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(54) Titre : AGENT DE TRAITEMENT D'AFFECTIONS DE LA VESSIE
(54) Title: AGENT FOR TREATMENT OF BLADDER TROUBLES

(57) Abrégé/Abstract:
In order to provide an agent for treatment of bladder troubles that has vesical mucosa epithelium dilatation promoting action and/or vesical mucosa healing action, and exhibits excellent curative effect for bladder troubles, in particular, non-bacterial intractable bladder troubles, the agent for treatment of bladder troubles is formulated with hyaluronic acid and/or a pharmaceutically acceptable salt thereof, preferably hyaluronic acid and/or a pharmaceutically acceptable salt thereof having the following physicochemical properties: (A) endotoxin content; 0.03 EU (endotoxin unit)/10 mg or less, (B) sulfur content; 0.01% or less as determined by coulometric titration, (C) iron content; 20 ppm or less, (D) protein content; 0.1% or less, and (E) weight average molecular weight; 500,000 or more, in an amount effective for promoting vesical mucosa epithelium dilatation and/or healing vesical mucosa, preferably in an amount of 0.2-0.6% by weight based on total amount of the agent.

Abstract

In order to provide an agent for treatment of bladder troubles that has vesical mucosa epithelium dilatation promoting action and/or vesical mucosa healing action, and exhibits excellent curative effect for bladder troubles, in particular, non-bacterial intractable bladder troubles, the agent for treatment of bladder troubles is formulated with hyaluronic acid and/or a pharmaceutically acceptable salt thereof, preferably hyaluronic acid and/or a pharmaceutically acceptable salt thereof having the following physicochemical properties:

(A) endotoxin content; 0.03 EU (endotoxin unit)/10 mg or less,

(B) sulfur content; 0.01% or less as determined by coulometric titration,

(C) iron content; 20 ppm or less,

(D) protein content; 0.1% or less, and

(E) weight average molecular weight; 500,000 or more,

in an amount effective for promoting vesical mucosa epithelium dilatation and/or healing vesical mucosa, preferably in an amount of 0.2-0.6% by weight based on total amount of the agent.

Specification

Agent for Treatment of Bladder Troubles

Technical Field

The present invention relates to an agent for treatment of bladder troubles. More precisely, it relates to an agent for treatment of bladder troubles having excellent curative effect for bladder troubles exerted through action for promoting vesical mucosa epithelium dilatation or action for healing vesical mucosa.

Background Art

Cystitis (bladder trouble) is caused by various kinds of causes. However, it is mostly caused by bacteria, and typical example of such cystitis is acute bacterial cystitis. Parsons, C. L. et al. have found that, when glycosaminoglycan (occasionally be abbreviated as GAG hereinafter) layer coating bladder is removed by acid treatment, bacterial adhesion to the bladder increases, and found that the amount of such adhesion of bacteria decreases when heparin, which is an exogenous GAG, is instilled into the bladder (J. Surg. Res. 25:324-329 (1978)). A later study has suggested

that exogenously administered heparin should coat migrating cells on the bladder surface, and act as a barrier for the bacteria and migrating cells (Infect. Immun. 24:552-557(1979)).

Non-bacterial intractable cystitis showing symptoms similar to those of acute bacterial cystitis but not responding to antibacterial agents has been known. Representative kinds of non-bacterial intractable cystitis are roughly classified into (1) interstitial cystitis, (2) eosinophilic cystitis, and (3) hemorrhagic cystitis. Interstitial cystitis is a disease frequently found in Europe and America, and it shows increased urinary frequency extending for ten months in middle-age or older females as main symptom and characterized by pain of suprapubic region when the bladder is filled. Interstitial cystitis causes decrease of bladder capacity, and petechia is observed in submucosal area when bladder wall expands, and there may be sometimes observed intravesical ulcer. As a pathological aspect, fibrillation of vesical mucosa is observed. As to the etiology of interstitial cystitis, there have been suggested collagen disease theory, autoimmune disease theory and the like, but it has not been fully elucidated. This disease is usually treated by instillation of dimethyl sulfoxide (DMSO), oxychlorosene sodium and the like. However, because effectiveness of these therapies can hardly be expected, vesical ectasia

is eventually performed as a surgical intervention in most cases. In addition, these therapies have drawbacks, for example, the instillation of DMSO may enhance intravesical irritation sensitivity, and makes exhalation have garlic odor, and instillation of oxychlorosene sodium requires anesthesia because it is accompanied with terrible pain.

While the etiology of interstitial cystitis has not been fully elucidated as described above, it has been suggested that its major cause might be aberration or defect of glycosaminoglycan coating the transitional epithelium of bladder (Eldrup J. 1983. British J. of Urology, 55:488). As one of analogues of glycosaminoglycan, sodium pentosanpolysulfate has been known, which has low molecular weight and low viscosity, and highly charged negatively. It has been suggested that interstitial cystitis may be treated by using the pentosanpolysulfate together with a steroid for oral administration or instillation into bladder, thereby preventing intravesical angiogenesis, leakage of cell membranes and leakage and exchange of blood capillaries (U. S. Patent Nos. 4,820,693 and 4,966,890). It has also been disclosed that the pentosanpolysulfate can inhibit adhesion of bacteria to transitional epithelium of bladder. Based on this, it has been proposed that interstitial cystitis may be treated by oral administration of the pentosanpolysulfate in a dose of

100 mg/day or more (U. S. Patent No. 5,180,715), and it is used as an agent for oral administration (trade name: Elmiron). This patent describes that interstitial cystitis or bacterial cystitis can be treated by administering pentosanpolysulfates to patents' bladders by irrigation, while it does not disclose specific experiments of such treatment.

Eosinophilic cystitis shows exactly the same symptoms as acute bacterial cystitis, and pyuria is a typical symptom of this disease. However, antibacterial agents are not effective to this disease, and the main component of pyuria is acidophils. This pathology is an allergic reaction to an agent having anti-allergic action such as tranilast. While eosinophilic cystitis is usually ameliorated by withdrawing administration of the causative agent, a steroid is effective when it is not readily ameliorated. When the steroid is not effective, bladder enucleation might be unavoidable.

Hemorrhagic cystitis is an affection accompanied by severe hematuria as the chief complaint, and as hemorrhagic cystitis there have been known, for example, radiation cystitis, which is observed in radiotherapy of uterine cancer, rectal cancer, bladder cancer and the like, and which makes vesical mucosa hemorrhagic labile, and is accompanied by hematuria; and drug cystitis, whose typical example is one causing uncontrollable cystorrhagia when treating malignancies with

chemotherapeutants such as cyclophosphamide.

For the treatment of radiation cystitis, intravesical electrocauterization or intravesical perfusion of aluminum potassium sulfate is effective for those in mild condition, whereas bladder enucleation or ectasia is used for severe cases. On the other hand, electrocauterization and intravesical perfusion of aluminum potassium sulfate are not effective for the drug cystitis, and intravesical instillation of formalin is used for its emergent cases. Bladders may shrink after the formalin instillation, and bladder ectasia is used for such cases.

As described above, any suitable therapies for non-bacterial intractable cystitis, in particular, for interstitial cystitis and hemorrhagic cystitis have not been developed yet. It has been known to use heparin, which is a GAG, or a pentosanpolysulfate, which is a synthesized analog of GAG, for the treatment of bacterial cystitis and interstitial cystitis. However, because the former has an average molecular weight of around 10,000, and the latter has a molecular weight of around 1,600-60,000 and they exhibit low viscosity, they can exhibit only poor activity for coating the highly viscous GAG layer in bladders. In addition, because they have a lot of sulfate groups in their molecules and hence exhibit strong anti-thrombogenic action, namely, hemorrhagic action, care must be taken for bleeding from

mucosa when they are used for the treatment of bacterial cystitis and interstitial cystitis, and they cannot be used for the treatment of hemorrhagic cystitis.

It has recently been reported that symptoms of interstitial cystitis were ameliorated by vesical instillation of aqueous solution of sodium hyaluronate (Proceedings of The American Urological Association, Vol.153, April 1995 supplement; WO96/25168). In this report, the symptom amelioration effect mentioned above was specifically confirmed only for the case where a solution formed by dissolving 40 mg of the hyaluronate in 50 ml of physiological saline (0.08% solution). However, according to the present inventors' supplementary examination, it was found that 0.08% hyaluronate solution could not prevent progress of fibrillation of bladders.

There has never been reported that hyaluronic acid and salts thereof have action for promoting vesical mucosa epithelium dilatation or action for healing vesical mucosa. There has also never been reported that a hyaluronic acid solution prevents fibrillation of bladder in non-bacterial intractable cystitis, or heals damaged vesical mucosa. Furthermore, effective concentration of the hyaluronic acid solution, molecular weight of hyaluronic acid, and other parameters for obtaining such actions as mentioned above have never been specifically examined.

Description of the Invention

The present invention has been completed under the circumstances described above, and in one broad aspect is directed to a medicament (also referred to in the specification of this application as an agent) for treatment of bladder troubles that has vesical mucosa epithelium dilatation promoting action and/or vesical mucosa healing action, and exhibits excellent curative effect for bladder troubles, in particular, non-bacterial intractable bladder troubles.

The present inventors conducted various studies, and as a result they found that, in the treatment of bladder troubles, the bladder troubles can be effectively treated by administering a solution of hyaluronic acid and/or a pharmaceutically acceptable salt thereof at a specific concentration directly into bladder, thereby promoting dilatation of vesical mucosa epithelium or healing vesical mucosa. The present invention has been completed on the basis of these findings.

Thus, in one embodiment, the present invention provides an agent for treatment of bladder troubles comprising a solution containing hyaluronic acid and/or a pharmaceutically acceptable salt thereof in an amount effective for promoting vesical mucosa epithelium dilatation and/or healing vesical mucosa.

The present invention also provides an agent for

treatment of bladder troubles comprising an aqueous solution containing 0.2-0.6% by weight of hyaluronic acid and/or a pharmaceutically acceptable salt thereof.

The present invention will be explained in detail hereinafter. Hyaluronic acid and/or pharmaceutically acceptable salts thereof contained in the solution constituting the agent for treatment of bladder troubles of the present invention will be explained first.

(1) Hyaluronic acid and/or pharmaceutically acceptable salts thereof

Hyaluronic acid and/or pharmaceutically acceptable salts thereof used for the agent for treatment of bladder troubles of the present invention are not particularly limited, so long as they can effectively treat bladder troubles when used as a solution of a certain concentration. As the hyaluronic acid and/or pharmaceutically acceptable salts thereof used for the agent for treatment of bladder troubles of the present invention, hyaluronic acid and/or a pharmaceutically acceptable salt thereof having action for promoting vesical mucosa epithelium dilatation and/or action for healing vesical mucosa are particularly preferred.

As the hyaluronic acid and/or pharmaceutically acceptable salts thereof used for the agent for treatment of bladder troubles of the present invention, hyaluronic acid and/or pharmaceutically acceptable salts thereof having the following physicochemical properties

can be specifically mentioned:

(A) endotoxin content; 0.03 EU (endotoxin unit)/10 mg or less,

(B) sulfur content; 0.01% or less as determined by coulometric titration,

(C) iron content; 20 ppm or less,

(D) protein content; 0.1% or less, and

(E) weight average molecular weight; 500,000 or more.

The properties (A) to (E) mentioned above are determined by methods commonly used by those skilled in the art for determining such physicochemical properties.

Specific examples of the hyaluronic acid and/or pharmaceutically acceptable salts thereof used for the agent for treatment of bladder troubles of the present invention include, for example, those having the aforementioned properties (A) to (D) and having a weight average molecular weight of around 500,000-4,000,000, preferably around 500,000-2,200,000, more preferably around 600,000-1,200,000, particularly preferably 800,000-1,200,000. If concentration and viscosity of the solution are selected adequately, hyaluronic acid and/or pharmaceutically acceptable salts thereof having a weight average molecular weight of around 1,500,000-2,200,000 or around 1,900,000-3,900,000 can also be used.

As the hyaluronic acid and/or pharmaceutically acceptable salts thereof used for the agent for

treatment of bladder troubles of the present invention, hyaluronic acid and/or pharmaceutically acceptable salts thereof having the aforementioned physicochemical properties (A) to (E) and having a limiting viscosity of 11.0-45 (dl/g) can also be mentioned.

As for the agent for treatment of bladder troubles of the present invention, if hyaluronic acid and/or pharmaceutically acceptable salts thereof having an endotoxin content; sulfated glycosaminoglycan content, iron content; protein content; weight average molecular weight; and/or limiting viscosity outside the above-defined ranges are used, it may cause exacerbation of inflammation and hemorrhage, allergic reactions and the like, or the desired effect may not be obtained.

As the pharmaceutically acceptable salts of hyaluronic acid used for the agent for treatment of bladder troubles of the present invention, pharmaceutically acceptable water-soluble metal salts of hyaluronic acid such as sodium hyaluronate, potassium hyaluronate, and calcium hyaluronate are usually used. Among these, sodium hyaluronate, which is used mainly for medical purposes including medicaments, medical devices and the like, is preferred. Origin of hyaluronic acid and/or pharmaceutically acceptable salts thereof, and production method therefor are not particularly limited. For example, they can be produced from chicken crest, animal umbilical cord, skin,

vitreous body or the like by extraction using any optional combination of heat treatment, organic solvent treatment, mincing, protease treatment and the like, and purification using any optional combination of salting out with ammonium sulfate, precipitation with organic solvents such as ethanol, fractional precipitation with quaternary ammonium salts, deproteination, absorption of impurities with absorbents (Celite*, activated carbon etc.), ultrafiltration, membrane filtration and the like (see, Japanese Published Examined Patent Application Nos. 61-8083, 61-60081, 61-21241, 6-8323, U. S. Patent Nos. 4,141,973, 5,449,104 etc.). They can also be produced by fermentation technique using microorganisms such as hemolytic streptococcus (genus Streptococcus) (see, U. S. Patent Nos. 4,946,780, 4,780,414 etc.).

