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(72) BERTLING, WOLF, DE
(71) NOVEMBER AKTIENGESELLSCHAFT GESELLSCHAFT FUR MOLEKULARE MEDIZIN, DE

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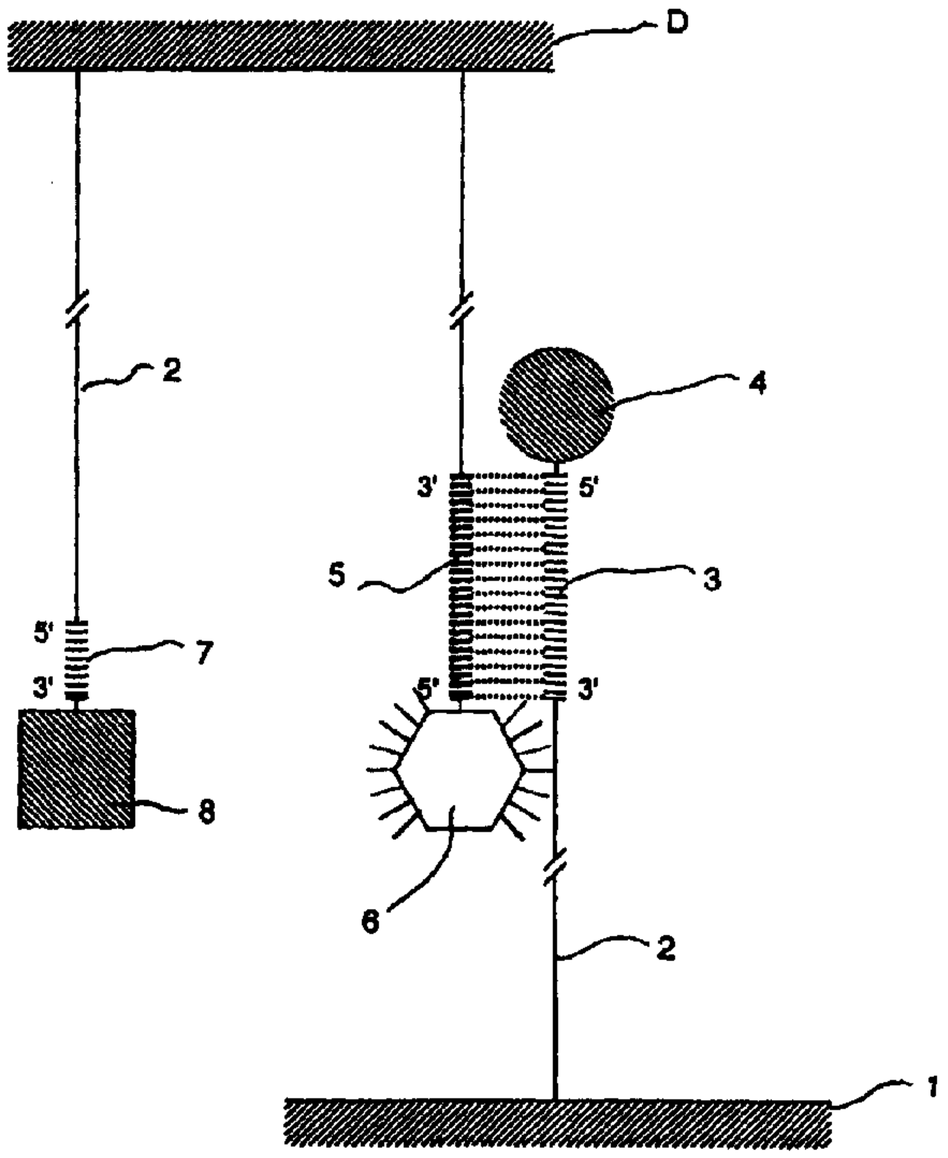
(54) **PROCEDE ET DISPOSITIF D'IDENTIFICATION D'UN MARQUAGE**

(54) **METHOD AND DEVICE FOR IDENTIFYING A TAG**



(57) Procédé d'identification d'un marquage présentant une première séquence nucléotidique (3) et fixé à un corps solide (1). La première séquence nucléotidique (3) est mise en contact avec une substance de détection comportant une deuxième séquence nucléotidique (5) correspondant à la première. La deuxième séquence nucléotidique (5) est liée par sa première extrémité à une phase solide D et présente à sa seconde extrémité un premier groupe fluorophore (6).

(57) The invention relates to a method for identifying a tag. Said tag is provided on a solid (1) and has a first nucleotide sequence (3). The first nucleotide sequence (3) is brought into contact with a detection agent which has a second nucleotide sequence (5) corresponding to the first. The second nucleotide sequence (5) is bound by its first end to a solid phase (D) and has a first flurophore group (6) at its second end.

PCT

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(71) Anmelder *(für alle Bestimmungsstaaten ausser US)*: NOVEMBER AG NOVUS MEDICATUS BERTLING GESELLSCHAFT FÜR MOLEKULARE MEDIZIN [DE/DE]; Ulrich–Schalk–Strasse 3 a, D–91056 Erlangen (DE).

(72) Erfinder; und
(75) Erfinder/Anmelder *(nur für US)*: BERTLING, Wolf [DE/DE]; Meisenweg 22, D–91056 Erlangen (DE).

(74) Anwalt: GASSNER, Wolfgang; Nägelsbachstrasse 49a, D–91052 Erlangen (DE).

(81) Bestimmungsstaaten: CA, JP, US, europäisches Patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

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(54) Title: METHOD AND DEVICE FOR IDENTIFYING A TAG

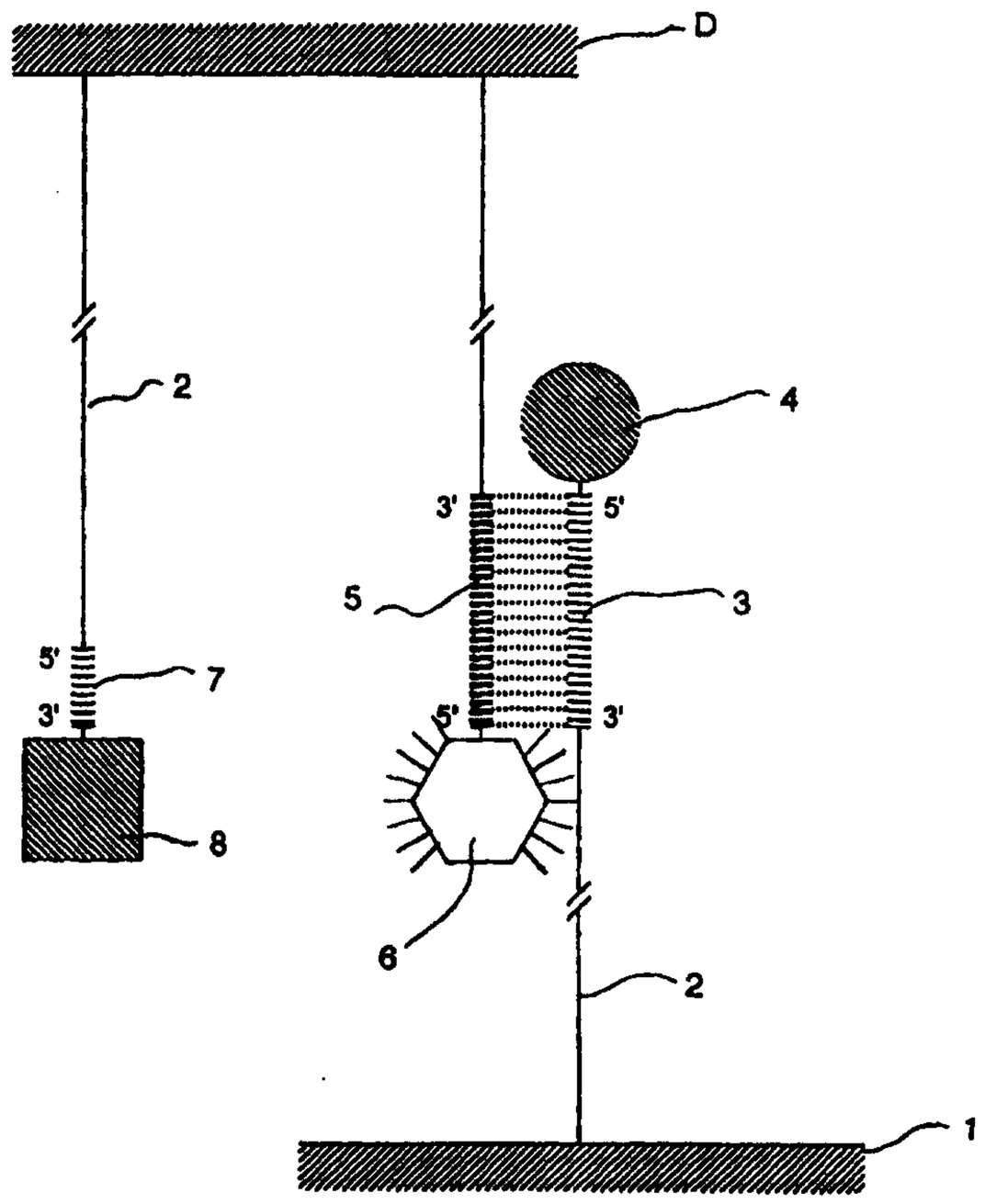
(54) Bezeichnung: VERFAHREN UND VORRICHTUNG ZUM IDENTIFIZIEREN EINER MARKIERUNG

(57) Abstract

The invention relates to a method for identifying a tag. Said tag is provided on a solid (1) and has a first nucleotide sequence (3). The first nucleotide sequence (3) is brought into contact with a detection agent which has a second nucleotide sequence (5) corresponding to the first. The second nucleotide sequence (5) is bound by its first end to a solid phase (D) and has a first flurophore group (6) at its second end.

(57) Zusammenfassung

Die Erfindung betrifft ein Verfahren zum Identifizieren einer an einem Festkörper (1) vorgesehenen eine erste Nukleotidsequenz (3) aufweisenden Markierung, wobei die erste Nukleotidsequenz (3) mit einem eine zur ersten korrespondierende zweite Nukleotidsequenz (5) aufweisenden Detektionsmittel in Kontakt gebracht wird, wobei die zweite Nukleotidsequenz (5) mit ihrem ersten Ende an eine feste Phase D gebunden ist und am zweiten Ende eine erste fluorophore Gruppe (6) aufweist.



Method and device for identifying a tag

The invention relates to a method and a device for identifying a tag provided on a solid and having a first nucleotide sequence.

US 4,996,143 discloses a method in which a first and a second primer are bound at a distance of from 2 to 7 nucleotides to the nucleotide sequence to be detected. The first and the second primer are each provided with a fluorophoreic molecule. In the binding state, as a consequence of the Förster effect there is a nonradiative energy transfer from one fluorophoreic molecule to the other. This causes a specific fluorescence.

US 5,607,834 discloses the use of a primer with a hairpin loop for detecting a nucleotide sequence. This entails a fluorophoreic molecule and a quencher being provided oppositely on the loop sections of the hairpin loop. The distance between the fluorophoreic molecule and the quencher make [sic] a nonradiative, fluorescence-quenching energy transfer possible. However, when the primer hybridizes with a complementary strand, the hairpin loop is opened. The fluorescence-quenching spatial relation between the fluorophoreic molecule and the quencher is altered. This makes it possible to observe a fluorescence.

