatives there-
 20 of; 8-Benzyl-7, (2-(ethyl(2-hydroxyethyl)amino)ethyl)theophylline (Bamiphylline);
 8-Cyclopentyl-3-(3-((4-(fluorosulfonyl)benzoyl)oxyl)propyl)-1-propylxanthine; 1,3-Dipropyl-8-(4-((2-aminoethyl)amino)carbonylmethyl-oxyphenyl)xanthine;
 25 8-(3-chlorostyryl)caffeine; 8-cyclopentyltheophylline;
 8-(noradamantan-3-yl)-1,3-dipropylxanthine (KW-3902);
 1,3-Dipropyl-8-(3-noradamantyl)-xanthine;
 1,3-Dipropyl-8-(4-sulphophenyl)-xanthine;
 1,3-Dipropyl-8-(2-amino-4-chlorophenyl)-xanthine;
 30 7 β -Hydroxyethyl-1,3-dimethylxanthine;
 7-(2,3-Dihydroxypropyl)-1,3-dimethylxanthine;
 8-Chloro-1,3-dimethylxanthine; 1,3,9-Trimethylxanthine;
 8-Propionic acid-1,3-dimethylxanthine;
 7,9-Dimethylxanthine; 8-Phenyl-1,3-dimethylxanthine;
 35 7-Acetic acid-1,3-dimethylxanthine; 9-Propylxanthine;

9-Methylxanthine; 8-Methylxanthine; 8-(p-Sulfophenyl)-1,3-dimethylxanthine; 1,9-Dimethylxanthine; hypoxanthine; fluoxetine; L-ornithine; azetazolamide; bumetanide; Tamoxifen and other estrogen antagonists, the calmodulin antagonist J8, calcium antagonists including nimodipine and nicardipine, Endothelin agonist, in particular sarafotoxin S6c (selective ETB receptor agonist); Dorzolamide (MK-507), sezolamide and MK-927 (thienothiopyran-2-sulfonamide derivatives carbonic anhydrase inhibitors), methazolamide, ethoxzolamide, leu-enkephalin or derivatives; and mixtures thereof.

In a preferred embodiment, the method comprises use of a mixture of two or more substances having an additive effect on the hypermetropia. In a still more preferred embodiment, the mixture has a synergistic effect. Both the additive effect and the synergistic effect may be measured by means of the methods described herein, such as by ERG.

Use of a mixture may also be preferred when minor total dosages of each of the substances are preferred compared to a higher total dosage of only one substance.

The active substance or mixture of substances according to the invention may be administered orally, parenterally, transdermally or transmucosally e.g. as an intranasal formulation or as eyedrops. In addition, a local application in the eye may result in a substantial local concentration of the substance in the retinal area due to the anatomical circumstances of the eye whereby a less systemic effect is achieved. This is e.g. the fact where the substance is applied topically to the eye and where the drainage of the substance is by the uveoscleral pathway.

The pharmaceutical composition according to the invention comprising the active substance or mixture of substances may be administered in the form of a pharmaceutical composition which is a tablet, a capsule, a sustained release capsule comprising micro capsules of the active ingredient, a sol-

ution or suspension, a device for transdermal application, or a suppository or implant, or in any other conventional formulation.

As the treatment or prevention of abnormal growth of the eye
5 is a longterm treatment to children, the active substance or mixture of substances may be incorporated into a general daily vitamin tablet or be incorporated into foodstuff, so-called functional foods, including softdrinks, milk, bread, etc.

10 It is also an aspect of the present invention, that the individual substances may be interchanged during a treatment period, or that one substance is administered in an individual dosage, e.g in the morning, and another substance is administered in the evening.

15 In a further aspect of the invention the active ingredient is derived from the biological natural sources. Several methylxanthines naturally occurs in plants. More than 60 plant species throughout the world have been identified as containing 1,3,7-trimethylxanthine, 1,7-dimethylxanthine, 1,3-dimethylxanthine,
20 thylxanthine, 1-methylxanthine, 3-methylxanthine, or 7-methylxanthine. These plants includes the species coffea, camellia, cola, paullinia (guarana), iles (maté), theobroma, citrus, geraniaceae, copermecia, phaseolul, mungo, soya bean, beta vulgaris, sugar cane, and xanthophycae. Since the active
25 substances often represents different steps in the synthesis or degradation of more complex methylxanthines, several xanthines may be present in the same plant in concentrations that variate with the season of the year.

Harvesting the plant at different periods of the season, and
30 using different extraction methods will result in extracts containing high concentration of certain methylxanthines with only minor contributions of other, and less desired methylxanthines. In a further embodiment of the invention, a extraction method is described that results in a composition

rich in 7-methylxanthine, taking advantage of the fact that 7-methylxanthines is more easily dissolved than the other methylxanthines.

Other embodiments relates to extraction of beta vulgaris, sugar canes, xanthophyceae and other plants comprising 7-methylxanthines.

Depending on the exact dissolving properties of the desired active ingredient, the extraction method may be adapted for the specific plant as well as for the desired active ingredient.

Examples of a suitable extraction method is given below in Examples 6 and 7.

In a still other aspect, the active ingredient for treating or preventing disease of the longitudinal growth of the eye may be produced by genetic engineering such as by transgenic plants expressing the active ingredient or by other biotechnological methods.

In addition to genetic manipulation, the plant material, for the purpose of increasing the yield of certain methylxanthines from the plant material, the plant may be subjected to treatment with substances that block or facilitate certain enzymes that determines the metabolism of the methylxanthines. As an example of this, treatment of coffee plants with allipurinol will result in accumulation of great amounts of 7-methylxanthine in the plant (Ashihara H (1996). Catabolism of caffeine and related purine alkaloids in leaves of coffea arabica; L. Planta 1998:334-339)

Registration of c-Wave at ERG in animals (e.g. rabbits) is a well-developed examination technique (Skoog, K.O., Nilsson, S.E.G., Acta Ophthalmol. (Kph), 52: 759-773 (1974), Jarkman, S., Skoog, K.O., Doc. Ophthalmol., 60: 383-392 (1985)). Also intraocular administration of test substances for test

animals is also a well-described technique (Textorius, O., Doc. Ophthalmol., 63:349-358 (1986)). Alternatively, test substances can be administered intravenously (Jarkman, S., Doc. Ophthalmol. 60: 375-382 (1985)).

- 5 In addition, it is possible to determine the effect of test substances on the standing potential and c-wave at ERG indirectly by means of an *in vitro* arrangement with a retinal pigment epithelium-choroidea preparation, and to determine the transepithelial potential (Kawasaki, K., Doc. Oph-
10 thalmol., 63: 375-381 (1986)).

By means of such examination techniques which can facilitate quick examinations it is according to the present invention possible to examine a large number of substances in various concentrations and to determine which substances have a
15 strong effect on the activity of the pigment epithelium of the retina.

Determination of the content of proteoglycanes in tissue samples can be performed by means of several well-developed methods (eg. Björnsson, S., Analytical Biochemistry 210, 282-
20 291 (1993)).

The content of the collagen specific amino acids (hydroxyproline, hydroxylysine, and proline) can be determined eg. by means of autoanalysis (Blumenkrantz, N., Clin. Biochem. 13: 177-183 (1980)) or by means of high pressure
25 liquid chromatography (HPLC) or other methods. A method for determination of the content of collagen specific amino acids in connective tissue by means of application of HPLC has been described in detail below.

A method for determination of the content of dermatane sulphate in percentages in relation to the other glycosaminoglycanes (cellulose acetate electroforese) has been described in
30 detail below.

WO 98/30900

PCT/DK98/00001

28

The following Table 1 shows a number of specific preferred xanthine substances according to the present invention effective for treating and/or preventing myopia, and in Table 2 for hypermetropia.

5

Table 1

Substances effective for treating and/or preventing myopia

-
- | | | | |
|----|-----|--|----------------------------|
| | 1. | 1,3-Bis(3-methylbut-2-butenyl-7-methylxanthine | |
| | 2. | 3,7-Dihydro-7-methyl-1,3-dipropyl-1H-purine-2,6-dione | |
| 10 | 3. | 7-Methyl-1,3-diprenylxanthine (7-methyl-1,3-dipropyl-xanthine ¹ | |
| | 4. | 7-Ethoxymethyl-1-(5-hydroxy-5-methylhexyl)3-methylxanthine | (Torbafylline, "HWA 448") |
| | 5. | 1-(5 hydroxy-5-methylhexyl)-3-methyl-7-propylxanthine | |
| 15 | | (A 80.2715) | |
| | 6. | 3,7-Dimethyl-1-(5-oxyhexyl)-xanthine | |
| | | (Pentoxifylline, "Trental") | |
| | 7. | 3,7-Dimethyl-1-(5-hydroxyhexyl)-xanthine | |
| | | (Hydroxypentoxifylline) | |
| 20 | 8. | 1-Hexyl-3,7-dimethylxanthine | (Pentifylline, "Cosaldon") |
| | 9. | 3,7-Dimethyl-1-proparglyxanthine | (DMPX) ²⁾ |
| | 10. | (E)-8-(3,4-Dimethoxystyryl)-1,3-dipropyl-7-methylxanthine | |
| | | (KF 17837) (Lisofylline) | |
| | 11. | 1-(5-Hydroxy-5-methylhexyl)-3-methylxanthine | |
| 25 | | (Albifylline, "HWA 138") | |
| | 12. | 3-Methyl-1-(5'-oxohexyl)-7-propylxanthine | |
| | | (Propentofylline, "HWA 285") | |
| | 13. | 1-(5-Hydroxyhexyl)-3,7-dimethylxanthine | (BL 194) |
| | 14. | (E)-1,3-dipropyl-8-(3,4-dimethoxystyryl)-7-methylxanthine | |
| 30 | | (KF 17.837) ¹⁾ | |
| | 15. | 1,3-di-n-butyl-7-(2'oxopropyl)-xanthine | (Denbufylline) |
| | 16. | 1-n-butyl-3-n-propylxanthine | (XT-044) |
| | 17. | 7-(2,3-dihydroxypropyl)-theophylline | (Dyphylline) |
| | 18. | 7-Methyl-8-(2-hydroxy-N-methylethylamino)-theophylline | |
| 35 | | (Cafaminol) | |
| | 19. | 7-(1,3-Dioxolan 2-ylmethyl)-theophylline | (Doxofylline) |
| | 20. | 7-(2-Hydroxyethyl)-1,3-dimethylxanthine | (Etofylline) |

WO 98/30900

PCT/DK98/00001

29

21. 7-(2-Hydroxypropyl)-1,3-dimethylxanthine (Proxiphylline)
22. Pyridoxine-O-(theophyllin-7-ylethyl) sulphate
(Pyridofylline)
23. 7-(2-(3-diethylcarbamoylpropionyloxy)ethyl)theophylline
5 (Suxamidofylline)
24. Piperazine bis (theophyllin-7-ylacetate) (Acepifylline)
25. 8-benzyl-7-(2-(N-ethyl-N-2-hydroxyethylamino)ethyl)theo-
phylline (Bamifylline)
26. 2-amino-2-methylpropan-1-ol theophyllinate (Bufylline)
- 10 27. 7-(2,3-Dihydroxypropyl)-1,3-dimethylxanthine
(Diprophylline)
28. 7-(2-diethylaminoethyl)-1,3-dimethylxanthine camphor 10
sulphonate (Etamiphylline Camsylate)
29. 3-Propylxanthine (Enprofylline)
- 15 30. 4-amino-8-chloro-1-phenyl-(1,2,4)-triazolo (4,3-
a) quinoxaline (CP 71366) ¹⁾

WO 98/30900

PCT/DK98/00001

30

Table 2

Substances effective for treating and preventing of hypermetropia

5	31. 1,3-Dipropyl-8-(2-(5,6-epoxynorbonyl)-xanthine	3)
	32. 8-Cyclopentyl-1,3-dipropylxanthine	(CPDPX) 4)
	33. 8-Sulphophenyltheophylline	
	34. 1,3-Dipropyl-8-(4-acrylate)phenylxanthine	(BW-A1433) 5)
10	35. (1-Propyl-11C)8-dicyclopropylmethyl-1,3-dipropylxanthine	
	(11C)KF15372	
	and 11C-ethyl and 11C-methyl derivatives thereof	All 4)
	36. 8-Benzyl-7, (2-(ethyl(2-hydroxyethyl)amino)ethyl)theophylline	(Bamiphylline)
15	37. 8-Cyclopentyl-3-(3-((4-(fluorosulfonyl)benzoyl)oxyl)propyl)-1-propylxanthine	3)
	38. 1,3-Dipropyl-8-(4-((2-aminoethyl)amino)carbonylmethoxyphenyl)xanthine	
	39. 8-(3-chlorostyryl)caffeine	
	40. 8-cyclopentyltheophylline	3)
20	41. 8-(noradamantan-3-yl)-1,3-dipropylxanthine	(KW-3902)
	42. 1,3-Dipropyl-8-(3-noradamantyl)-xanthine	3)
	43. 1,3-Dipropyl-8-(4-sulphophenyl)-xanthine	
	44. 1,3-Dipropyl-8-(2-amino-4-chlorophenyl)-xanthine	
	45. Hypoxanthine	
25	1) A2 antagonist	
	2) Selective A2 antagonist	
	3) A1 antagonist	
	4) Selective A1 antagonist	
	5) A3 receptor antagonist	

30

Table 3

Effect of xanthines on [³H]ryanodine binding to skeletal RyR

	Bound [³ H]ryanodine, % of control	
	+3mmol/l	+1mmol/l
35	AMP	AMPPCP

WO 98/30900

PCT/DK98/00001

31

	Control (-xanthine)	100	100	100
	1-Hexyl-3,7-dimethylxanthine (pentifylline)	549±33*	449±28*	414±47*
5	1,7-Dimethyl- xanthine	240±27*	378±34*	360±28*
	1,3-Dipropyl-7-methyl- xanthine	220±23*	326±30*	295±23*
	7-Propylxanthine	216±25*	234±14*	270±12*
10	7β-Chloroethyl-1,3-dimethyl- xanthine	220±27*	256±34*	230±9*
	3,7-Dimethyl-1-propargyl- xanthine	193±15*	201±33*	230±6*
	3-Propylxanthine	211±13*	203±14*	226±14*
	7-Methylxanthine	204±25*	214±15*	225±11*
15	1-(5-Oxohexyl)-3,7-dimethyl- xanthine (pentoxy- fylline)	187±16*	185±25*	188±5*
	1,3,7-Trimethylxanthine (caffeine)	155±22*	164±11*	182±18*
20	1-Methylxanthine	153±15*	167±16*	179±22*
	3-Isobutyl-1-methyl- xanthine	137±16*	139±11*	175±20*
	1,3-Dimethylxanthine (theophylline)	136±29*	148±8*	170±25*
25	3,7-Dimethylxanthine (theobromine)	124±22*	136±8*	160±22*
	3,9-Dimethyl- xanthine	146±26*	141±11*	143±20*
	3-Methylxanthine	155±24*	137±10*	134±9*
30	8-Cyclopentyl-1,3-dimethyl- xanthine	155±24*	142±14*	133±6*
	7β-Hydroxyethyl-1,3-dimethyl- xanthine	113±13*	134±15*	124±15*
35	7-(2,3-Dihydroxypropyl)-1,3-di- methylxanthine	100±16	123±16*	117±9*
	8-Chloro-1,3-dimethyl- xanthine	98±25	108±14	116±17
	1,3,9-Trimethyl-			

WO 98/30900

PCT/DK98/00001

32

	xanthine	103±18	103±15	105±7
	8-Propionic acid-1,3-dimethyl-			
	xanthine	113±13	100±11	102±5
	7,9-Dimethyl-			
5	xanthine	99±14	100±15	102±13
	8-Phenyl-1,3-dimethyl-			
	xanthine	109±11	103±14	99±18
	7-Acetic acid-1,3-dimethyl-			
	xanthine	93±15	99±12	98±19
10	9-Propylxanthine	102±24	97±23	96±12
	9-Methylxanthine	96±11	85±18	98±10
	8-Methylxanthine	109±9	90±11	95±20
	8- (p-Sulfophenyl) -1,3-di-			
	methylxanthine	95±7	91±16	92±17
15	1,9-Dimethyl-			
	xanthine	76±13	86±18	84±14

(Specific [³H]ryanodine binding was determined as described in Materials and Methods in Liu et AL., Structure-activity Relationship of Xanthines and Skeletal Muscle Ryanodine Receptor/Ca²⁺ Release Channel; Pharmacology 1997;54:135-143. Xanthine concentrations were 1.5 mmol/l. Control [³H]ryanodine binding values (-xanthines) were 0.55 ± 0.05, 0.73 ± 0.08 and 1.69 ± 0.12 pmol/mg protein in the absence and presence of 3 mmol/l AMP or 1 mmol/l AMPPCP, respectively.

* Significantly different from control at p < 0.05.)

From the above Table 3 it is seen that nonpolar residues in positions, 1, 3 and 7 stimulated [³H] ryanodine binding, whereas nonpolar residues in positions 8 and 9 counteracted the effect.

According to the present invention, substances which have an effect on the [³H] ryanodine binding of at least 130%, preferable more than 150% of that of the control xanthine are effective for treating or preventing disease related to excessive longitudinal growth of the eye, and substances which have an effect on the [³H] ryanodine binding of less

WO 98/30900

PCT/DK98/00001

33

than 130%, preferable less than 100% of that of the control xanthine are effective for treating or preventing disease of insufficient longitudinal growth of the eye.

According to the present invention, the substances disclosed in Table 3 having the highest binding effect are most preferred for treating of myopia, such as the substance showing an effect on the [³H] ryanodine binding of more than 200% compared to the control xanthine, and includes the substance Pentifylline. The substances disclosed in Table 3 having the lowest binding effect are most preferred for treating of hypermetropia, such as the substance showing an effect on the [³H] ryanodine binding of less than 110% compared to the control xanthine, and includes the substance 1,9-dimethyl-xanthine.

