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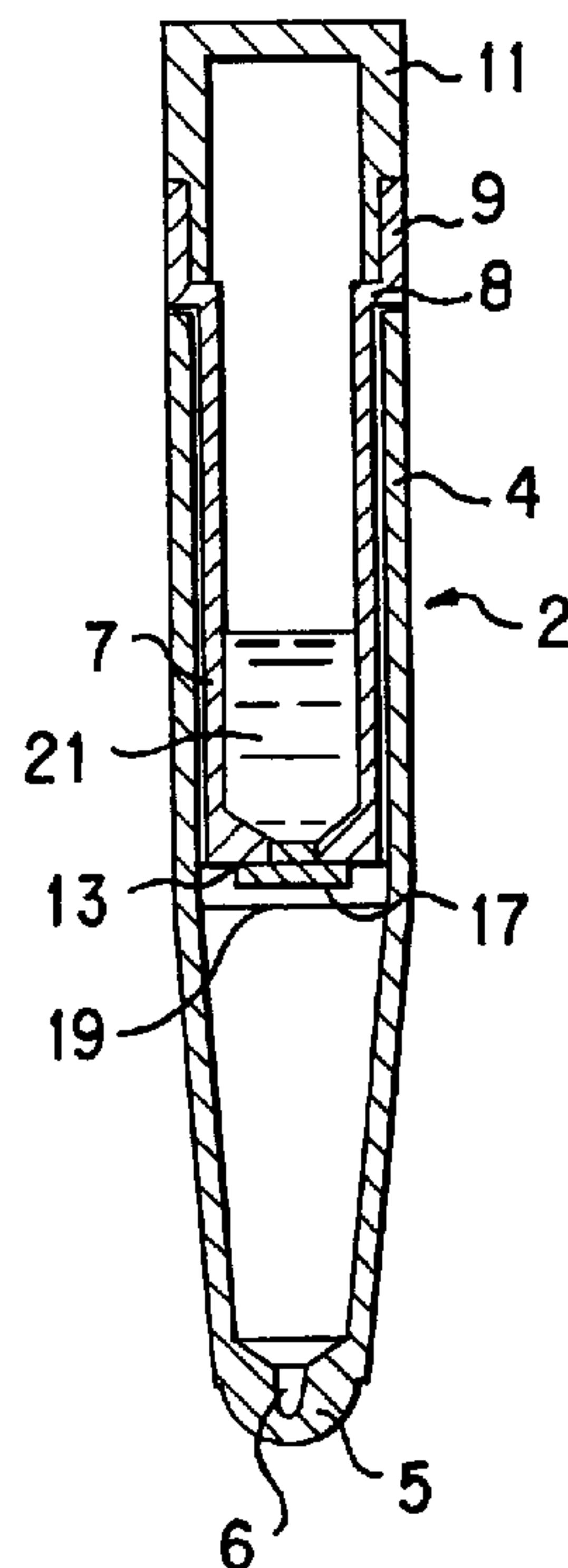
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(54) **PRELEVEMENT ORAL POUR ANALYSE IMMUNOLOGIQUE**

(54) **ORAL COLLECTION FOR IMMUNOASSAY**



(57) Procédé de prélèvement d'immunoglobulines et d'autres analytes dans la cavité orale à des fins d'analyses immunologiques et autres. Un tampon absorbant est utilisé pour prélever un spécimen présentant une teneur élevée en immunoglobulines ou autres analytes. Ce spécimen peut être soumis à une technique d'analyse immunologique de base, que l'on peut utiliser comme outil pour le dépistage de maladies chez un patient.

(57) A method for collecting immunoglobulins and other analytes from the oral cavity for immunological and other testing. An absorbent pad is used to collect a specimen having a high concentration of immunoglobulins or other analytes. The specimen can be subjected to a basic immunological testing technique which can be used as a tool for screening a patient for diseases.

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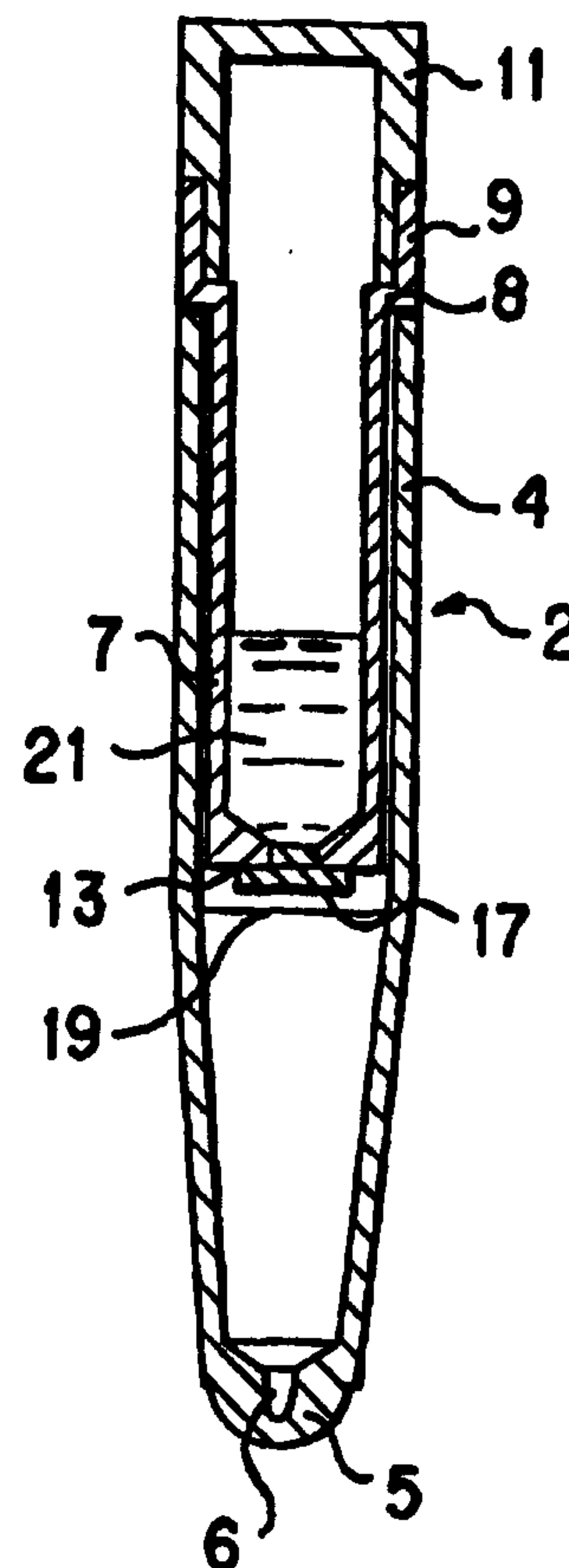
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(54) Title: ORAL COLLECTION FOR IMMUNOASSAY

(57) Abstract

A method for collecting immunoglobulins and other analytes from the oral cavity for immunological and other testing. An absorbent pad is used to collect a specimen having a high concentration of immunoglobulins or other analytes. The specimen can be subjected to a basic immunological testing technique which can be used as a tool for screening a patient for diseases.