Some of such hyaluronic acid and/or pharmaceutically acceptable salts thereof are commercially available, and hence those made as preparations for medical use such as for medicaments, whose concentration of hyaluronic acid and/or pharmaceutically acceptable salts thereof and the like are adjusted depending on the purpose, can be selected from them and used for the agent for treatment of bladder troubles of the present invention. Preferred examples of such commercially available hyaluronic acid and/or pharmaceutically acceptable salts thereof include, as sodium hyaluronate satisfying the above

*Trade-mark

properties (A) to (D), ARTZ* (weight average molecular weight; 600,000-1,200,000), OPEGAN* (weight average molecular weight; 600,000-1,200,000), and OPEGAN Hi (weight average molecular weight; 1,900,000-3,900,000) produced by SEIKAGAKU CORPORATION, Hyalein* (weight average molecular weight; 600,000-1,200,000) produced by Santen Pharmaceutical Co., Ltd., OPELEAD* (weight average molecular weight; 1,530,000-2,130,000) produced by Shiseido Co., Ltd., Healon* (weight average molecular weight; 1,900,000-3,900,000) produced by Kabi Pharmacia Inc. and the like.

(2) Agent for treatment of bladder troubles of the present invention

The agent for treatment of bladder troubles of the present invention comprises a solution containing hyaluronic acid and/or a pharmaceutically acceptable salt thereof in an amount effective for promoting vesical mucosa epithelium dilatation and/or healing vesical mucosa. The term amount effective for promoting vesical mucosa epithelium dilatation and/or healing vesical mucosa of hyaluronic acid and/or a pharmaceutically acceptable salt thereof contained in the solution constituting the agent for treatment of bladder troubles of the present invention means an amount sufficient for the solution to act on vesical mucosa epithelium and promote its dilatation, and/or act

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on vesical mucosa and heal it or reproduce it as an agent for treatment of bladder troubles. Specifically, while it depends on the kinds of hyaluronic acid and/or pharmaceutically acceptable salts thereof contained in the solution, the concentration of hyaluronic acid and/or pharmaceutically acceptable salts thereof in the solution is, for example, around 0.1-0.8% by weight, preferably around 0.2-0.6% by weight, more preferably around 0.3-0.45% by weight, even more preferably around 0.35-0.45% by weight, and particularly preferably about 0.4% by weight.

As for the agent for treatment of bladder troubles of the present invention, when the content of hyaluronic acid and/or a pharmaceutically acceptable salt thereof in the solution is less than 0.2%, the agent may not be effectively used as the agent for treatment of bladder troubles of the present invention, because, for example, the action for promoting vesical mucosa epithelium dilatation becomes insufficient. When the content exceeds 0.6% by weight, difficulty of urination may be caused because of high viscosity.

Upon administration of the agent at an effective dose within the range defined above, vesical mucosa epithelium dilatation is promoted and the mucosa is healed and reproduced at damaged or excoriated regions of vesical mucosa epithelium caused by bladder troubles.

In non-bacterial intractable bladder troubles, patients suffer from pain when bladder is filled because

nerve is usually denuded due to excoriation of vesical mucosa epithelium, and patients suffer from increased urinary frequency because of decreased bladder capacity caused by fibrillation of bladders. The agent for treatment of bladder troubles of the present invention can indirectly ameliorate such pain and prevent progress of vesical fibrillation through the aforementioned actions.

The solution containing hyaluronic acid and/or a pharmaceutically acceptable salt thereof that constitutes the agent for treatment of bladder troubles of the present invention preferably have a pH value of about 6-8, and a relative osmotic pressure to body fluid is around 0.9-1.2 (substantially isotonic as to body fluid or physiological saline).

By adjusting the pH of the solution in the agent for treatment of bladder troubles of the present invention to the aforementioned range of around 6-8, its acrimony for vesical mucosa made sensitive can be reduced. When the pH is less than 6, acrimony for vesical mucosa may become too strong. When the pH is more than 8, acrimony for vesical mucosa may become too strong, healing and reproduction of vesical mucosa may be retarded, and the preventive effect for the vesical fibrillation may be invalidated.

By adjusting the relative osmotic pressure of the solution in the agent for treatment of bladder troubles

of the present invention to the aforementioned range of around 0.9-1.2, its acrimony for vesical mucosa can be reduced as in the adjustment of pH. When the relative osmotic pressure is less than 0.9, difficulty of urination may be caused by increased viscosity. When the relative osmotic pressure is more than 1.2, acrimony for vesical mucosa may become too strong.

For the agent for treatment of bladder troubles of the present invention comprising a solution containing hyaluronic acid and/or a pharmaceutically acceptable salt thereof, the solution containing hyaluronic acid and/or a pharmaceutically acceptable salt thereof is preferably an aqueous solution having an apparent viscosity of 10-1500 mPa·s as determined at a shear rate of 9.6 sec^{-1} at 20°C.

By adjusting the apparent viscosity of the aqueous solution constituting the agent for treatment of bladder troubles of the present invention to around 10-1500 mPa·s, it becomes possible to obtain appropriate degree of the effect for healing vesical mucosa without causing difficulty of urination. When the apparent viscosity of the aqueous solution is less than 10 mPa·s, the effect for healing vesical mucosa may become insufficient. When the apparent viscosity of the aqueous solution exceeds 1500 mPa·s, difficulty of urination may be caused.

The present inventors found that administration of

5% acetic acid to bladders of experimental animals excoriates vesical mucosa epithelium and irreversibly promotes fibrillation of the bladders, and as a result, the bladder capacity is lowered, and that the symptoms of such animals as treated as mentioned above are quite similar to those of non-infectious intractable bladder troubles of humans. Moreover, they also found that, using the aforementioned animal models, medicaments effective for treatment of non-infectious intractable bladder troubles can be screened by evaluating the following parameters: (a) bladder capacity, (b) bladder dry weight or amount of intravesical hydroxyproline (amount of collagen), and (c) amount of intravesical trypan blue adhesion or area of trypan blue adhesion to the excoriation region of vesical mucosa, which reflect (A) degree of vesical expansion, (B) degree of fibrillation of bladder proper muscle coat, and (C) degree of dilatation of vesical mucosa epithelium and/or degree of healing of vesical mucosa, respectively.

The term "5% acetic acid" herein used refers to a solution containing acetic acid at a concentration of 5%.

Specific examples of the solution containing hyaluronic acid and/or a pharmaceutically acceptable salt thereof, which constitutes the agent for treatment of bladder troubles of the present invention, include such a solution prepared so that bladder capacity of a

rabbit which has been subjected to vesical mucosa excoriation treatment by introducing 5% acetic acid into bladder and retaining it for 10 minutes, and then a treatment by introducing the solution to the bladder every day for seven days after the excoriation of the vesical mucosa should be 2 to 3 times as large as bladder capacity of a rabbit which has been subjected to the same vesical mucosa excoriation treatment with 5% acetic acid as mentioned above and a treatment by introducing phosphate buffered physiological saline instead of the solution in the same manner; such a solution prepared so that bladder dry weight of a rabbit which has been subjected to the same vesical mucosa excoriation treatment with 5% acetic acid as mentioned above and then the same treatment by introducing the solution as mentioned above should be 0.6 to 0.9 times as large as bladder dry weight of a rabbit which has been subjected to the same vesical mucosa excoriation treatment with 5% acetic acid as mentioned above and the same treatment by introducing phosphate buffered physiological saline instead of the solution as mentioned above; and the like.

Specific examples of the solution containing hyaluronic acid and/or a pharmaceutically acceptable salt thereof, which constitutes the agent for treatment of bladder troubles of the present invention, further include such a solution prepared so that amount of

intravesical trypan blue adhesion of a rabbit which has been subjected to vesical mucosa excoriation treatment by introducing 5% acetic acid to bladder and retaining it for 10 minutes, then a treatment by introducing the solution to the bladder every day for seven days after the excoriation of the vesical mucosa, and then a treatment by introducing 0.5% aqueous trypan blue solution to the bladder to obtain trypan blue adhesion should be 0.5 to 0.7 times as large as amount of intravesical trypan blue adhesion of a rabbit which has been subjected to the same vesical mucosa excoriation treatment with 5% acetic acid as mentioned above, then a treatment by introducing phosphate buffered saline instead of the solution in the same manner, and then the same trypan blue adhesion treatment as mentioned above; such a solution prepared so that trypan blue adhesion area in vesical mucosa excoriation region of a rabbit which has been subjected to the same vesical mucosa excoriation treatment with 5% acetic acid as mentioned above, then the same treatment with the solution as mentioned above, and then the same trypan blue adhesion treatment as mentioned above should be 0.25 to 0.45 times as large as trypan blue adhesion area in vesical mucosa excoriation region of a rabbit which has been subjected to the same vesical mucosa excoriation treatment with 5% acetic acid as mentioned above, then a treatment by introducing phosphate buffered saline

instead of the solution in the same manner, and then the same trypan blue adhesion treatment as mentioned above; and the like.

Specific examples of the solution containing hyaluronic acid and/or a pharmaceutically acceptable salt thereof further include such a solution prepared so that amount of intravesical hydroxyproline of a rabbit which has been subjected to the same vesical mucosa excoriation treatment with 5% acetic acid as mentioned above, and then the same treatment with the solution as mentioned above should be 0.8 to 0.9 times as large as amount of intravesical hydroxyproline of a rabbit which has been subjected to the same vesical mucosa excoriation treatment with 5% acetic acid as mentioned above, and then a treatment by introducing phosphate buffered saline instead of the solution in the same manner; and the like.

The solution containing hyaluronic acid and/or a pharmaceutically acceptable salt thereof, which constitutes the agent for treatment of bladder troubles of the present invention, such as those specifically mentioned above, is a solution selected based on evaluation of the action for promoting vesical mucosa epithelium dilatation and/or action for healing vesical mucosa of the solution in bladders of rabbits whose vesical mucosa has been excoriated by introducing 5% acetic acid into their bladders and retaining it for 10

minutes as a model, more specifically, selected based on the following indexes, action for increasing bladder capacity, action for decreasing bladder dry weight, action for decreasing amount of intravesical trypan blue adhesion or trypan blue adhesion area at vesical mucosa excoriated region, action for decreasing amount of intravesical hydroxyproline and the like of the solution, which indexes are determined by comparing results obtained in the model when the solution is used and result obtained when phosphate buffered saline is used in the same manner instead of the solution. These indexes will be explained hereinafter.

The rabbit model whose vesical mucosa is excoriated, referred to as rabbit vesical mucosa trouble model hereinafter, will be explained first. It has been known that acetic acid has mucosa acrimony, and gastric mucosa trouble models prepared by using acetic acid have widely been used for tests of medicaments and the like. Therefore, in the present invention, a rabbit vesical mucosa trouble model was created in which vesical mucosa is excoriated by applying acetic acid to the vesical mucosa as described above, and factors representing the action for promoting vesical mucosa epithelium dilatation and/or the action for healing vesical mucosa measured in this model were used as indexes for selecting solutions constituting the agent for treatment of bladder troubles of the present invention as

described above.

The rabbit vesical mucosa trouble model is prepared, for example, as follows. A bladder catheter is inserted into bladder of rabbit under general anesthesia obtained by a usual method, and then residual urine in the bladder is drained using an infusion pipe of a suitable size. After the drainage of residual urine, the inside of the bladder is washed with sufficient amount of physiological saline. Then, a solution comprising acetic acid diluted with distilled water to a concentration of 5% is introduced into the bladder in an amount sufficiently filling the bladder, and retained for 10 minutes so that vesical mucosa should be excoriated. Subsequently, the acetic acid is drained, and intravesical irrigation is performed with a sufficient amount of physiological saline.

In the present invention, while the rabbit vesical mucosa trouble model treated with 5% acetic acid for ten minutes is used as the standard, any similar models may be used for evaluation tests of various drug substances. In such cases, acetic acid concentration of the acetic acid solution used for excoriation of vesical mucosa is not limited to 5%, and retention time of the acetic acid solution in bladder is not limited to ten minutes. These factors may be appropriately modified as required according to the circumferential conditions. Though kind, age represented by week-basis, and sexuality of

rabbits used for the model production are not particularly limited, preferred example of the rabbits include JW male rabbits of 12-14 week old and the like. Animals other than rabbit can also be used.

The factors for evaluating action for promoting vesical mucosa epithelium dilatation and/or action for healing vesical mucosa upon applying the pharmaceutical solution to bladder of the aforementioned vesical mucosa trouble model will be explained hereinafter.

If the bladder treated with acetic acid as described above is not given any appropriate therapeutic treatment, severe inflammation should be induced after the excoriation of vesical mucosa epithelium, and fibrillation of vesical proper muscle coat should be caused after a few days, which makes bladder expansion very difficult. In contrast, if any effective curative treatment is given, dilatation of vesical mucosa epithelium should be promoted, and fibrillation of vesical proper muscle coat should be prevented because of healing of vesical mucosa, thereby the expansion function of bladder should be improved.