DE 195 81 489 T1 describes a method for identifying and detecting components in a multicomponent mixture. This entails use of a marker which has at least two fluorophoreic groups which are bound to a basic framework and are in an energy-transfer relation. The known method is suitable for separation methods such as electrophoresis, chromatography or the like. It is unsuitable for identifying a tag provided on a solid phase.

The fluorescence energy transfer in hybrids between fluorophore-labeled oligonucleotides is described from Cardullo, R.A. et al.; Detection of nucleic acid hybridization by nonradiative fluorescence resonance energy transfer; Proc. Natl. Acad. Sci. USA 85 (1988) 8790-8794. Use of the fluorescent energy transfer for identifying tags is not mentioned therein.

The known methods are not particularly sensitive. They can be used only in solution. They are unsuitable for simple and rapid identification of nucleotide sequences bound to a solid.

It is an object of the present invention to eliminate the advantages of the prior art. It is particularly intended to indicate a method and a device with which simple and rapid identification of a tag provided on a solid and having a first nucleotide sequence is possible. It is particularly intended that the identification be possible in the dry state, that is to say without preparing a solution.

This object is achieved by the features of Claims 1 and 10. Expedient embodiments are evident from the features of Claims 2 to 9 and 11 to 22.

The invention provides a method for identifying a tag provided on a solid and having a first nucleotide sequence, wherein the first nucleotide sequence is brought into contact with a detection means having a second nucleotide sequence corresponding to the first, wherein the second nucleotide sequence is bound by its first end to a solid phase and has on the second end a first fluorophoreic group. - This makes simple and rapid identification of a nucleotide sequence provided on a solid possible. Preparation of a solution is unnecessary for this.

According to one embodiment feature, the first and the second nucleotide sequence hybridize on being brought into contact. This may advantageously entail releasing the first nucleotide sequence from an existing hybridization with a third nucleotide sequence. A fluorescence reaction can be induced on hybridization of the first and second nucleotide sequence. This makes simple identification possible.

According to another embodiment feature, the first nucleotide sequence can, on being brought into contact, be moved by the action of a magnet toward the first detection means. The bringing into contact can also be promoted by applying a gel or a, preferably viscous, liquid to the detection means and/or the tag.

A protective layer covering the first nucleotide sequence is expediently removed before the being brought into contact, in which case the tag is advantageously heated to a temperature of more than 50°C. After the being brought into contact, the tag is expediently cooled to a temperature of less than 50°C.

The invention further provides a device for identifying a tag provided on a solid and having a first nucleotide sequence using a detection means having a second nucleotide sequence corresponding to the first, wherein the second nucleotide sequence is bound by its first end to a solid phase and has on the second end a first fluorophoreic group. Such a device makes simple and rapid identification of a nucleotide sequence provided on a solid possible. Preparation of a solution is unnecessary for this.

It is advantageous for a first end of the first nucleotide sequence to be bound via spacer molecules

covalently to the solid. Such a tag is particularly durable.

A magnetic means is expediently bound to a second end of the first nucleotide sequence. This may be a magnetic plastic, Fe_2O_3 particles or the like. The size of the magnetic means is preferably from 20 nm to 200 nm. It is also possible to use plastics which are superparamagnetic from a chain length of 4 monomers onward. Magnetic means produced from such plastics may have a size of a few Angström [sic].

According to another embodiment feature, the first end of the second nucleotide sequence is bound via spacer molecules to the solid phase. The detection means may have a third nucleotide sequence which is designed to correspond at least in sections to the second nucleotide sequence. A first end of the third nucleotide sequence can likewise be bound via spacer molecules to the solid phase.

The spacer molecules preferably have a length of from 0.2 to 1 μ. The solid phase of the detection means may be produced from a transparent material, for example plastic, glass or quartz. A further possibility is to provide a layer produced from water-binding substances, for example polyethylene glycol, glycerol or the like, on the surface of the solid phase.

It is expedient to provide a second fluorophoreic group or a quencher on a second end of the third nucleic acid. The second and the third nucleic acid sequences are expediently in the hybridized state for the identification so that an interaction develops between the first fluorophoreic molecule and the quencher. The interaction which develops causes either a quenching of a fluorescence of a first fluorophoreic molecule or else a particularly strong fluorescence reaction as a

consequence of a direct or nonradiative energy transfer from the first to the second fluorophoreic molecule or vice versa. As soon as the spatial relation between the first fluorophoreic molecule and the second fluorophoreic molecule or the quencher is changed it is possible to observe a change in the fluorescence. The change in the fluorescence serves to identify the first nucleotide sequence.

It has proven particularly advantageous for the first, second and the third nucleic acid sequence to be formed from a deoxyribonucleic acid (=DNA) or peptic nucleic acid (=PNA). Other, in particular nuclease-resistant nucleic acid derivatives are likewise conceivable.

The detector means may further have a light source and/or a fluorescence detector. It may further be provided with a unit for generating a heat pulse, preferably an IR laser, NMR, microwave or ultrasonic pulse. It is possible in this way briefly to heat the tag.

Examplary embodiments of the invention are explained in detail by means of the drawing. These show in

Fig. 1 a diagrammatic representation of a tag,

Fig. 2 a diagrammatic view of part of a detector,

Fig. 3 a-c the effect of a magnetic field on a tag shown in Fig. 1,

Fig. 4 a diagrammatic view of part of the surface of the detector,

Fig. 5 the tag shown in Fig. 1 in contact with the detector shown in Fig. 3,

Fig. 6 a-c the detection process,

Fig. 7 a, b a second tag in the nonhybridized and in the hybridized state,

Fig. 8 a, b a third tag in the nonhybridized and in the hybridized state,

Fig. 9 a, b a fourth tag in the nonhybridized and in the hybridized state,

Fig. 10 a, b a fifth tag in the nonhybridized and in the hybridized state,

Fig. 11 the fluorescence of a detector nucleotide (1) without tag nucleotide and (2) with tag nucleotide,

Fig. 12 a a slide coated with tag nucleotide and

Fig. 12 b the tag nucleotide shown in Fig. 12 a in the hybridization state with a detector nucleotide.

Fig. 1 shows a first tag in the nonhybridized state. A spacer molecule 2 is covalently bound to the surface of a solid 1, for example a banknote. The spacer molecule has a length of from 5 to 10,000 nm. The 3′ end of a PNA 3 is bound to the free end of the spacer molecule 2. This PNA consists of about 10 to 50 bases. A magnetizable particle 4 is bound to the 5′ end of the PNA 3. This may be a polymer group with a diameter of from 20 to 200 nm.

Fig. 2 shows a diagrammatic view of part of a detector. A second PNA 5 is bound via the spacer molecule 2 to a solid phase D which is made of plastic. A first

fluorophoreic group 6 is bound to the 5′ end of the second PNA 5. A third PNA 7, which is designed to correspond in sections to the second PNA 5, is bound by its 5′ end via another spacer molecule 2 to the solid phase D. A second fluorophoreic molecule or a quencher 8 is bound to the 3′ end of the third PNA 7.

The first fluorophoreic molecule 6 may be an acceptor group and the second fluorophoreic molecule may be a donor group. The acceptor group may be a 6-carboxy-tetramethyl-rhodamine and the donor group may be a 6-carboxy-fluorescein. Further suitable donor/acceptor pairs are evident from the table below:

Donor	Acceptor
Fluorescein	Fluorescein
Fluorescein	Tetramethylrhodamine
IAEDANS = (5-((((2-iodo-acyl)amino)ethyl)amino)-naphathalene-1sulfonic acid	Fluorescein DABCYL (4-dimethylaminoazobenzen-4′-sulfoylochloride)
EDANS (5-((2-aminomethyl)-amino)naphthalene-1-sulfonic acid	DABCYL (4-dimethylaminoazo-benzen-4′-sulfoylochloride)
BODOPY FL	BODIPY FL

It is, of course, possible to interchange the first and the second fluorophoreic molecule. According to another embodiment feature, the second fluorophoreic molecule may be replaced by a quencher 8.

Suitable quencher/fluorophore pairs are evident from the following table:

Quencher	**Fluorophore**
DABCYL	Coumarin
DABCYL	EDANS
DABCYL	Fluorescein

DABCYL	Lucifer yellow
DABCYL	Bodipy
DABCYL	Eosin
DABCYL	Tetramethylrhodamine
DABCYL	Texas red
DABCYL	Erythrosin

Fig. 3 a to c show the behavior of the tag under the influence of a magnetic field. When an appropriately oriented magnetic field is applied, the magnetic particles 4 are moved away from the surface of the solid 1.

Fig. 4 again shows the surface of the detector shown in Fig. 2. It is evident from this that on the surface of the solid phase D third PNA 7 provided with second fluorophoreic molecules or quenchers 8 are bound in excess via spacer molecules 2 to the surface. This ensures that, in the case where a quencher 8 is used - the fluorescence of the first fluorophoreic molecule 6 is quenched as long as the detector is not in contact with a first PNA 3 designed to correspond to the second PNA 5.

Fig. 5 shows the tag shown in Fig. 1 in contact with the detector shown in Fig. 3. The first PNA 3 hybridizes with the second PNA 5 of the detector. The third PNA 7 is separated from the second PNA 5. There is no longer an interaction between the first fluorophoreic molecule 6 and quencher 8. This elicits a fluorescence reaction. In the case where a second fluorophoreic molecule is used it is possible to observe an altered fluorescence. The binding between second PNA and the third PNA 7 can be loosened by increasing the temperature before bringing the tag into contact with the detector. This is done by briefly heating the tag, for example using an IR laser pulse.

Fig. 6 a to c again show diagrammatically the detection process. Firstly, as shown in Fig. 6 a, the detector is brought near the tag. The tag is subsequently heated briefly and a magnetic field is applied. This moves the first PNA 3 toward the solid phase D. The PNA 3 hybridizes with the second PNA 5 which has been released from the third PNA 7 by the heat pulse. The hybridization results in a fluorescence reaction. After detection thereof by a suitable fluorescence detector, the magnetic field is reversed where appropriate and thus the detection process is terminated.

Fig. 7 a and b show a second embodiment of a 20 [sic] detector. In this case, the second PNA 5 and the third PNA 7 are provided on one and the same nucleotide strand. Hybridization of the second PNA 5 with the third PNA 7 leads to formation of a loop. The first fluorophoric molecule 6 [lacuna] the quencher 8 develop an interaction. In the example shown in Fig. 8 a, the second PNA 5 is provided on one branch and the third PNA 7 is provided on another branch of a branched molecule. Fig. 9 a once again shows the previously described design of the detector in which the second PNA 5 and the third PNA 7 are bound on two separate strands to the solid phase D.

Fig. 7b, 8b and 9b each show the hybridization state.