15

Table 4

Effect of xanthines on [³H]ryanodine binding to skeletal RyR in the presence of 1 mmol/l AMPPCP

20	Bound [³ H]ryanodine	
	pmol/mg protein	% of control
<i>Monosubstituted xanthines</i>		
	Control (-xanthine)	1.69±0.12 100
25	7-Propyl-	4.57±0.20 270±12*
	3-Propyl-	3.82±0.23 226±14*
	7-Methyl-	3.80±0.19 225±11*
	1-Methyl-	3.02±0.38 179±22*
	3-Methyl-	2.26±0.15 134±9*
30	9-Propyl-	1.62±0.20 96±12
	9-Methyl-	1.66±0.17 98±10
	8-Methyl-	1.61±0.33 95±20
<i>Disubstituted xanthines</i>		
35	Control (-xanthine)	1.69±0.12 100
	1,7-Dimethyl-	6.08±0.47 360±28*

WO 98/30900

PCT/DK98/00001

34

	3-Butyl-1-methylxanthine	2.96±0.33	175±20*
	1,3-Dimethyl- (theophylline)	2.87±0.42	170±25*
	3,7-Dimethyl- (theobromine)	2.70±0.37	160±22*
5	3,9-Dimethyl-	2.42±0.34	143±20*
	7,9-Dimethyl-	1.73±0.22	102±13
	1,9-Dimethyl-	1.42±0.23	84±14
	<i>1-Substituted 3,7-dimethylxanthines</i>		
10	3,7-Dimethyl- xanthine (control)	2.70±0.37	100
	1-Hexyl- (pentifylline)	6.99±0.80	259±24*
	1-Propargyl-	3.88±0.10	144±3*
	1- (5-Oxoethyl) - (pentoxyl- fylline)	3.18±0.08	118±3*
15	1-Methyl- (caffeine)	3.07±0.30	114±2
	<i>7-Substituted 1,3-dimethylxanthines</i>		
	1,3-Dimethyl- xanthine (control)	2.87±0.42	100
20	7β-Chloroethyl-	3.88±0.16	135±6*
	7-Methyl- (caffeine)	3.07±0.301	107±4
	7β-Hydroxyethyl-	2.10±0.26	73±4*
	7- (2,3-Dihydroxypropyl) -	1.97±0.16	69±6*
25	7-Acetic acid-	1.65±0.32	57±6*
	<i>8-Substituted 1,3-dimethylxanthines</i>		
	1,3 Dimethyl- xanthine (control)	2.87±0.42	100
30	8-Cyclopentyl-	2.25±0.28	78±8
	8-Chloro-	1.96±0.13	68±4*
	8-Propionic acid-	1.73±0.09	60±3*
	8-Phenyl-	1.67±0.31	58±9*
	8- (p-Sulfophenyl) -	1.55±0.28	54±6*
35			
	<i>Others</i>		
	1,3-Dipropyl-7-methyl- xanthine	4.96±0.39	

1,3,9-Trimethylxanthine 1.77±0.12

([³H]ryanodine-binding measurements were determined as described in Materials and Methods in Liu et AL., Structure-activity Relationship of Xanthines and Skeletal Muscle Ryanodine Receptor/Ca²⁺ Release Channel; Pharmacology, 1997; 54:135-143. Xanthine concentrations were 1.5 mmol/l. The control bound [³H]ryanodine for each subgroup was set equal to 100. Results are means ± SD of 3-4 separate experiments. * Significantly different from controls at p<0.05.)

10 From this Table 4, the effect of the individual substituents to the xanthine molecule structure is clearly demonstrated.

Xanthines which is substituted in one or more of position 1, 3, and 7 (of the general formula of xanthine shown i Fig. 3) with any of the following substituents: hydroxy, halogen, triflourmethyl, lower alkyl (C₁₋₆), tertiary amino/alkoxy are preferred for the treatment or prevention of myopia.

On the other hand, when the xanthine is substituted in one or more of position 8 and 9 with these substituents, the substance is useful for treatment or prevention of hypermetropia, especially if the xanthine is only substituted in these positions.

When the xanthine is substituted in both positions 7 and 9, such as in 7,9-Dimethylxanthine, the double bond between the nitrogen atom and carbon atom as shown in the general formula (I) is hydrogenated as appears from formula III.

In the present context, the term "C₁₋₂₀-alkyl" is intended to mean a linear, cyclic or branched hydrocarbon group having 1 to 20 carbon atoms, such as methyl, ethyl, propyl, isopropyl, cyclopropyl, butyl, tert-butyl, iso-butyl, cyclobutyl, pentyl, cyclopentyl, hexyl, cyclohexyl, hexadecyl, heptadecyl, octadecyl, nonadecyl. Analogously, the term "C₁₋₆-alkyl" is intended to mean a linear, cyclic or branched

hydrocarbon group having 1 to 6 carbon atoms, such as methyl, ethyl, propyl, iso-propyl, pentyl, cyclopentyl, hexyl, cyclohexyl.

Preferred examples of "C₁₋₆-alkyl" are methyl, ethyl, propyl, iso-propyl, butyl, tert-butyl, iso-butyl, pentyl, cyclopentyl, hexyl, cyclohexyl, in particular methyl, ethyl, propyl, iso-propyl, tert-butyl, iso-butyl and cyclohexyl.

Similarly, the terms "C₂₋₂₀-alkenyl", "C₄₋₂₀-alkadienyl", and "C₆₋₂₀-alkatrienyl" are intended to mean a linear, cyclic or branched hydrocarbon group having 2 to 20, 4 to 20, and 6 to 20, carbon atoms, respectively, and comprising one, two, and three unsaturated bonds, respectively. Analogously, the term "C₂₋₆-alkenyl" is intended to mean a linear, cyclic or branched hydrocarbon groups having 2 to 6 carbon atoms and comprising one double bond.

Examples of alkenyl groups are vinyl, allyl, butenyl, pentenyl, hexenyl, heptenyl, octenyl, heptadecaenyl. Examples of alkadienyl groups are butadienyl, pentadienyl, hexadienyl, heptadienyl, heptadecadienyl. Examples of alkatrienyl groups are hexatrienyl, heptatrienyl, octatrienyl, and heptadecatrienyl.

The term "C₂₋₂₀-alkynyl" is intended to mean a linear or branched hydrocarbon group having 2 to 20 carbon atoms and comprising a triple bond. Examples hereof are ethynyl, propynyl, butynyl, octynyl, and dodecaynyl. Analogously, the term "C₂₋₆-alkynyl" is intended to mean a linear or branched hydrocarbon groups having 2 to 6 carbon atoms and comprising one triple bond, such as ethynyl, propynyl, butynyl, pentynyl, and hexynyl.

In the present context the term "alkoxy" means alkyl-oxy, and "halogen" means fluoro, chloro, bromo, iodo.

In the present context, i.e. in connection with the terms "alkyl", "alkenyl", "alkadienyl", "alkatrienyl", "alkynyl",

and "alkoxy", the term "optionally substituted" is intended to mean that the group in question may be substituted one or several times, preferably 1-3 times, with group(s) selected from hydroxy (which when bound to an unsaturated carbon atom may be present in the tautomeric keto form), C₁₋₆-alkoxy (i.e. alkyl-oxy), C₂₋₆-alkenyloxy, carboxy, oxo (forming a keto or aldehyde functionality), C₁₋₆-alkoxycarbonyl, C₁₋₆-alkylcarbonyl, formyl, aryl, aryloxycarbonyl, aryloxy, arylcarbonyl, heteroaryl, heteroaryloxycarbonyl, heteroaryloxy, heteroarylcarbonyl, amino, mono- and di(C₁₋₆-alkyl)amino; carbamoyl, mono- and di(C₁₋₆-alkyl)aminocarbonyl, amino-C₁₋₆-alkyl-aminocarbonyl, mono- and di(C₁₋₆-alkyl)amino-C₁₋₆-alkyl-aminocarbonyl, C₁₋₆-alkylcarbonylamino, guanidino, carbamido, C₁₋₆-alkanoyloxy, sulphono, C₁₋₆-alkylsulphonyloxy, nitro, sulphanyl, C₁₋₆-alkylthio, trihalogen-C₁₋₆-alkyl, halogen such as fluoro, chloro, bromo or iodo, where aryl and heteroaryl may be substituted as specifically described below for "optionally substituted aryl and heteroaryl".

In the present context the term "aryl" is intended to mean a fully or partially aromatic carbocyclic ring or ring system, such as phenyl, naphthyl, 1,2,3,4-tetrahydronaphthyl, anthracyl, phenanthracyl, pyrenyl, benzopyrenyl, fluorenyl and xanthenyl, among which phenyl is a preferred example.

The term "heteroaryl" is intended to mean a fully or partially aromatic carbocyclic ring or ring system where one or more of the carbon atoms have been replaced with heteroatoms, e.g. nitrogen, sulphur, and/or oxygen atoms. Examples of such heteroaryl groups are oxazolyl, isoxazolyl, thiazolyl, isothiazolyl, pyrrolyl, imidazolyl, pyrazolyl, pyridinyl, pyrazinyl, pyridazinyl, piperidinyl, coumaryl, furyl, quinolyl, benzothiazolyl, benzotriazolyl, benzodiazolyl, benzooxazolyl, phthalazinyl, phthalanyl, triazolyl, tetrazolyl, isoquinolyl, acridinyl, carbazolyl, dibenzazepinyl, indolyl, benzopyrazolyl, phenoxazonyl. Preferred heteroaryl groups are pyridinyl, benzopyrazolyl, and imidazolyl.

In the present context, i.e. in connection with the terms "aryl" and "heteroaryl", the term "optionally substituted" is intended to mean that the group in question may be substituted one or several times, preferably 1-5 times, in particular 1-3 times) with group(s) selected from hydroxy (which when present in an enol system may be represented in the tautomeric keto form), C₁₋₆-alkyl, C₁₋₆-alkoxy, oxo (which may be represented in the tautomeric enol form), carboxy, C₁₋₆-alkoxycarbonyl, C₁₋₆-alkylcarbonyl, formyl, aryl, aryloxy, aryloxycarbonyl, arylcarbonyl, heteroaryl, amino, mono- and di(C₁₋₆-alkyl)amino; carbamoyl, mono- and di(C₁₋₆-alkyl)aminocarbonyl, amino-C₁₋₆-alkyl-aminocarbonyl, mono- and di(C₁₋₆-alkyl)amino-C₁₋₆-alkyl-aminocarbonyl, C₁₋₆-alkylcarbonylamino, guanidino, carbamido, C₁₋₆-alkanoyloxy, sulphono, C₁₋₆-alkylsulphonyloxy, nitro, sulphanyl, dihalogen-C₁₋₄-alkyl, trihalogen-C₁₋₄-alkyl, halogen such as fluoro, chloro, bromo or iodo, where aryl and heteroaryl representing substituents may be . Preferred examples are hydroxy, C₁₋₆-alkyl, C₁₋₆-alkoxy, carboxy, C₁₋₆-alkoxycarbonyl, C₁₋₆-alkylcarbonyl, aryl, amino, mono- and di(C₁₋₆-alkyl)amino, and halogen such as fluoro, chloro, bromo or iodo, wherein aryl and heteroaryl may be substituted as above.

The pharmaceutical preparation according to the present invention may be formulated in accordance with conventional pharmaceutical practice with pharmaceutical excipients conventionally used for the preparation of oral, topical and systemical formulations. In addition, many of the substances are well known medical formulations.

The concentration of the substance or mixture of substances may be present in the medicament in an amount of 0.001-99%, typically 0.01-75%, more typically 0.1-20%, especially 1-10% by weight of the medicament, however, for many of the substances are already available for pharmaceutical use, and generally, the substances are then to be used in ordinary clinical dosages and concentrations known in the literature.

In another aspect of the invention, the dosage is correlated to an effect similar to the effect of the testes drugs of the present invention and which appears from the examples. This correlation may be performed by the measuring methods as
5 described en the present text.

The preparation of the invention may also contain other additives such as emulsifieres, stabilizing agents, preservatives, etc.

The invention is furthermore described in the claims and
10 rendered probable in the following examples.

List of Examples

Example 1 Materials and Methods

Example 2 Biochemical changes in rabbit sclera after long term treatment with Fluoxetine, Teofylamin, Caffeine and L-cysteine.
15

Example 3 Effect of myopia progression in a human by treatment with caffeine and L-cysteine.

Example 4 Biochemical changes in rabbit sclera after long-term treatment with 7-methylxanthine, Theobromine, Acetazolamide, and L-ornithine.
20

Example 5 Effect on proteoglycanes compared with effect on [³H] ryanodine binding of different xanthines.

Example 6 Extraction method for substance from natural source.

25 **Example 7** Extraction method for substance from natural source.

Example 8 Experiments under investigation:

Comments to the Examples

On the basis of the biochemical results in Example 2 it is
30 likely that a treatment of myopia with caffeine will be more efficient than L-cystine as a moderate dosage of caffeine unambiguously affects all parameters appropriately.

WO 98/30900

PCT/DK98/00001

40

In humans caffeine has a relatively long half-life period (approximately 5 hours). After administration of a dosage of caffeine, the serum concentration of caffeine is therefore dominant, however, with an essential contribution of the pharmacologically active metabolites 1,7-dimethylxanthine (paraxanthine), 3,7-dimethylxanthine (theobromine), and 1,3-dimethylxanthine (theophylline), 1-methylxanthine, 3-methylxanthine, and 7-methylxanthine (heteroxanthine).

In rabbits the half-life period for caffeine is short (approximately 1 hour) and the most essential contribution for the serum concentration comes from the metabolite 1,7-dimethylxanthine whereas the serum concentration of caffeine will be essentially lower.

The strong effect of caffeine on the biochemical composition of sclera, as it appears from Example 2, is believed to be due to the above-mentioned metabolites of caffeine.

Example 3 demonstrates that substances identified according to Example 2 of having specific effects on the biochemical composition of sclera of a non-human mammal, the rabbit, in fact are able to prevent inappropriate growth of the eye in a human.

Example 4 shows that the caffeine metabolite theophylline (1,3-dimethylxanthine) partly reduces c-wave at ERG and the standing potential of the eye, and partly reduces the content of proteoglycans in sclera. Theophylline does increase the content of the collagen specific amino acids hydroxyproline and proline, but it reduces the content of hydroxylysine. In total theophylline therefore has an inhibiting effect on the content of connective tissue components and thus a directly opposite effect as compared to the closely familiar substance caffeine.

Caffeine is transformed after administration partly to theophylline which works against the stimulating effect of

caffeine on the scleral tissue. In order to avoid this weakening effect those metabolites can be employed for caffeine which is **not** transformed into theophylline.

Theophylline only differs from caffeine by not having a 7-methyl group. Other appropriate substances for treatment of myopia among caffeine derivatives or analogues with a 7-methyl group include. 1,7-dimethylxanthine (paraxanthine), and 7-methylxanthine (heteroxanthine) are especially interesting, however also 3-methylxanthine and 1-methylxanthine are believed to be effective against myopia.

Other 7-methyl xanthines which can be effective for treatment of myopia can involve derivatives of xanthine having substituents in other positions than the 7-position. In a similar way, xanthines derivatives not having a methyl group in the 7-position may be useful for the treatment of hypermetropia.

Example 1

Materials and Methods

Test animal: Charles River Deutschland Chinchilla Bastard female rabbits.

Number of animals: 6 test animals and 6 control animals

Age: approximately 8 weeks at the commencement of the test.

Weight: 1.4-1.9 kg at the commencement of the test.

Lightness/Darkness cycle: 12/12 hours.

Water: ad libitum.

Cages: The animals live in identical cages made of stainless steel (Scanbur), length 1 m, depth 0.52 m, height 0.5 m.

Food: Stanrab standard diet from Special Diet Services, P.O. Box 705, Witham Essex CM8 3AD, UK (further details, see Table A).

Amount of food: Increasing from 75 g per day at the commencement of the test up to 130 g after three months.

WO 98/30900

PCT/DK98/00001

42

Experimental drinking water 1 contained theophyllin in a dose equivalent of 27 mg increasing to 63 mg (added as theophyllin and ethylendiamin in a dose of 33 to 77 mg) daily.

Experimental drinking water 2 contained fluoxetine (Fontex,
5 Lilly) in a dose equivalent of 2 mg increasing to 5 mg daily.

Experimental diet 1 was added caffeine (1,3,7-trimethyl-xanthine) 500 mg per kg Stanrab standard diet equivalent of a daily caffeine dose of 37.5 g increasing to 65 g per day.

Experimental diet 2 was added 4.5 g L-cystine per kg Stanrab
10 standard diet equivalent of a daily L-cystine dose of 338 mg increasing to 585 mg per day. With respect to the supplement of L-cystine, the normal intake per day of the standard diet is 218 mg increasing to 377 mg L-cystine.

Experimental diet 3 was added 7-methylxanthine 500 mg per kg
15 Stanrab standard diet equivalent of a daily 7-methylxanthine dose of 37.5 g increasing to 65 g per day.

Experimental diet 4 was added theobromine (3,7-dimethyl-xanthine) 500 mg per kg Stanrab standard diet equivalent of a daily theobromine dose of 37.5 g increasing to 65 g per day.

Experimental diet 5 was added acetazolamide 500 mg per kg
20 Stanrab standard diet equivalent of a daily acetazolamide dose of 37.5 g increasing to 65 g per day.

Experimental diet 6 was added 4.5 g L-ornithine per kg Stanrab standard diet equivalent of a daily L-ornithine dose of
25 338 mg increasing to 585 mg per day.

Collection of sample: Sclera biopsies are taken for analysis for proteoglycane content, distribution between the individual glycosaminoglycans and the content of collagen specific amino acids. Four tests from each animal: Two samples from
30 the front, 5 x 10 mm along limbus (without cornea), and two

samples from the back, punch 10 mm corresponding to the center of the retina. Furthermore, tests are taken for electron microscopy (EM) from two animals in each group.

Analyses: Samples for proteoglycane analysis are frozen down immediately. The samples are analyzed by Wieslab AB, IDEON, S-223 70 Lund, Sweden, by means of a method which has been described by Björnsson, S., Analytical Biochemistry 210, 282-291 (1993). The method comprises extraction by 20 microlitres of 4 M guanidine-HCl, 50 Mm sodium acetate per mg wet weight over night by room temperatures. The extracts are centrifuged in order to remove debris. 20 microlitres of supernatant are reduced 1/10 in extraction buffer and 20 microlitres of reduced supernatant are mixed with 10 microlitres of reagent 1 (see Björnsson, S., Analytical biochemistry 210, 282-291 (1993)) on a shaking table for 15 minutes. 200 microlitres of reagent 2 are added and the tests are incubated for 60 minutes in order to precipitate the proteoglycans. The proteoglycane/alcan blue precipitate is transferred to 96-wells MillBlotD apparatus and washed with 2 x 200 microlitres of 40% DMSO, 0.05 M of $MgCl_2$. The colour intensity of the wells are quantitated by microtech E3 scanner and Scan Analysis software (Biosoft, Cambridge, U.K.). Chondroitine-6-sulphate (Sigma C4384) is used as the calibrator. All samples are analyzed in duplicate.

Samples for analysis for content of collagen specific amino acids are frozen down immediately. The amino acid content is determined by HPLC-chromatography. The tissue bits are delipidized with acetone at 4°C in 3 x 24 hours, then with acetone:diethylether 1:1 for 24 hours, and finally 1 hour of standing with pure diethylether.