Therefore, degree of expansion function of bladder can be evaluated by measuring bladder capacity. In other words, the larger the bladder capacity of the aforementioned rabbit vesical mucosa trouble model after the bladder of the model is applied with a solution of various agents, the more the bladder function is

recovered. Further, degree of fibrillation of vesical proper muscle coat can be evaluated by measuring bladder dry weight and intravesical amount of hydroxyproline (collagen). That is, the larger the bladder dry weight or the smaller the intravesical amount of hydroxyproline of the aforementioned rabbit vesical mucosa trouble model after the bladder of the model is applied with a solution of various agents, the stronger the ability of preventing fibrillation of vesical proper muscle coat of the solution.

Furthermore, because it has been known that trypan blue, an acidic living tissue staining agent, can stain damaged mucosa well, degree of dilatation of vesical mucosa epithelium or degree of healing of vesical mucosa can be evaluated by measuring amount of intravesical trypan blue adhesion or trypan blue adhesion area at excoriation region of vesical mucosa. That is, the smaller the amount of intravesical trypan blue adhesion, or the trypan blue adhesion area at excoriation region of vesical mucosa after the bladder of the model is applied with a solution of various agents, the more the solution can promote dilatation of vesical mucosa epithelium or healing of vesical mucosa.

In the present invention, results obtained by daily irrigation of a solution containing hyaluronic acid and/or a pharmaceutically acceptable salt thereof for 7 days to a bladder of the aforementioned rabbit vesical

mucosa trouble model and results obtained by daily irrigation of phosphate buffered saline (PBS) to the same for 7 days are compared, and solutions of hyaluronic acid and/or a pharmaceutically acceptable salt thereof resulting a bladder capacity within 2 to 3 times of that obtained with PBS, bladder dry weight within 0.6-0.9 times of that obtained with PBS, amount of intravesical trypan blue adhesion within 0.5-0.7 times of that obtained with PBS, trypan blue adhesion area at vesical mucosa excoriation region within 0.25-0.45 times of that obtained with PBS, and/or amount of intravesical hydroxyproline within 0.8-0.9 times of that obtained with PBS are selected as a preferred solution for the agent for treatment of bladder troubles of the present invention.

Those solutions of hyaluronic acid and/or a pharmaceutically acceptable salt thereof not resulting, when those solutions of hyaluronic acid and/or a pharmaceutically acceptable salt thereof are administered to bladder of the aforementioned vesical mucosa trouble model, a bladder capacity within 2 to 3 times of that obtained with PBS, bladder dry weight within 0.6-0.9 times of that obtained with PBS, amount of intravesical trypan blue adhesion within 0.5-0.7 times of that obtained with PBS, trypan blue adhesion area at vesical mucosa excoriation region within 0.25-0.45 times of that obtained with PBS, and/or amount of

intravesical hydroxyproline within 0.8-0.9 times of that obtained with PBS may not be effectively used as the agent for treatment of bladder troubles of the present invention. Therefore, solutions resulting the values of the evaluation factors within the ranges defined above are preferred for the agent for treatment of bladder troubles of the present invention.

The agent for treatment of bladder troubles of the present invention can be produced by dissolving hyaluronic acid and/or a pharmaceutically acceptable salt thereof in a suitable solvent so that a content within the aforementioned range can be obtained. Examples of the solvent include water, buffers, physiological saline, water containing a water-soluble organic solvent such as dimethyl sulfoxide and the like. In the present invention, water or physiological saline is preferably used. The agent for treatment of bladder troubles of the present invention may also be provided as powder of hyaluronic acid and/or a pharmaceutically acceptable salt thereof or the like, so that it can be prepared upon use into a solution having a concentration, apparent viscosity, pH, relative osmotic pressure and the like within the ranges defined above.

The agent for treatment of bladder troubles of the present invention may contain, in addition to the above-explained hyaluronic acid and/or a pharmaceutically acceptable salt thereof and a solvent, optional

ingredients as required. Examples of the optional ingredients include, for example, pharmaceutically acceptable known antiinflammatory agents, analgesics, vitamins, antibacterial agents, growth factors, adhesion factors, buffers, stabilizers, inorganic salts and the like. Because the concentration of hyaluronic acid and/or a salt thereof in the solution is relatively low (e.g., 0.1-0.8% by weight, preferably 0.2-0.6% by weight), the hyaluronic acid and the like may be degraded into lower molecular weight compounds when they are subjected to heat sterilization or stored for a long term. In such a case, it is necessary to use a means for preventing the degradation of hyaluronic acid and the like into lower molecular weight compounds, which may be a known one. Examples of such means include, for example, lowering metal ion (e.g., iron ions) content in hyaluronic acid and or salts thereof to be used to 20 ppm or less, preferably 10 ppm or less (see, U. S. Patent No. 5,559,104), adding known stabilizers capable of preventing the degradation of hyaluronic acid and the like such as citric acid, citric acid salts, and glycerin (see, Japanese Patent Application Laid-open Nos. 8-104642 and 9-176020) and the like.

The agent for treatment of bladder troubles of the present invention is an agent to be directly administered to bladders, and it can be administered by a method usually used for medicaments usually

administered directly to bladders. For example, administration method using a intravesical catheter may be used.

As for doses of the agent for treatment of bladder troubles of the present invention, for example, when a phosphate buffered physiological saline containing 0.2-0.6% by weigh of sodium hyaluronate having an endotoxin content of 0.03 EU (endotoxin unit)/10 mg or less, sulfur content of 0.01% or less as determined by coulometric titration, iron content of 20 ppm or less, protein content of 0.1% or less, and weight average molecular weight of 600,000-1,200,000 is used as the agent for treatment of bladder troubles, it may be administered once to seven times a week at a dose of 50 ml per single administration.

The agent for treatment of bladder troubles of the present invention can be widely used for bladder troubles in generic sense so long as the troubles are those whose symptoms can be improved by dilatation of vesical mucosa epithelium and/or healing of vesical mucosa. It is particularly effective for treatment of non-bacterial intractable bladder troubles, for example, hemorrhagic cystitis such as radiation cystitis caused by radiotherapy of uterine cancer, rectal cancer, cystocarcinoma, prostate cancer and the like and drug cystitis caused by chemotherapeutants for treatment of malignancy, e.g., cyclophosphamide, mitomycin, platinum

chelates such as cisplatin, methotrexate, bleomycin hydrochloride, and bleomycin sulfate, stimulation therapy agents for treatment of rheumatism such as auranofin; interstitial cystitis; eosinophilic cystitis; neurogenic increased urinary frequency, and the like.

Because the agent for treatment of bladder troubles of the present invention has action for promoting vesical mucosa epithelium dilatation, and heals vesical mucosa damaged by cystitis, thereby exhibiting excellent action for inhibiting fibrillation of bladder, bladder troubles can be effectively treated by the agent. Further, because the agent for treatment of bladder troubles of the present invention does not show anti-thrombogenic activity, it can be used without causing adverse drug reactions such as becoming easy to bleed.

As for safety of hyaluronic acid (drug toxicity, and noninflammability), many experiments have been already performed, and the safety has been confirmed.

For practical use, storage, transportation or the like, the agent for treatment of bladder troubles may be put in a commercial package which comprises a container containing therein the agent and a written matter describing indications of the agent and how to use it.

Brief Description of the Drawings

Fig. 1 is a graph representing bladder capacity in 5% acetic acid-induced rabbit cystitis models for control and groups administered with agents for treatment of bladder troubles.

Fig. 2 is a representative photograph of dissected bladder showing trypan blue adhesion to vesical mucosa

in a 5% acetic acid-induced rabbit cystitis model for control.

Fig. 3 is a representative photograph of dissected bladder showing trypan blue adhesion to vesical mucosa in a 5% acetic acid-induced rabbit cystitis model for a group administered with agent for treatment of bladder troubles 1.

Fig. 4 is a representative photograph of dissected bladder showing trypan blue adhesion to vesical mucosa in a 5% acetic acid-induced rabbit cystitis model for a group administered with agent for treatment of bladder troubles 2.

Fig. 5 is a representative photograph of dissected bladder showing trypan blue adhesion to vesical mucosa in a 5% acetic acid-induced rabbit cystitis model for a group administered with agent for treatment of bladder troubles 3.

Fig. 6 is a graph representing ratios of damaged vesical area (trypan blue adhered area/total intravesical area) in 5% acetic acid-induced rabbit cystitis models for control and groups administered with agents for treatment of bladder troubles.

Fig. 7 is a graph representing bladder dry weight in 5% acetic acid-induced rabbit cystitis models for control and groups administered with agents for treatment of bladder troubles.

Fig. 8 is a graph representing amount of trypan

blue adhesion to damaged vesical area in 5% acetic acid-induced rabbit cystitis models for control and groups administered with agents for treatment of bladder troubles.

Fig. 9 is a graph representing amount of intravesical hydroxyproline (collagen) in 5% acetic acid-induced rabbit cystitis models for control and groups administered with agents for treatment of bladder troubles.

Fig. 10 shows photographs of stained bladder tissue of 5% acetic acid-induced rabbit cystitis model for control. (a) is a photograph of hematoxylin-eosin stained tissue in 10 magnifications, and (b) is a photograph of Azan stained tissue in 10 magnifications.

Fig. 11 shows photographs of stained bladder tissue of 5% acetic acid-induced rabbit cystitis model for a group administered with agent for treatment of bladder troubles 1. (a) is a photograph of hematoxylin-eosin stained tissue in 10 magnifications, and (b) is a photograph of Azan stained tissue in 10 magnifications.

Fig. 12 shows photographs of stained bladder tissue of 5% acetic acid-induced rabbit cystitis model for a group administered with agent for treatment of bladder troubles 2. (a) is a photograph of hematoxylin-eosin stained tissue in 10 magnifications, and (b) is a photograph of Azan stained tissue in 10 magnifications.

Fig. 13 shows photographs of stained bladder tissue

of 5% acetic acid-induced rabbit cystitis model for a group administered with agent for treatment of bladder troubles 3. (a) is a photograph of hematoxylin-eosin stained tissue in 10 magnifications, and (b) is a photograph of Azan stained tissue in 10 magnifications.

Best Mode for Carrying Out the Invention

The present invention will be further explained more specifically with reference to the following examples.

Examples 1-3

Agents for treatment of bladder troubles were prepared by dissolving sodium hyaluronate having an endotoxin content of 0.006 EU (endotoxin unit)/10 ml, sulfur content of 0.005% as determined by coulometric titration, iron content of 4.6 ppm, protein content of 0.01%, and weight average molecular weight of 890,000 in PBS (phosphate buffered saline) so that the content of the sodium hyaluronate should be 0.1% by weight for Example 1, 0.2% by weight for Example 2, and 0.4% by weight for Example 3. The agents for treatment of bladder troubles provided in Examples 1-3 are hereinafter referred to as agent for treatment of bladder troubles 1, agent for treatment of bladder

troubles 2, and agent for treatment of bladder troubles 3, respectively. Limiting viscosity and relative osmotic pressure of the agents for treatment of bladder troubles provided above and PBS were measured. The results are shown in Table 1.

Table 1

	Composition	Limiting viscosity (dl/g)	Relative osmotic pressure
Example 1	0.1% Sodium hyaluronate solution in PBS	15.7	1.04
Example 2	0.2% Sodium hyaluronate solution in PBS	15.7	1.04
Example 3	0.4% Sodium hyaluronate solution in PBS	15.8	1.06
Control	PBS	-	1.03

<Evaluation of agents for treatment of bladder troubles of the present invention using acetic acid-induced rabbit cystitis model>

Using the agents for treatment of bladder troubles provided in Examples 1-3, action for promoting vesical mucosa epithelium dilatation or action for healing vesical mucosa of the agents for treatment of bladder troubles of the present invention for the 5% acetic

acid-induced rabbit cystitis model were examined, and the results were used as evaluation of bladder trouble curative effect.

1) Preparation of rabbit cystitis model

Eleven rabbit cystitis models for each of the above-provided agents for treatment of bladder troubles 1-3 and control (PBS), 44 rabbits in total, were prepared as follows

Forty four 12-14 day-old JW male rabbits (CONV.) having a body weight of about 3 kg were intravenously introduced with 5 mg/kg of ketamine and 2 mg/kg of xylazine for general anesthesia. Then, a bladder catheter provided with a balloon of 8 Fr size was inserted into bladders, and residual urine in the bladders was drained using a 20 ml infusion pipe. After the drainage of residual urine, inside of the bladders was washed twice with 20 ml of physiological saline. Then, 10 ml of solution comprising acetic acid (Wako Pure Chemical Industries, Co., Ltd.) diluted with distilled water to a concentration of 5% was introduced into the bladders, and retained for 10 minutes to induce cystitis. After the cystitis induction, the acetic acid was drained, and intravesical irrigation was performed with 20 ml of physiological saline.

2) Administration test of agents for treatment of

bladder troubles

Each of the agents for treatment of bladder troubles 1-3 provided in the above examples and PBS for control was introduced into bladders of rabbits of each group induced with cystitis as described above using a catheter once a day in an amount of 40 ml immediately after the cystitis induction, 20 ml on day 1 after the induction, and 10 ml on day 2 to day 6, and the introduced agents or PBS were retained for 10 minutes for each time. After pulling out the catheter after the retention of ten minutes, test solution or PBS remained in bladders were drained ad libitum.