2180025**ORAL COLLECTION FOR IMMUNOASSAY**

The present invention relates to the field of immunological testing. In particular, a system for analyzing immunoglobulins and other substances derived from mucosal transudate is disclosed.

Because of the association between immunoglobulins of the blood and saliva, as well as the occurrence of secretory IgA peculiar to salival fluid, antigen-antibody tests have been conducted on the saliva to assess the value of such tests as a screening tool for diseases.

Collection of saliva from the salivary glands is complicated by the low volumes secreted, the diverse anatomic dispersion of the glands, and the relatively high viscosity of the fluid. Most techniques for collection involve the use of capillary tubes, suction into micropipettes, chewing on paraffin or aspiration into syringes. These methods, however, are limited in that viscosity of the saliva makes the recovery of bubble-free material by these techniques difficult. Other methods of collection have been suggested to eliminate or at least reduce the quantity of bubbles in the sample. Among such methods include collecting saliva in the mouth by direct absorption with a sponge or flexible wad of osmotic membrane. After absorption, the saliva can be separated from the absorptive material by centrifugation or by compressing the absorptive material. However, absorption is generally accomplished by using cotton, nylon, or polyester as the absorptive material. These materials can non-specifically bind proteins which can result in an undesirably low recovery of immunoglobulins.

Testing of salivary specimens has not been extensively developed. In addition to problems with collection, the samples collected by the known methods typically contain about 0.01-0.1% of the immunoglobulin found in blood serum. Because of the highly reduced immunoglobulin content of saliva, it has been necessary to use more accurate antigen-antibody assay methods in screening patients for disease. Parry et al., "Rational Programme for Screening Travellers for Antibodies to Hepatitis A Virus", The Lancet, June 25, 1988, have discussed such methods

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and have found that the more accurate IgG-capture radioimmunoassay (GACRIA) test is preferable to avoid false indications which may occur in less sensitive methods. Of course, more sensitive testing procedures usually require added time and expense to achieve the test results.

The immune system of the mouth not only interacts with the general immune system of the body, but also has its own centralized center for antigen-antibody response. Within the oral cavity is found extraoral lymph nodes and intraoral lymphoid aggregations. The extraoral lymph nodes are involved in the drainage of the oral mucosa, gum and teeth. However, the function of the intraoral lymphoid tissue is little understood.

The extraoral lymph nodes include a fine network of lymph capillaries which are superficially located in the mouth, palate, cheeks, lips, gingiva, and pulp of the teeth. The capillaries join to larger lymph vessels which originate from a network deep in the muscle of the tongue and other structures. An antigen can gain entry into the oral lymphatic system directly through the capillaries or be transported there by phagocytes. Once inside the network, the antigen can induce an immune response.

Included in the intraoral lymphoid tissue are generally four distinct tissue aggregations: (a) the tonsils, (b) scattered submucosal lymphoid aggregations, (c) salivary gland lymphoid tissue, and (d) gingival lymphoid tissue.

The tonsils (palatine and lingual) primarily produce B-cells and T-cells which are generally contained within a cap of lymphocytes and plasma cells. Antigen typically gains entry into the tonsils through a distinct epithelial region wherein the antigen can come into contact with the T- and B-cells to stimulate an immune response. The predominant type of antibody formed in the tonsils is found to be IgG followed, in order, by IgA, IgM, IgD and IgE.

Scattered submucosal lymphoid cells have not been extensively studied. These cell masses are histologically similar to tonsillar tissue.

Both the major salivary glands (parotid, submandibular and sublingual) and the minor salivary glands have been found to

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contain lymphocytes and plasma cells. Most of the plasma cells secrete IgA and some IgG or IgM. The IgA synthesized in the salivary glands has a dimeric structure. This type of IgA is referred to as secretory IgA (sIgA) and is the major immunoglobulin component in saliva.

Both T-cells and B-cells are found in the gingival lymphoid tissue. In subjects having clinically normal gingival tissue, T-cells predominate. During an infectionary period, such as during the development of gingivitis, B-cells have been found to predominate.

Plasma cells are also found in the gingival lymphoid tissue. Clusters of these cells are generally located near the blood vessels and predominantly produce IgG. To a lesser extent, IgA and IgM are also manufactured. More importantly, Brandtzaeg et al. in, Human Saliva: Clinical Chemistry and Microbiology edited by Jorma O. Tenovuo, have shown that the immunoglobulins from the secretions from the gingival tissue area are directly related to the immunoglobulins found in the blood.

In order to eliminate or greatly reduce the problems inherent in antigen-antibody analysis of salival fluid, we previously proposed a method for collecting immunoglobulins from the oral cavity with the aid of a hypertonic solution as a rinse. See EP 0 418 739 A1. We also previously proposed the use of a collecting pad, which has been treated with a hypertonic solution, in the oral cavity to absorb oral immunoglobulin for immunological testing. See WO 91/13355.

We have now found that an untreated pad can be used to collect a sufficient quantity of oral immunoglobulin for immunological testing. The use of the pad results in a yield of immunoglobulins greater than would be expected and can incorporate basic antigen-antibody testing techniques as a screening tool for diseases. We have also found that the present invention can be used to collect substances other than immunoglobulins for testing. In fact, the invention has been successfully used to collect substances having molecular weights ranging from about 176 (cotinine) to about 950,000 (IgM). There is no limit to the size of the molecule which can be collected using the present

invention. If the molecule can pass through the walls of the capillaries and other oral tissue, it can be collected using the present invention. The pad is contacted with the oral mucosa without chewing.

More specifically, the present invention provides a method of immunological testing which comprises:

(a) obtaining a mucosal transudate sample to be tested by

10 (i) inserting a substantially salt-free absorbent pad into the oral cavity,

(ii) contacting the pad with the oral mucosa without masticating said pad,

(iii) removing the pad from the oral cavity;

(b) subjecting the sample to at least one immunological test known to reveal the presence or absence of at least one immunologically active analyte, and

(c) analyzing the test results for the presence or absence of the analyte.