Then they are dried to constant weight by standing in vacuum exsiccator for 1.5 hours with the pump on and after closure standing over night.

WO 98/30900

PCT/DK98/00001

44

The dried tissue with constant weight is minced into very fine particles with a pair of scissors, approximately 20 per piece of tissue. The small flakes from each original piece of tissue are divided into two equally sized portions, one of which is employed for hydrolysis and the other one is kept for reference. The constant dry weight of each portion is known.

The finely divided tissue is hydrolyzed in Pyrex glass tubes for 24 hours at 114°C, and subsequently the hydrolysates are dried (removal of remnants from of hydrochloric acid) by standing in vacuum exsiccator at 40°C over night. The dry residue of hydrolysate is resolubilized in 320 microliter of 0.1 N hydrochloric acid by standing in ultrasound bath for 10 minutes vortexing and then standing over night at 4°C surrendered by a final wortexing and centrifugation.

From each resolubilized hydrolysate 3 aliquots of each 8 microlitres and 3 aliquots of 40 µl are taken and transferred to separate polypropylene tubes in which 20 microlitres of a solution of internal standard substances citrulline and 2 aminobutyric acid were added where the further method takes place.

The taken aliquots from hydrolysate are dried in a vacuum exsiccator at 35°C over night and subsequently submitted to derivatization with phenylisothiocyanate.

The resulting phenylisothiocarbamates are transferred to the HPLC instrument for separation and quantification. A new set of calibration curves is established, 1 curve for each of the 19 amino acids, prior to the analysis of the samples from one investigation.

The concentration range of standard samples exceed the ranges of amino acid concentrations in the unknowns. Hydroxyproline and proline are quantitated from the 8 microliter aliquots,

and hydroxylysine is quantitated from the 40 microliter aliquots.

The curves were straight with a high coefficient of correlation.

Samples for analysis of the distribution between the various
5 glycosaminoglycans are immediately put in acetone. The
analysis is made with a method described by Olsen, E.B., Acta
Orthop. Scand. 60 (1), 23-25 (1989), comprising delipidization,
drying, digestion with pronase. The glycosaminoglycans
are separated in the procedure comprising centyltrimethyl
10 ammonium bromide. The glycosaminoglycane centyltrimethyl
ammonium bromide complex is washed with ethanol saturated
with NaCl in order to remove centyltrimethyle ammonium bromide.
The cleaned Na-glycosaminoglycans are dried and resolubilized
in NaOH and distilled water for further analysis.
15 The individual glycosaminoglycans are separated by means of
cellulose acetate electroforese, and the relative content of
the essential glycosaminoglycans are measured by means of
optical scanning.

EM is made on ultra thin cuts from the center part of the
20 biopsies after fixation in 4% of glutaraldehyde in cacodylate
buffer with 7.5% sucrose at 4°C over night, dehydrated in
ethanol and moulded in epoxy resin. The cuts are stained with
saturated uranyl acetate solution in 50% of ethanol for 1
hour, followed by lead citrate (Reynolds) for 3 minutes for
25 routine electron microscopy.

Ultra thin cuts are collected on gold nets and stained by
means of periodine acid-silver proteinate technique for
glucoproteins (PAS) and with 0.1% of ruthenium red solution
in 0.1 M ammonia for acidic glycosaminoglycans.

30 The results of longterm treatment with each of the 8 substances
specified above are shown in Example 2 (treatment for 12 weeks)
and Example 4 (treatment for 10 weeks).

WO 98/30900

PCT/DK98/00001

46

No significant differences between control and treatment groups was identified with respect to bodyweight and weight of eyes.

Example 2

- 5 Long time treatment of 3 month with each of the four substances, Caffeine, L-cysteine, Teofylamin, and Fluoxetine and the effect on the biochemical changes in rabbit sclera

Table 5

10 Content of collagen specific amino acids in nanomol/mg tissue (dried weight defatted weight) from anterior sclera (Wilcoxon test. * $p < 0.05$), and the increase (Inc.) or decrease (Decr.) compared to the control.

		Hydroxyproline		Hydroxylysine		Proline	
	Control	604	+/-50	41	+/-12	886	+/-29
15	Fluoxetine	619	+/-40	33	+/-11	896	+/-21
	Teofylamin	653	+/-39	28	+/-10	958	+/-23
	Caffeine	688	+/-36*	39	+/-19	993	+/-18*
	L-cystine	599	+/-41	45	+/-10	885	+/-22
		Hydroxyproline		Hydroxylysine		Proline	
20		Inc.	Decr.	Inc.	Decr.	Inc.	Decr.
	Fluoxetine	-	-	-	20%	-	-
	Teofylamin	8%	-	-	32%	8%	-
	Caffeine	14%	-	-	-	12%	-
	L-cystine	-	-	-	-	-	-

25

Table 6

Content of collagen specific amino acids in nanomol/mg tissue (dried weight and defatted weight) from posterior sclera (Wilcoxon test.), and the increase (Inc.) or decrease (Decr.) compared to the control.

		Hydroxyproline	Hydroxylysine	Proline
30	Control	547 +/-141	43 +/-12	810 +/-86

WO 98/30900

PCT/DK98/00001

47

	Fluoxetine	640	+/-31	41	+/-13	927	+/-15
	Teofylamin	643	+/-74	28	+/-15	902	+/-53
	Caffeine	668	+/-61	37	+/-16	947	+/-38
	L-cystine	596	+/-39	43	+/-8	867	+/-14
5		Hydroxyproline		Hydroxylysine		Proline	
		Inc.	Decr.	Inc.	Decr.	Inc.	Decr.
	Fluoxetine	17%	-	-	-	15%	-
	Teofylamin	18%	-	-	35%	11%	-
	Caffeine	22%	-	-	14%	17%	-
10	L-cystine	9%	-	-	-	7%	-

Table 7

Content of proteoglycans in μg PG/mg tissue (wet weight) (Wilcoxon test. * $p < 0.05$), and the increase or decrease compared to the control.

15		Anterior sclera		Posterior sclera	
	Control	2.7 +/-0.2		2.7 +/-0.2	
	Fluoxetine	2.2 +/-0.4*		2.6 +/-0.2	
	Teofylamin	2.4 +/-0.5		2.7 +/-0.3	
	Caffeine	3.2 +/-0.8		3.2 +/-0.5	
20	L-cystine	3.5 +/-0.5*		3.5 +/-0.4*	
		Anterior sclera		Posterior sclera	
		Increase	Decrease	Increase	Decrease
	Fluoxetine	-	19%	-	-
	Teofylamin	-	11%	-	-
25	Caffeine	19%	-	19%	-
	L-cystine	29%	-	29%	-

Conclusion:

Fluoxetine: Treatment with fluoxetine (a serotonin re-uptake inhibitor) for three months decreased the content of proteoglycans in anterior sclera by 19%. Hydroxylysine in the anterior sclera is decreased with 20% and hydroxyproline and

WO 98/30900

PCT/DK98/00001

48

proline are increased with 17% and 15%, respectively, in the posterior sclera.

Teofylamin: Treatment with Teofylamin for 3 months reduces the content of proteoglycanes in sclera by 11% (at the front)

- 5 Caffeine: Treatment with caffeine for three months increases the content of proteoglycanes in sclera by 19% (to the front) and 19% (to the back). The content of the collagen specific amino acids hydroxyproline and proline are increased by 22% and 17%, respectively, in sclera tests in the back of the
10 eye. Accordingly, the example shows that treatment of a young mammal with caffeine in a moderate dosage results in a strong increase in the content of proteoglycanes as well as in collagen specific amino acids in sclera.

- L-cystine: Treatment of young rabbits for three months with
15 L-cystine increases the content of proteoglycanes in the sclera by 29% in both the front and in the back of eye. The content of the collagen specific amino acids hydroxyproline and proline are increased by 9% and 7%, respectively, in sclera samples from the back of the eye. Accordingly, the
20 example shows that treatment with a relative large dosage of L-cystine also increases the content of proteoglycanes, but that the effect on the content of collagen specific amino acids is ambiguous.

Example 3

- 25 **Myopia progression stopped by treatment with caffeine and L-cystine**

- A boy presented with myopia at the age of 9 years. Earlier examination showed no ametropia. He had severely progrediating myopia and was treated with a combination of caffeine
30 (100 mg/day) and L-cystine (200 mg/day) for three months followed by a control period of three months with no treatment, and subsequently a new treatment period followed by a

WO 98/30900

PCT/DK98/00001

49

control period, etc. In total 4 treatment periods and 4 control periods alternated.

Measurement of the axial length of the eye was made by means of Auto Axial Biometer AL-010, Shin-Nippon.

- 5 Table 8 and Table 9 shows the average growth in the axial length of both eyes in the 4 treatment periods and the 4 control periods, respectively.

Table 8

10 Treatment with caffeine 50 mg × 2 and L-cystine 100 mg × 2 (tablets) from day 0-90, day 161-289, and 412-570.

Axial length in mm RE = right eye, LE = left eye.

	Day	RE	LE
	0	23.74	23.70
	57	23.86	23.58
15	118	24.06	23.83
	161	24.14	24.07
	227	24.21	24.03
	260	24.26	24.10
	289	24.24	24.22
20	350	24.43	24.44
	392	24.56	24.47
	412	24.59	24.39
	447	24.63	24.53
	476	24.73	24.52
25	503	24.74	24.53
	541	24.72	24.49
	570	24.71	24.55

Table 9

30 Average growth during treatment and control periods, respectively.

	Axial length growth	n
Caffeine + L-cysteine (100 + 200 mg/day)	0.089 mm	4
Control period	0.253 mm	4

Conclusion: Example 3 confirms that treatment with a combination of caffeine and L-cystine actually is able to inhibit the myopia progression in a child as a significant effect of the treatment with a factor of 3 (inhibition of growth) in relation to no treatment is demonstrated in study.

Example 4

The effect of the caffeine metabolite 7-methylxanthine (heteroxanthine) 50 mg per animal daily on scleras content of proteoglycanes and collagen specific amino acids

10 The effect of the caffeine metabolite 3,7-dimethylxanthine (theobromine) 50 mg per animal daily on scleras content of proteoglycanes and collagen specific amino acids.

The effect of acetazolamide 50 mg per animal daily on scleras content of proteoglycanes and collagen specific amino acids.

15 The effect of 500 mg per animal daily of L-ornithine on scleras content of proteoglycanes and collagen specific amino acids.

Conclusion: No significant differences were found as regards the content of proteoglycans in the sclera samples, but there was a near significant increase in 7-methylxanthine treated posterior sclera, and a decrease in theobromine treated posterior sclera.

Significant higher content of all collagen specific amino acids, except hydroxylysine in anterior sclera, was found in all sclera samples from theobromine (3,7-dimethylxanthine) treated animals. Animals treated with 7-methylxanthine showed significantly higher content of hydroxyproline and proline in posterior sclera. Treatment with acetazolamide reduced the content of hydroxyproline and proline in anterior sclera significantly.

Table 10

WO 98/30900

PCT/DK98/00001

51

Content of proteoglycanes in μg PG/mg tissue (wet weight)

	anterior sclera	posterior sclera
Control	2.9 +/-0.5	2.9 +/-0.4
7-methylxanthine	2.5 +/-0.5	3.3 +/-0.3
5 3,7-dimethylxanthine	2.6 +/-0.7	2.5 +/-0.4
Acetazolamide	2.9 +/-0.4	2.8 +/-0.8
L-ornithine	3.0 +/-1.1	3.0 +/-0.9

		Anterior sclera		Posterior sclera	
		Increase	Decrease	Increase	Decrease
10	7-methylxanthine	-	14%	14%	-
	3,7-dimethylxanthine	-	10%	-	14%
	Acetazolamide	-	-	-	-
	L-ornithine	-	-	-	-

Table 11

15 Content of collagen specific amino acids in nanomol/mg tissue (dried weight defatted weight) from anterior sclera.
(Wilcoxon test. * $p < 0.05$)

	Hydroxyproline	Hydroxylysine	Proline
Control	638 +/-24	45 +/-10	928 +/-56
20 7-methylxanthine	667 +/-73	45 +/-3	951 +/-111
3,7-dimethylxanthine	751 +/-53*	49 +/-6	1073 +/-78*
Acetazolamide	596 +/-27*	41 +/-4	843 +/-47*
L-ornithine	605 +/-37	37 +/-4	875 +/-48

Table 12

25 Content of collagen specific amino acids in nanomol/mg tissue (dried weight and defatted weight) from posterior sclera.
(Wilcoxon test. * $p < 0.05$)

	Hydroxyproline	Hydroxylysine	Proline
Control	622 +/-59	41 +/-5	864 +/-67
30 7-methylxanthine	719 +/-81*	44 +/-7	1018 +/-108*
3,7-dimethylxanth.	741 +/-18*	49 +/-4*	1029 +/-45*

WO 98/30900

PCT/DK98/00001

52

Acetazolamide	563 +/-91	38 +/-6	781 +/-125
L-ornithine	611 +/-24	38 +/-5	871 +/-35

		Hydroxyproline		Hydroxylysine		Proline	
		Inc.	Decr.	Inc.	Decr.	Inc.	Decr.
5	7-methylxanthine	16%	-	7%	-	10%	-
	3,7-dimethylxanth.	19%	-	20%	-	20%	-
	Acetazolamide	-	10%	-	7%	-	10%
	L-ornithine	-	-	-	7%	-	-

Example 5

- 10 **Effect on proteoglycanes compared with effect on [³H ryanodine binding of different xanthines**

Comparison of effect on proteoglycanes content of posterior sclera in % to control with effect on [³H ryanodine binding in % compared to control.

15

Table 13

Effect on proteoglycan content of posterior sclera in % compared to control	Effect on [³ H ryanodine binding in % compared to control
---	---

20	Caffeine	119	240
	Theophylline	100	136
	7-methylxanthine	114	204
	Theobromine	86	124
	(Theobromine = 3,7-dimethylxanthine)		

25 **Example 6****Extraction method**

The plant material must either be extracted immediately after harvesting or stored at -20°C until extraction take place,

WO 98/30900

PCT/DK98/00001

53

and preferable, the material is freeze-dried before the cold storage. The material is finely ground and boiled for 20 minutes with 0.0125 N sulphuric acid. After cooling the extract is applied to a column packed with siliceous earth
5 (Extralut, Merck), 700 p per litre of extract, and after 10 minutes the column is eluted with a 4-fold volume of chloroform. The chloroform phase is eluted to dryness and chloroform condensed and collected for re-use. The residue contains the methylxanthines.

10 Example 7

The plant material must either be extracted immediately after harvesting or stored at -20°C until extraction can take place, and preferable, the material must be freeze-dried before the cold storage. The material is finely ground and
15 boiled for 20 minutes with =.1 N hydrochloric acid. After cooling the extract is filtered and applied to a column packed with polyvinylpyrrolidone powder, and after 10 minutes the column is eluted with water. The fraction of the eluate which has a high concentration of the methylxanthine in
20 question is collected and evaporated to a standardized concentration of this substance.

Example 8

Experiments under investigation:

A) The effect of the caffeine metabolite 3,7-dimethylxanthine
25 (theobromine) on c-wave by ERG and the standing potential of the eye is under investigation.

B) The effect of the caffeine metabolite 7-methylxanthine (heteroxanthine) on c-wave by ERG and the standing potential of the eye is under investigation.

30 C) Pilot projects

WO 98/30900

PCT/DK98/00001

54

Substances to be tested for treatment or preventing of myopia:

- 1) Caffeine and L-cystine (dosages as described)
- 2) 7-methylxanthine 250 mg
- 5 3) Pentifylline 250 mg

Substances to be tested for treatment or preventing of hypermetropia:

- 4) 1,9-dimethylxanthine 250 mg
- 5) theobromine 250 mg
- 10 (may be used once or twice daily equivalent of 10 or 20 mg per kg per day with a body weight of 25 kg)

The Test conditions:

Caffeine and L-cystine are to be given as tablets for the inhibition of the progression of myopia in a group of
15 children aged 10-14.

The background of the test

The content of L-cystine in the food varies depending on whether the protein need is covered by e.g. fish (approximately 350 mg L-cystine per day for a child weighing 30 kg)
20 or wheat bread (approximately 700 mg L-cystine per day) (Garrow, J.S.: Human Nutrition and Dietetics, Churchill Livingstone, 1993). L-cystine is absorbed through the intestine, the part that is not used for the building up of the proteins of the body is burnt on equal terms with e.g. sugar.

25 Caffeine is a well-known component of e.g. coffee, tea, chocolate and cola soft drinks. One cup of coffee contains 100-150 mg of caffeine, one cup of tea approximately 60 mg, one cup of cocoa approximately 5 mg, and one glass of cola approximately 20 mg. Only a very small part of the caffeine
30 in cola comes from the cola nut, primarily it is a matter of added caffeine. Thus it has been calculated that the total consumption of caffeine in cola in the U.S.A. corresponds to the amount deriving by way of decaffeinating coffee in the

country (James, J.E.: Caffeine and Health, Academic Press, 1991).

From the above foods children in the U.S.A. aged 10-17 consume approximately 1.5 mg of caffeine per kg weight per day, i.e. for a child of 30 kg approximately 45 mg per day (Albeit, M.L.: Journal of the American Dietetic Association, 88, 466-471 (1988). A Finnish examination showed that approximately 40% of children aged 12 consumed one or more cups of coffee per day. An even larger percentage consumed tea or hot chocolate every day (55<, E.: Social Science and Medicine, 26, 259-264 (1988).

L-cystine is an important factor in the stabilisation of the collagen molecules in the sclera as it forms the basis of sulphurous cross-links. Therefore, it is possible that, in a period with heavy body weight increase, a relative lack of L-cystine can lead to an unstable collagen and thus participate in developing myopia.

Therefore it is possible that supplementing the diet with L-cystine can inhibit the development of myopia.

Candidates of substances applicable for treatment of such a relatively benign condition as myopia in so many children must necessarily be free of almost any side effects.

Caffeine and L-cystine comply with this condition.

Treating a 9-year old boy with severely increasing myopia (Example 3) also implies an effect as the axial longitudinal growth over a period of three months was reduced from 0.253 mm to 0.089 mm with caffeine 100 mg + L-cystine 200 mg per day. The treatment showed no side effects.

The normal longitudinal growth of the human eye for the age of 3-14 amounts to approximately 0.1 mm per year, but there is much variation. In approximately 15% the axial longitudinal growth is thus >0.4 mm per year (Sorsby, A., Med. Res.