3) Evaluation of action for promoting vesical mucosa epithelium dilatation or action for healing vesical mucosa (cystitis curative effect)

In the rabbits administered with the agents for treatment of bladder troubles 1-3 obtained in the examples as described above or PBS alone, the action for promoting vesical mucosa epithelium dilatation or the action for healing vesical mucosa (cystitis curative effect) were evaluated on day 7 after the cystitis induction based on the following evaluation items. Ten rabbits were used for the evaluation of the evaluation items (a)-(e), one remaining rabbit was used for the evaluation of the item (f).

(a) Determination of amount of trypan blue injectable to inside of bladder (bladder capacity)

On day 7 after the cystitis induction, residual urine in bladders of rabbits was drained, and 20 ml of preliminarily prepared 0.5% aqueous solution of trypan blue (Wako Pure Chemicals Industries, Co., Ltd.) was gradually introduced into the bladders of rabbits. When the solution overflowed from the top of penises, injection limit amount was calculated from amount of the remained trypan blue aqueous solution, and that was recorded as bladder capacity.

From the results of the above determination, mean and standard deviation of bladder capacity of 10 animals in each administration group were calculated and represented as a graph (Fig. 1). The mean values of bladder capacity were 8 ml for the control group, 17.8 ml for the agent for treatment of bladder troubles 1 administration group, 16.3 ml for the agent for treatment of bladder troubles 2 administration group, and 17 ml for the agent for treatment of bladder troubles 3 administration group. The indication "**" in Fig. 1 means that the differences between the results of the test groups and the control group is significant with $p \leqq 0.01$ when linearity of the measured data was confirmed by a re-sampling method (Multitest Procedure), and the difference of the mean values was tested by Dunnett multiple comparison test.

From the mean values mentioned above and Fig. 1, it is clear that bladder capacity of any of the rabbits of 5% acetic acid-induced rabbit cystitis model administered with the agents for treatment of bladder troubles 1-3 obtained in the above examples was significantly larger than bladder capacity of the rabbits administered with PBS. This difference of bladder capacity is considered to be caused by the fact that, in the control group, severe inflammation was caused after excoriation of vesical mucosa epithelium by acetic acid, and the vesical proper muscle coat caused fibrillation on day 7, thereby expansion became very difficult, whereas, in the agents for treatment of bladder troubles 1-3 administration groups, the fibrillation of vesical proper muscle coat was inhibited by the administration of the therapeutic agents, thereby bladder expansion function was improved.

(b) Photography of trypan blue adhesion to damaged area of bladder and determination of bladder trouble area ratio

The 0.5% trypan blue aqueous solution was introduced into bladders of the rabbits to the injection limit amounts as described above, and retained for 5 minutes. Then, rabbits were killed by bleeding, and the bladders were excised. The excised bladders were dissected along extracystic blood vessels, and washed by

floating them in physiological saline. After the washing, the bladders were opened, and fixed with pins so that intravesical walls should be upside, and each bladder was photographed as a whole at the same magnification (Figs. 2-5). Figs. 2-5 are representative photographs among photographs of dissected bladders of 10 animals representing trypan blue adhesion to vesical mucosa, with Fig. 2 being for the control group, Fig. 3 for the agent for treatment of bladder troubles 1 administration group, Fig. 4 for the agent for treatment of bladder troubles 2 administration group, and Fig. 5 for the agent for treatment of bladder troubles 3 administration group.

It has known that trypan blue, an acidic living tissue staining agent, can readily stain damaged mucosa From the aforementioned photographs, it has been revealed that the trypan blue adhered area of vesical mucosa, i.e., size of the damaged area of vesical mucosa is largest in the control, where trypan blue adhered to almost all of the vesical inner surface, and it becomes smaller in the order of the agent for treatment of bladder troubles 1 administration group, where trypan blue adhered area was about half of that observed in the control group, the agent for treatment of bladder troubles 2 administration group, and the agent for treatment of bladder troubles 3 administration group.

From the aforementioned photographs representing

trypan blue adhesion to the vesical mucosa, total intravesical areas and the damaged areas adhered with trypan blue, which were stained blue, were also determined, and bladder trouble area ratio (%) was calculated according to the following equation:

Bladder trouble area ratio (%) = (trypan blue adhered area/total intravesical area) × 100

From the results of the above calculation, mean and standard deviation of bladder trouble area ratio of 10 animals in each administration group were calculated, and represented as a graph (Fig. 6). The mean values of bladder trouble area ratio were 74.77% for the control group, 33.2% for the agent for treatment of bladder troubles 1 administration group, 28.15% for the agent for treatment of bladder troubles 2 administration group, and 21.84% for the agent for treatment of bladder troubles 3 administration group. The indication "**" in Fig. 6 means that the differences between the results of the test groups and the control group is significant with $p \leqq 0.01$ when linearity of the measured data was confirmed by a re-sampling method (Multitest Procedure), and the difference of the mean values was tested by Dunnett multiple comparison test.

From the mean values mentioned above and Fig. 6, it is clear that bladder trouble area ratio of any of the

rabbits of 5% acetic acid-induced rabbit cystitis model administered with the agents for treatment of bladder troubles 1-3 obtained in the above examples was significantly smaller than bladder trouble area ratio of the rabbits administered with PBS. Further, significant linearity between the bladder trouble area ratio and the amount of sodium hyaluronate contained in the agents for treatment of bladder troubles administered, i.e., dose-dependency, was observed. These results are considered to show that, by administering the agents for treatment of bladder troubles containing sodium hyaluronate in the amounts mentioned above to the 5% acetic acid-induced rabbit cystitis models, dilatation of vesical mucosa epithelium may be promoted, and vesical mucosa damaged by acetic acid may be healed.

(c) Determination of bladder dry weight

After the photography in the above (b), the bladders were individually lyophilized, macerated, and weighed.

From the results of the weighing, mean and standard deviation of bladder dry weight of 10 animals in each administration group were calculated and represented as a graph (Fig. 7). The indication "**" in Fig. 7 means that the differences between the results of the test groups and the control group is significant with $0.01 \leqq p \leqq 0.05$ when linearity of the measured data was

confirmed by a re-sampling method (Multitest Procedure), and the difference of the mean values was tested by Dunnett multiple comparison test.

From Fig. 7, it is clear that bladder dry weight of any of the rabbits of 5% acetic acid-induced rabbit cystitis model administered with the agents for treatment of bladder troubles 1-3 obtained ion the above examples was smaller than bladder dry weight of the rabbits administered with PBS.

(d) Determination of amount of trypan blue adhesion to damaged area of bladders

The bladders after the above weighing were digested with 0.25% actinase, a part of the digest was filtered to afford supernatants, and absorbance of these supernatants at 582 nm was measured. From the obtained values, amounts of trypan blue adhesion were calculated.

From the results of the above determination, mean and standard deviation of amounts of trypan blue adhesion of 10 animals in each administration group were calculated and represented as a graph (Fig. 8). The mean values of amount of trypan blue adhesion were 507 μg/bladder for the control group, 341 μg/bladder for the agent for treatment of bladder troubles 1 administration group, 264 μg/bladder for the agent for treatment of bladder troubles 2 administration group, and 265 μg/bladder for the agent for treatment of

bladder troubles 3 administration group. The indications "*" and "**" in Fig. 8 mean that the differences between the results of the test groups and the control group are significant with $0.01 \leqq p \leqq 0.05$ and $p \leqq 0.01$, respectively, when linearity of the measured data was confirmed by a re-sampling method (Multitest Procedure), and the difference of the mean values was tested by Dunnett multiple comparison test..

From the mean values mentioned above and Fig. 8, it is clear that amount of trypan blue adhesion of any of the rabbits of 5% acetic acid-induced rabbit cystitis model administered with the agents for treatment of bladder troubles 1-3 was significantly smaller than amount of trypan blue adhesion of the rabbits administered with PBS. Further, significant linearity between the bladder trouble area ratio and the amount of sodium hyaluronate contained in the agent for treatment of bladder troubles administered, i.e., dose-dependency, was observed. These results are considered to show that, by administering the agents for treatment of bladder troubles containing sodium hyaluronate in the amounts mentioned above to the 5% acetic acid-induced rabbit cystitis models, dilatation of vesical mucosa epithelium can be promoted, and vesical mucosa damaged by acetic acid can be healed.

(e) Quantitative assay of intravesical hydroxyproline

(collagen)

A part of the actinase digest obtained above was hydrolyzed with hydrochloric acid. Amount of hydroxyproline formed by the hydrolysis (amount of collagen) was assayed by a conventional manner, and amount of intravesical hydroxyproline (amount of collagen) was calculated.

From the results of the above assay, mean and standard deviation of amount of hydroxyproline of 10 animals in each administration group were calculated and represented as a graph (Fig. 9). The indication "*" in Fig. 9 means that the differences between the results of the test groups and the control group are significant with of $0.01 \leqq p \leqq 0.05$, when linearity of the data was confirmed by a re-sampling method (Multitest Procedure), and the difference of the mean values was tested by Dunnett multiple comparison test.

From Fig. 9, it is clear that amount of intravesical hydroxyproline of any of the rabbits of 5% acetic acid-induced rabbit cystitis model administered with the agents for treatment of bladder troubles 1-3 was smaller than amount of intravesical hydroxyproline of the rabbits administered with PBS. These results are considered to show that, by administering the agents for treatment of bladder troubles containing sodium hyaluronate in the amounts mentioned above to the 5% acetic acid-induced rabbit cystitis models, fibrillation

of bladders can be prevented.

(f) Histopathological examination

On day 7 after the cystitis induction, after remained urine in bladders of rabbits was drained, 10 ml of preliminarily prepared 10% phosphate buffered formalin solution was introduced into the bladders of rabbits, then the rabbits were killed by bleeding, and the bladders were removed. The removed bladders were fixed by placing them in 10% phosphate buffered formalin solution when they were still in the inflated state, and histopathological examination was performed by hematoxylin-eosin staining and Azan staining methods.

Microphotographs (10 magnifications) of vesical tissues stained with hematoxylin-eosin or Azan are shown in Figs. 10-13. Fig. 10 shows photographs of stained vasical tissue for the control group, Fig. 11 for the agent for treatment of bladder troubles 1 administration group, Fig. 12 for the agent for treatment of bladder troubles 2 administration group, and Fig. 13 for the agent for treatment of bladder troubles 3 administration group. In these figures, (a) indicates photographs of hematoxylin-eosin staining in 10 magnifications, and (b) indicates photographs of Azan staining in 10 magnifications.

Fig. 10 (a) indicates that, in the bladder of the control group, no vesical mucosa epithelium was

recognized for total inner surface of the bladder, massive necrotic tissues projecting into the cavity were observed, amorphous matters were observed on the surfaces of the massive necrotic tissues, and severe cellular infiltration was seen in the lining. Fig. 10 (b) indicates that, in the bladder of the control group, formation of collagen fibers, which are stained blue with Azan, was observed in the total inner surface of the bladder, and an image which may be called as bladder induration was provided.

Fig. 11 (a) indicates that, in the bladder of the agent for treatment of bladder troubles 1 administration group, while massive necrotic tissues were observed like in the control group, formation of vesical mucosa epithelium not seen in the control group was recognized, and, though mucosa epithelium cell alignment was irregular, necrotic image was not observed. Fig. 11 (b) indicates that, in the bladder of the agent for treatment of bladder troubles 1 administration group, an image that can be called as bladder induration was seen for the apparent granulation region like the control group.

Fig. 12 (a) indicates that, in the bladder of the agent for treatment of bladder troubles 2 administration group, massive necrotic tissues projecting into the bladder were observed in a wider range compared with the agent for treatment of bladder troubles 1 administration

group. However, Fig. 12 (b) indicates that, in the bladder of the agent for treatment of bladder troubles 2 administration group, though a bladder induration image was observed, condition of mucosal epithelium was substantially normal.

Fig. 13 (a) indicates that, in the bladder of the agent for treatment of bladder troubles 3 administration group, no massive necrotic tissue projecting into the bladder was observed at all. Fig. 13 (b) indicates that, in the bladder of the agent for treatment of bladder troubles 3 administration group, a bladder induration image was not observed.

In summary, any of the bladders of the rabbits of the to 5% acetic acid-induced rabbit cystitis models after administered with the agents for treatment of bladder troubles 1-3 provided in the above examples exhibited smaller histopathological difference relative to bladders of normal rabbits compared with the bladders of the rabbits after administered with PBS. Furthermore, in the agent for treatment of bladder troubles 3 administration group where the agent contained 0.4% by weight of sodium hyaluronate, markedly good results were provided, i.e., no massive necrotic tissue was observed. The results of the histopathological examination are considered to support the results of the determination of bladder capacity, bladder dry weight, trypan blue adhesion area, amount of

trypan blue adhesion, and amount of hydroxyproline (amount of collagen).

As seen from the results mentioned above, bladder capacity, trypan blue adhesion area, amount of trypan blue adhesion, amount of hydroxyproline, and bladder weight could be improved, and healing and improving effects of vesical mucosa due to vesical mucosa epithelium dilatation were observed in the histopathological examination when the agents for treatment of bladder troubles 1-3 provided in the above examples were administered to 5% acetic acid-induced rabbit cystitis models. Therefore, it can be considered that the agent for treatment of bladder troubles of the present invention can provide effective therapy for bladder troubles by promoting vesical mucosa epithelium dilatation, and healing vesical mucosa, thereby inhibiting fibrillation.