20 Fig. 1 is a longitudinal section of one embodiment of a container for storing the pad;

Fig. 2 is a longitudinal section of the embodiment of Fig. 1 with the pad and holder shown;

Fig. 3 is an elevational view of another embodiment of a container useful in the present invention with the cap removed;

Fig. 4 is a top plan view of the container of Fig. 3;

Fig. 5 is a longitudinal cross-sectional view of

the container of Fig. 4 taken along the line 5--5 of Fig. 4;

Fig. 6 is an elevational view of a cap for use with the container shown in Fig. 3;

Fig. 7 is a top plan view of the cap of Fig. 6; and

Fig. 8 is a cross-sectional view of the cap taken along the line 8--8 of Fig. 7.

10 The present invention is concerned with collecting oral immunoglobulins for immunological testing and other substances for testing. A pad is used to collect a specimen having a high concentration of immunoglobulins or the other substances. High levels of immunoglobulins from the oral cavity are considered to be concentrations in excess of 50 μ g total Ig per ml. The specimen can be subjected to a basic testing technique which can be used as a tool for screening a patient for diseases or for the presence of certain foreign substances.

Representative molecules which have been successfully collected by the use of the present invention are:

20	<u>Analyte</u>	<u>Molecular Weight</u>
	Cotinine	176
	Glucose	180
	Theophylline	180
	Cocaine	303
	Beta2-microglobulin	11,818
	Hepatitis B surface antigens	24,000
	Beta-human chorionic gonadotropin	37,900

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IgG -- human antibody	150,000
Total IgG (antigen not specified)	
HIV-1	
Hepatitis A	
Hepatitis B	
Rubeola (measles)	
Syphilis non-treponemal antigen	
IgA -- human antibody	160,000
Total IgA (antigen not specified)	
IgM -- human antibody	950,000
Total IgM (antigen not specified)	
Hepatitis A	
Hepatitis B	

In order to minimize degradation in a collected specimen, container in which the pad is stored after use the hypertonic solution of the present invention can include a preservative. Such a preservative can act to inhibit proteolytic enzymatic activity which can be responsible for the destruction of antibody molecules. Compounds contemplated as a preservative include anti-bacterial agents, anti-fungal agents, bacteriostatic agents, fungistatic agents, and enzyme inhibitors. In a preferred embodiment benzoic acid, sorbic acid or the salts thereof are used as anti-fungal agents. As bacteriostatic agents, salts in high concentration and compounds capable of maintaining the hypertonic solution at low pH are contemplated. Such salts include thimerosal (or merthiolate), phenyl mercuric acetate, phenyl mercuric nitrate and sodium azide. Other preferred preservatives include preservatives which are typically used in medicines and mouthwashes. Examples include ethyl alcohol and chlorhexidine gluconate. Another class of preferred anti-microbial agents are detergents which can be used as topical germicides or in mouthwashes. An example is benzalkonium chloride. It is preferred to use these preservatives in a range of about 0.01% to about 0.2% by weight.

In the present invention, a pad is used to absorb mucosal secretions from the oral cavity. The pad is made of an absorbent material which can be effectively placed into the oral cavity. A plastic or carbohydrate material such as cellulose can be used as the absorbent material, but a thick, absorbent cotton paper is preferred. An example of a thick, absorbent cotton paper is product #300 manufactured by Schleicher and Schuell in Keene, New

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Hampshire. The pad is preferably not in the form of a foam or sponge, although foam or sponge could be used.

Most materials from which the pad is made can non-specifically bind protein. Thus, some immunoglobulins can undesirably bind to the pad and it is desired to block proteins from binding to the pad by using a blocking agent. Non-specific binding is not normally a problem in the collection of blood samples since blood contains its own blocking agent (i.e., human serum albumin).

To reduce non-specific binding in the collection of oral specimens, a blocking agent can be added to the hypertonic solution to be incorporated into the pad. A blocking agent is generally a soluble protein which is used to prevent non-specific binding of another protein to a solid surface. Compounds which can be added as blocking agents include albumin and gelatin, but any water soluble, non-toxic protein can be used as a blocking agent as long as the protein does not adversely affect antibody molecules. It is preferred to use bovine gelatin. In general, blocking agents can be made into a solution at a concentration of between about 0.01% and 0.2% by weight. The solution is then incorporated into the pad by soaking or spraying followed by drying.

To collect a substance from the oral cavity, the pad can be placed into the mouth with the aid of a holder. The pad and holder are shown in the drawing. The pad holder 1 can be a hollow, plastic stick having a groove 2 at one end. The pad 3 is inserted into the groove and the holder can be manipulated to place the pad into the oral cavity, preferably between the lower gums and cheek. Placement of the pad between the lower cheek and gums facilitates absorption of secretions originating from gingival lymphoid tissue as well as secretions from submucosal lymphoid tissue and salivary gland lymphoid tissue. It is preferable that the specimen be collected by rubbing the pad back and forth between the gums and cheek for about ten seconds and then holding the pad in position for about two minutes. The pad should not be chewed since chewing stimulates salivation which is undesirable.

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After the specimen has been collected, the pad is stored in a container until immunological testing can be performed. One type of container is shown in Figs. 1 and 2. It is desired that the container have a centrifuge tube as an outer portion of the container, and that an inner portion of the container have an inner tube which mounts into the centrifuge tube. The pad is to be placed into the inner tube, and the contents therein are secured by a tube cap.

Reference is had to Fig. 1 wherein there is shown an assembly which has been modified for use with the instant invention. The container 2 comprises a centrifuge tube 4 having a tapered lower end or base 5 with a downwardly tapering recess 6 in which solid matter accumulates upon centrifugation; an upper tube or container 7 having a radially outwardly projecting annular flange 8 and a cylindrical upper portion 9 at its upper end; and a plug or stopper 11. The cylindrical portion 9 and stopper 11 are of the same size and shape as the upper part of centrifuge tube 4 so they are flush with the outer surface of the centrifuge tube and the assembly presents a uniform appearance, although this feature is not important to the practice of the instant invention. In the floor 13 at the bottom of container 7 is a bore 17 to allow liquid to flow from container 7 to centrifuge tube 4 when the complete assembly is centrifuged. The container 7 is made of any suitable material such as polyethylene, glass, etc. Similarly, the stopper 11 is made of any suitable material such as polyethylene as is well-known in the art.

There is a removable plug 19 in bore 17. The plug could be made of any suitable material such as wax, a plastic, etc. A suitable quantity of a preservative solution 21 is placed in the container 7.

Once the pad is placed in the inner tube, a preservative solution 21 is added. Such a preservative solution can act to inhibit enzymatic activity which can be responsible for the destruction of antibody molecules or can function as an anti-microbial agent.