WO 98/30900

PCT/DK98/00001

56

Counc. Spec. Rep. Ser., No. 301, London, 1962). Particularly this group develops myopia.

c. Test persons

Boys and girls aged 10-14 with progreding myopia.

- 5 Inclusion: 3 x 12 myopic persons, 10-14 years old, glass strength > -1.25.

Exclusion: Severe ordinary disease (e.g. asthma, epilepsy, diabetes, physical disease), severe congenital myopia, other severe eye diseases (e.g. congenital cataract, ceratoconus,
10 chronic iritis, glaucoma).

The test persons were recruited from practising ophthalmologists.

As myopia develops in the childhood it is necessary to apply children in the test.

15 e. Method

Pilot test.

12 persons are treated with tablets of caffeine, 50 mg in the morning and 50 mg in the evening for 6 months.

12 persons are treated with tablets of L-cystine, 100 mg in
20 the morning and 100 mg in the evening for 6 months.

12 persons are treated with tablets of caffeine, 50 mg, and tablets of cystine, 100 mg in the morning and in the evening for 6 months.

Then a control period of 6 months follows with no treatment.

25 The level of myopia progression for the two periods is compared for each participant.

Furthermore, the myopia progression in the treatment period is compared with existing material concerning myopia progression in children of the same age group (e.g. Jensen, H.: Myopia progression in young school children, Acta Ophthal.,
5 Suppl. 200, Vol 69, 1991).

During the test the following examinations are made:

1. Status at the commencement of the test.

a. Subjective measuring of glass strength (ordinary glass determination)

10 b. Refraction determination with auto refractor (objective determination of the glass strength) in cyclogylcycloplegy (cancelling the accommodation reflex with cyclogyl drops).

15 c. Measuring the axial length with Shin-Nippon axial length measurer. The patient is dripped with local anaestheticum, and an ultra sound probe is put on the eye by means of a tonometer set at 15 mmHg. The axial length is stated in mm by the apparatus by 2 digits.

20 Three measurements are made and the average is calculated.

2. Measurement of the axial length after two months (as 1c).

3. Measurement of the axial length after 4 months (as 1c).

4. Status after 6 months (as 1a-c).

5. Measurement of the axial length after 8 months (as 1c).

25 6. Measurement of the axial length after 10 months (as 1c).

7. Status after 12 months (as 1a-c).

WO 98/30900

PCT/DK98/00001

58

Data identifying the patient is destroyed at the end of the test.

WO 98/30900

PCT/DK98/00001

59

Table A

STANDARD RABBIT DIET (STANRAB)					
5	Crude Oil	%	3.1	Glycine	% 1.39
	Crude Protein	%	16.7	Aspartic acid	% 1.14
	Crude Fibre	%	14.8	Glutamic acid	% 3.01
	Ash	%	8.1	Proline	% 1.18
	N.F.E.	%	47.3	Serine	% 0.70
10	Dig, Crude Oil	%	2.8	Hydroxyproline	% -
	Dig, Crude Protein	%	14.9	Hydroxylysine	% -
	Tot, Dietary Fibre	%	31.9	Alanine	% 0.11
	Pectin	%	2.4	Calcium	% 0.83
	Hemicellulose	%	14.7	Total Phosphorous	% 0.61
15	Cellulose	%	11.7	Phytate Phosphorous	% 0.36
	Lignin	%	3.1	Available Phosphorous	% 0.25
	Starches	%	22.7	Sodium	% 0.25
	Sugars	%	7.5	Chlorine	% 0.36
	Gross Energy	mj/kg	14.8	Magnesium	% 0.41
20	Dig, Energy	mj/kg	9.1	Potassium	% 1.52

WO 98/30900

PCT/DK98/00001

60

5

10

15

Met, Energy	mj/kg	8.2	Iron	mg/kg	194.0
Myrleteoleic acid	%	0.02	Copper	mg/kg	17.0
Palmitoileic acid	%	0.09	Manganese	mg/kg	93.0
Oleic acid	%	0.79	Zinc	mg/kg	47.0
Linoleic acid	%	0.75	Cobalt	mcg-/kg	535.0
Linolenic acid	%	0.18	Iodine	mcg-/kg	665.0
Arachidonic acid	%	0.15	Selenium	mcg/-kg	217.0
Clupanodonic acid	%	-	Fluorine	mg/kg	24.0
Lauric acid	%	0.03	Retinol	mcg-/kg	47473.0
Myristic acid	%	0.18	Vitamin A	iu/kg	156991.0
Palmitic acid	%	0.37	Cholecalciferol	mcg-/kg	37.6
Stearic acid	%	0.07	Vitamin D3	iu/kg	1504.0
Arginine	%	1.21	α -Tocopherol	mg/kg	62.0
Lysine	%	0.91	Vitamin E	mg/kg	68.2
Methionine	%	0.35	Vitamin B1	mg/kg	10.6

WO 98/30900

PCT/DK98/00001

61

5

10

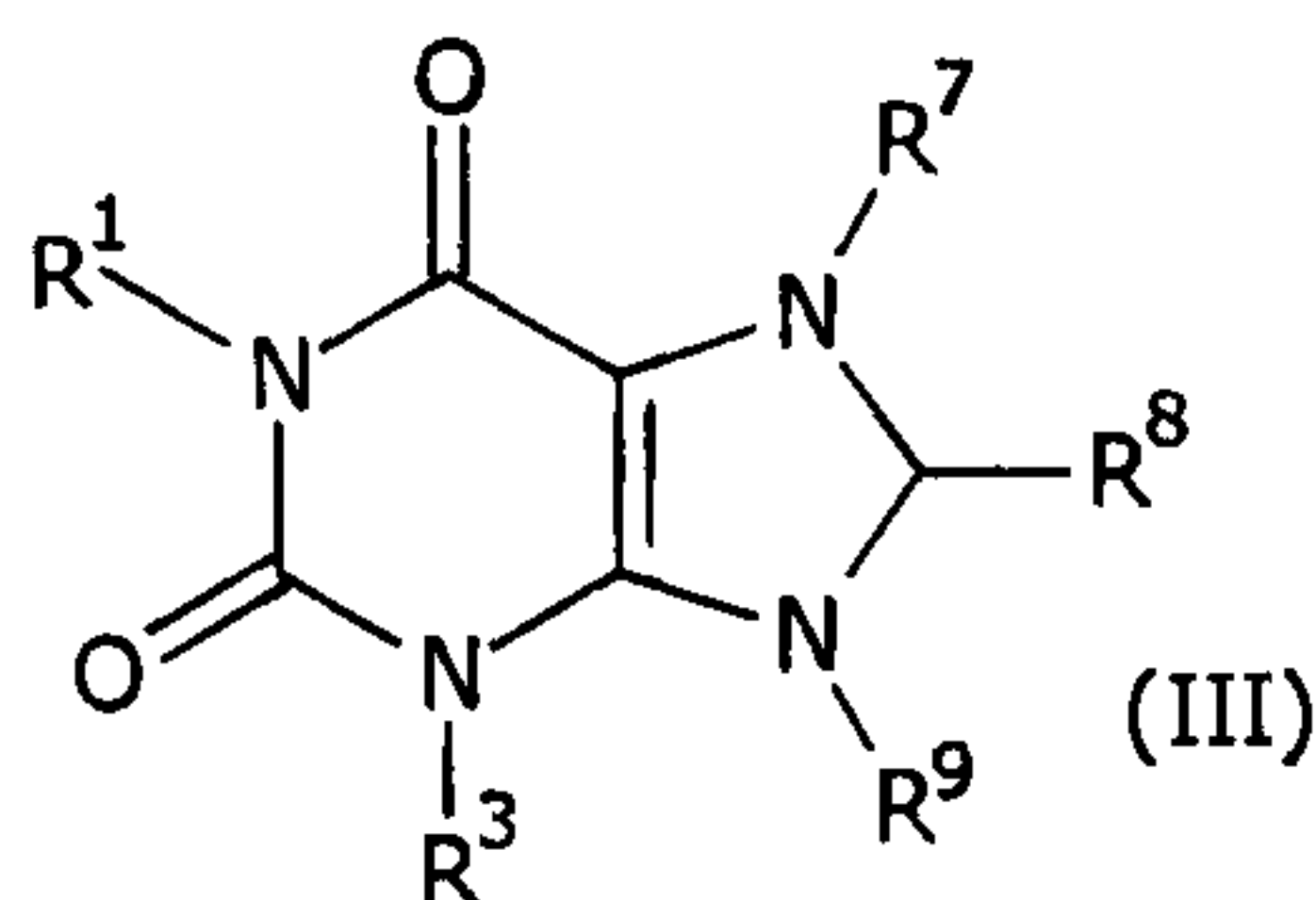
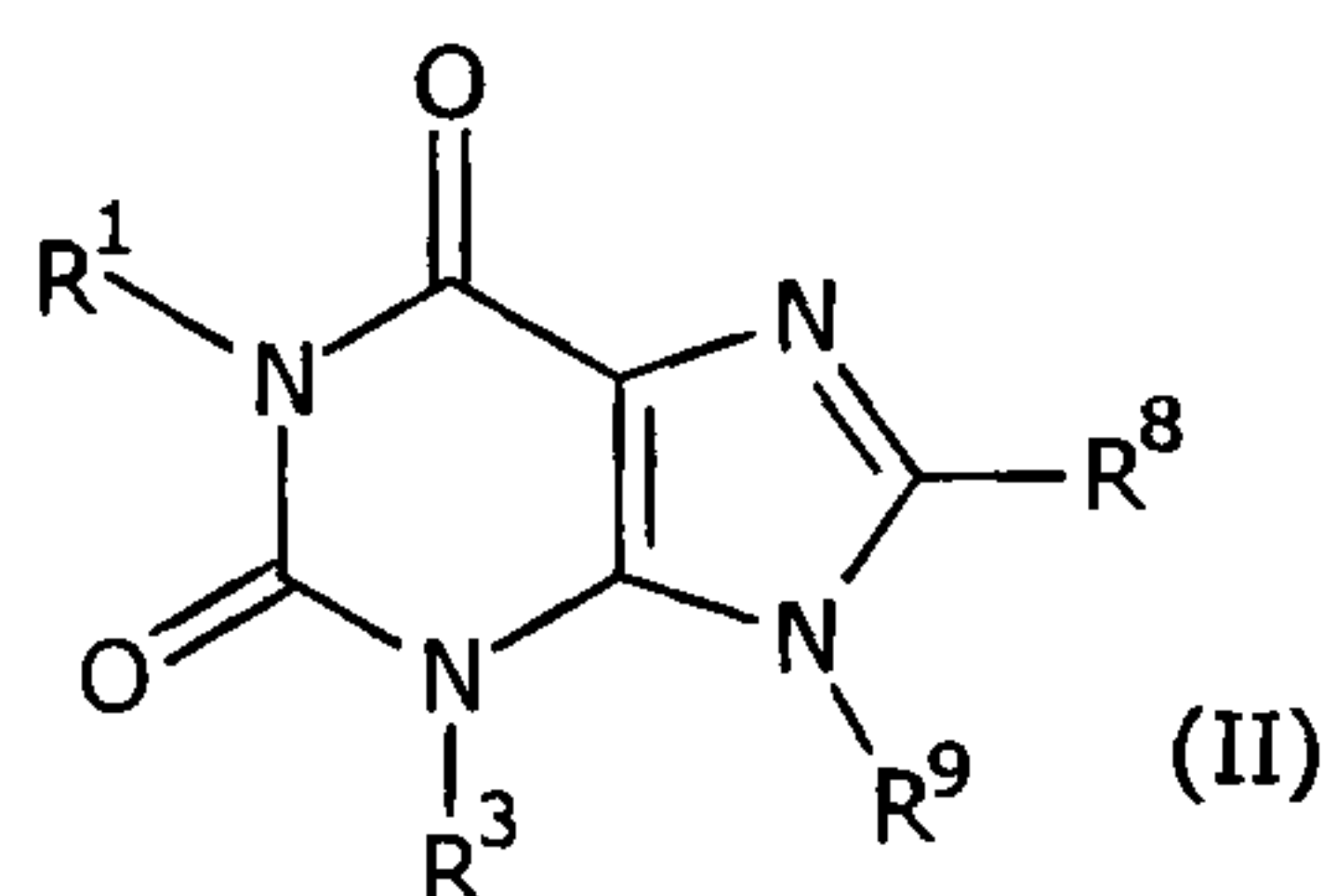
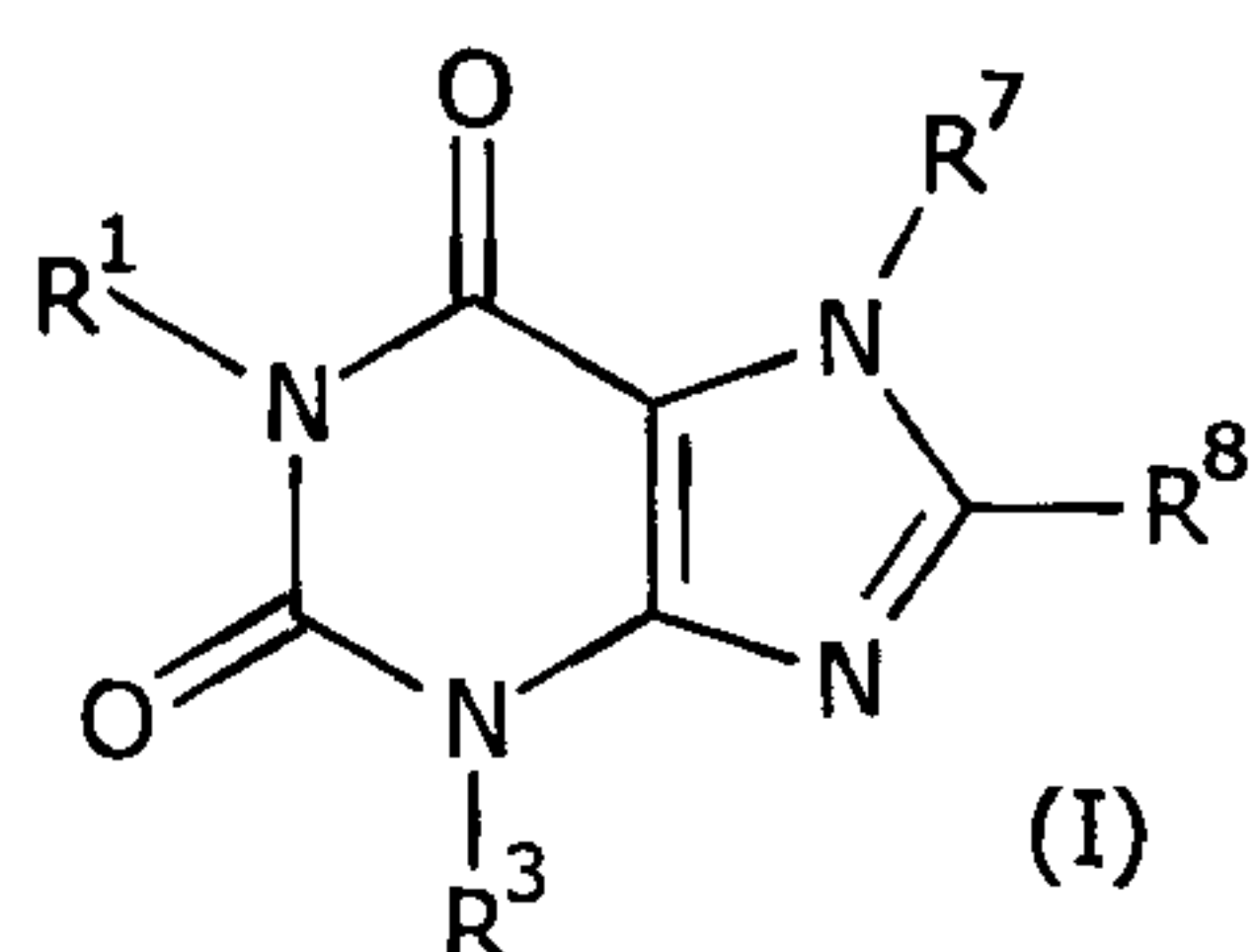
Cystine	%	0.2 9	Vitamin B2	mg/kg	14.2
Tryptophan	%	0.2 8	Vitamin B6	mg/kg	7.6
Histidine	%	0.4 4	Vitamin B12	mcg- /kg	11.0
Threonine	%	0.6 8	Vitamin C	mg/kg	113.0
Isoleucine	%	0,7 4	Vitamin K3	mg/kg	63.2
Leucine	%	1.2 8	Folic acid	mg/kg	2.1
Phenylalanine	%	0.8 2	Nicotinic acid	mg/kg	73.8
Valine	%	0.8 8	Pantothenic acid	mg/kg	34.7
Tyrosine	%	0.6 2	Choline	mg/kg	1151.0
Taurine	%	-	Inositol	mg/kg	1515.0
			Biotin	mcg- /kg	344.0
			p-aminobenzoic acid	mg/kg	-
			β -Carotene	mg/kg	99.2
			Xanthophyl	mg/kg	-

1. All values calculated to a nominal 10% moisture content.
2. Values are total calculated values.
3. 1 mcg Retinol nid 3.3 i.u. vitamin A.
- 5 4. Total Retinol content includes the Retinol equivalent of β -Carotene.
5. 1 mcg Cholecalciferol = 40 i.u. vitamin D3 activity.
6. 1 mcg β -Carotene = 1.6 i.u. vitamin A activity.
7. 1 mg α -Tocopherol = 1.1 i.u. vitamin E activity.
- 10 8. 1 MJ = 239.23 Calories

What is claimed is:

1. Use of a compound of the general formula I, II or III

5



wherein R1, R3, R7, and R9 are independently selected from the group consisting of hydrogen, optionally substituted C1-20-alkyl, optionally substituted C2-20-alkenyl, optionally substituted C4-20-alkadienyl, optionally substituted C6-20-alkatrienyl, optionally substituted C2-20-alkynyl, optionally substituted C1-20-alkoxycarbonyl, optionally substituted C1-20-alkylcarbonyl, formyl, optionally substituted aryl selected from the group consisting of optionally substituted phenyl, optionally substituted naphthyl, optionally substituted 1,2,3,4-tetrahydronaphthyl, optionally substituted anthracyl, optionally substituted phenanthracyl, optionally substituted pyrenyl, optionally substituted benzopyrenyl, optionally substituted fluorenyl and optionally substituted xanthenyl, optionally substituted heteroaryl selected from the group consisting optionally substituted oxazolyl, optionally substituted isoxazolyl, optionally substituted thiazolyl, optionally substituted isothiazolyl, optionally substituted pyrrolyl, optionally substituted imidazolyl, optionally substituted pyrazolyl, optionally substituted pyridinyl, optionally substituted pyrazinyl, optionally substituted pyridazinyl, optionally substituted piperidinyl, optionally substituted coumaryl, optionally substituted furyl, optionally substituted quinolyl, optionally substituted benzothiazolyl, optionally substituted benzotriazolyl, optionally substituted benzodiazolyl, optionally substituted benzoxazolyl, optionally substituted phthalazinyl, optionally substituted phthalanyl, optionally substituted triazolyl, optionally substituted tetrazolyl, optionally substituted isoquinolyl, optionally substituted acridinyl, optionally substituted carbazolyl, optionally substituted dibenzazepinyl, optionally substituted indolyl, optionally substituted benzopyrazolyl and optionally substituted phenoxazonyl and halogen