Fig. 10a shows another examplary embodiment. In this case, the second PNA 5 is directly bound by its 3′ end to the solid phase D. The first fluorophoreic molecule 6 is located at the 5′ end. The third PNA 7 is bound by its 5′ end directly to the solid phase D. In this case, a second fluorophoreic molecule 8 is located at the 3′ end of the third PNA 7. In the case of hybridization, the second PNA 5 and the third PNA 7 bind to the first PNA 3 in such a way that a spatial relation between the

first 6 and the second fluorophoreic molecule 8 results. In this examplary embodiment, according to the Förster effect, there is a nonradiative transfer of energy from the second 8 to the first fluorophoreic molecule 6 and thus an enhanced fluorescence reaction.

Example 1:

Design and synthesis of a tag nucleotide and a detector nucleotide

An oligonucleotide consisting of 32 nucleotides and having the following sequence is synthesized as tagging means:

5'-CCAAGC CTGGAGGGATGATACTTT GCGCTTGG-3'

An oligonucleotide consisting of 32 nucleotides and complementary to the sequence of the tagging means or nucleotide is synthesized as detection means:

3'-X-GGTTCG GACCTCCCTACTATGAAACG CGAACC-6FAM-5'

X = dT(C2-DABCYL)

The synthesis mixture comprises 0.2 µmol. The oligonucleotides are purified by HPLC.

The six terminal nucleotides within an oligonucleotide are complementary to one another. It would therefore be possible for these nucleotides to undergo folding back.

GACCTCCCT

3'-X-GGTTCG A

5'-6FAM-CCAAGC C

GCAAAGTAT

In this folding back, the 5'-6FAM group on the detector nucleotide is in the immediate neighborhood of the 3'-DABCYL group. With appropriate excitation of the 6FAM group (absorption maximum 496 nm, emission maximum 516 nm come [sic]), nonradiative energy transfer of the absorbed energy to the DABCYL group can occur. The fluorescence of the 6FAM group is greatly reduced thereby in this state.

When the detector means or nucleotide is brought together with the tag nucleotide there is hybridization of the complementary sequences of the two molecules:

5'-CCA AGC CTG GAG GGA TGA TAC TTT GCG CTT GG-3'
3'-X-GGT TCG GAC CTC CCT ACT ATG AAA CGC GAA CC-6FAM-5'

In the hybridized structure, the 5'-6FAM is spatially separate from the 3'-X group of the detector nucleotide. This means that energy transfer between the 6FAM and the DABCYL group is no longer possible. With appropriate excitation of the 6FAM group, accordingly, there is unimpaired fluorescence of the 6FAM group.

To detect a fluorescence energy transfer, detector nucleotide and tag nucleotide are dissolved in 10 mM tris-Cl, 1 mM EDTA, pH 8 (TE). To 100 µl of a 1 µM solution of the detector nucleotide are added the same volume of a 2 µM solution of the tag nucleotide or the same volume of TE. To detect the hybridization, the fluorescence of the solution is determined with excitation at 496 nm and emission at 516 nm. Mixing the nucleotides causes the fluorescence to increase by a factor of about nine (Fig. 11). The increase in the fluorescence shows the augmentation of the intramolecular fluorescence energy transfer of the detector nucleotide because of a hybridization of detector nucleotide and tag nucleotide.

Example 2:
Determination of detection sensitivity of hybrids of detector nucleotides and tag nucleotides

To determine the detection sensitivity of hybrids of detector nucleotides and tag nucleotides, the fluorescence of a decreasing concentration series of hybrids of tag nucleotide and detector nucleotide is measured. This is done by placing drops, each with a volume of 1 µl, of a 1 µM solution of the tag nucleotide on a slide. The same volume of a decreasing concentration series of the detector nucleotide is added to each drop. The concentrations of detector nucleotide are 1000, 500, 200, 100, 50, 20, 10, 5, 0 nM. To determine the background, the same concentration series of the detector nucleotide are added to 1 µl portions of TE. The samples are examined under a fluorescence microscope using a FAM-specific filter block with 500 × magnification.

It was still possible to detect a specific fluorescence owing to the hybridization of detector nucleotide and tag nucleotide after addition of a detector nucleotide solution with a concentration of 5 nM to the tag nucleotide solution. Without tag nucleotide, the fluorescence of the detector nucleotide was visible as far as a concentration of 50 nM.

Example 3:
Hybridization of tag nucleotide and detector nucleotide in the dry state

To detect a hybridization of tag nucleotide and detector nucleotide in the dry state, a 1 µm solution of the tag nucleotide in TE is prepared. In addition, 4,6-diamidino-2-phenylindoles (DAPI) in a concentration of 2ng/µl is added to the solution for visualization of the tag nucleotide. DAPI is a dye which binds DNA and

fluoresces in the bound state. The fluorescence of DAPI can be separated by appropriate filters from the fluorescence of the FAM group and be detected.

1 µl of the tag nucleotide solution is applied with a 2 µl pipette in the form of lines to a polished slide. The liquid is dried at room temperature. Fig. 12 a shows a slide coated in this way. The tag nucleotide is evident in the form of the pale areas. On a second slide, 5 µl of a 20 nM solution of the detector nucleotide is applied on an area of about 40 mm^2 and likewise left to stand at room temperature until dry. The slides are then laid one on top of another with the sides carrying the tag nucleotides and pressed together with a pressure of about 400 N/cm^2 for one minute. The slides are then examined under a fluorescence microscope with 500 × magnification. The applied tag nucleotide can be detected on the basis of the DAPI stain. The detector nucleotide is undetectable in the nonhybridized state. In the hybridized state, the detector nucleotide can be detected on the basis of the FAM-specific fluorescence. The fluorescence of the detector nucleotide can be observed only in the region of the applied tag nucleotide (Fig. 12 b). The observed fluorescence of the detector nucleotide in the region of the applied tag nucleotide is a demonstration of the hybridization of the tag nucleotide with the detector nucleotide.

List of reference numbers

1.	Solid
2	Spacer molecule
3	First PNA
4	Magnetic group
5	Second PNA
6	First fluorophoreic molecule
7	Third PNA
8	Second fluorophoreic molecule or quencher
D	Solid phase

Patent Claims

1. Method for identifying a tag provided on a solid (1), which tag has a first nucleotide sequence (3) bound to the solid,

wherein the first nucleotide sequence (3) is brought into contact with a detection means,

wherein the detection means has a solid phase (D) to which a second nucleotide sequence (5) corresponding to the first nucleotide sequence (3) is bound by its first end and wherein the second nucleotide sequence (5) has on the second end a first fluorophoric group (6),

wherein the first (3) and the second nucleotide sequence (5) hybridize on being brought into contact,

and wherein a fluorescence reaction is induced on hybridization of the first (3) and second nucleotide sequence (5).

2. Method according to any of the preceding claims, wherein, before or on being brought into contact, the first nucleotide sequence (3) is released from a hybridization with a third nucleotide sequence (7).

3. Method according to any of the preceding claims, wherein the first nucleotide sequence (5) [sic] is, on being brought into contact, moved by the action of a magnet toward the detection means.

4. Method according to any of the preceding claims, wherein a protective layer covering the first

nucleotide sequence (3) is removed before the being brought into contact.

5. Method according to any of the preceding claims, wherein the tag is heated to a temperature of more than 50°C before the being brought into contact.

6. Method according to any of the preceding claims, wherein the tag is cooled to a temperature of less than 50°C after the being brought into contact.

7. Method according to any of the preceding claims, wherein the hybridization between the first (3) and the second nucleotide sequence (5) is assisted by the action of light of a predetermined wavelength.

8. Device for identifying a tag provided on a solid (1), which tag has a first nucleotide sequence (3) bound to the solid,

using a detection means which has a second nucleotide sequence (5) corresponding to the first nucleotide sequence (3), wherein the second nucleotide sequence (5) is bound by its first end to a solid phase (D) and has on the second end a first fluorophoric group (6),

so that the first (3) and the second nucelotide sequence (5) hybridize when the detection means comes into contact with the tag, and a fluorescence reaction can be induced on hybridization of the first (3) and the second nucleotide sequence (5).

9. Device according to Claim 8, wherein a first end of the first nucleotide sequence is bound via spacer molecules (2) covalently to the solid (1).

10. Device according to either of Claims 8 or 9, wherein a magnetic means (4) is bound to a second end of the first nucleotide sequence (3).

11. Device according to any of Claims 8 to 10, wherein the detection means comprises a magnet.

12. Device according to any of Claims 8 to 11, wherein the first end of the second nucleotide sequence (5) is bound via spacer molecules (2) to the solid phase (D).

13. Device according to any of Claims 8 to 12, wherein the detection means has a third nucleotide sequence (7) which is designed to correspond at least in sections to the first (3) or second nucleotide sequence (5).

14. Device according to Claim 13, wherein a first end of the third nucleotide sequence (7) is bound via spacer molecules (2) to the solid phase (D).

15. Device according to either of Claims 13 or 14, wherein a second fluorophoric group or a quencher is provided on a second end of the third nucleotide sequence.

16. Device according to any of Claims 13 to 15, wherein the second and the third nucleotide sequence are in the hybridized state for the identification, so that an interaction can develop between the first fluorophoric molecule and the second fluorophoric molecule or the quencher or can be interrupted.

17. Device according to any of Claims 13 to 16, wherein the first (3), second (5) and third nucleotide sequence (7) is formed from DNA or PNA.

18. Device according to any of Claims 8 to 17, wherein a layer produced from water-binding substances is provided on the surface of the solid phase.

19. Device according to any of Claims 8 to 18, wherein the detector means has a light source and/or a fluorescence detector.

20. Device according to any of Claims 18 to 19, wherein the detector means has a unit for generating a heat pulse, preferably an IR laser, NMR, microwave or ultrasonic pulse.

Fetherstonhaugh & Co.
Ottawa, Canada
Patent Agents

SEQUENCE PROTOCOLS

<110> november AG Novus Medicatus Bertling Gsellschaft [sic] für Molekulare Medium