Industrial Applicability

The agent for treatment of bladder troubles of the present invention has an action for promoting vesical mucosa epithelium dilatation and an action for healing vesical mucosa, prevents progress of fibrillation of bladder, and recovers expansion function of bladder, and therefore it exhibits excellent curative effect against bladder troubles.

72689-98

CLAIMS:

1. A medicament in the form of an aqueous solution to be applied directly into bladder for treatment of a non-bacterial bladder trouble selected from the group consisting of interstitial cystitis, eosinophilic cystitis, hemorrhagic cystitis and neurogenic increased urinary frequency, comprising:

0.3-0.45% by weight of hyaluronic acid or a pharmaceutically acceptable salt thereof; and

an appropriate liquid carrier,

wherein the aqueous solution has a pH value of about 6 to 8, a relative osmotic pressure to body fluid of 0.9 to 1.2 and an apparent viscosity of 10 to 1500 mPa·s as determined at a shear rate of 9.6 sec^{-1} at 20°C.

2. The medicament of claim 1, wherein the aqueous solution comprises hyaluronic acid or a pharmaceutically acceptable salt thereof in an amount of 0.35-0.45% by weight.

3. The medicament of claim 1 or 2, wherein the hyaluronic acid or a pharmaceutically acceptable salt thereof has the following physicochemical properties:

(A) endotoxin content: 0.03 endotoxin unit/10 mg or less,

(B) sulfur content: 0.01% or less as determined by coulometric titration,

(C) iron content: 20 ppm or less,

(D) protein content: 0.1% or less, and

72689-98

(E) weight average molecular weight: 500,000 or more.

4. The medicament of claim 1, 2 or 3, wherein the hyaluronic acid or a pharmaceutically acceptable salt thereof has a weight average molecular weight of around 500,000-2,200,000.

5. The medicament of claim 1, 2 or 3, wherein the hyaluronic acid or a pharmaceutically acceptable salt thereof has a weight average molecular weight of around 600,000-1,200,000.

6. The medicament of claim 1, 2 or 3, wherein the hyaluronic acid or a pharmaceutically acceptable salt thereof has a weight average molecular weight of around 800,000-1,200,000.

7. The medicament of claim 1, 2 or 3, wherein the hyaluronic acid or a pharmaceutically acceptable salt thereof has a limiting viscosity of 11.0-45 dl/g.

8. The medicament of any one of claims 1 to 7, which has an action for inhibiting fibrillation of bladder.

9. The medicament of any one of claims 1 to 7, wherein the hemorrhagic cystitis is hemorrhagic cystitis caused by radiotherapy or chemotherapeutant.

10. A commercial package comprising:

a container containing therein the medicament of any one of claims 1 to 9, and a written matter describing that the medicament is to be given inside of a patient's bladder for promoting vesical mucosa epithelium dilatation and healing vesical mucosa.

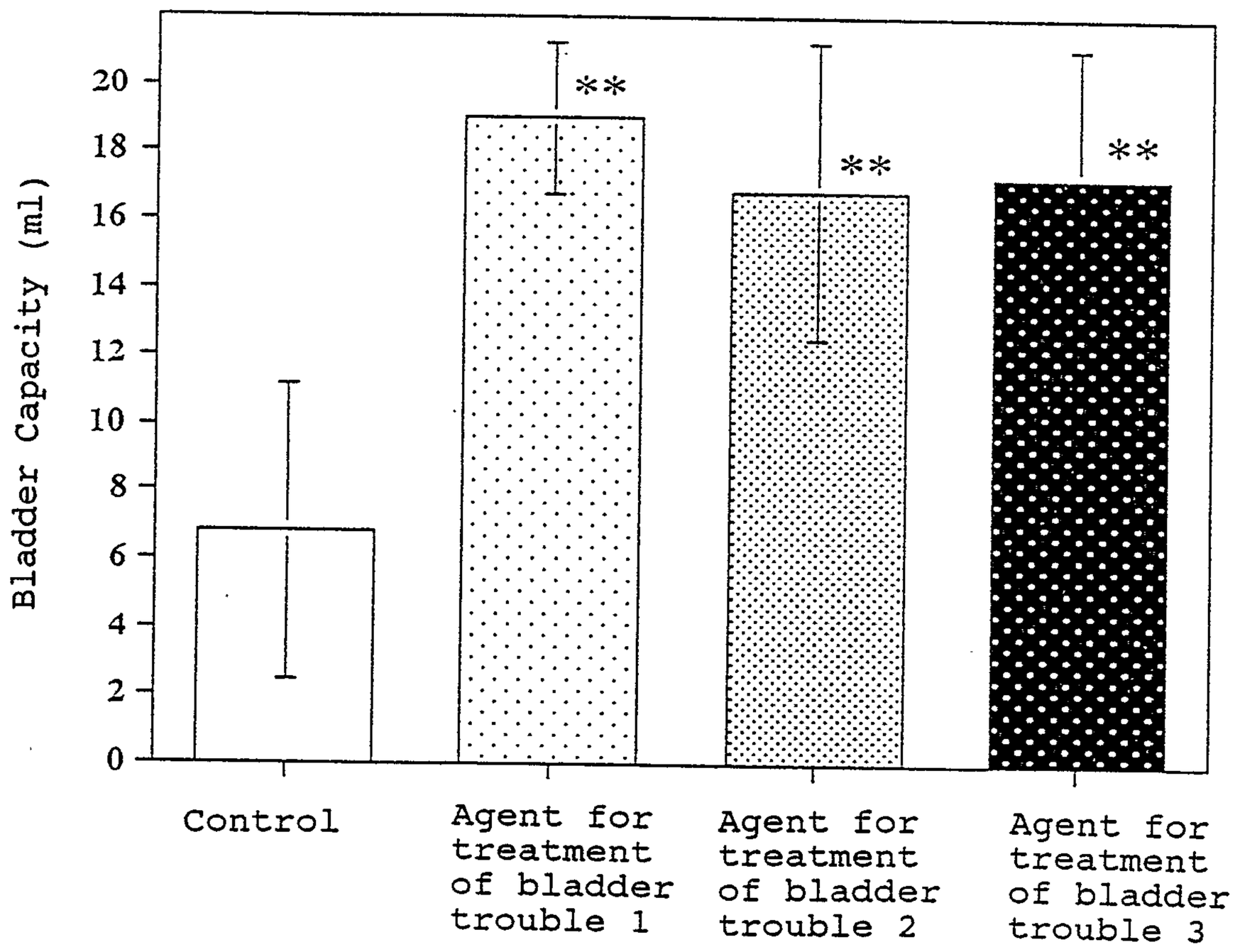

Bladder Capacity (ml)
0
2
4
6
8
10
12
14
16
18
20
**
**
**
Control
Agent for treatment of bladder trouble 1
Agent for treatment of bladder trouble 2
Agent for treatment of bladder trouble 3

FIG. 1

FIG. 2

FIG. 3

FIG. 4

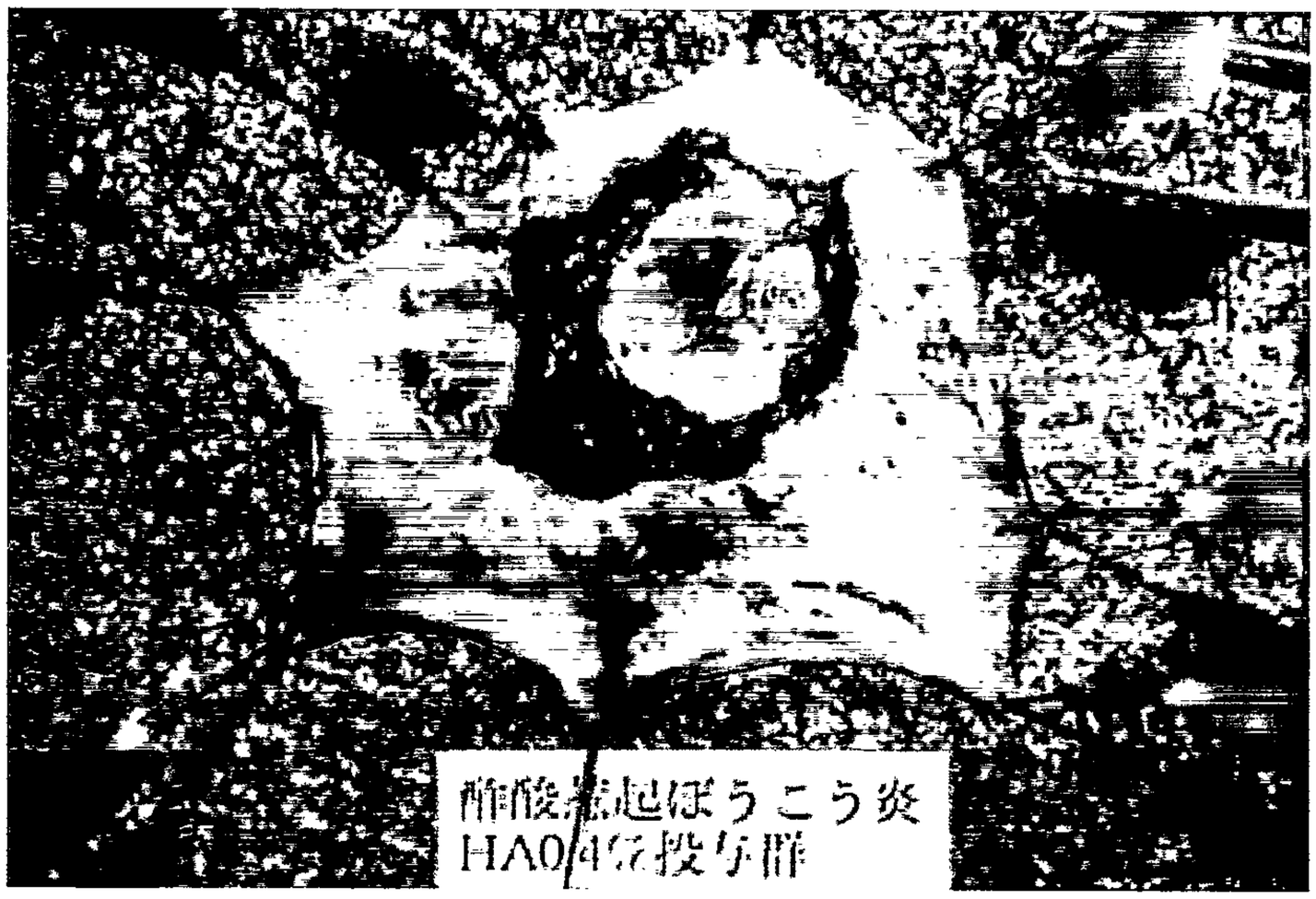


FIG. 5

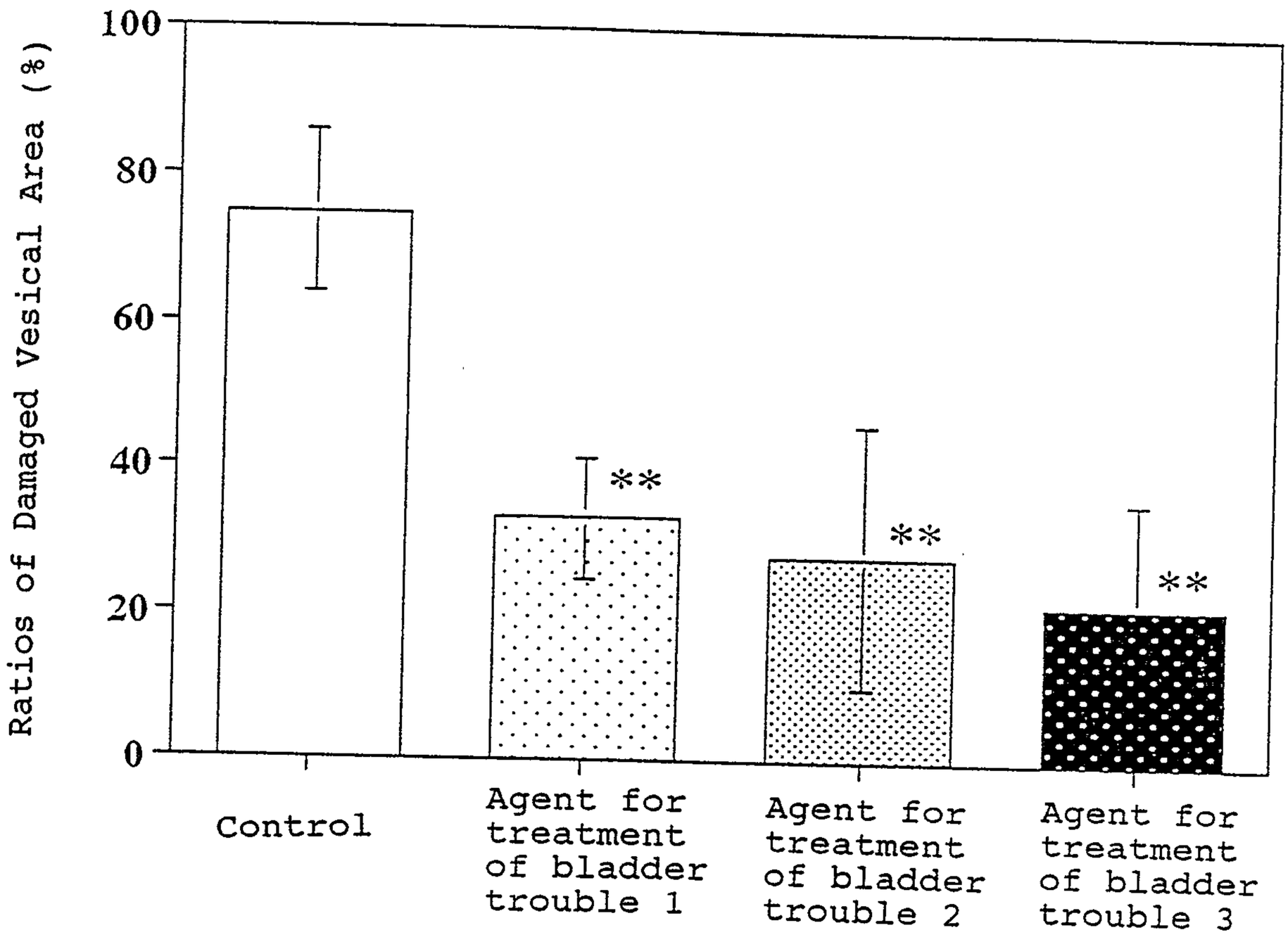

Ratios of Damaged Vesical Area (%)
100
80
60
40
20
0
**
**
**
Control
Agent for treatment of bladder trouble 1
Agent for treatment of bladder trouble 2
Agent for treatment of bladder trouble 3