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Compounds contemplated for use in the inner tube as a preservative include anti-bacterial agents, anti-fungal agents, bacteriostatic agents, fungistatic agents, and enzyme inhibitors. As an antibacterial agent, it is preferred to use chlorhexidine gluconate or thimerosal.

The preservative solution to be used in the inner tube can contain one or a combination of the preservatives. In general, the preservatives are included in a concentration which limits microbial contamination and does not adversely effect the immunoglobulins absorbed into the pad.

The preservative solution to be used in the inner tube can also contain a detergent which improves removal of antibody from the pad during centrifugation. Tween 20 (polyoxyethylene sorbitan monooleate) is a preferred detergent since it can also prevent non-specific binding of antibody to a solid surface. It is preferred to use a combination comprising about 0.01%-0.2% chlorhexidine gluconate and 0.2%-0.7% Tween 20. A combination comprising about 0.1% chlorhexidine gluconate and 0.5% Tween 20 is most preferred.

After the preservative solution is added to the inner tube, the tube cap is inserted into the container to seal in the contents. The pad can be stored in this manner for several days until immunological testing can be initiated.

In this embodiment, the pad 3 on holder 1 is used as already described. After the pad 3 is removed from the user's mouth, stopper 11 is removed from container 7 and the pad is placed within the container 7. The holder 1 is broken off at a point outside the mouth of container 7 so it will project upwardly from the container. Then the stopper 11 is replaced. Since stopper 11 is hollow, it will securely seal the container 7 with the broken end of holder 1 extending into it. Holder 1 is preferably scored at a suitable location to provide for easy breaking. When the pad 3 is inserted in container 7, it will absorb at least a part of preservative solution 21.

The pad 3 is stored in container 7 until testing can be initiated. At the laboratory, the container 7, with the stopper 11 securely in place, is inverted, the seal 19 of wax or other

suitable substance is removed, and the container 7 is placed in a centrifuge tube 4. The complete assembly 2 is then centrifuged whereby all the liquid, including preservative solution, saliva, etc., is drawn down through bore 17 into the centrifuge tube 4. Testing is then performed using known techniques.

To simplify the collection and analysis of an oral specimen using the pad collection system, a kit can be provided. The kit can include a combination of the pad and implements used to collect and prepare the oral specimen for analysis. One preferred embodiment of the kit includes the treated pad 3 and pad holder 1; the container 7 having the stopper 11 and the storage preservative 21. Optionally, a centrifuge tube 4 could be included.

Another container for storing collected substances for subsequent testing according to the present invention comprises an open upper end adapted to be sealed with a removable stopper and a lower end having an opening communicating the interior of the container with the outside, the opening being selectively sealed by a frangible nipple during storage of the substances and unsealed for removal of the collected substances for subsequent testing. For both comfort and safety of the user, the distal end of the frangible nipple is enlarged, preferably in the form of a ball. The open upper end is preferably sealed with a screw-on "plug seal" cap. In addition, the floor of the container, which slopes towards the opening in the center thereof, has a plurality of upstanding webs to prevent the pad described in

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U.S. Patent No. 5,103,836 from resting on the bottom of the container and blocking the opening at the bottom.

The container is dimensioned to permit its insertion into a standard 15 ml. conical centrifuge tube. This allows centrifugation after the nipple is broken off, thereby transferring the vial contents into the centrifuge tube.

The container is intended to hold a volume of preservative liquid (from about 0.5 ml. to about 2.0 ml.). When the pad is placed in the container, the preservative is absorbed into the pad. Since the container with the preservative will be stored for up to a year without significant loss of liquid volume, the

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water transmission rate must be low. The container is fabricated of any suitable plastic such as polyethylene, polycarbonate, polystyrene, PET (polyethylene terephthalate), polypropylene, EPC (ethylene propylene copolymer), and the like. Polycarbonate is the least desirable due to excessive "breathing," that is, allowing moisture to escape. The preferred material is polypropylene.

Referring to Figs. 3 and 4, the container of this embodiment, generally designated by the numeral 10, comprises an elongated body portion 12. Body portion 12 is generally cylindrical although, it is preferably slightly tapered from the upper end to the floor 15. The upper end 16 is open and is provided with a screw thread 18 to accommodate the cap.

A nipple 20 extends downwardly from the floor 15. The floor 15 preferably has a slight slope at an angle α from the outside to the center. Angle α is preferably about 20° . At the center of the inside of the floor 15 is a depression 22, preferably "V" shaped and preferably forming an angle of about 88.50° . The depression 22 causes the base end of nipple 20 to be weakened, thereby allowing the same to break off when sufficient pressure is applied. A bulb 24 is formed at the distal end of nipple 20 to make it easier and safer to break off the frangible nipple 20. In order to prevent the pad which will be inserted in the container 10 from resting on the floor 15 at the hold created by breaking off nipple 20, a plurality of upstanding webs 26 are provided on floor 15. Preferably, there are four webs 26.

Turning now to Figs. 5, 6, and 7, there is shown a cap, generally designated by the numeral 28 for sealing the open upper end 16 of container 10. Cap 28 is of the type commonly known as a "plug seal" cap. Cap 28 has internal screw threads 30 to cooperate with threads 18 on the container. Annular depression 32 creates an annular space 34 bounded by annular depression 32 and the inside of outer wall 36. The wall of the container thus fits into annular space 34 and creates a perfect seal. The outside surface of cap 28 is knurled as shown at 38 to facilitate removal and replacement of the cap.

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The container 10 is used in the same manner as the containers described above with respect to Figs. 1 and 2. The following examples show the effectiveness of the pad of the present invention.

EXAMPLE 1

Comparison of IgG in Saliva versus Mucosal Transudate
The purpose of this study was to compare the levels of IgG in saliva versus mucosal transudate.

Oral samples were collected from ten subjects. The subjects collected saliva by chewing on the "absorbent body" sold as part of the Sarstedt Salivette™ kit with a cotton plug as an absorbent body until the cotton plug became saturated with saliva. Approximately 4 hours later, the subject then collected mucosal transudate by collecting oral fluid using the treated pad described in WO 91/13355. After the saliva or transudate was collected, the liquid was removed from the cotton plug and treated pad by centrifugation. The oral specimens were analyzed for the presence of IgG antibody using an enzyme immunoassay.

Table 1 shows the levels of IgG collected by the cotton plug versus the treated pad.

Table 1

Subject Number	Saliva IgG ($\mu\text{g/ml}$)	Transudate IgG ($\mu\text{g/ml}$)
1	1.6	38.3
2	3.2	60.3
3	0.7	21.0
4	1.8	48.5
5	2.4	135
6	4.9	86.7
7	1.1	72.3
8	1.3	31.1
9	3.0	63.1
10	<u>4.1</u>	<u>37.5</u>
Mean IgG	2.4	59.4

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Saliva samples collected by chewing on an "absorbent body" of the Sarstedt Salivette™ kit contain approximately 25 times less IgG antibody than samples collected by the treated pad of WO 91/13355.