<120> Method and device for identifying a tag

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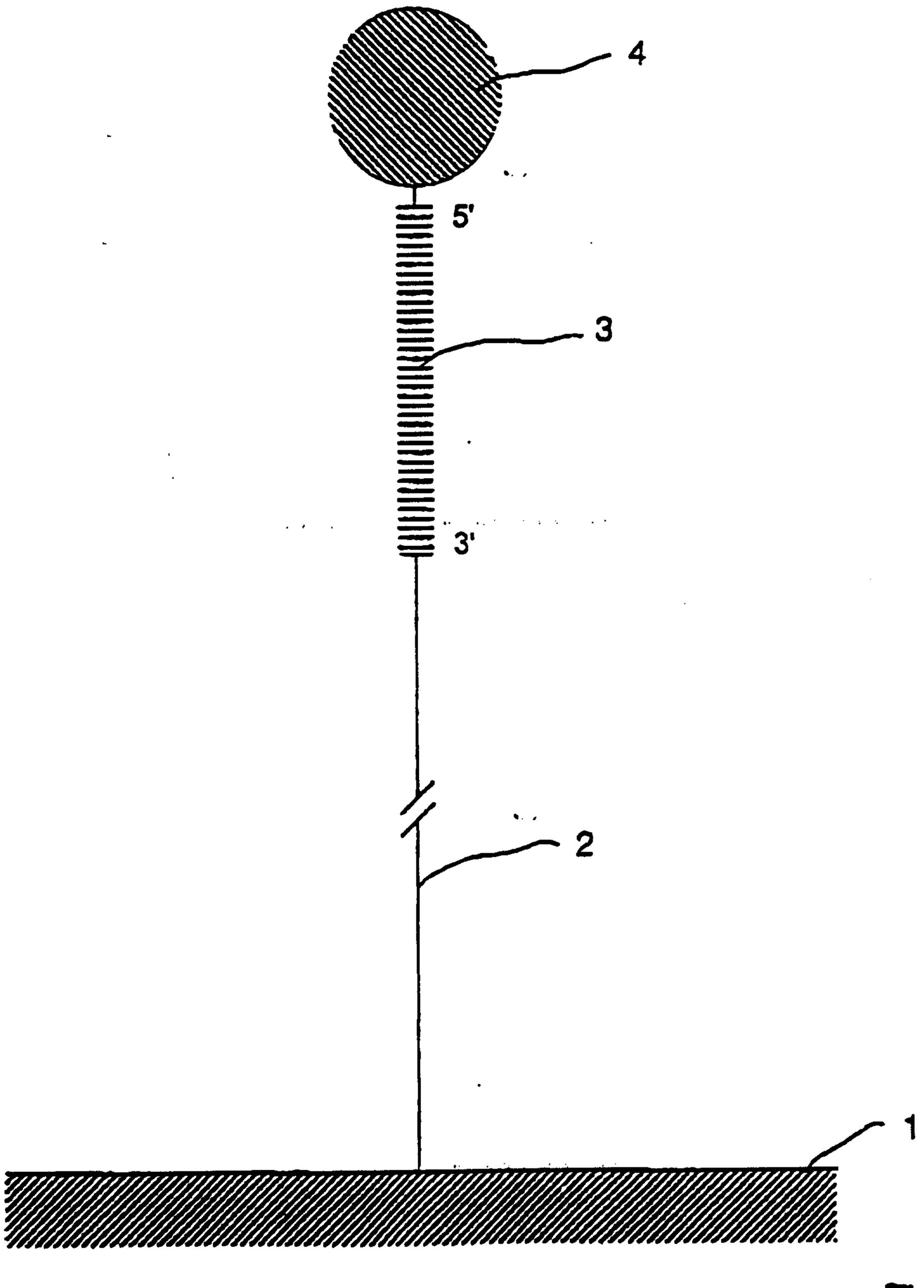