FIG. 6

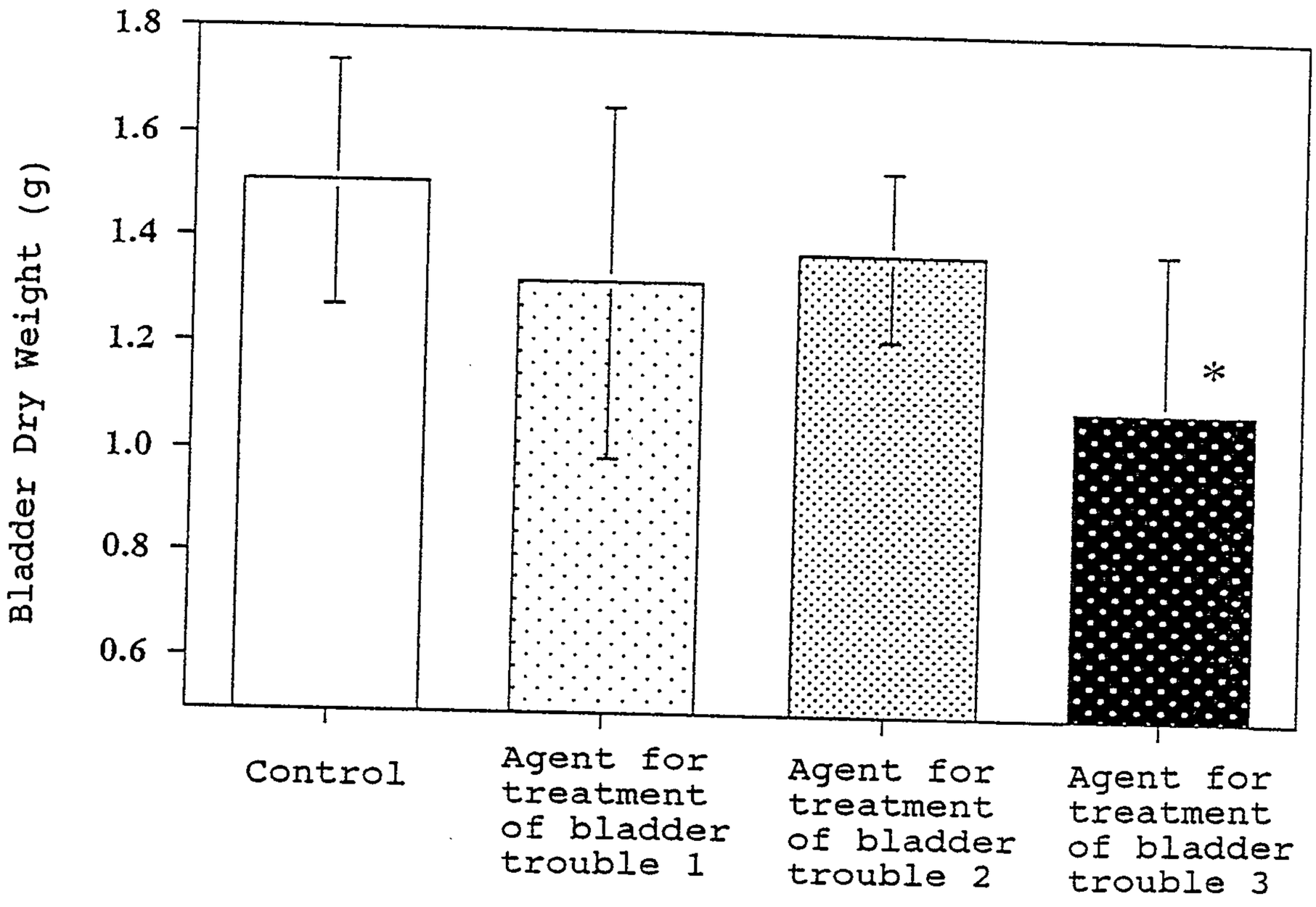

Bladder Dry Weight (g)
1.8
1.6
1.4
1.2
1.0
0.8
0.6
*
Control
Agent for treatment of bladder trouble 1
Agent for treatment of bladder trouble 2
Agent for treatment of bladder trouble 3

FIG. 7

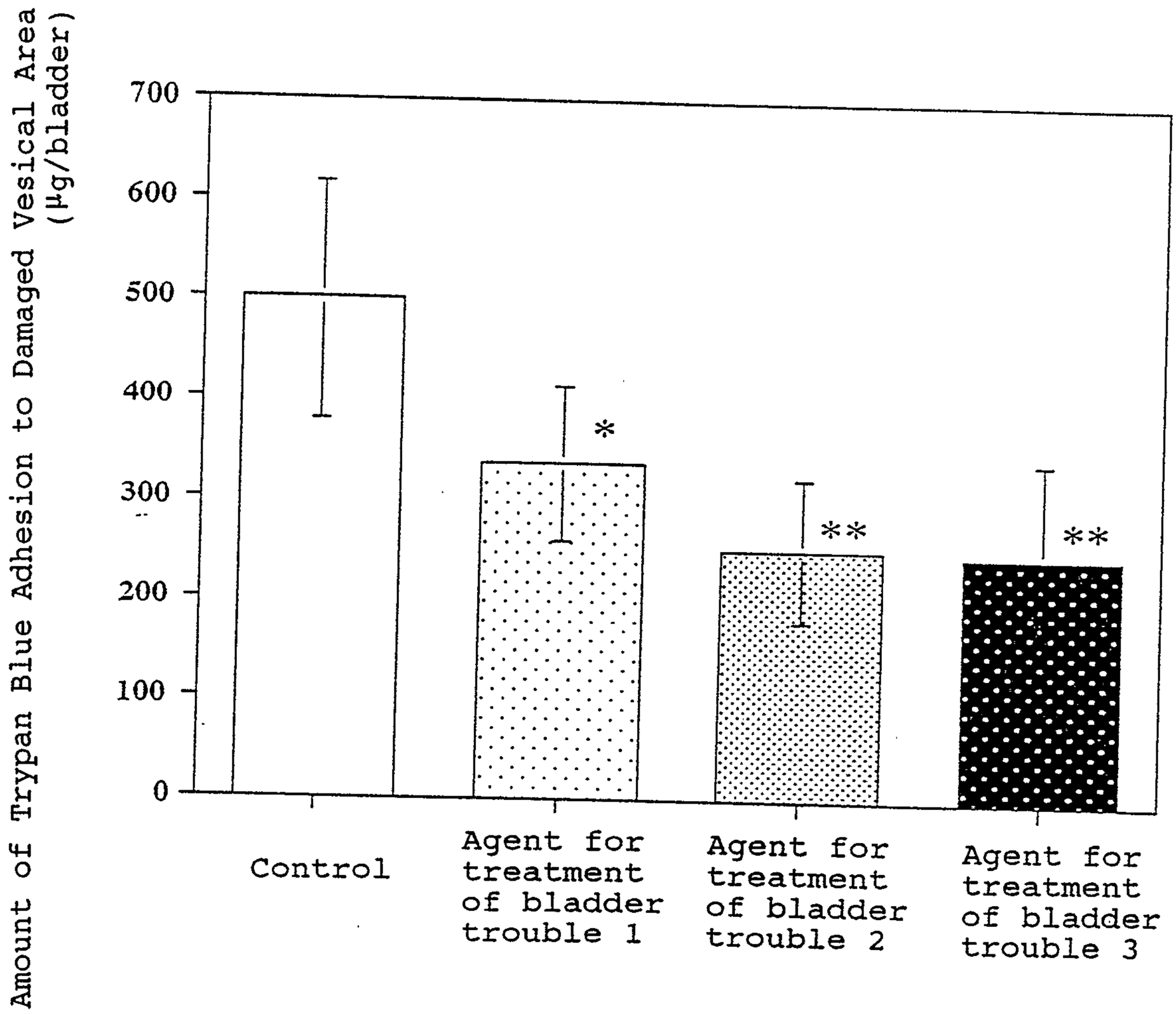

Amount of Trypan Blue Adhesion to Damaged Vesical Area
(μg/bladder)
700
600
500
400
300
200
100
0
*
**
**
Control
Agent for treatment of bladder trouble 1
Agent for treatment of bladder trouble 2
Agent for treatment of bladder trouble 3