EXAMPLE 2

Tests comparing the treated pad of WO 91/13355 and the untreated pad according to the instant invention were run.

A total of four pads were used for oral specimen collection from each voluntary participant, two of the pads being treated and two untreated. Each collection (two like pads collected simultaneously) lasted two minutes. The participant rinsed his/her mouth with tap water after the initial collection and waited at least one-half hour before the second collection. Participants in the study were divided into two groups. Half of the participants initially collected an oral sample using two untreated pads, followed later by two treated pads. The other participants collected with two treated pads first, and two untreated pads later.

After collection the participant inserted the pad into an empty vial which has been labeled with the specimen number and the type of pad used for collection (treated, or untreated). The stick was snapped-off and the vial capped tightly. The specimen remained at 4°C before and during centrifugation. Once centrifuged, the oral specimens collected by the two like pads from each participant were pooled. Specimens were stored at 4°C.

Each specimen pool was assayed using Epitope, Inc.'s EIA for Total IgG to quantitate the IgG present in each specimen.

2180025Table 2

Subject Number	IgG ($\mu\text{g/ml}$)	
	Untreated Pads	Treated Pads
1*	6.7	9.1
2*	4.9	7.8
3	4.6	11.2
4	12.4	23.9
5*	10.2	26.4
6*	9.2	43.9
7	5.3	45.4

* Untreated pads collected first.

Specimens collected with the treated pads contained higher levels of immunoglobulin G (IgG) than those collected with the untreated pads, as analyzed by an in-house IgG-specific enzyme immunoassay (EIA).

From the analysis of the matter collected by the pads of WO 91/13355 and the matter collected by the Sarstedt "absorbent body", it is concluded that the pad collected the IgG from mucosal transudate and not saliva, and that the difference is significant.

From the analysis of the matter collected by the treated pads of WO 91/13355 and the untreated pads of the instant invention, it is concluded that while the untreated pads collected less IgG than the treated pads, the untreated pads still collected significantly more IgG than would be expected to be collected from saliva (5.3 $\mu\text{g/ml}$ vs. 2.4 $\mu\text{g/ml}$).

THE EMBODIMENTS OF THE INVENTION IN WHICH AN EXCLUSIVE PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:

1. A method of immunological testing which comprises:
 - (a) obtaining a mucosal transudate sample to be tested by
 - (i) inserting a substantially salt-free absorbent pad into the oral cavity,
 - (ii) contacting the pad with the oral mucosa without masticating said pad,
 - (iii) removing the pad from the oral cavity;
 - (b) subjecting the sample to at least one immunological test known to reveal the presence or absence of at least one immunologically active analyte, and
 - (c) analyzing the test results for the presence or absence of the analyte.
2. The method of claim 1, wherein said pad is preserved after removal from the oral cavity for subsequent removal of collected mucosal transudate from the pad for testing.
3. The method of claim 1, wherein said oral cavity is a human oral cavity.
4. A method of preferentially collecting mucosal transudate from an oral cavity for testing comprising the steps of:
 - (a) inserting a substantially salt-free absorbent pad into the oral cavity,

(b) contacting the pad with the oral mucosa without masticating said pad,

(c) removing the pad from the oral cavity, and

(d) preserving the pad for subsequent removal of the collected mucosal transudate from the pad for testing.

5. The method of claim 2, 3 or 4, wherein the pad is stored in a container when the pad is removed from the oral cavity.

6. The method of claim 5, wherein the container includes a preservative solution.

7. The method of claim 6, wherein the preservative solution includes chlorhexidine gluconate, thimerosal or sodium azide.

8. The method of claim 7, wherein the preservative solution includes chlorhexidine gluconate.

9. The method of claim 5, wherein the container comprises an open upper end sealed with a removable stopper and a lower end having an opening communicating the interior of the container with the outside, said opening being selectively sealed during storage of said pad and unsealed for said removal of said collected mucosal transudate for subsequent testing.

10. The method of claim 9, wherein said opening is selectively sealed by a frangible nipple.
11. The method of any one of claims 1 to 4, wherein said mucosal transudate contains analytes having a molecular weight from about 176 to about 950,000 daltons.
12. The method of claim 11, wherein said analytes are selected from the group consisting of cotinine, glucose, theophylline, cocaine, beta 2-microglobulin, hepatitis B surface antigens, beta-human chorionic gonadotropin, and immunoglobulins, and mixtures thereof.
13. The method of claim 11, wherein said analytes are immunoglobulins.
14. The method of claim 13, wherein said immunoglobulins are selected from the group consisting of IgG, IgA, and IgM.
15. The method of claim 13, wherein said immunoglobulins are antibodies against at least one of a member of the group consisting of HIV-1, hepatitis A, hepatitis B, rubeola, and syphilis non-treponemal antigen.
16. The method of claim 2, wherein said testing is by immunological testing.

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17. The method of claim 16, wherein said immunological test is an ELISA test.