Fig. 1

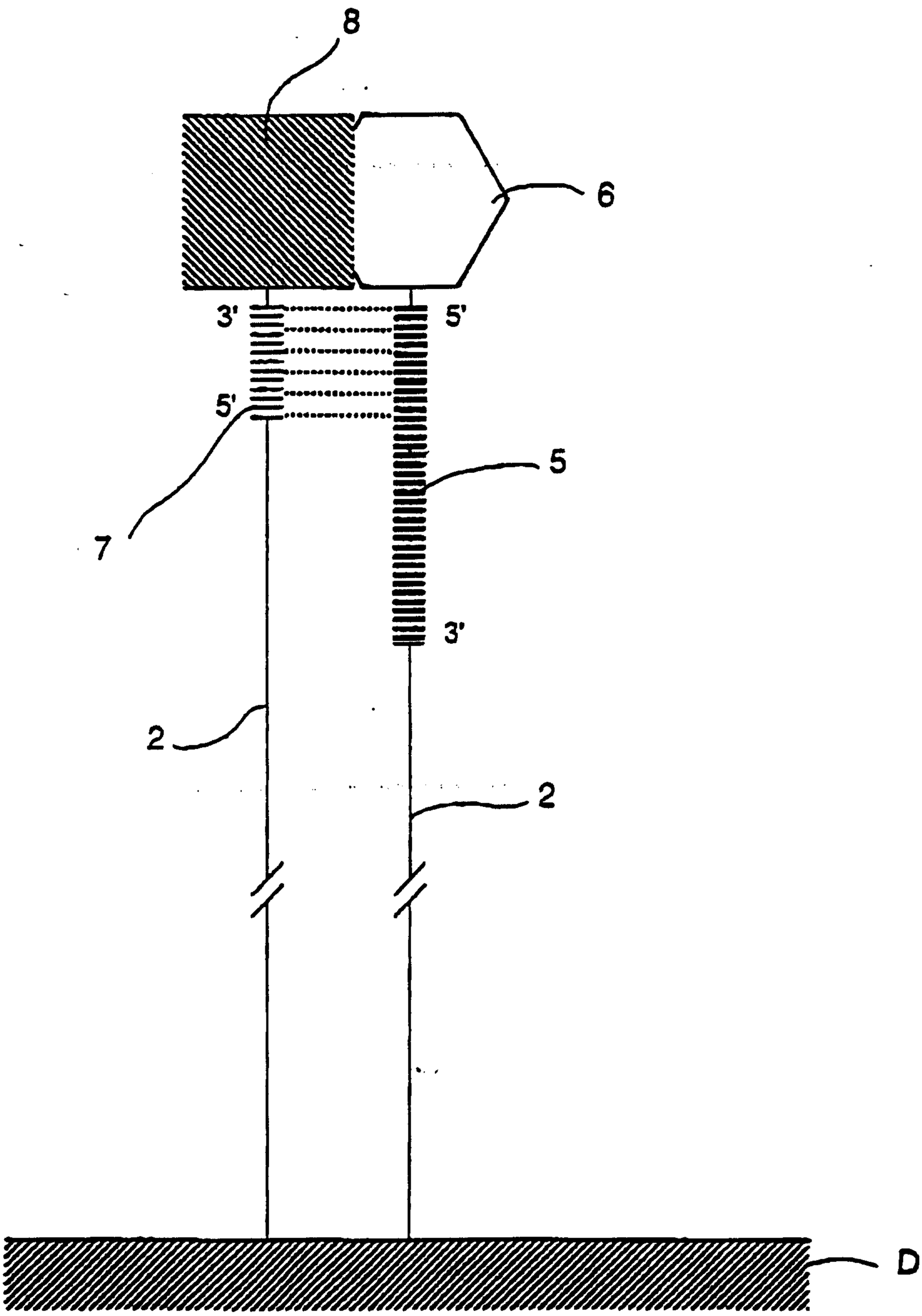


Fig. 2

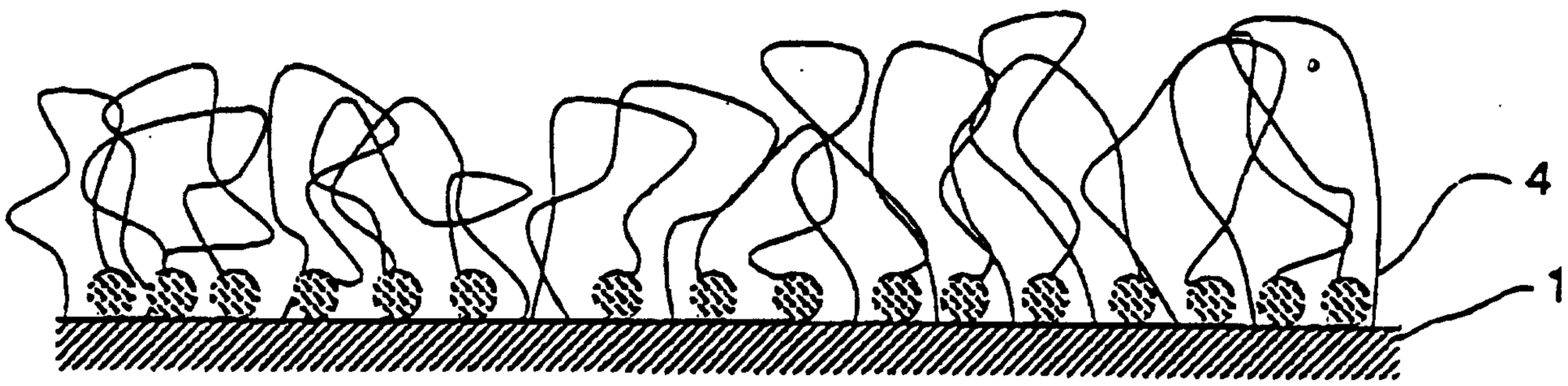


Fig. 3a

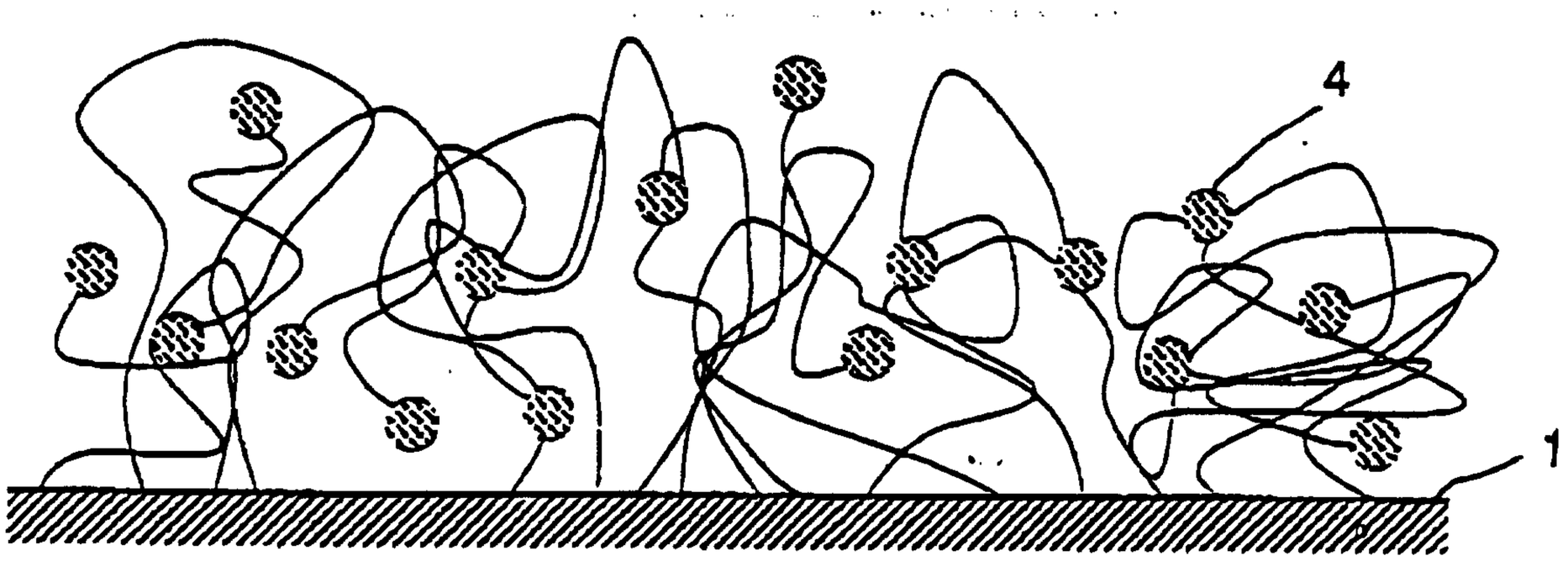


Fig. 3b

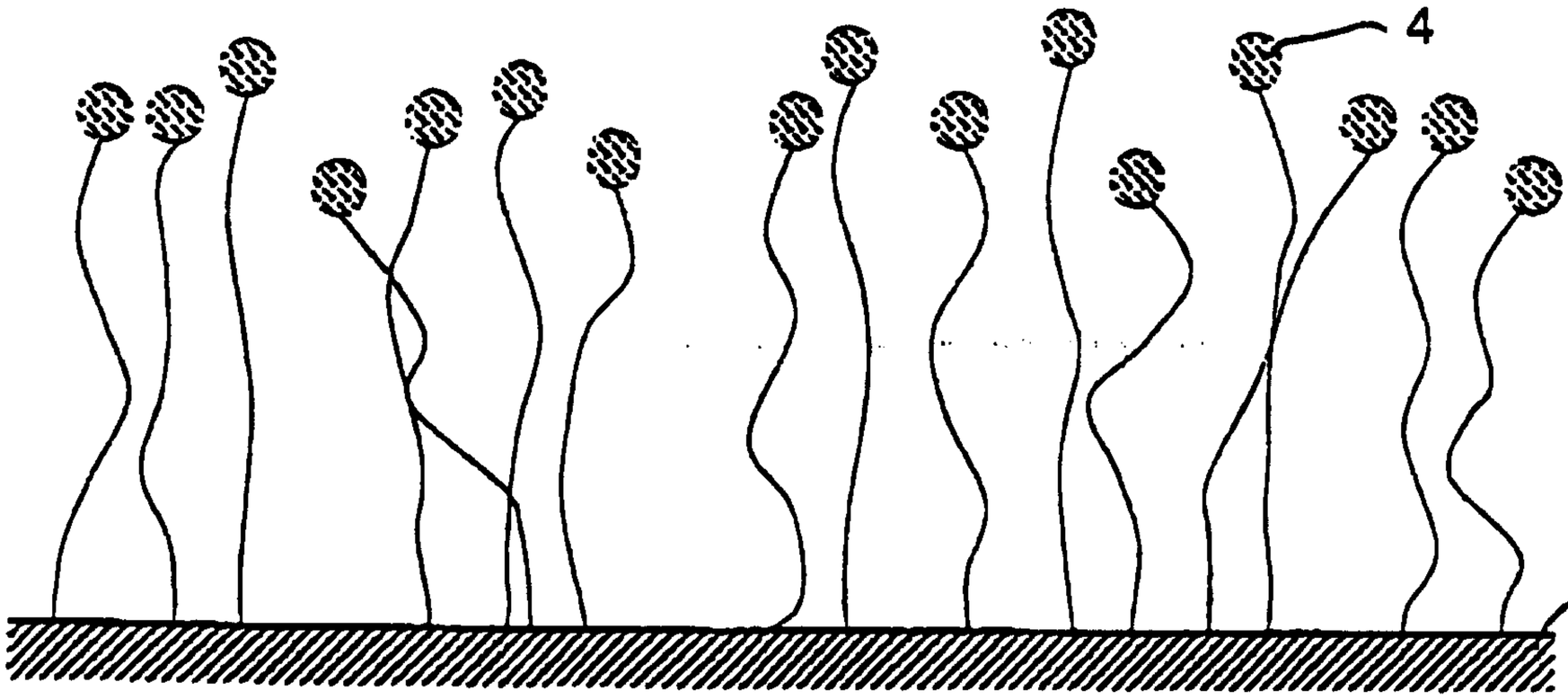


Fig. 3c

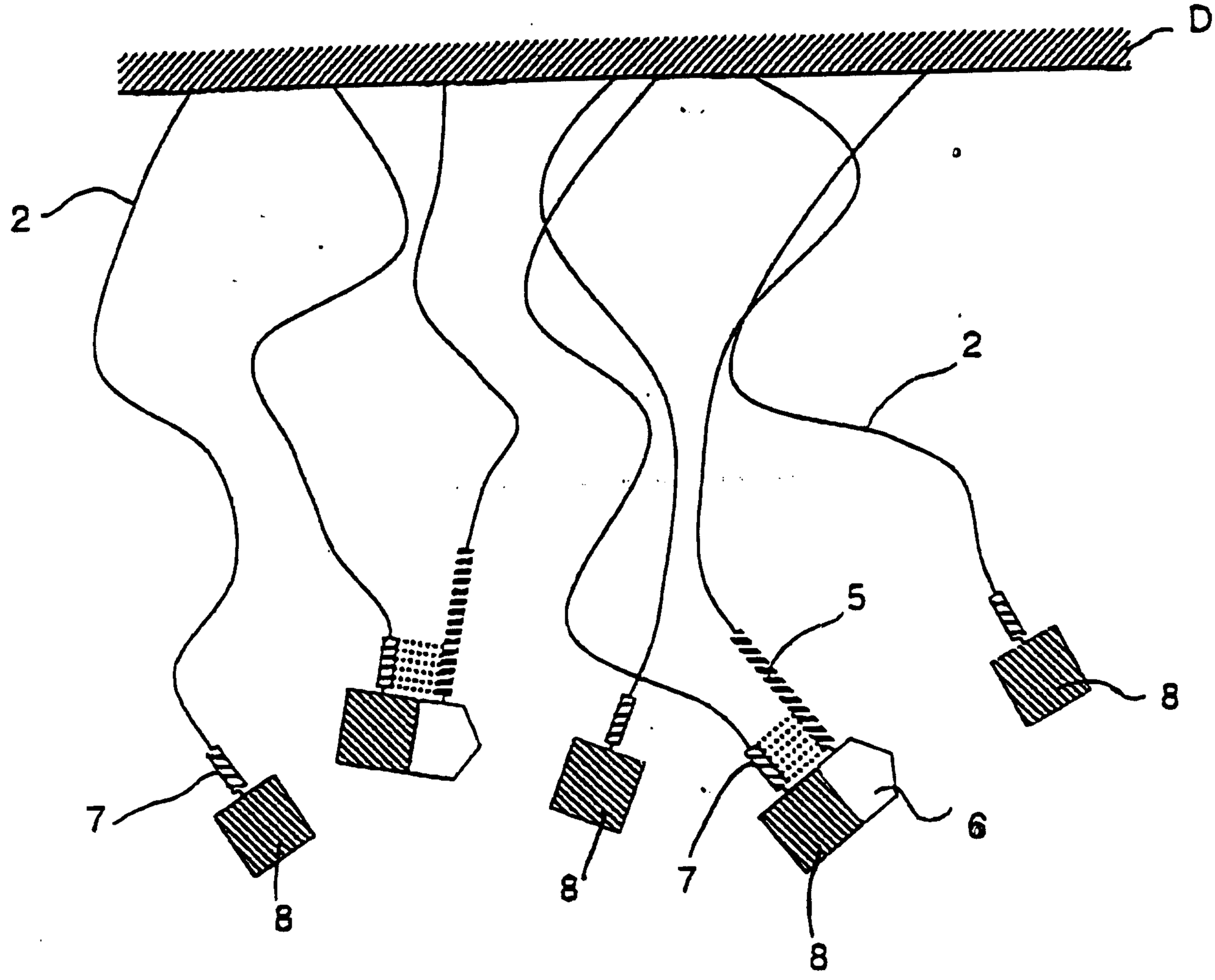


Fig. 4

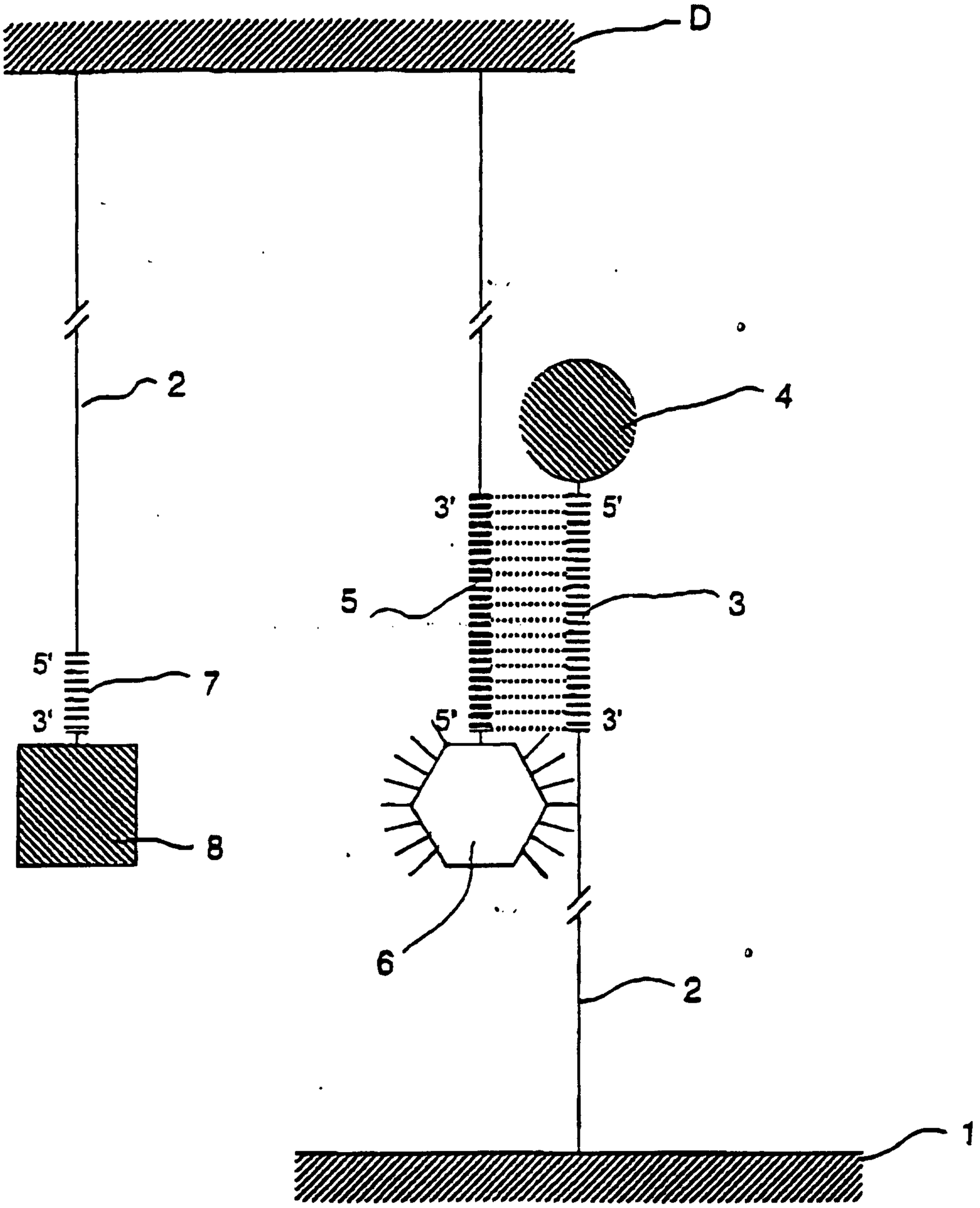