FIG. 8

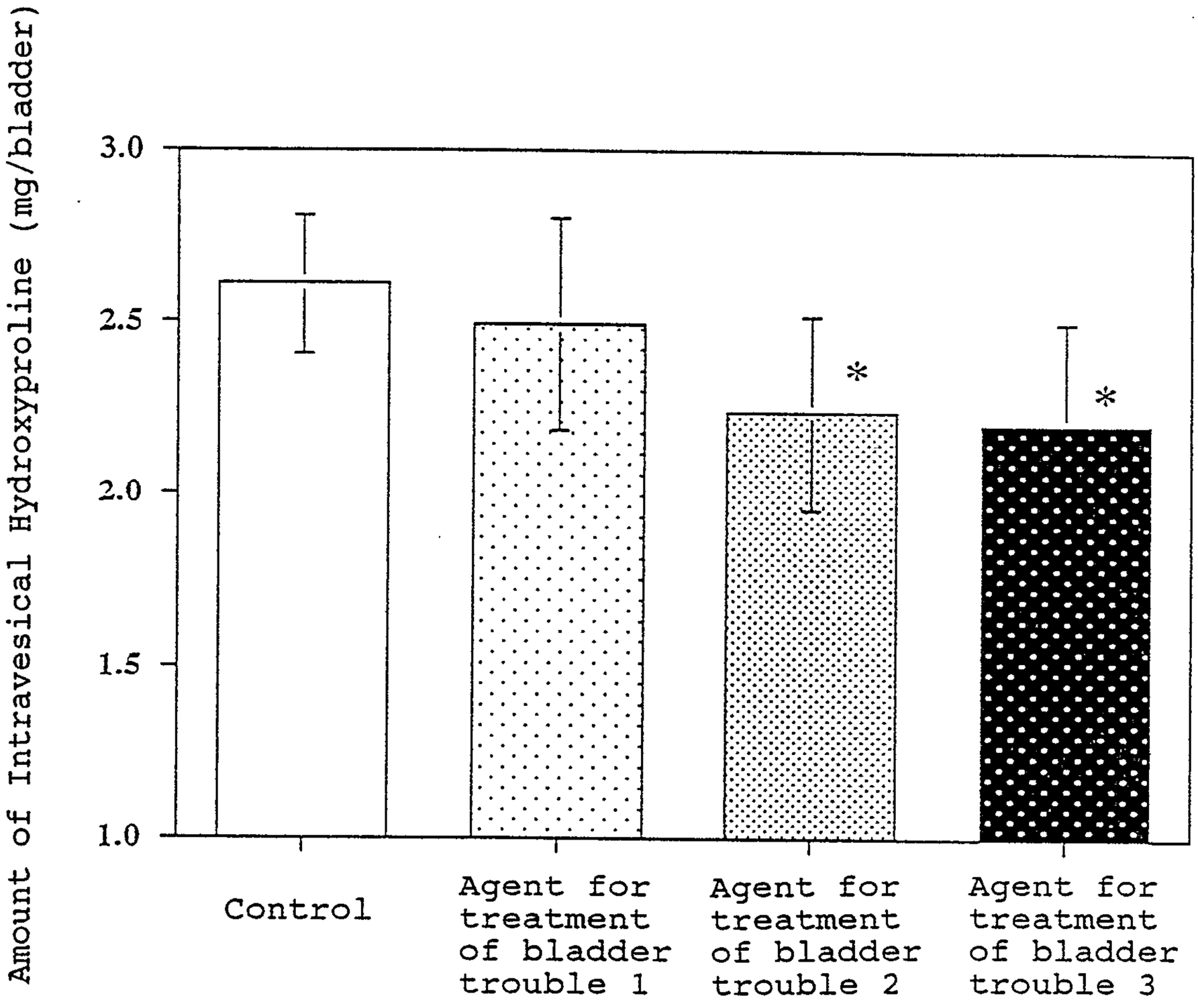
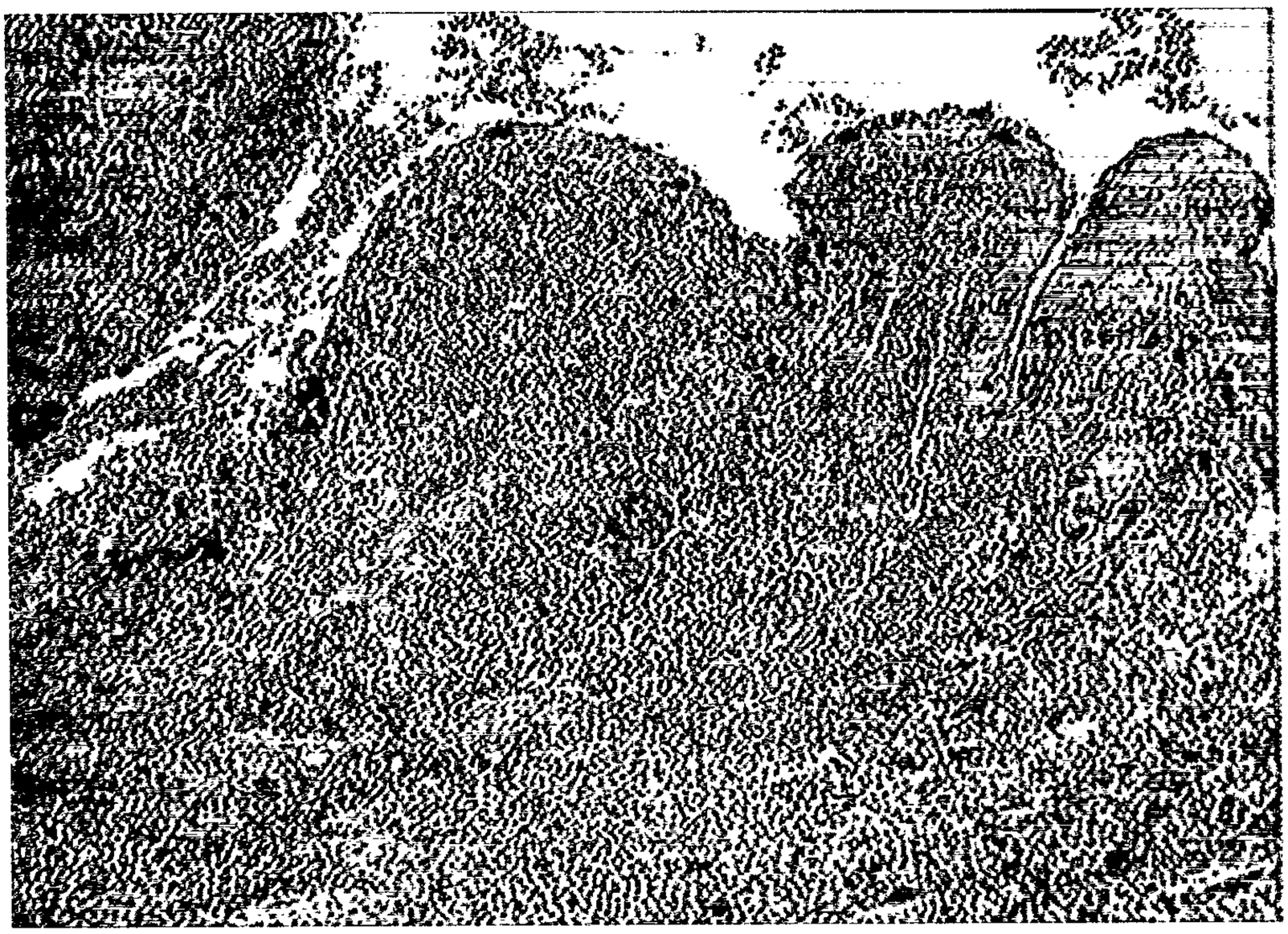
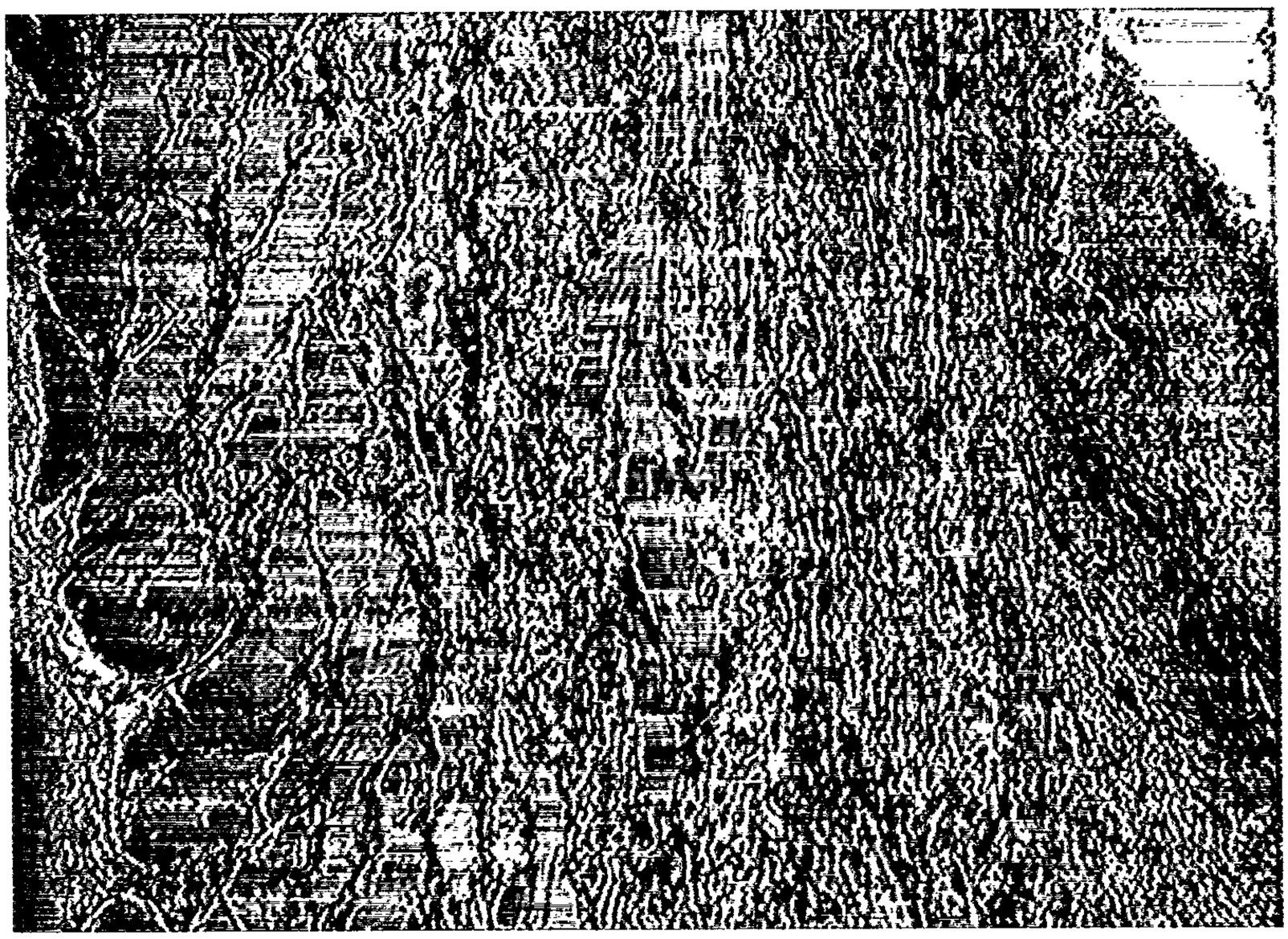

Amount of Intravesical Hydroxyproline (mg/bladder)
3.0
2.5
2.0
1.5
1.0
*
*
Control
Agent for treatment of bladder trouble 1
Agent for treatment of bladder trouble 2
Agent for treatment of bladder trouble 3

FIG. 9

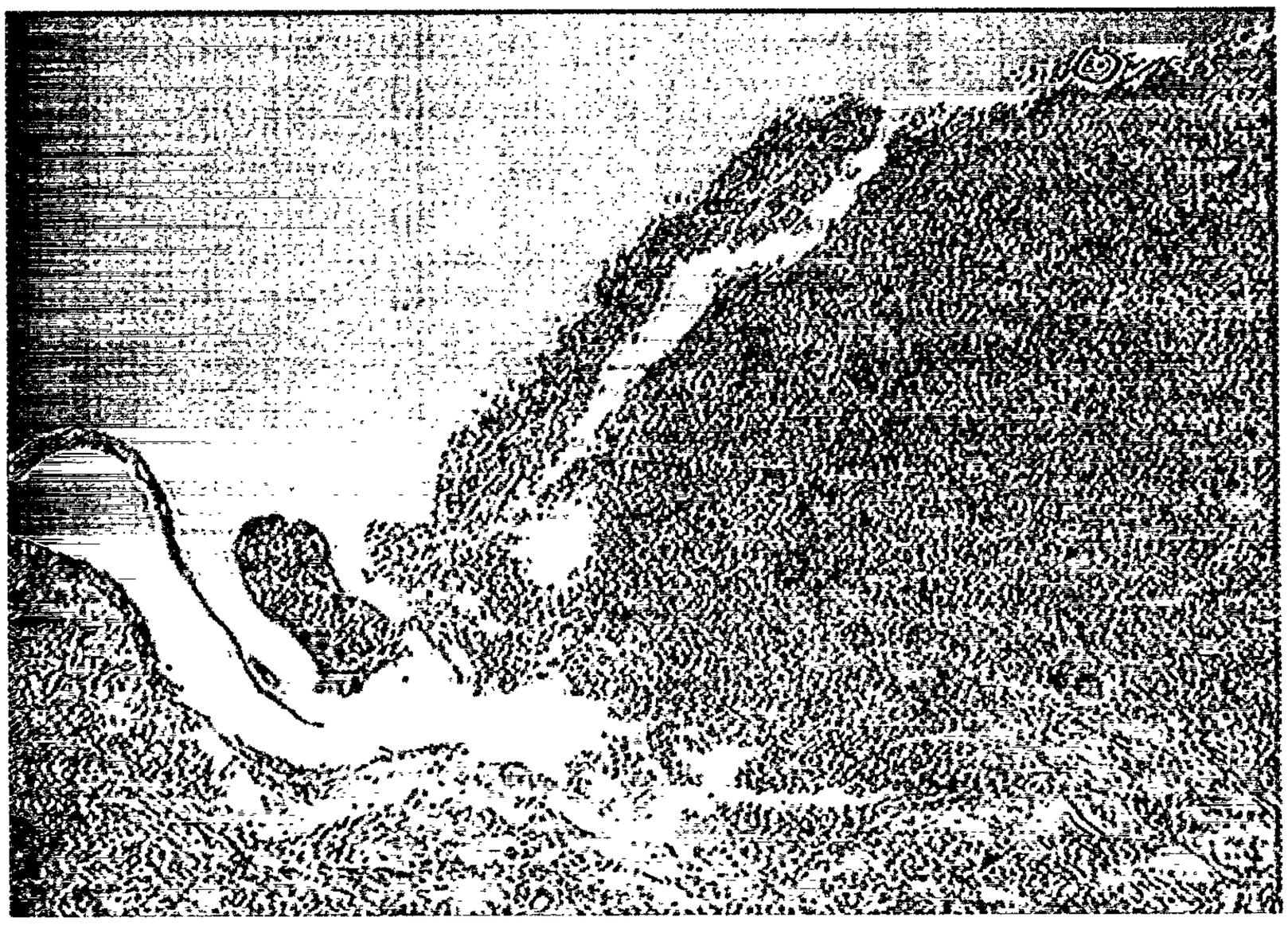


(a)