18. The method of any one of claims 1 to 4, wherein the pad is made of a carbohydrate material.

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PATENT AGENTS

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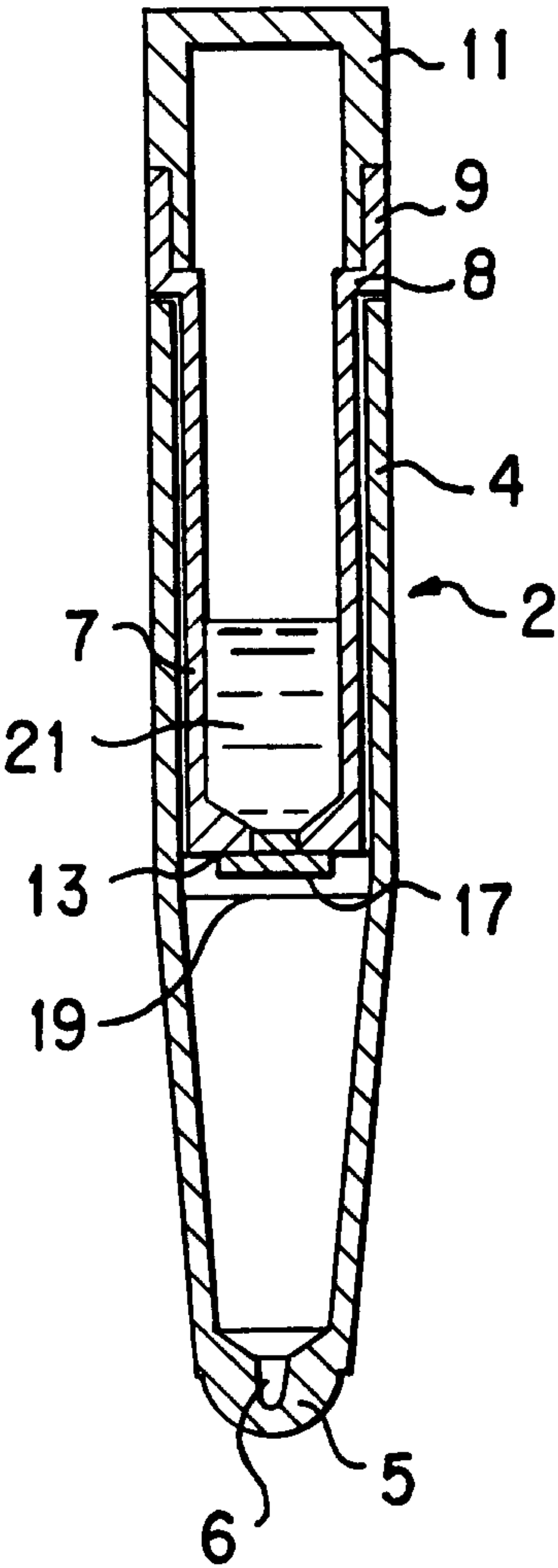