Fig. 5

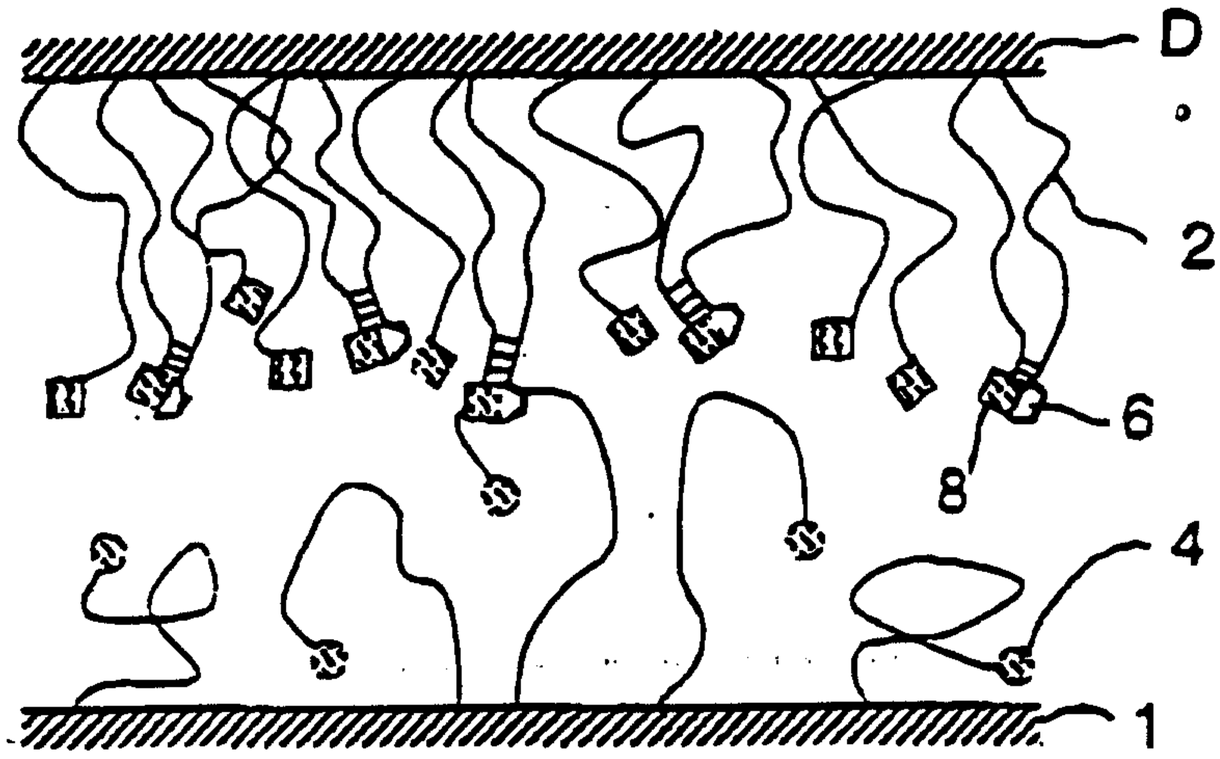


Fig. 6a

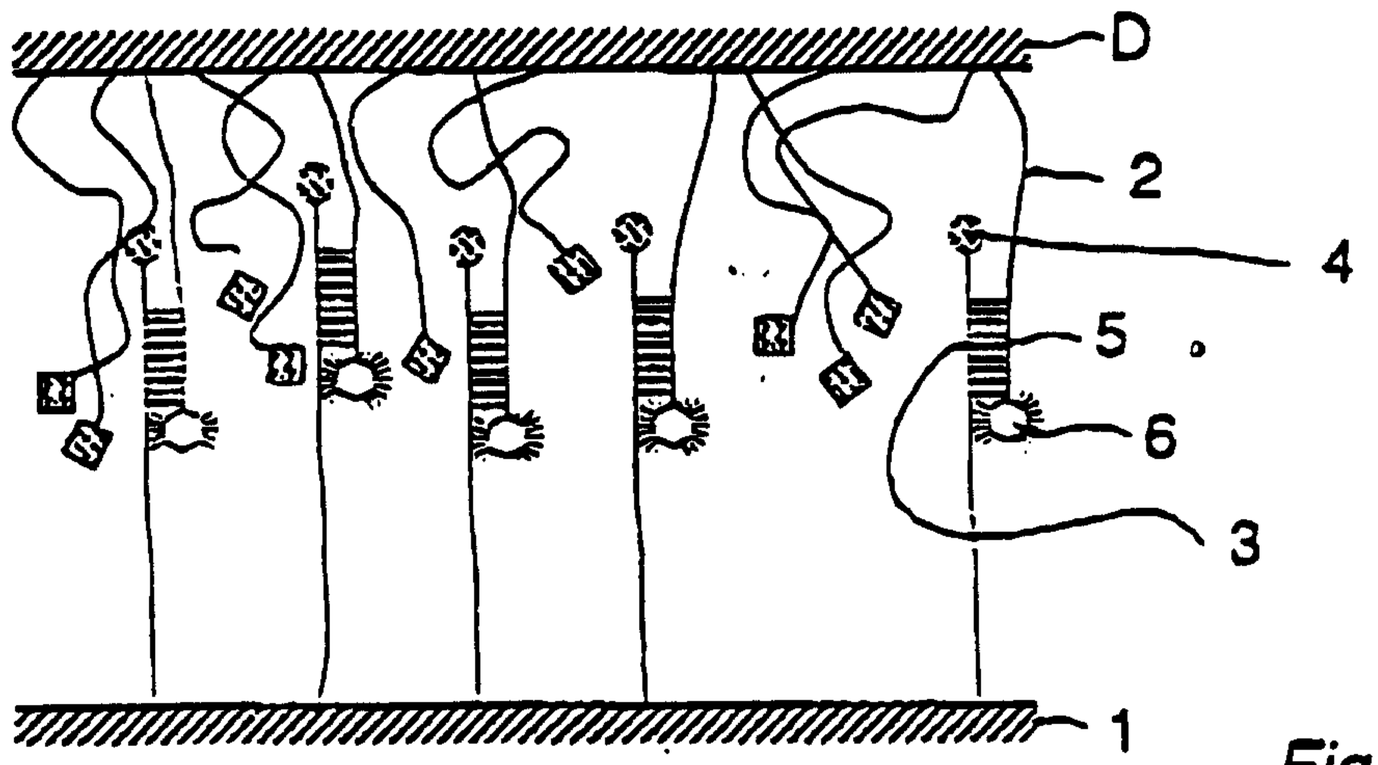


Fig. 6b

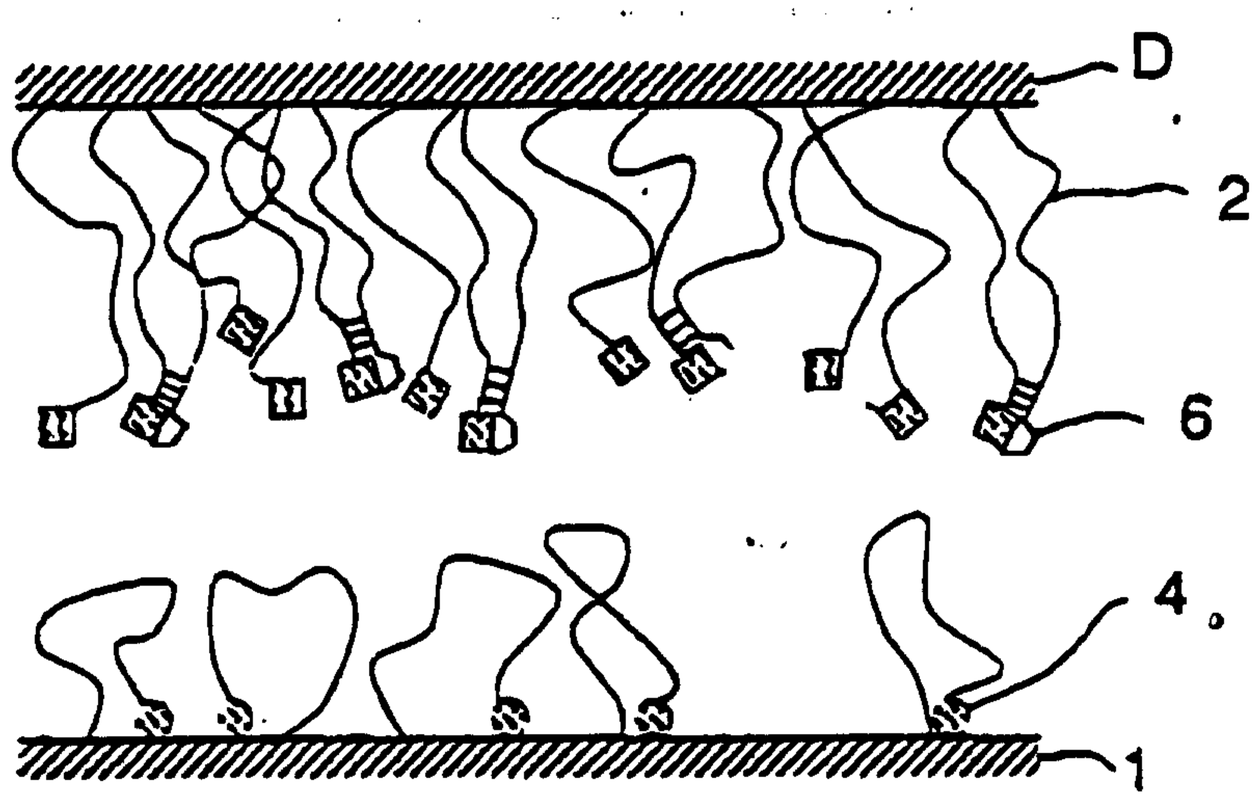


Fig. 6c

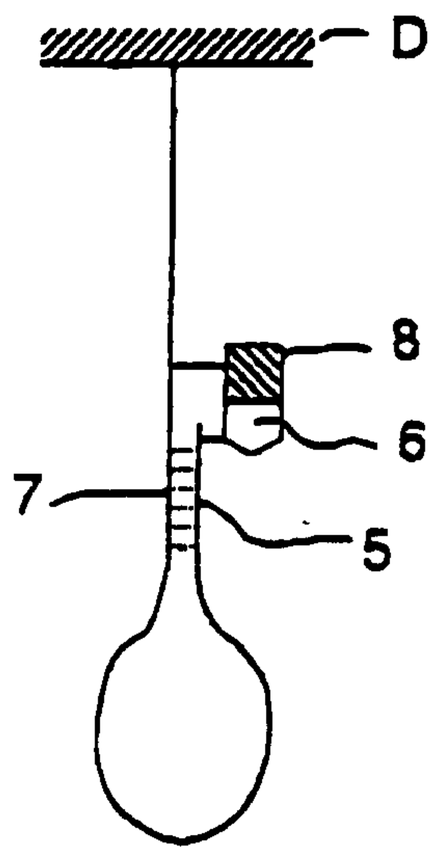


Fig. 7a

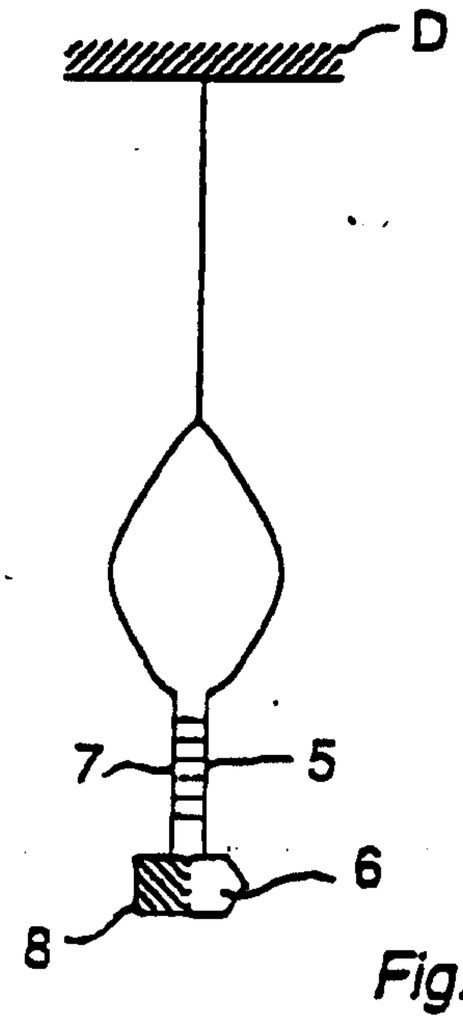


Fig. 8a