30

and

R8 is selected from the group consisting of hydrogen, optionally substituted C1-20-alkyl, optionally substituted C2-20-alkenyl, optionally substituted C4-20-alkadienyl, optionally substituted C6-20-alkatrienyl, optionally substituted C2-20-alkynyl, optionally substituted C1-20-alkoxy, optionally substituted C2-20-alkenyloxy, carboxy, hydroxy, optionally substituted C1-20-alkoxycarbonyl, optionally substituted C1-20-alkylcarbonyl, formyl, optionally substituted aryl selected from the group consisting of optionally substituted phenyl, optionally substituted naphthyl, optionally substituted 1,2,3,4-tetrahydronaphthyl, optionally substituted anthracyl, optionally substituted phenanthracyl, optionally substituted pyrenyl, optionally substituted benzopyrenyl, optionally substituted fluorenyl and optionally substituted xanthenyl, optionally substituted heteroaryl selected from the group consisting of optionally substituted oxazolyl, optionally substituted isoxazolyl, optionally substituted thiazolyl, optionally substituted isothiazolyl, optionally substituted pyrrolyl, optionally substituted imidazolyl, optionally substituted pyrazolyl, optionally substituted pyridinyl, optionally substituted pyrazinyl, optionally substituted pyridazinyl, optionally substituted piperidinyl, optionally substituted coumaryl, optionally substituted furyl, optionally substituted quinolyl, optionally substituted benzothiazolyl, optionally substituted benzotriazolyl, optionally substituted benzodiazolyl, optionally substituted benzoxazolyl, optionally substituted phthalazinyl, optionally substituted phthalanyl, optionally substituted triazolyl, optionally substituted tetrazolyl, optionally substituted isoquinolyl, optionally substituted acridinyl, optionally substituted carbazolyl, optionally substituted dibenzazepinyl, optionally substituted indolyl, optionally substituted benzopyrazolyl and optionally substituted phenoxazonyl, amino, mono- and di(C1-20-alkyl)amino, carbamoyl, mono- and di(C1-20-alkyl)aminocarbonyl, amino-C1-20-alkyl-aminocarbonyl, mono- and di(C1-20-alkyl)amino-C1-20-alkyl-aminocarbonyl, optionally substituted C1-20-alkylcarbonylamino, guanidino, carbamido, optionally substituted C1-20-alkanoyloxy, sulphonyloxy, optionally substituted C1-20-alkylsulphonyloxy, nitro, sulphonyl, optionally substituted C1-20-alkylthio, and halogen,

for the preparation of a medicament for treating or preventing disorders of the eye related to the axial length of the eye.

2. Use according to claim 1, wherein R1, R3, R7, and R9 are independently selected from the group consisting of hydrogen, optionally substituted C1-6-alkyl, optionally substituted C2-6-alkenyl, optionally substituted C2-6-alkynyl, optionally substituted C1-6-alkylcarbonyl, formyl, optionally substituted aryl selected from the group consisting of optionally substituted phenyl, optionally substituted naphthyl, optionally substituted 1,2,3,4-tetrahydronaphthyl, optionally substituted anthracyl, optionally substituted phenanthracyl, optionally substituted pyrenyl, optionally substituted benzopyrenyl, optionally substituted fluorenyl and optionally substituted xanthenyl, optionally substituted heteroaryl selected from the group consisting of optionally substituted oxazolyl, optionally substituted isoxazolyl, optionally substituted thiazolyl, optionally substituted isothiazolyl, optionally substituted pyrrolyl, optionally substituted imidazolyl, optionally substituted pyrazolyl, optionally substituted pyridinyl, optionally substituted pyrazinyl, optionally substituted pyridazinyl, optionally substituted piperidinyl, optionally substituted coumaryl,

optionally substituted furyl, optionally substituted quinolyl, optionally substituted benzothiazolyl, optionally substituted benzotriazolyl, optionally substituted benzodiazolyl, optionally substituted benzoxazolyl, optionally substituted phthalazinyl, optionally substituted phthalanyl, optionally substituted triazolyl, optionally substituted tetrazolyl,
 5 optionally substituted isoquinolyl, optionally substituted acridinyl, optionally substituted carbazolyl, optionally substituted dibenzazepinyl, optionally substituted indolyl, optionally substituted benzopyrazolyl and optionally substituted phenoxazonyl,

and

10

R8 is selected from the group consisting of hydrogen, optionally substituted C1-6-alkyl, optionally substituted C2-6-alkenyl, optionally substituted C2-6-alkynyl, optionally substituted C1-6-alkoxy, optionally substituted C2-6-alkenyloxy, carboxy, hydroxy, optionally substituted C1-6-alkoxycarbonyl, optionally substituted C1-6-alkylcarbonyl,
 15 formyl, optionally substituted aryl selected from the group consisting of optionally substituted phenyl, optionally substituted naphthyl, optionally substituted 1,2,3,4-tetrahydronaphthyl, optionally substituted anthracyl, optionally substituted phenanthracyl, optionally substituted pyrenyl, optionally substituted benzopyrenyl, optionally substituted fluorenyl and optionally substituted xanthenyl, optionally substituted heteroaryl selected
 20 from the group consisting of optionally substituted oxazolyl, optionally substituted isoxazolyl, optionally substituted thiazolyl, optionally substituted isothiazolyl, optionally substituted pyrrolyl, optionally substituted imidazolyl, optionally substituted pyrazolyl, optionally substituted pyridinyl, optionally substituted pyrazinyl, optionally substituted pyridazinyl, optionally substituted piperidinyl, optionally substituted coumaryl, optionally substituted furyl, optionally substituted quinolyl, optionally substituted benzothiazolyl,
 25 optionally substituted benzotriazolyl, optionally substituted benzodiazolyl, optionally substituted benzoxazolyl, optionally substituted phthalazinyl, optionally substituted phthalanyl, optionally substituted triazolyl, optionally substituted tetrazolyl, optionally substituted isoquinolyl, optionally substituted acridinyl, optionally substituted carbazolyl,
 30 optionally substituted dibenzazepinyl, optionally substituted indolyl, optionally substituted benzopyrazolyl and optionally substituted phenoxazonyl, amino, mono- and di(C1-6-alkyl)amino, carbamoyl, mono- and di(C1-6-alkyl)aminocarbonyl, optionally substituted C1-6-alkylcarbonylamino, guanidino, carbamido, optionally substituted C1-6-alkanoyloxy, sulphono, optionally substituted C1-6-alkylsulphonyloxy, nitro, sulphonyl, optionally substituted C1-6-alkylthio, and halogen.
 35

3. Use according to claim 2, wherein R1, R3, R7 and R9 are independently selected from the group consisting of hydrogen, optionally substituted C1-6-alkyl, optionally substituted C2-6-alkenyl, optionally substituted C2-6-alkynyl, optionally substituted aryl selected from
 40 the group consisting of optionally substituted phenyl, optionally substituted naphthyl, optionally substituted 1,2,3,4-tetrahydronaphthyl, optionally substituted anthracyl, optionally substituted phenanthracyl, optionally substituted pyrenyl, optionally substituted benzopyrenyl, optionally substituted fluorenyl and optionally substituted xanthenyl,

and

- R8 is selected from the group consisting of hydrogen, optionally substituted C1-6-alkyl, optionally substituted C2-6-alkenyl, optionally substituted C2-6-alkynyl, optionally substituted C1-6-alkoxy, optionally substituted C2-6-alkenyloxy, carboxy, hydroxy, optionally substituted aryl selected from the group consisting of optionally substituted phenyl, optionally substituted naphthyl, optionally substituted 1,2,3,4-tetrahydronaphthyl, optionally substituted anthracyl, optionally substituted phenanthracyl, optionally substituted pyrenyl, optionally substituted benzopyrenyl, optionally substituted fluorenyl, and optionally substituted xanthenyl, optionally substituted heteroaryl selected from the group consisting of optionally substituted oxazolyl, optionally substituted isoxazolyl, optionally substituted thiazolyl, optionally substituted isothiazolyl, optionally substituted pyrrolyl, optionally substituted imidazolyl, optionally substituted pyrazolyl, optionally substituted pyridinyl, optionally substituted pyrazinyl, optionally substituted pyridazinyl, optionally substituted piperidinyl, optionally substituted coumaryl, optionally substituted furyl, optionally substituted quinolyl, optionally substituted benzothiazolyl, optionally substituted benzotriazolyl, optionally substituted benzodiazolyl, optionally substituted benzoxazolyl, optionally substituted phthalazinyl, optionally substituted phthalanyl, optionally substituted triazolyl, optionally substituted tetrazolyl, optionally substituted isoquinolyl, optionally substituted acridinyl, optionally substituted carbazolyl, optionally substituted dibenzazepinyl, optionally substituted indolyl, optionally substituted benzopyrazolyl, and optionally substituted phenoxazonyl, amino, nitro, sulphanyl, and halogen.
4. Use of one or more substances selected from caffeine; 3,7-dimethylxanthine (theobromine), 1,7-dimethylxanthine (paraxanthine), 7-methylxanthine (heteroxanthine), 3-methylxanthine; 1-methylxanthine, isobutylmethylxanthine (IBMX); 1-Hexyl-3,7-dimethylxanthine (pentifylline); 1,7-Dimethyl-xanthine; 1,3-Dipropyl-7-methyl-xanthine; 7-Propylxanthine; 7 β -Chloroethyl-1,3-dimethylxanthine; 3,7-Dimethyl-1-propargyl- xanthine; 3-Propylxanthine; 1-(5-Oxohexyl)-3,7-dimethylxanthine (pentoxifylline); 3-Isobutyl-1-methylxanthine; 3,9-Dimethylxanthine 8-Cyclopentyl-1,3-dimethylxanthine; 1,3-Bis(3-methylbut-2-butenyl-7-methylxanthine; 3,7-Dihydro-7-methyl-1,3-dipropyl-1H-purine-2,6-dione; 7-Methyl-1,3-diprenylxanthine(7-methyl-1,3-dipropyl-xanthine; 7-Ethoxymethyl-1-(5-hydroxy-5-methylhex-methylxanthine (Torbafylline, "HWA 448"); 1-(5 hydroxy-5-methylhexyl)-3-methyl-7-propylxanthine (A 80.2715); 3,7-Dimethyl-1-(5-oxyhexyl)-xanthine (Pentoxifylline, "Trental®");

- 3,7-Dimethyl-1-(5-hydroxyhexyl)-xanthine (Hydroxypentoxifylline);
 1-Hexyl-3,7-dimethylxanthine (Pentifylline, "Cosaldon");
 3,7-Dimethyl-1-propargylxanthine (DMPX);
 (E)-8-(3,4-Dimethoxystyryl)-1,3-dipropyl-7-methylxanthine
 5 (KF 17837)(Lisofylline);
 1-(5-Hydroxy-5-methylhexyl)-3-methylxanthine
 (Albifylline, "HWA 138");
 3-Methyl-1-(5'-oxohexyl)-7-propylxanthine
 (Propentofylline, "HWA 285");
 10 1-(5-Hydroxyhexyl)-3,7-dimethylxanthine (BL 194);
 (E)-1,3-dipropyl-8-(3,4-dimethoxystyryl)-7-methylxanthine (KF 17.837);
 1,3-di-n-butyl-7-(2'oxopropyl)-xanthine (Denbufylline);
 1-n-butyl-3-n-propylxanthine (XT-044);
 7-(2,3-dihydroxypropyl)-theophylline (Dyphylline);
 15 7-Methyl-8-(2-hydroxy-N-methylethylamino)-theophylline (Cafaminol);
 7-(1,3-Dioxolan 2-ylmethyl)-theophylline (Doxofylline);
 7-(2-Hydroxyethyl)-1,3-dimethylxanthine (Etofylline);
 7-(2-Hydroxypropyl)-1,3-dimethylxanthine (Proxiphylline);
 Pyridoxine-O-(theophyllin-7-ylethyl)sulphate (Pyridofylline);
 20 7-(2-(3-diethylcarbamoylpropionyloxy)ethyl)theophylline (Suxamidofylline);
 Piperazine bis (theophyllin-7-ylacetate)(Acepifylline);
 8-benzyl-7-(2-(N-ethyl-N-2-hydroxyethylamino)ethyl)theo-phylline (Bamifylline);
 2-amino-2-methylpropan-1-ol theophyllinate (Bufylline);
 7-(2,3-Dihydroxypropyl)-1,3-dimethylxanthine (Diprophylline);
 25 7-(2-diethylaminoethyl)-1,3-dimethylxanthine camphor 10 sulphonate (Etamiphylline
 Camsylate);
 3-Propylxanthine (Enprofylline); and mixtures thereof;

for the preparation of a medicament for treating or preventing myopia of a human eye
 30 wherein the myopia is caused by the length of the eye being too long.

5. Use according to claim 4 wherein the caffeine or the derivative thereof is to be used in a dosage of 7.5-750 mg 1-4 times daily.

- 35 6. Use of one or more substances selected from theophylline; xanthine; 1,9-dimethylxanthine;
 1,3-Dipropyl-8-(2-(5,6-epoxynorbonyl)-xanthine;
 8-Cyclopentyl-1,3-dipropylxanthine (CPDPX);
 8-Sulphophenyltheophylline;
 40 1,3-Dipropyl-8-(4-acrylate)phenylxanthine (BW-A1433);
 (1-Propyl-11C)8-dicyclopropylmethyl-1,3-dipropylxanthine
 (11C)KF15372 and 11C-ethyl and 11C-methyl derivatives thereof;
 8-Benzyl-7,(2-(ethyl(2-hydroxyethyl)amino)ethyl)theo-phylline (Bamiphylline);
 8-Cyclopentyl-3-(3-((4-(fluorosulfonyl)benzoyl)oxy)pro-pyl)-1-propylxanthine;

- 1,3-Dipropyl-8-(4-((2-aminoethyl)amino)carbonylmethyl-oxyphenyl)xanthine;
 8-(3-chlorostyryl)caffeine;
 8-cyclopentyltheophylline;
 8-(noradamantan-3 yl)-1,3-dipropylxanthine (KW-3902);
- 5 1,3-Dipropyl-8-(3-noradamantyl)-xanthine;
 1,3-Dipropyl-8-(4-sulphophenyl)-xanthine;
 1,3-Dipropyl-8-(2-amino-4-chlorophenyl)-xanthine;
 7 β -Hydroxyethyl-1,3-dimethylxanthine;
 7-(2,3-Dihydroxypropyl)-1,3-dimethylxanthine;
- 10 8-Chloro-1,3-dimethylxanthine;
 1,3,9-Trimethylxanthine;
 8-Propionic acid-1,3-dimethylxanthine;
 7,9-Dimethylxanthine;
 8-Phenyl-1,3-dimethylxanthine;
- 15 7-Acetic acid-1,3-dimethylxanthine;
 9-Propylxanthine; 9-Methylxanthine;
 8-Methylxanthine; 8-(p-Sulfophenyl)-1,3-dimethylxanthine;
 1,9-Dimethylxanthine; hypoxanthine; and mixtures thereof for the preparation of a medicament for treating or preventing hypermetropia of a human eye.
- 20
7. Use according to claim 6 wherein the substances are to be used in a dosage of 7.5-750 mg 1-4 times daily.
8. Use according to any one of claims 1-7 wherein the substance is to be used
- 25 systematically.
9. Use according to any one of claims 1-7 wherein the preparation is in the form suitable for application on mucosa or for application on nasal mucosa.
- 30 10. Use according to claim 9, wherein the preparation is in the form of eye drops, eye salve, eye gel, an eye insert, a nasal insert, a nasal drop or spray, a nasal ointment or gel.
11. Use according to any one of claims 1-7 and 9-10 wherein the preparation is in the form suitable for topical application on skin.
- 35
12. Use according to claim 11, wherein the preparation is in the form of a powder, paste, ointment, lotion, gel, cream, emulsion, solution, suspension, spray, sponge, strip, plaster, pad, or dressing.
- 40 13. Use according to any one of claims 1-7 wherein the preparation is in a form suitable for implantation, injection or systemic administration.

14. Use according to any one of claims 1-13 wherein the substance or the mixture of substances is present in the medicament in an amount of 0.001-99% by weight of the medicament.

- 5 15. Use according to any one of the claims 1-14 wherein the substance or the mixture of substances are extracted from natural sources.