FIG. 1

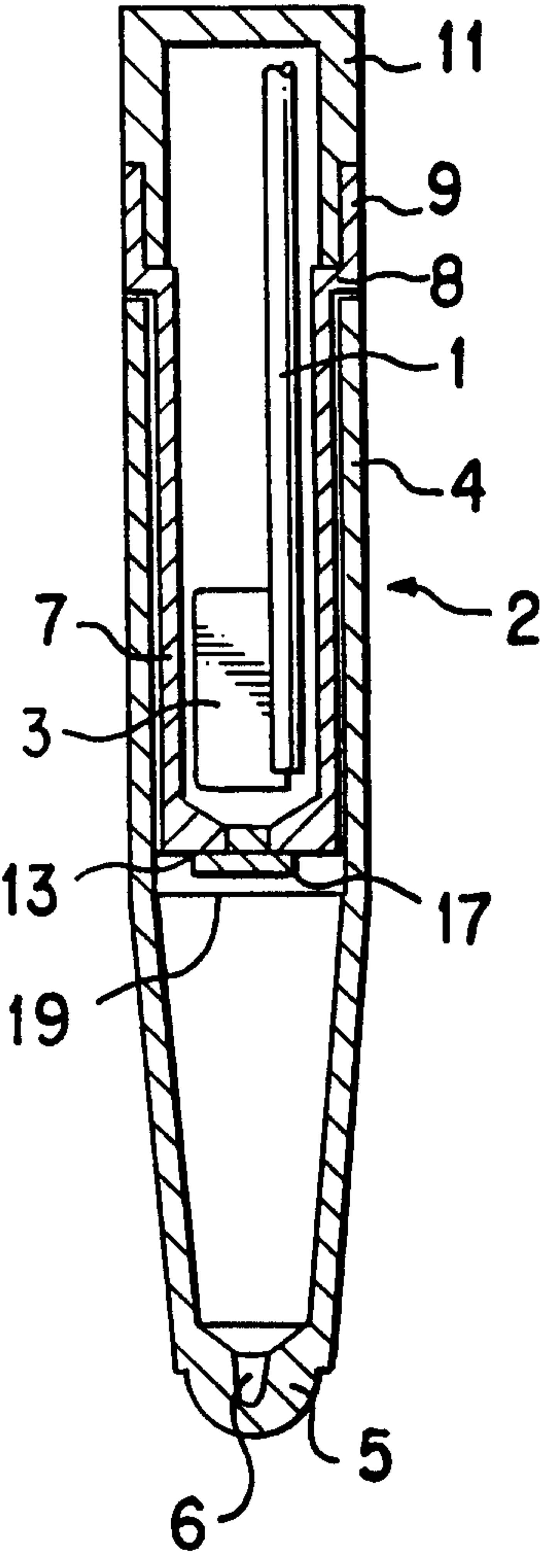


FIG. 2

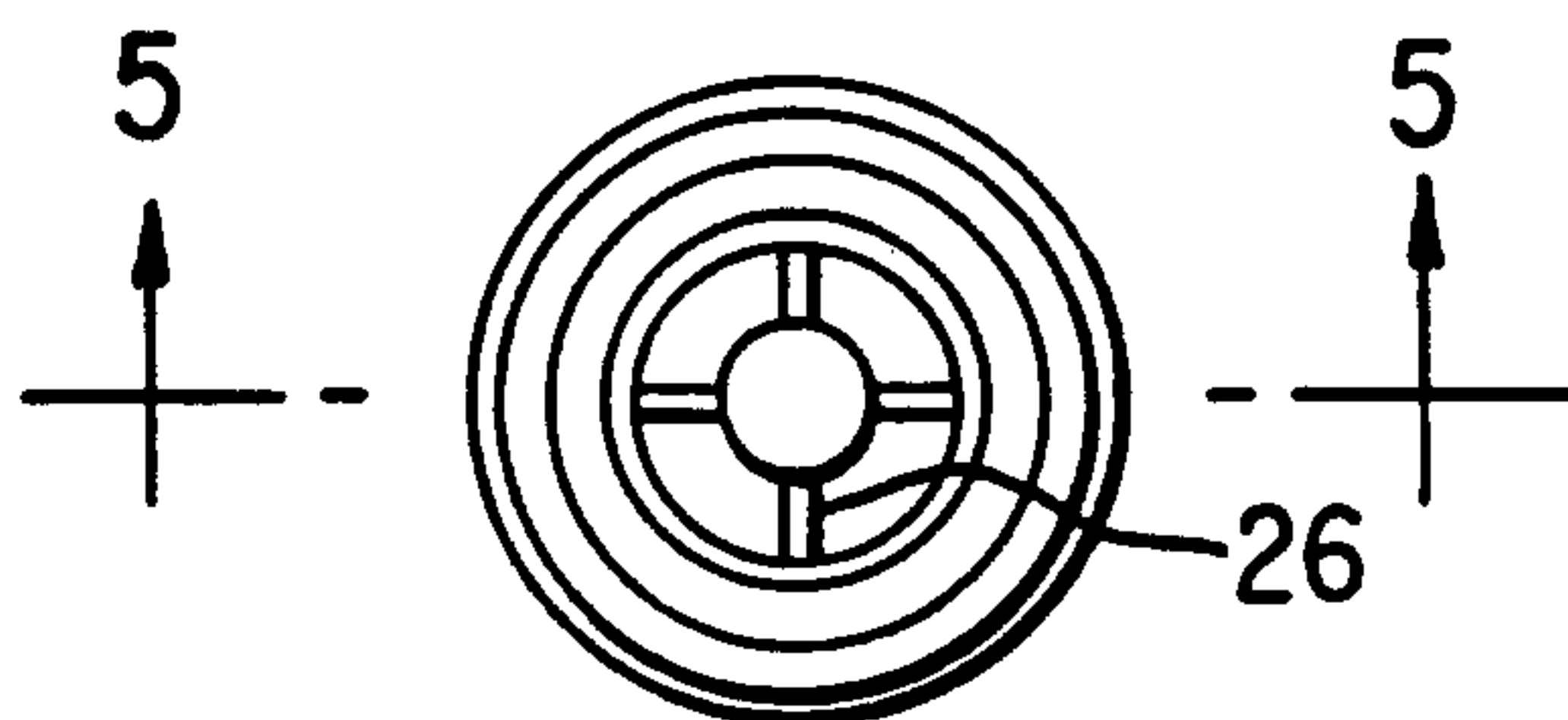


FIG. 4

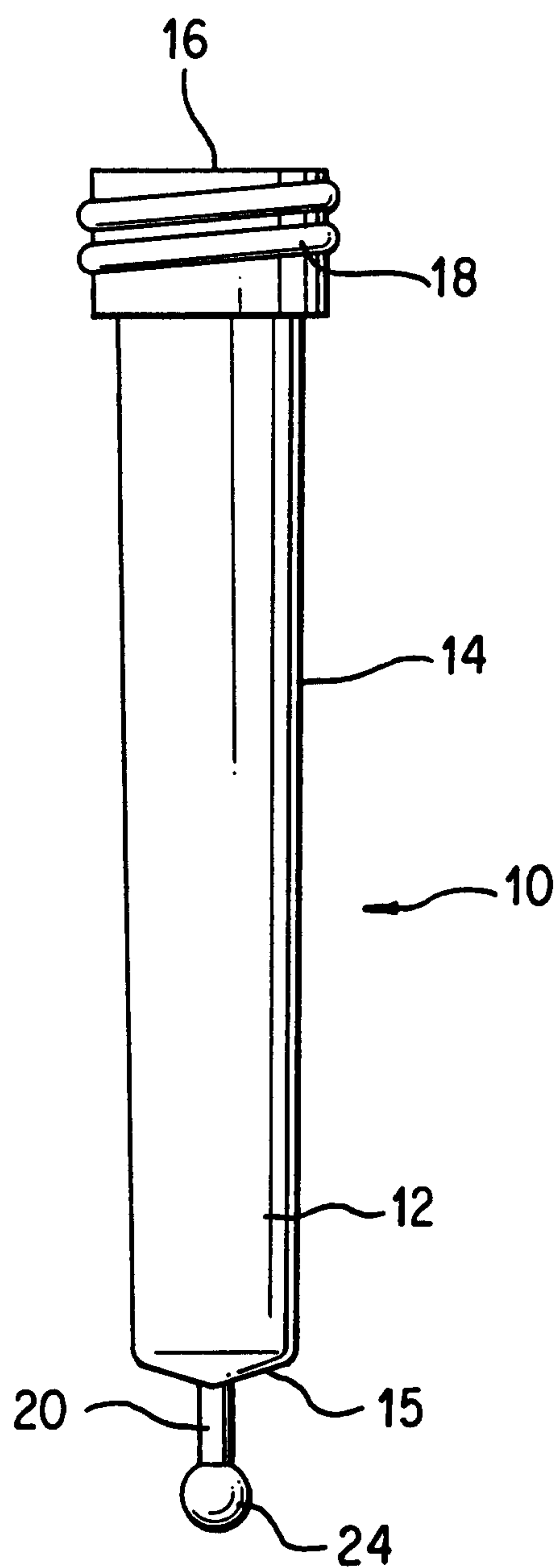


FIG. 3

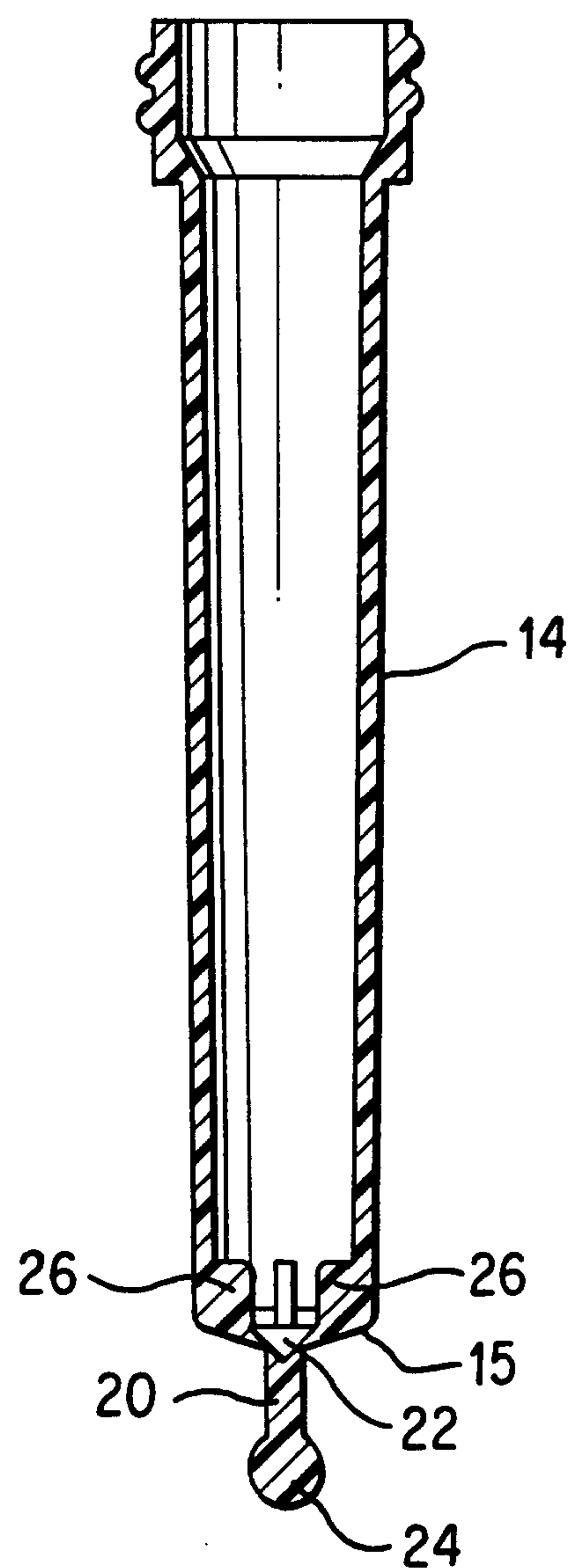