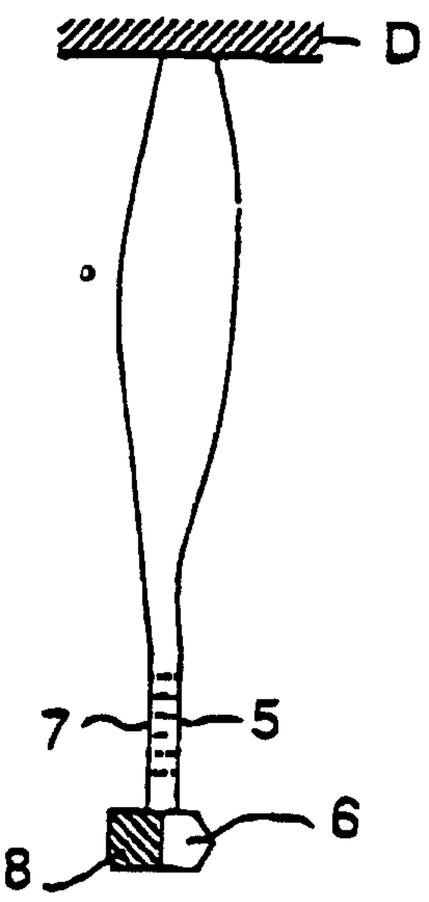


Fig. 9a

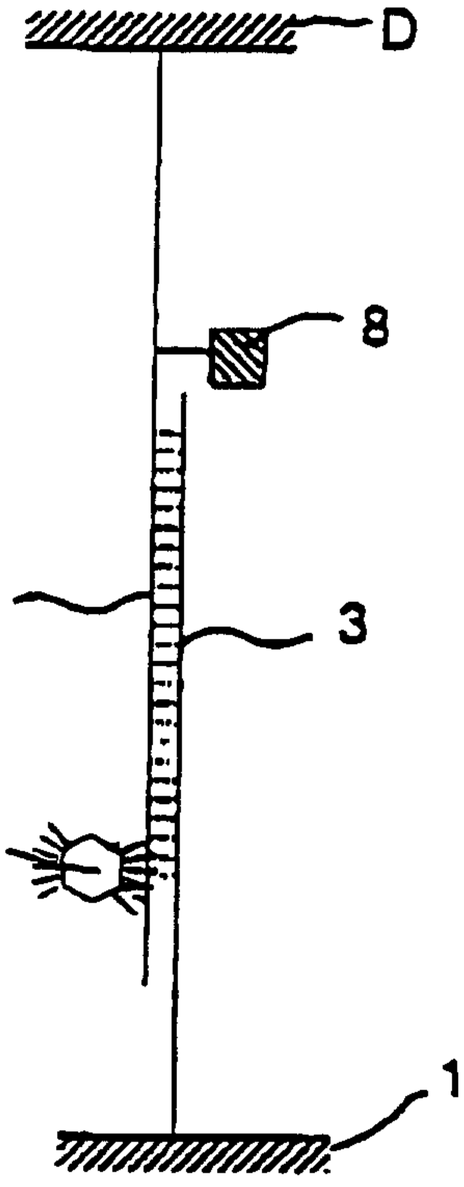


Fig. 7b

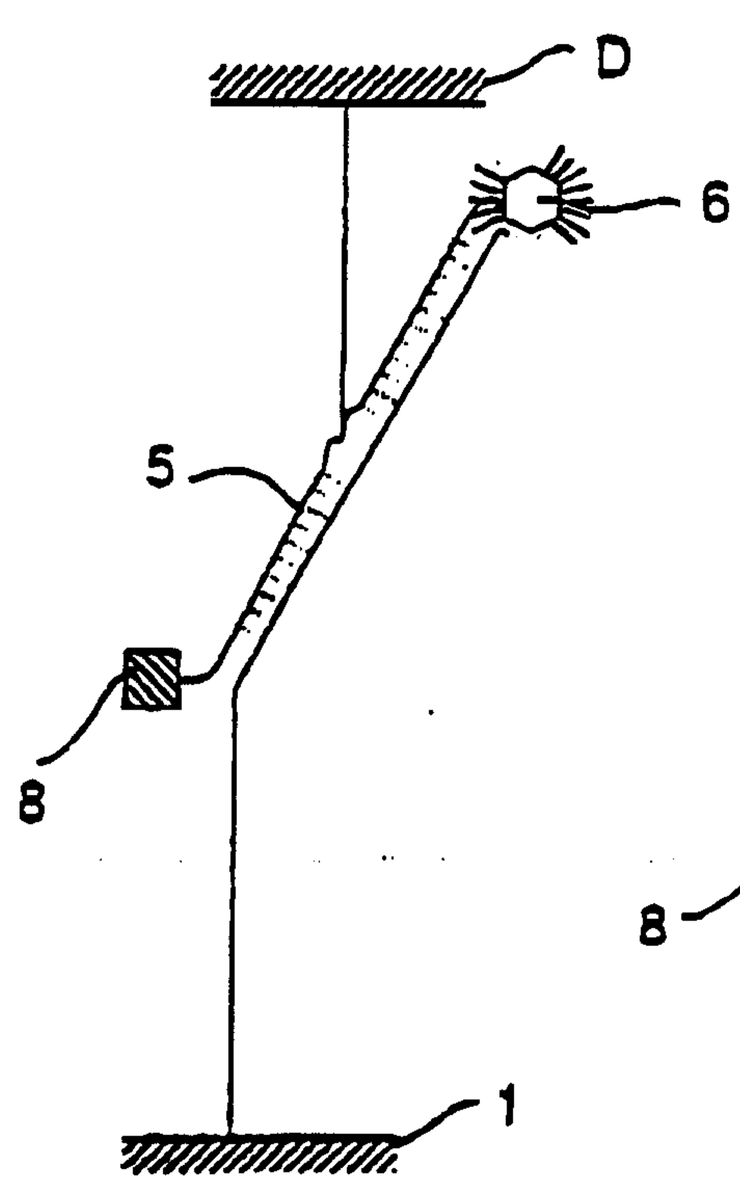


Fig.8b

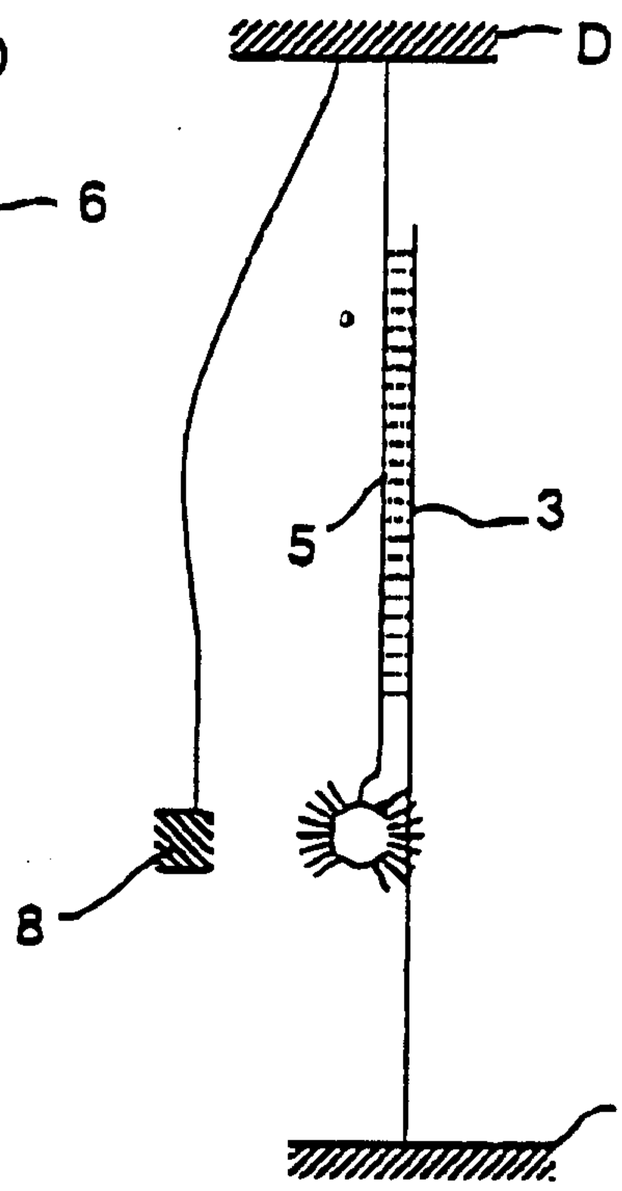


Fig.9b

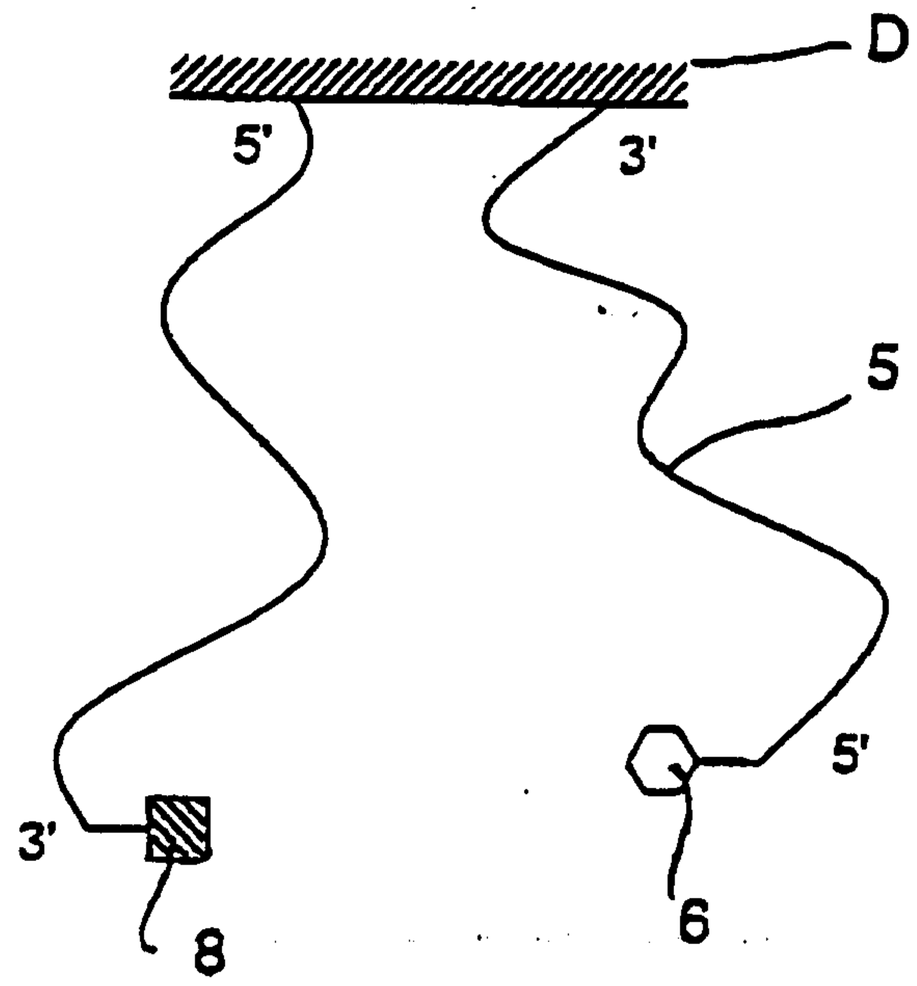


Fig. 10a

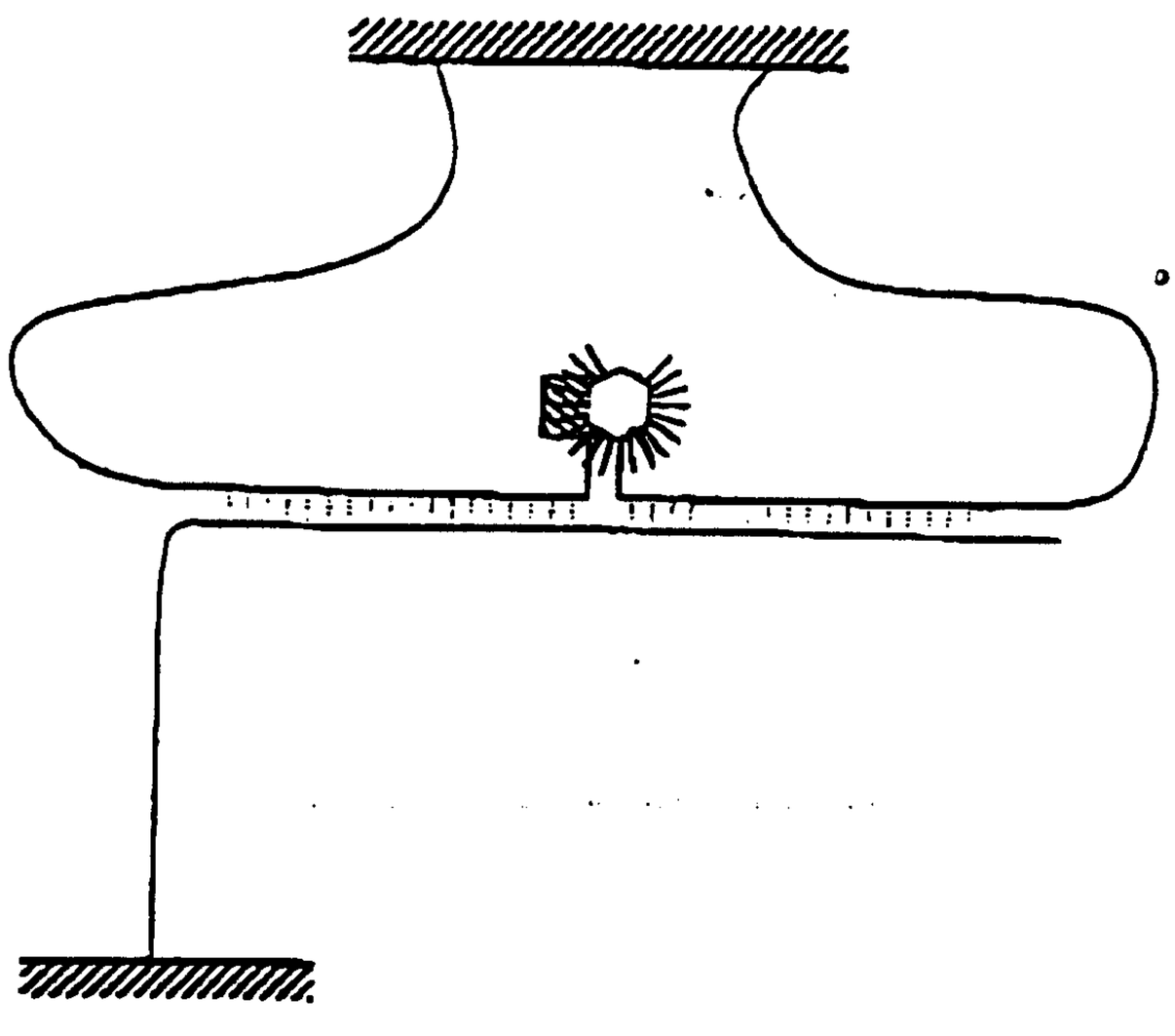