10 TOR_LAW\ 6388732\1

1/12

Growth of axial length of child (age 10 years at day 0)

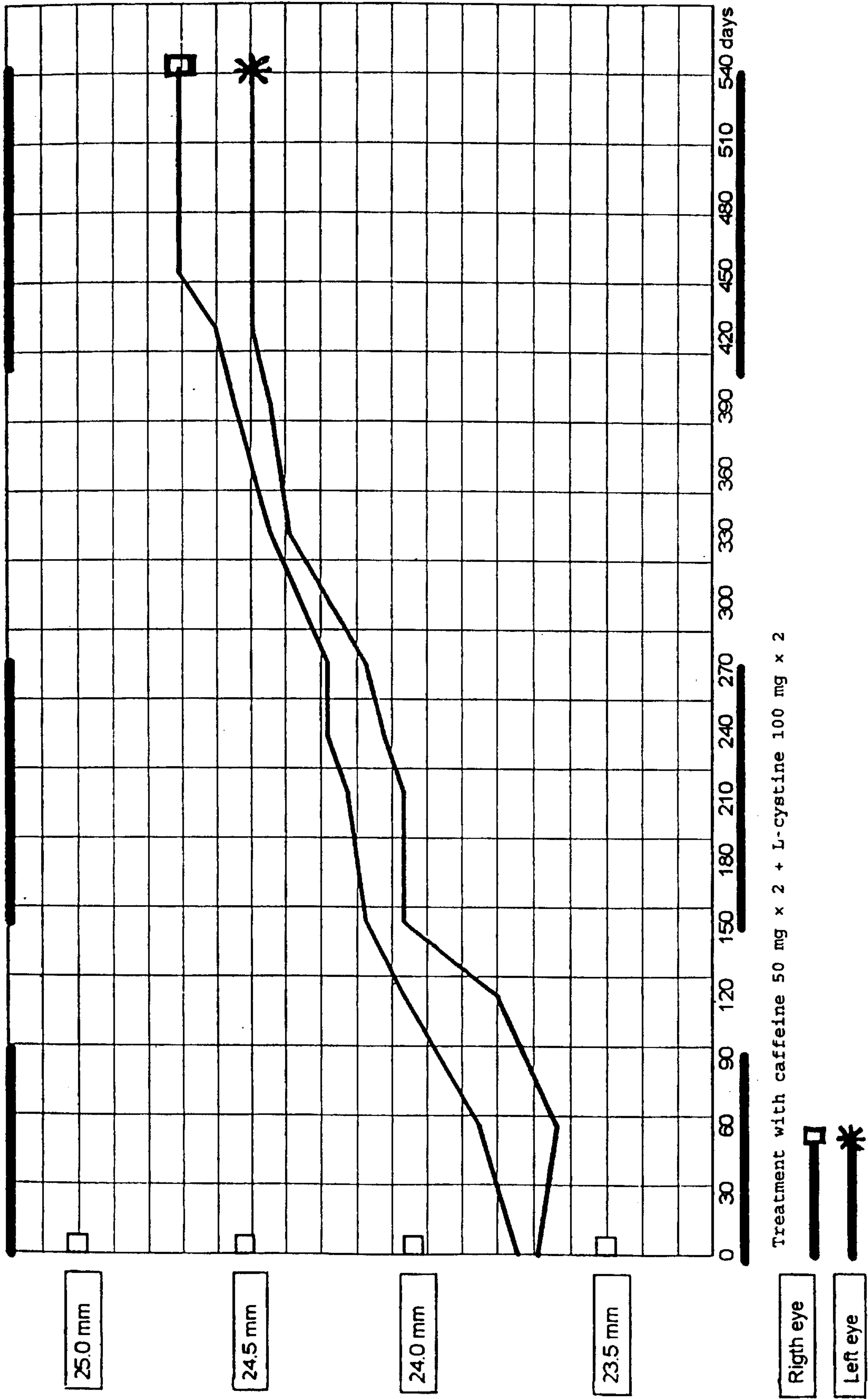


Fig. 1

WO 98/30900

PCT/DK98/00001

2/12

Vial 65	Sample Type:	Unknown
Injection 1	Volume:	8.00
Sample Name:	Run Time:	22.0 min
Date Acquired:	Date Processed:	18-03-97 13.18.28
Acq Meth Set:		
Processing Method:		

Rbeye7A2-2
18-03-97 04.37.05
Hypersil3um47endelig_MS
HypersilCollagen2aba_PM

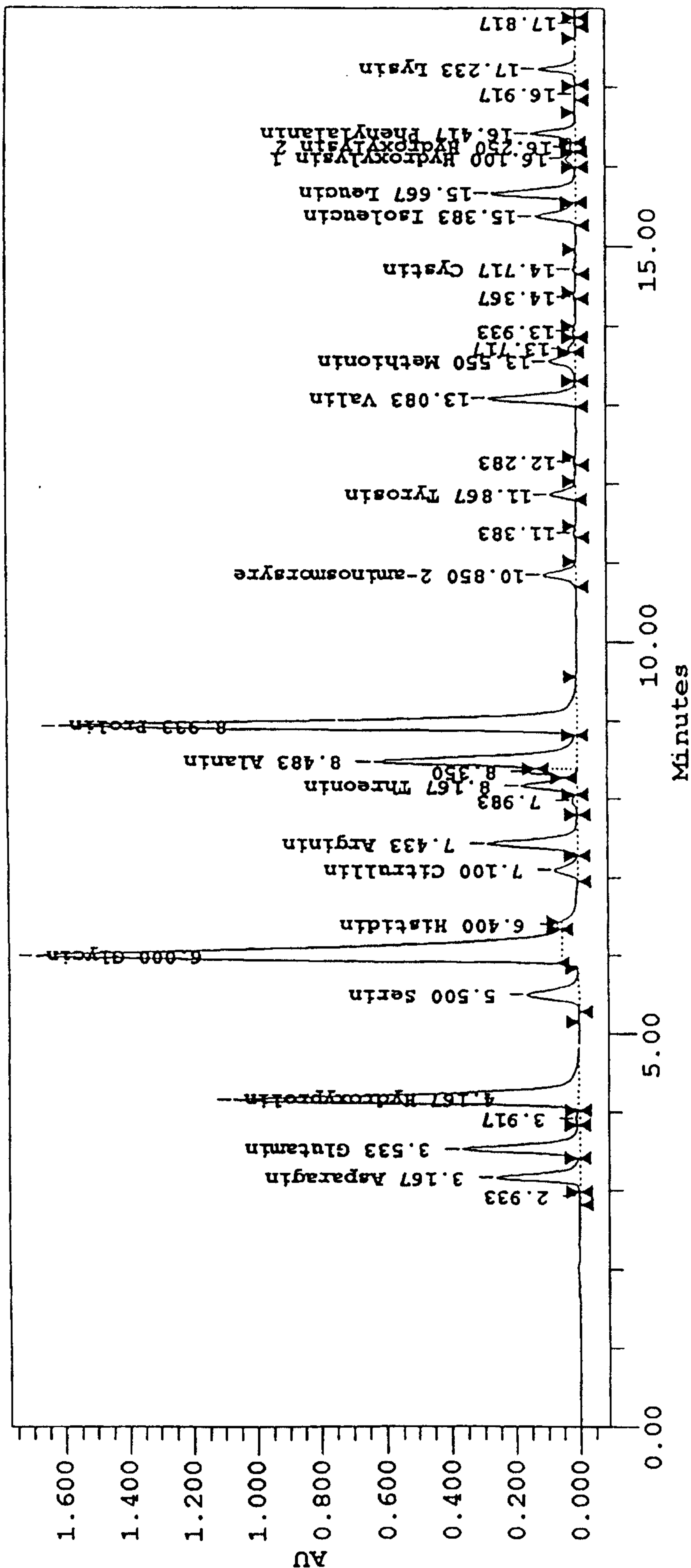
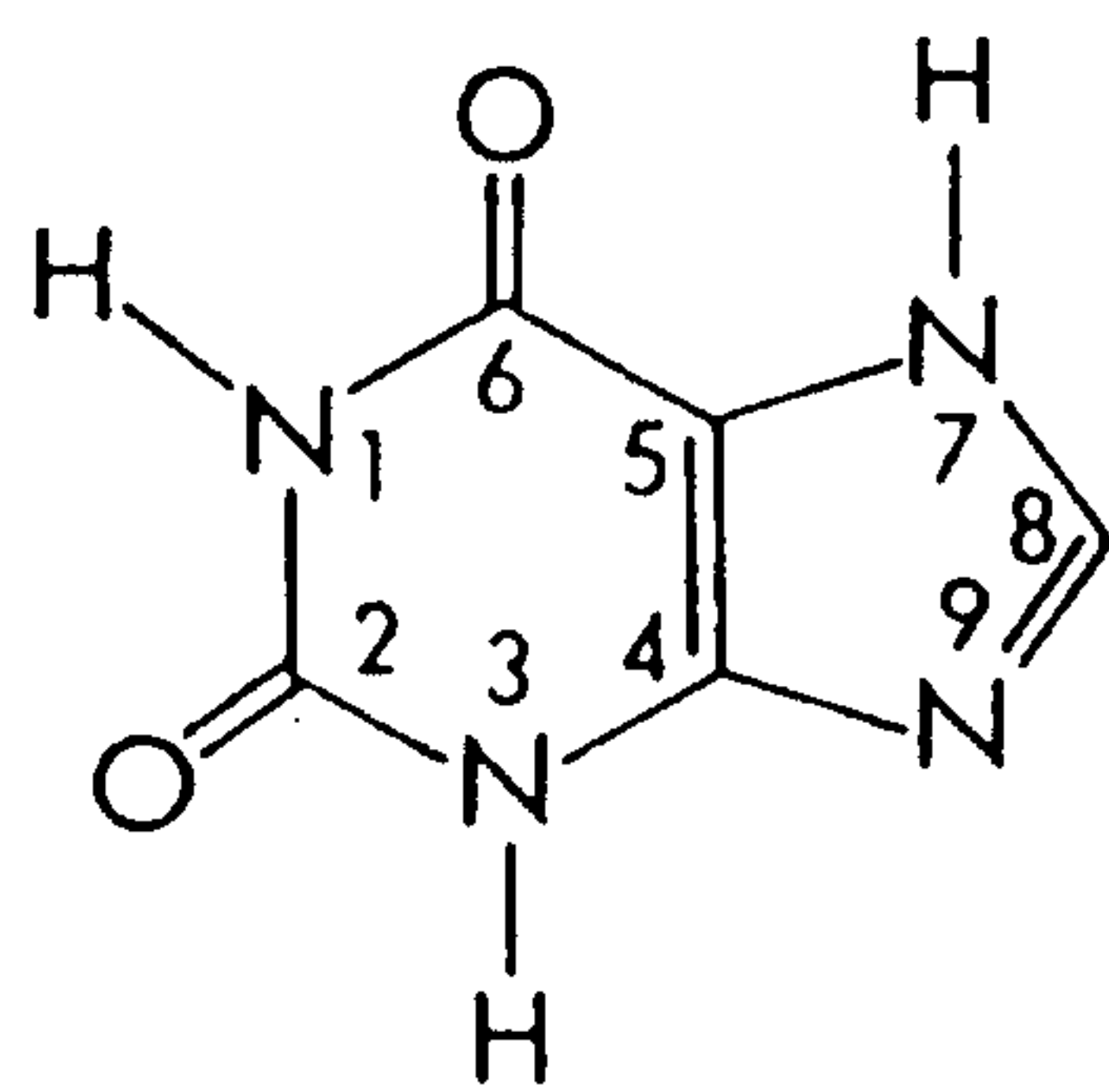
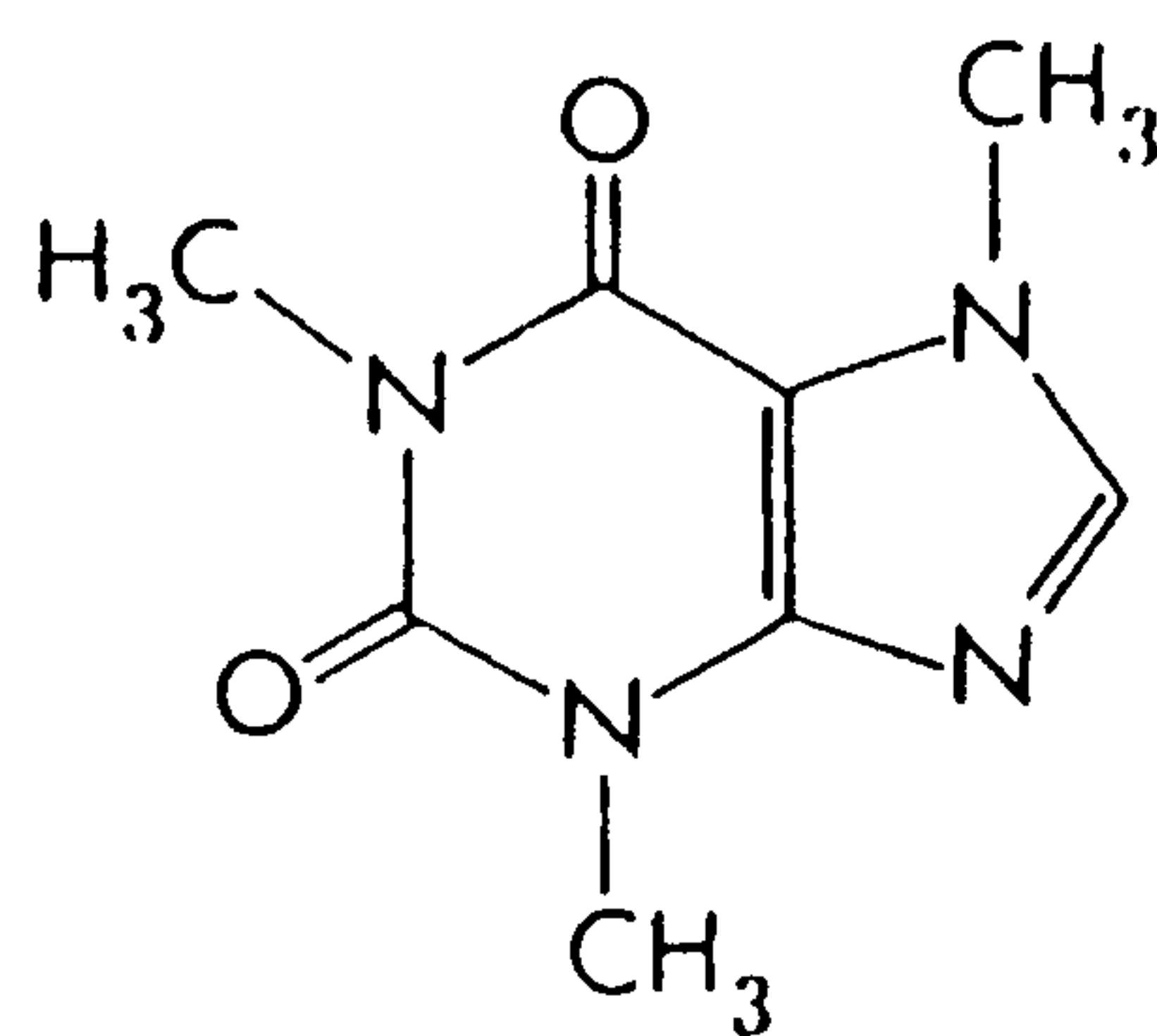