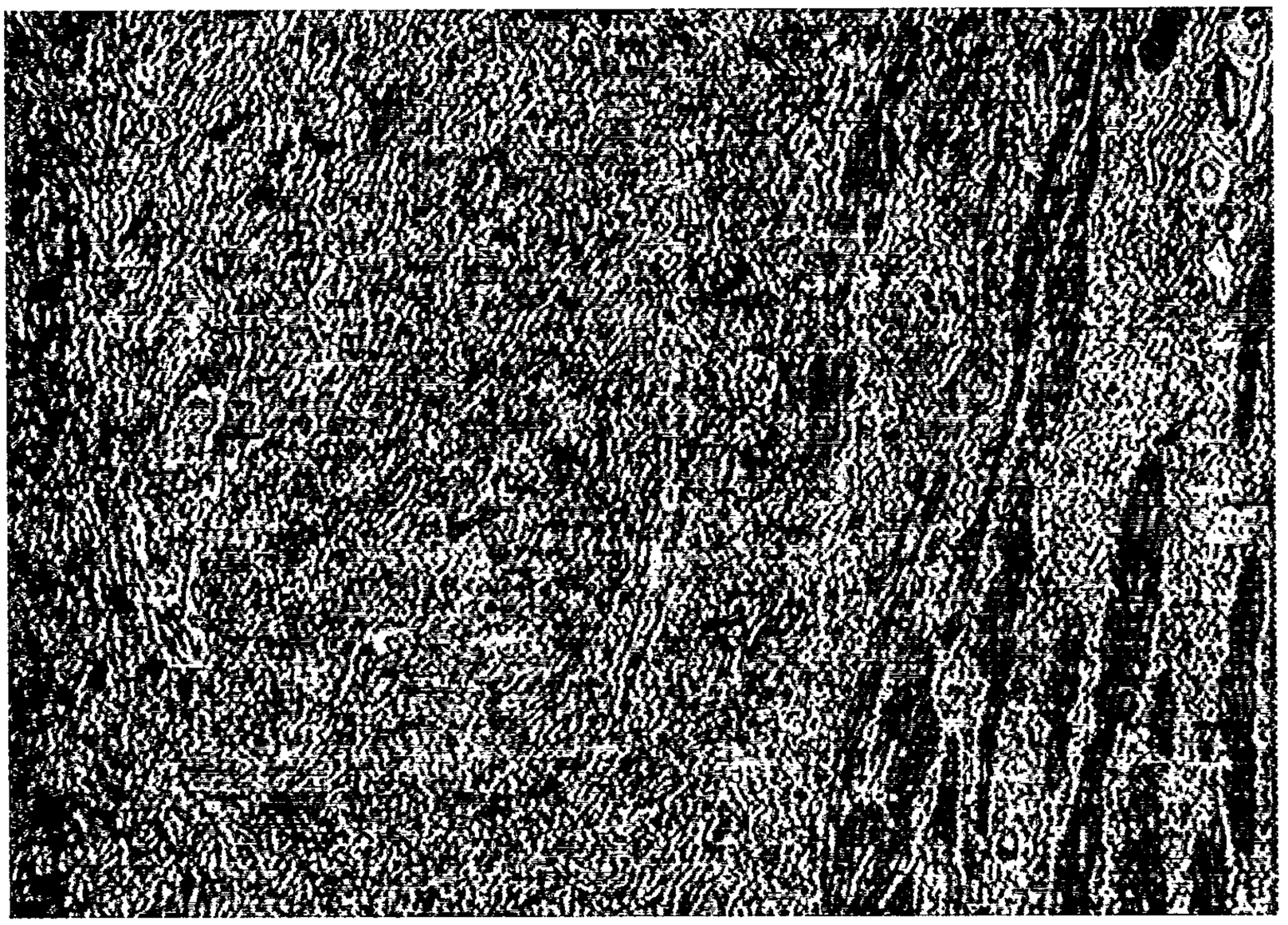


(b)

FIG. 10

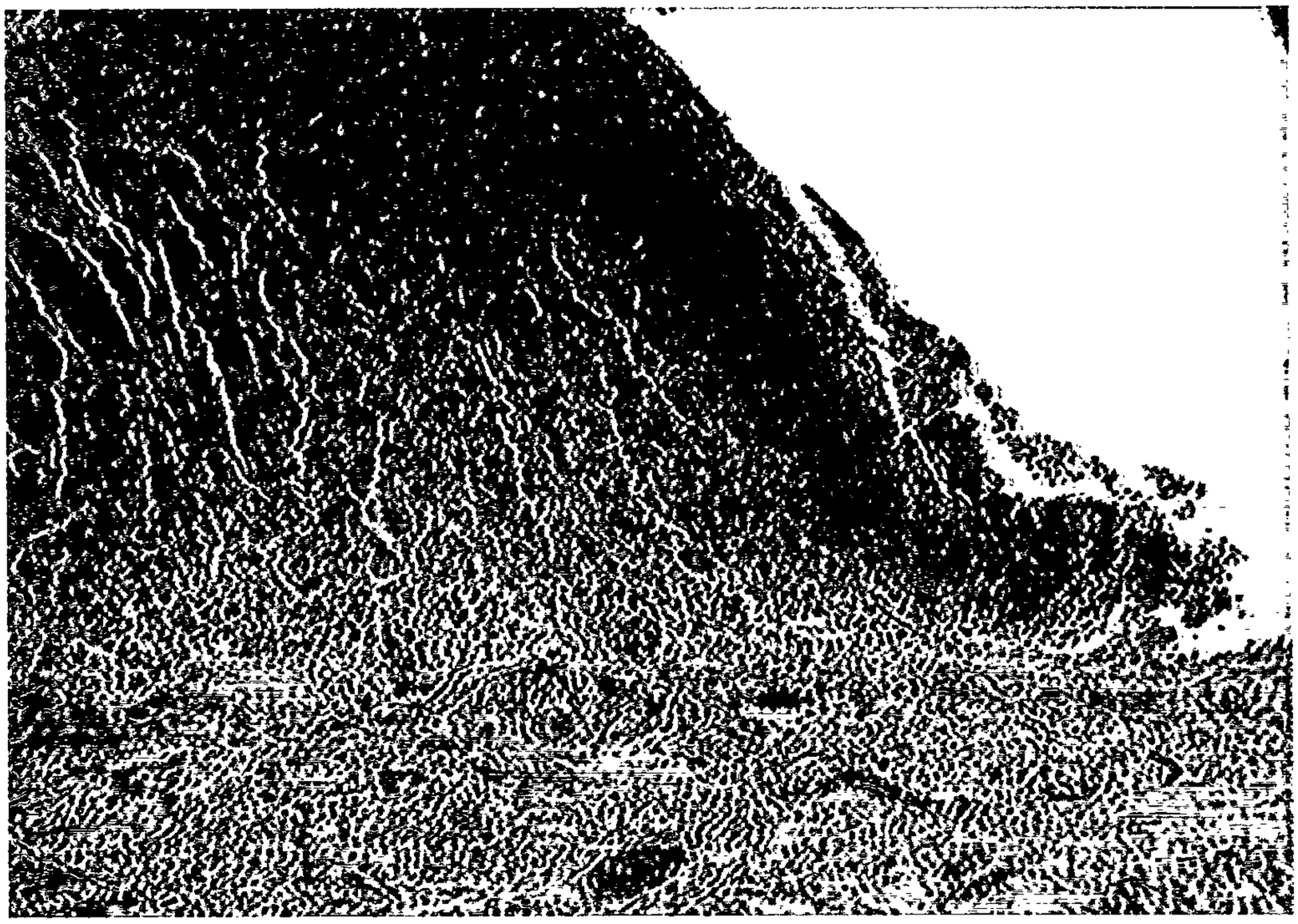


(a)

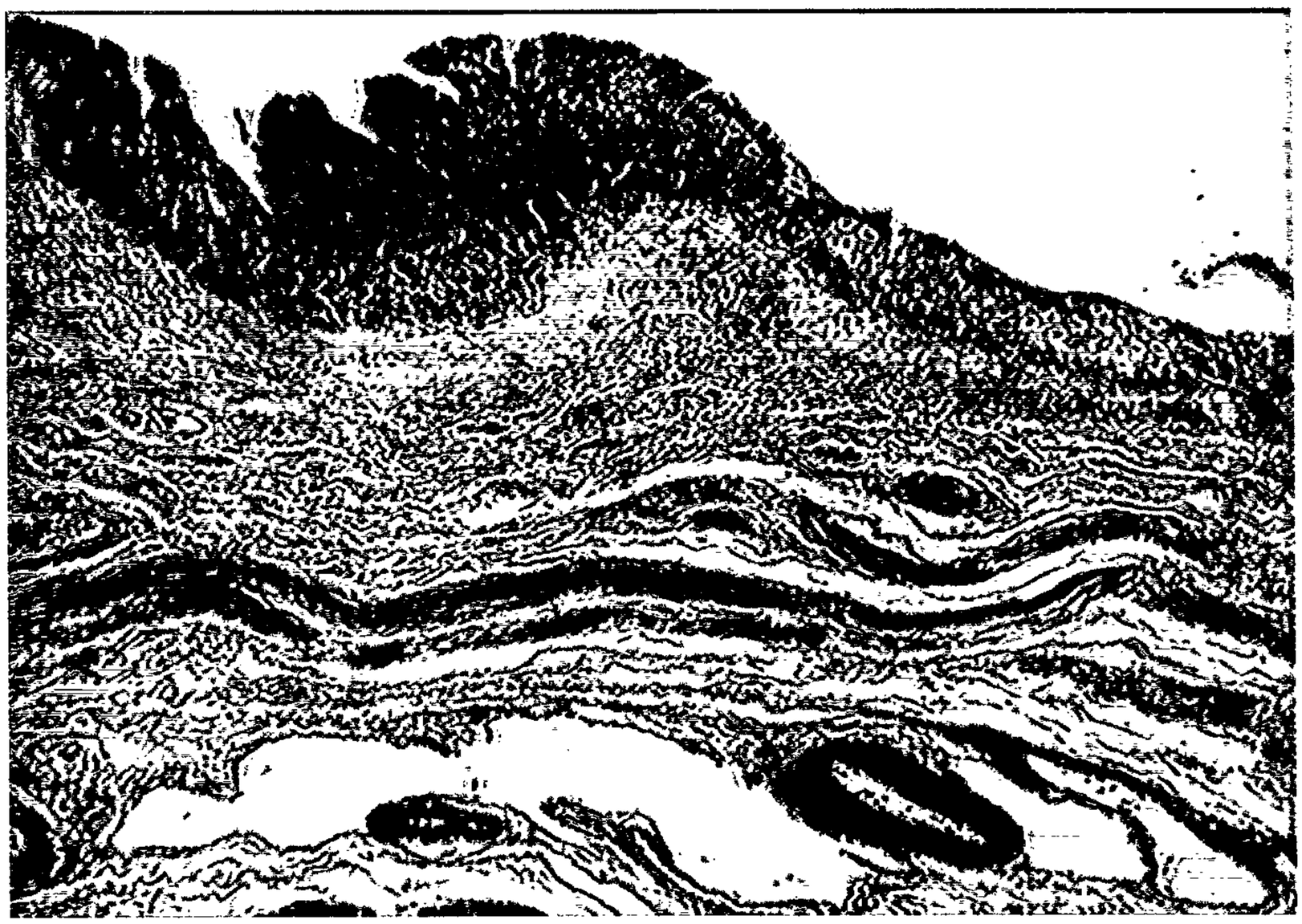


(b)

FIG. 11



(a)



(b)

FIG. 12

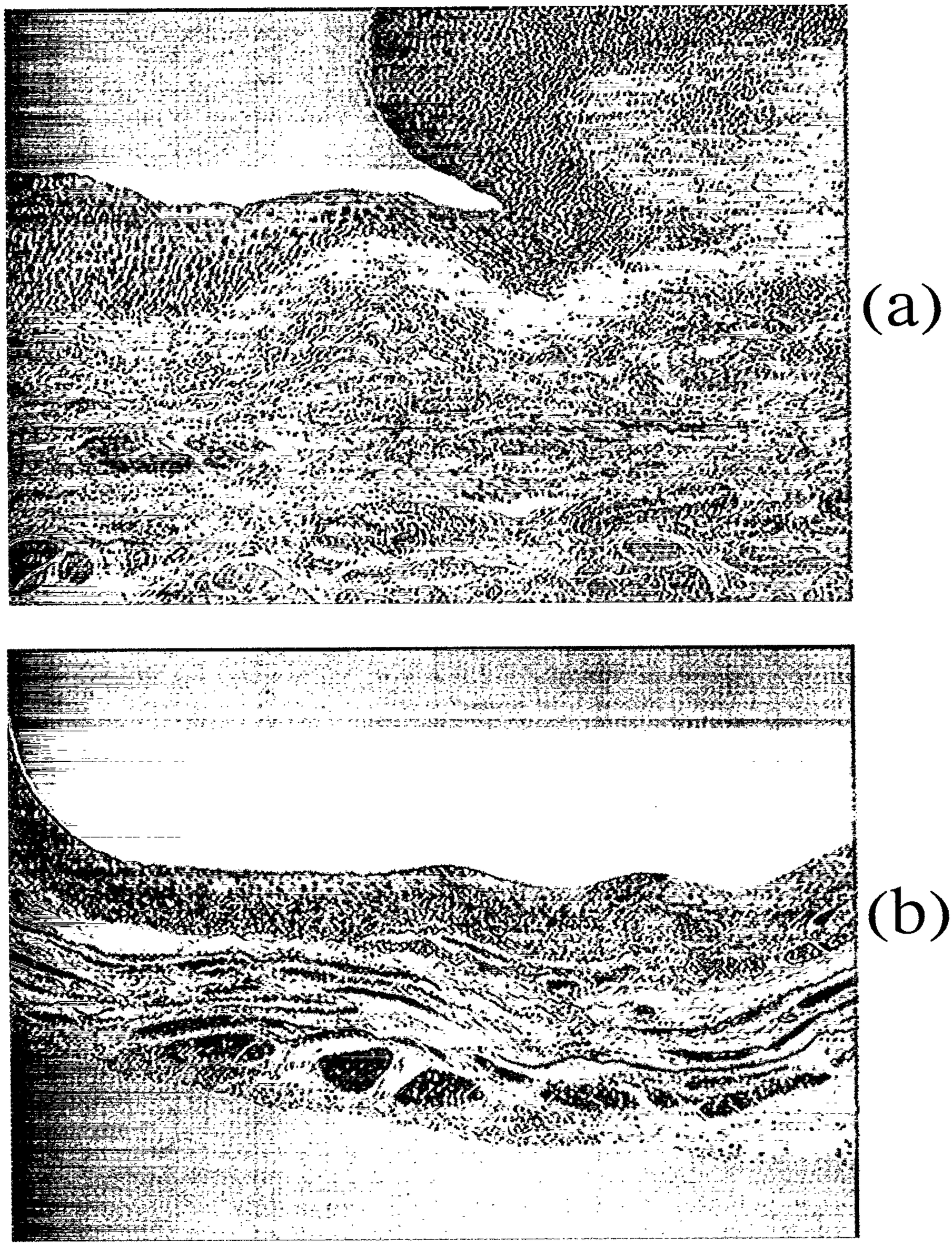


FIG. 13