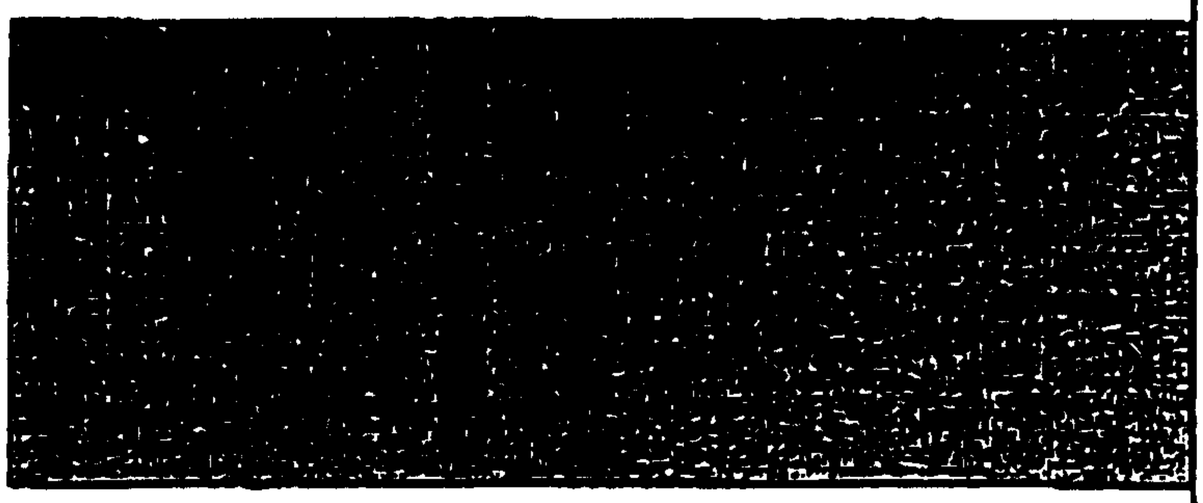


Fig. 10b

Fig. 11



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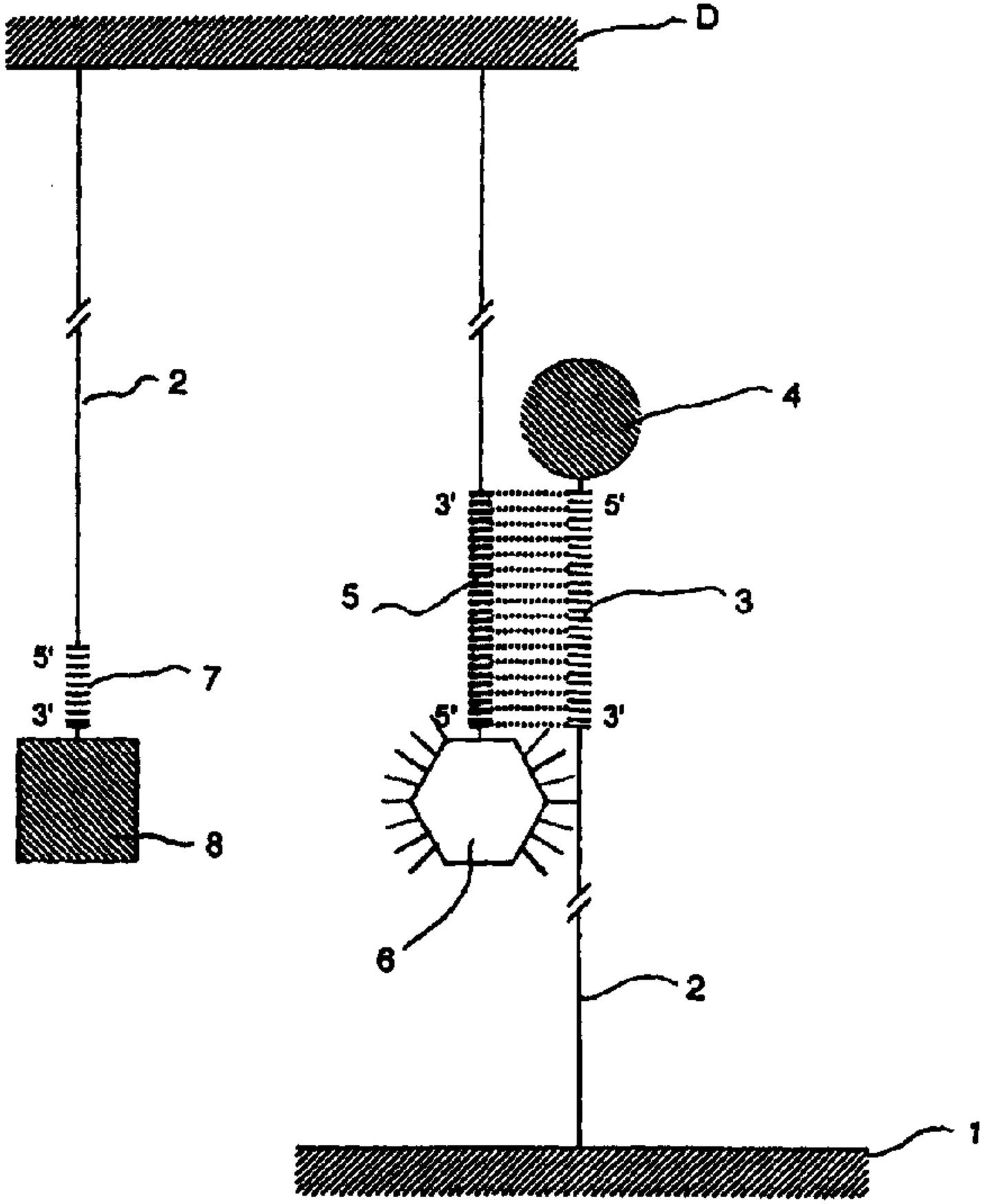
(DOCUMENT AU 10 IÈME ÉTAGE AIRE 5 DANS LA SECTION DE LA

PRÉPARATION DES DOSSIERS)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