Fig. 2

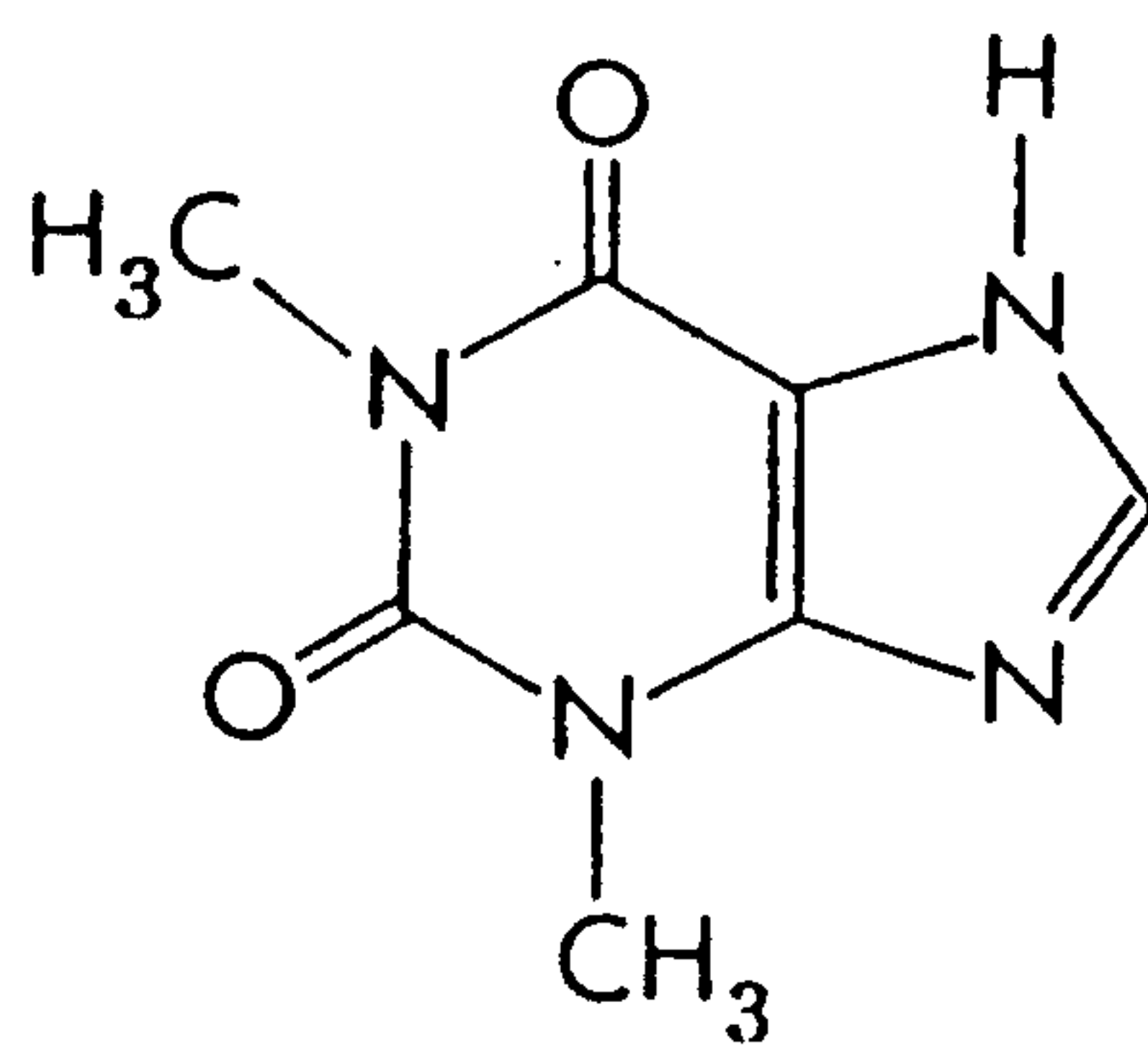
3/12



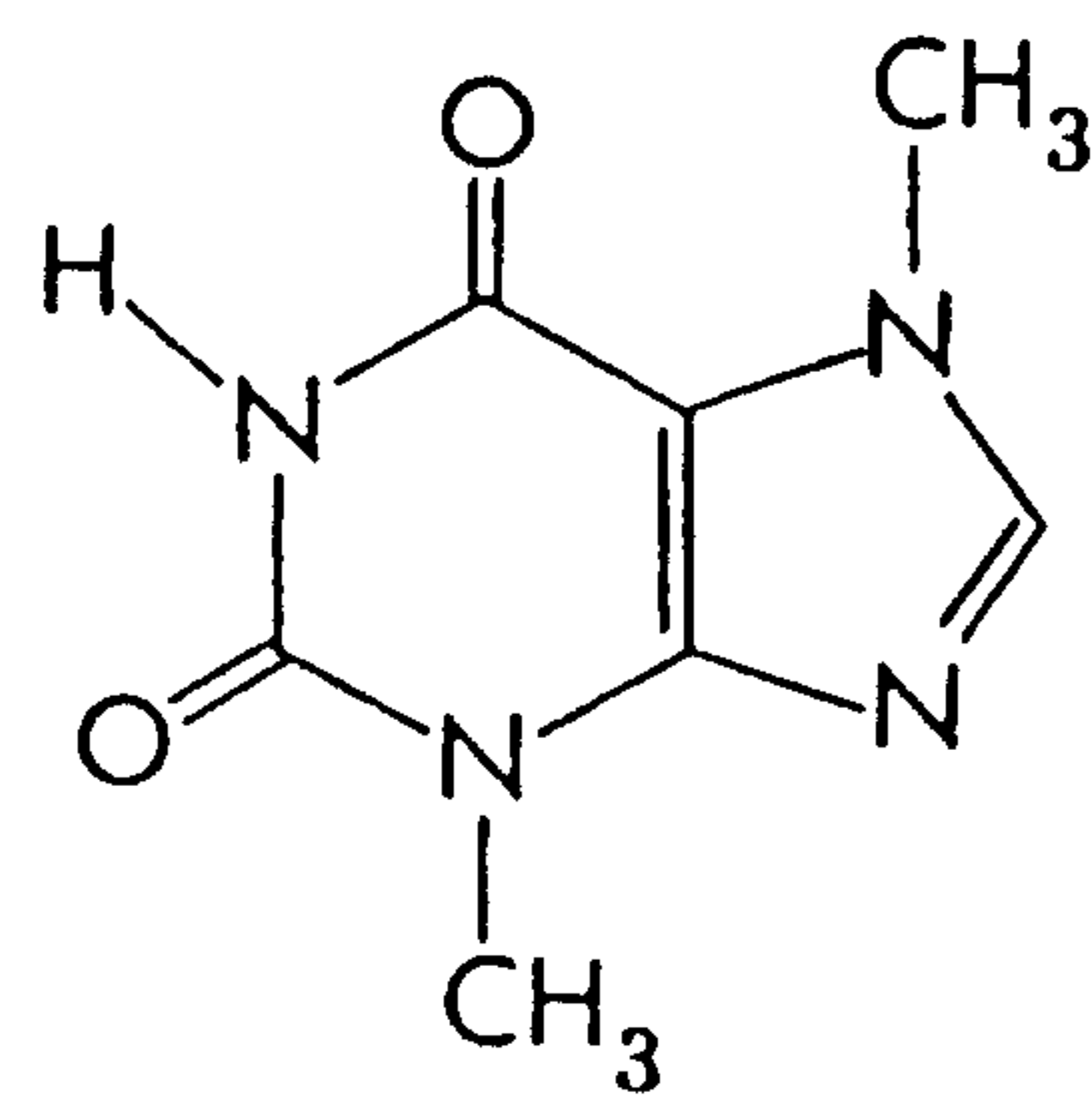
Xanthine



Caffeine



Theophylline



Theobromine

Fig. 3

4/12

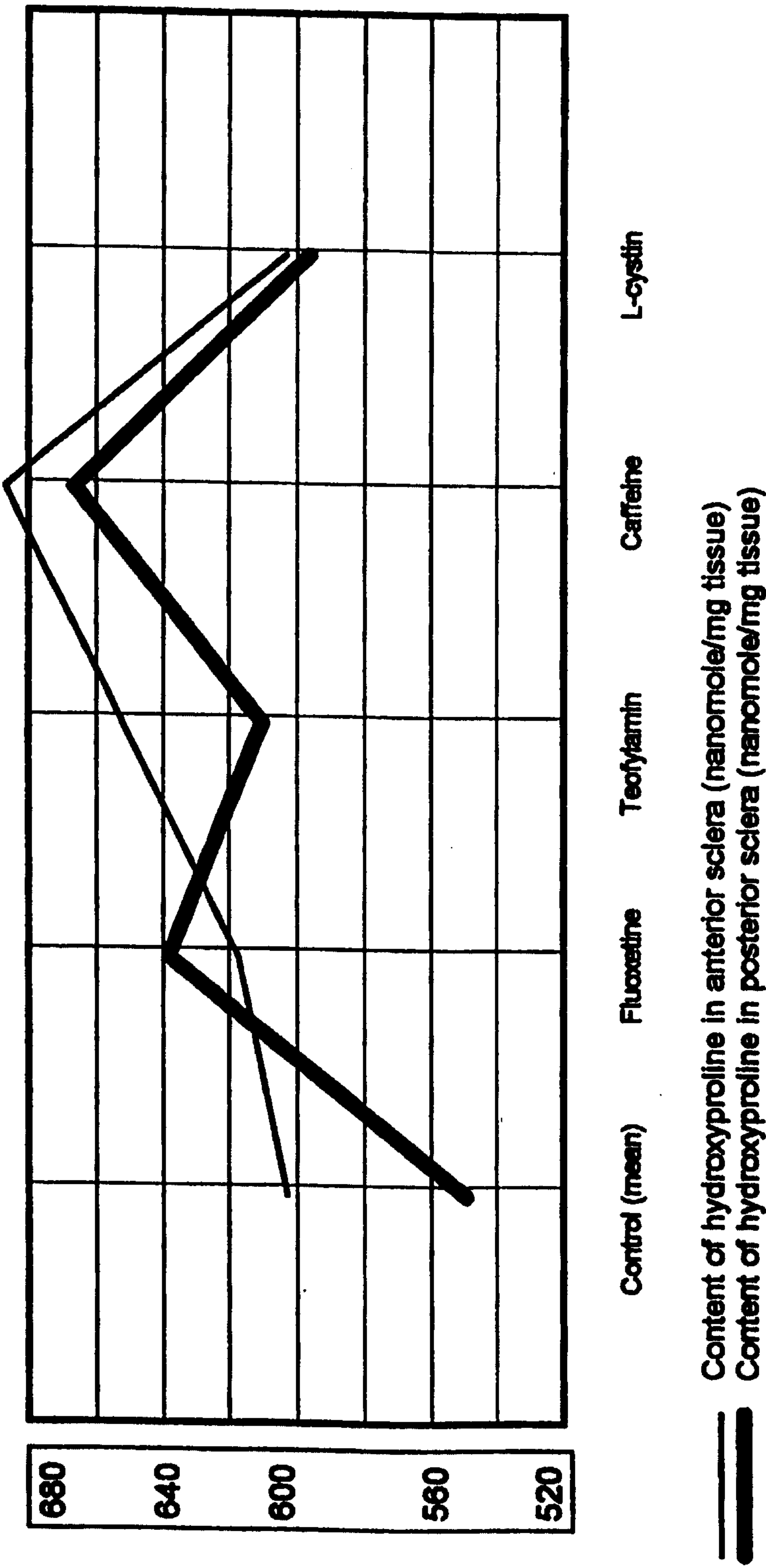


Fig. 4

5/12

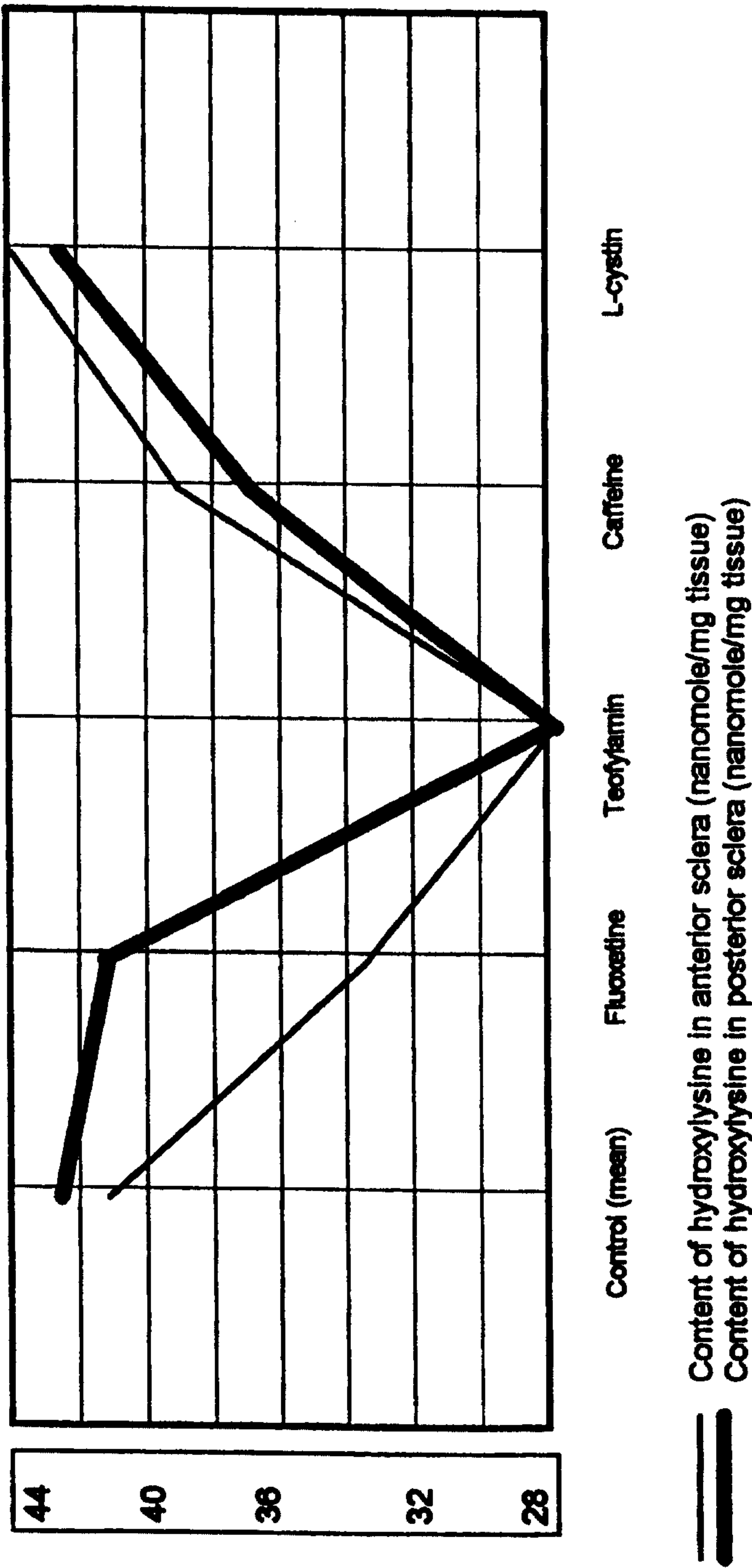


Fig. 5

6/12

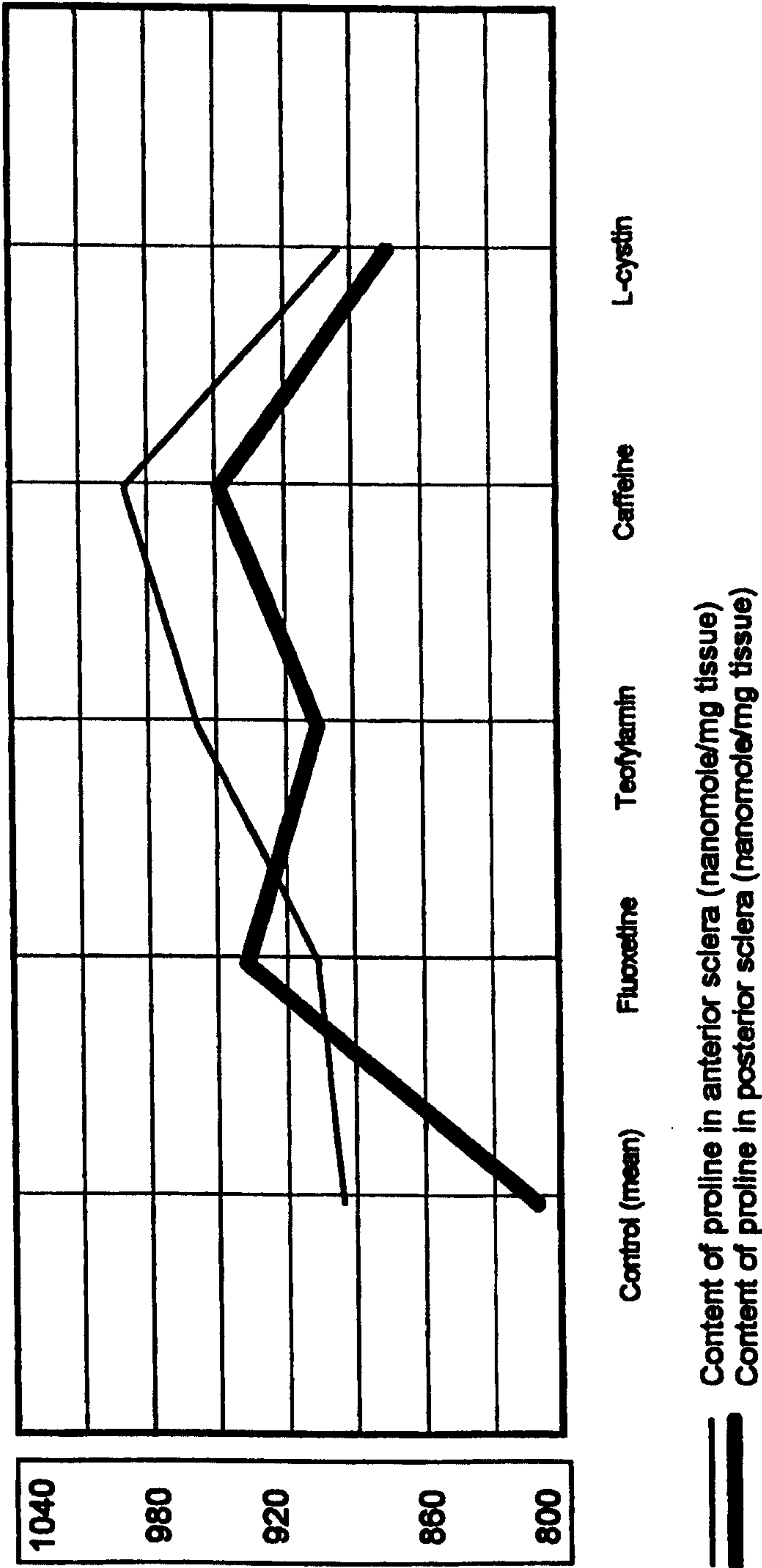


Fig. 6

7/12

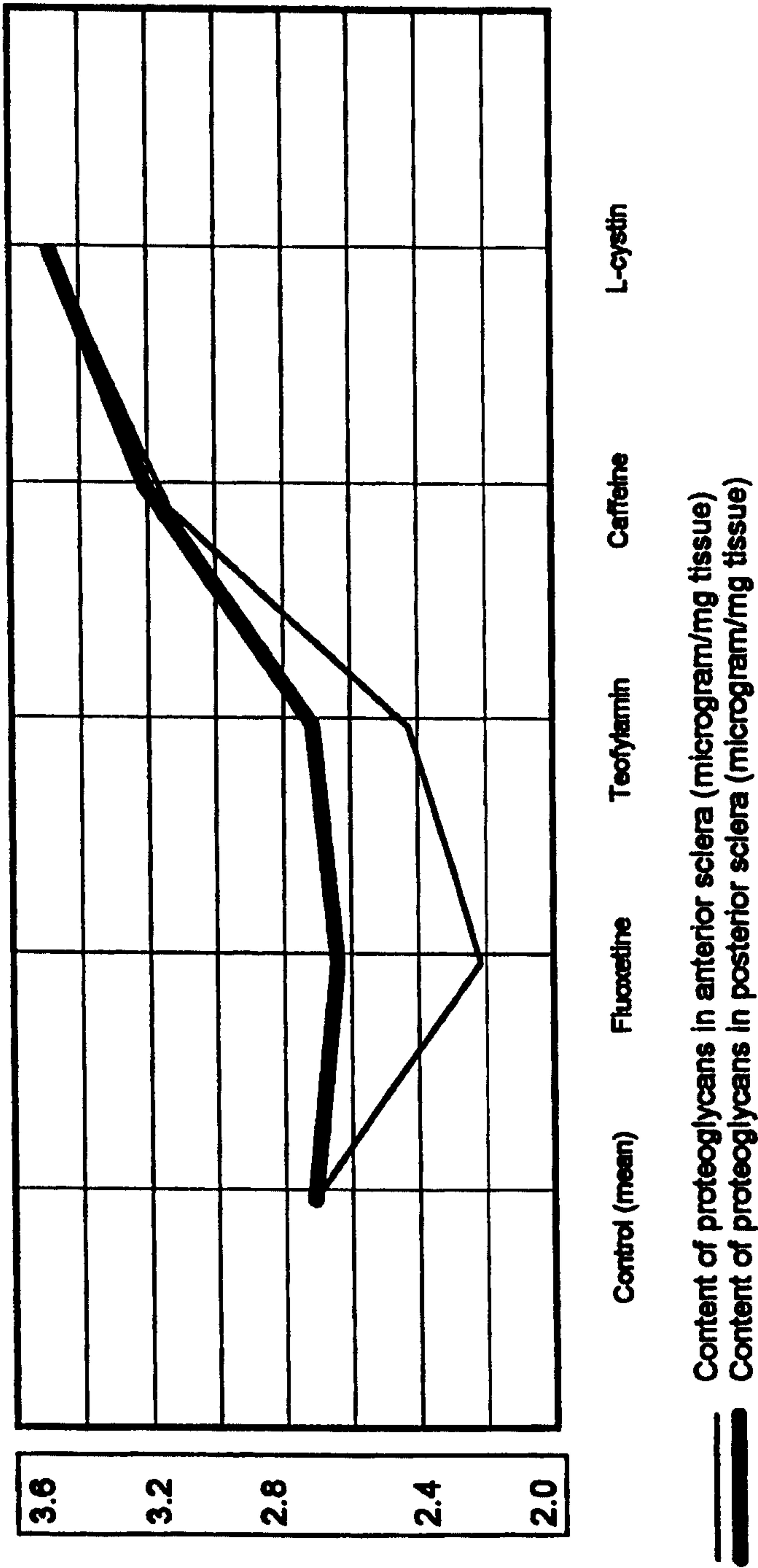


Fig. 7

8/12

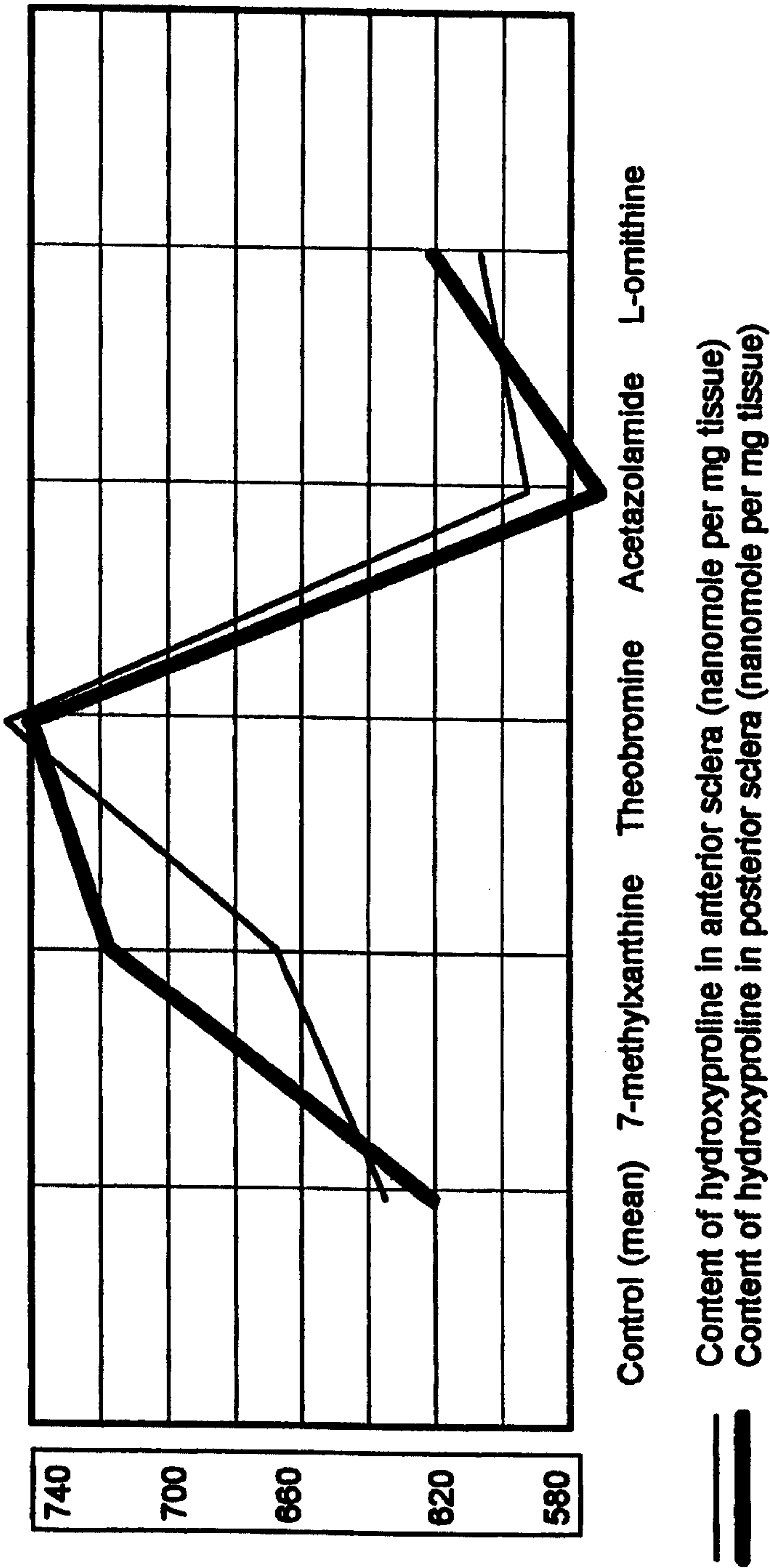


Fig. 8

9/12

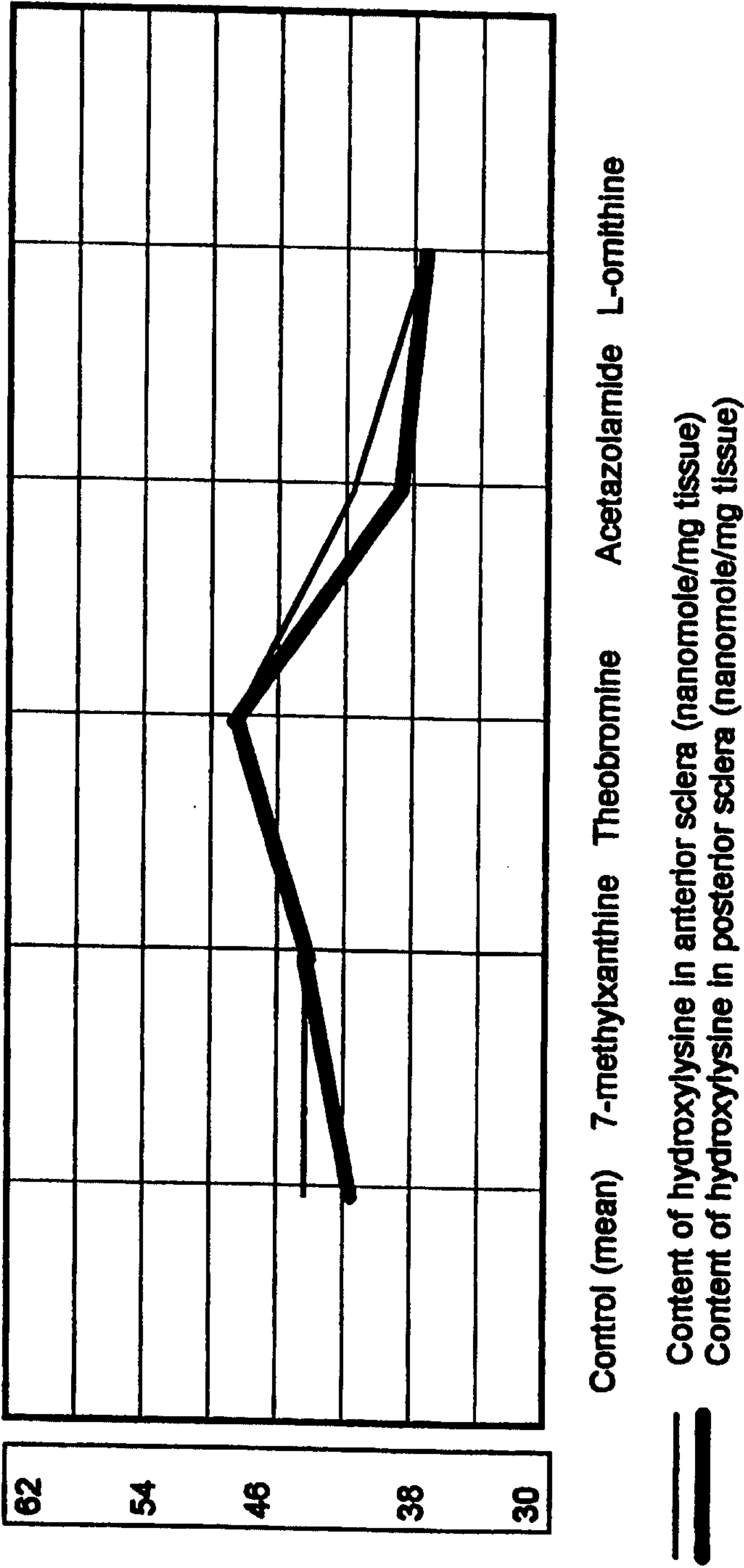


Fig. 9

10/12

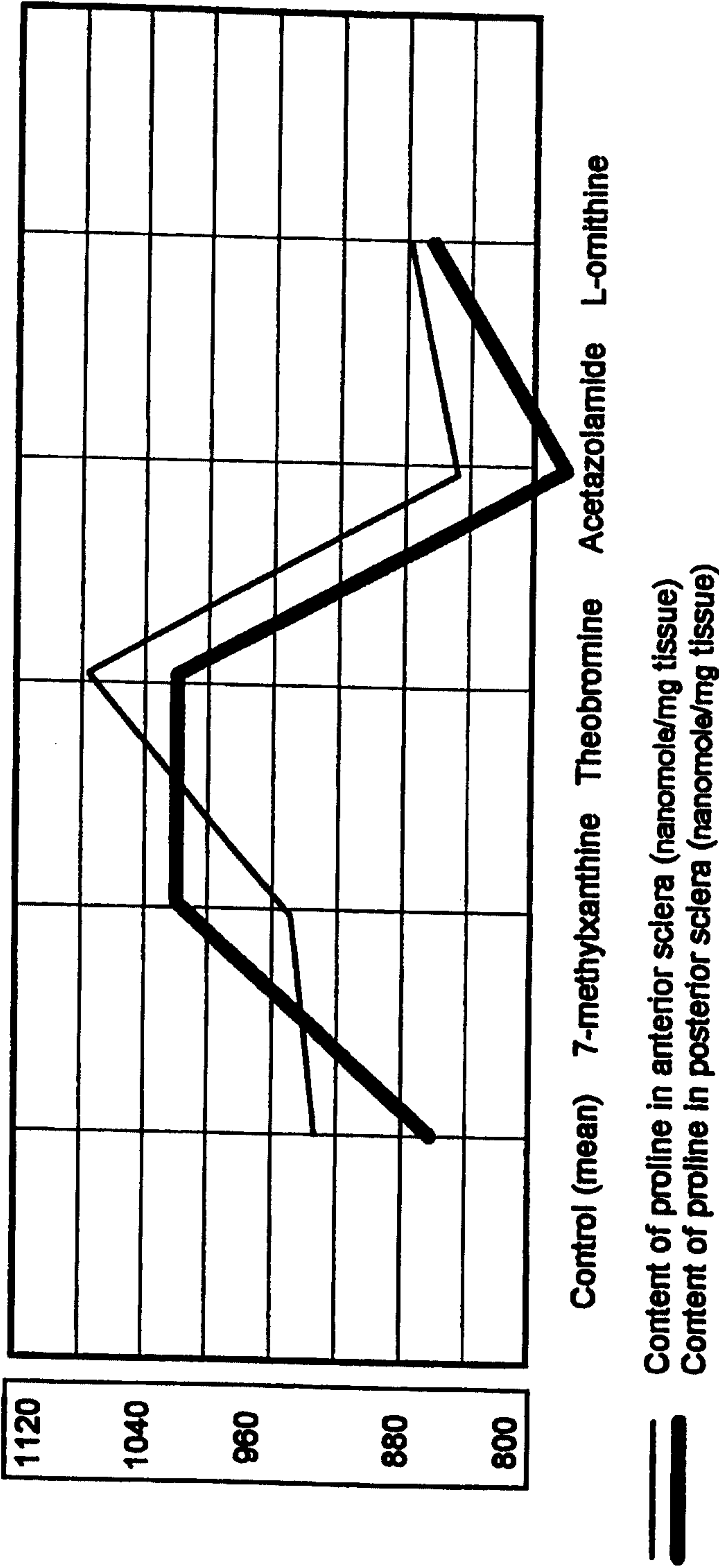


Fig. 10

11/12

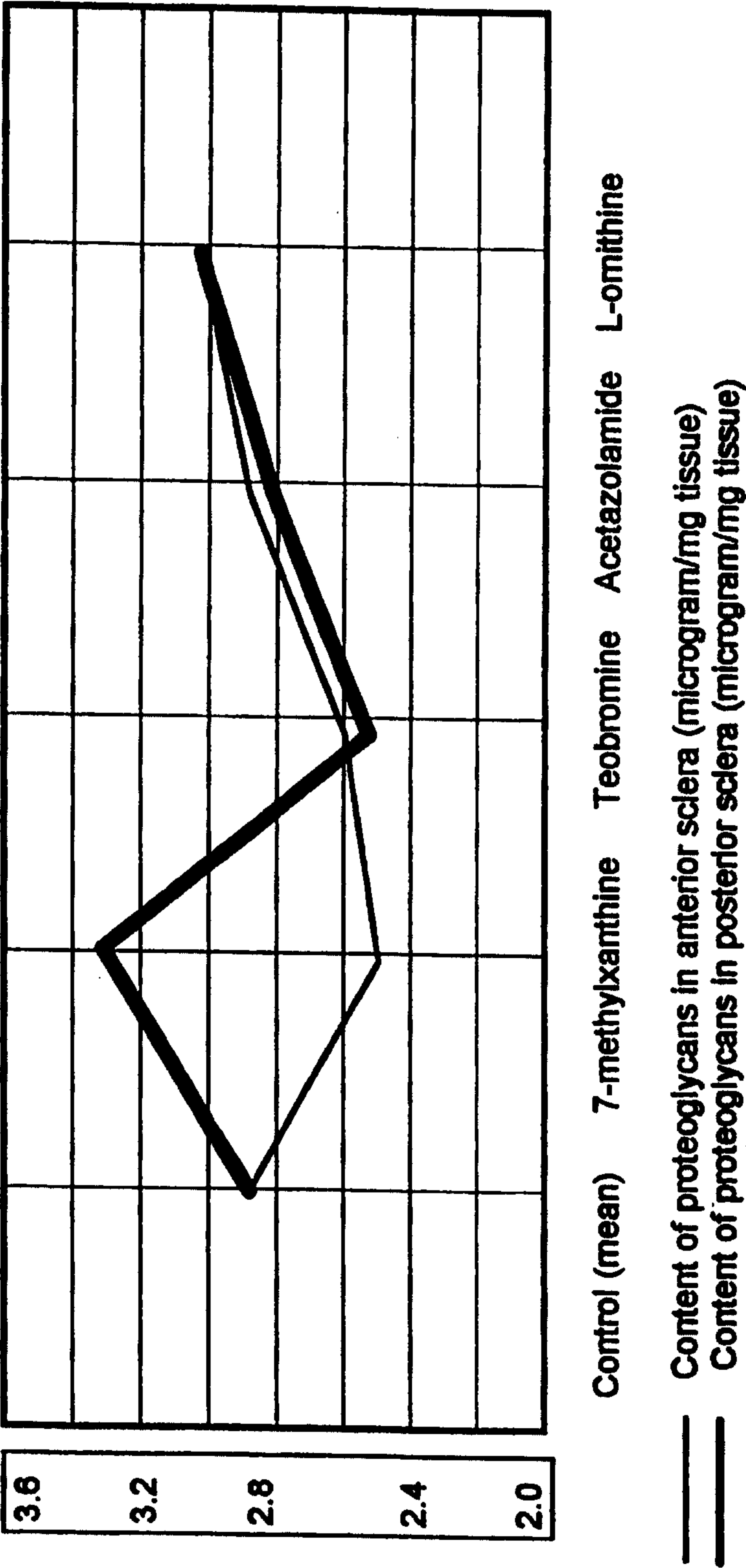
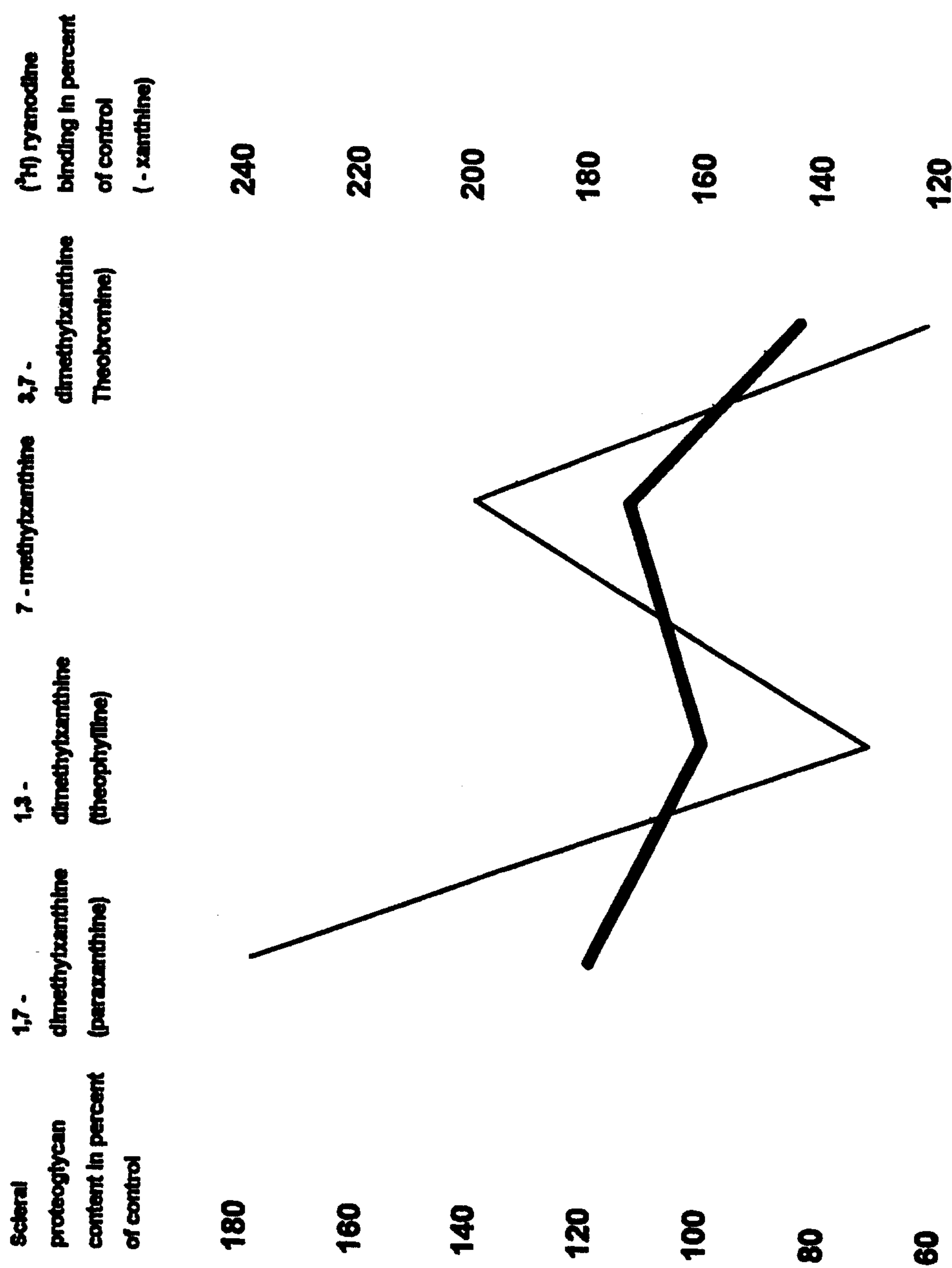


Fig. 11

12/12



shows complete correlation between effect of different methylxanthines on content of proteoglycans in posterior sclera (—) and effect on ³H ryanodine binding compared to that of - xanthine (—)

Caffeine is represented by 1,7 - dimethylxanthine, since this metabolite dominates in the serum of rabbits after feeding with caffeine.

A ³H ryanodine binding exceeding 130% of - xanthine is apparently needed to produce a positive effect on scleral content of proteoglycans

Fig. 12