FIG. 5

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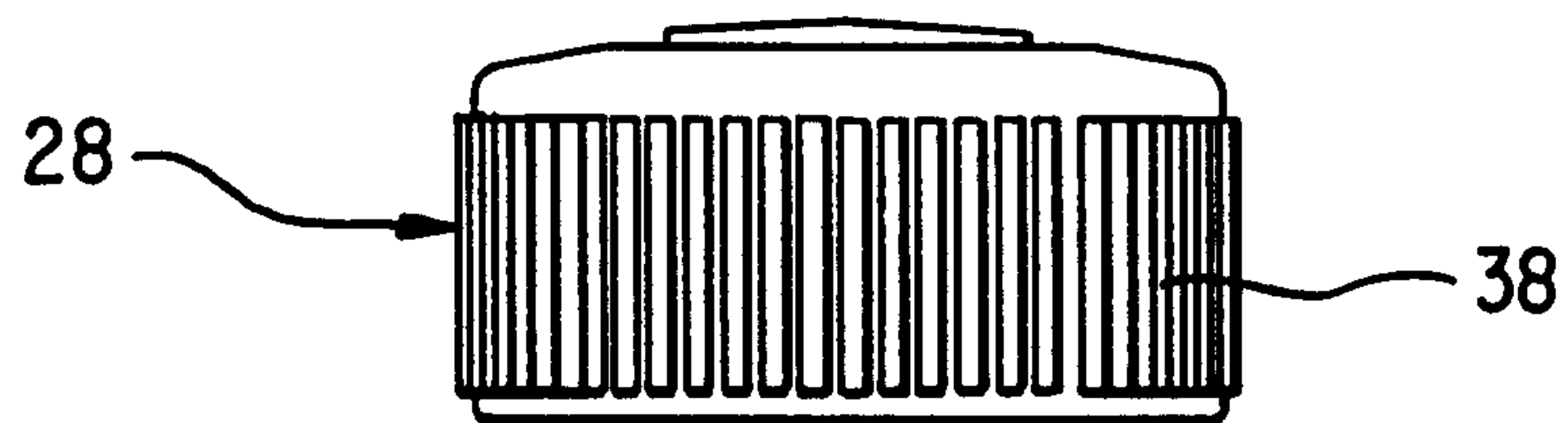


FIG. 6

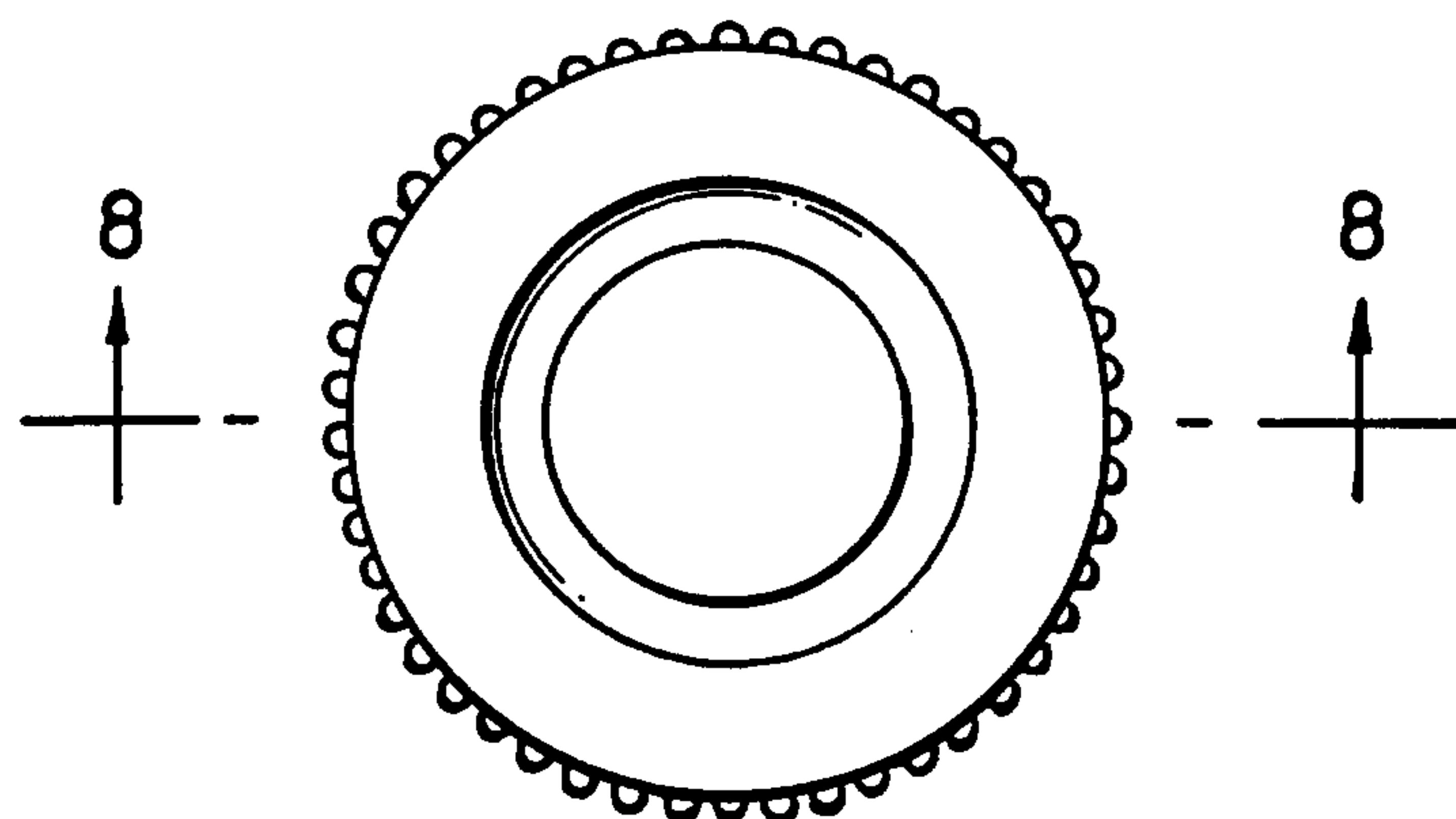


FIG. 7

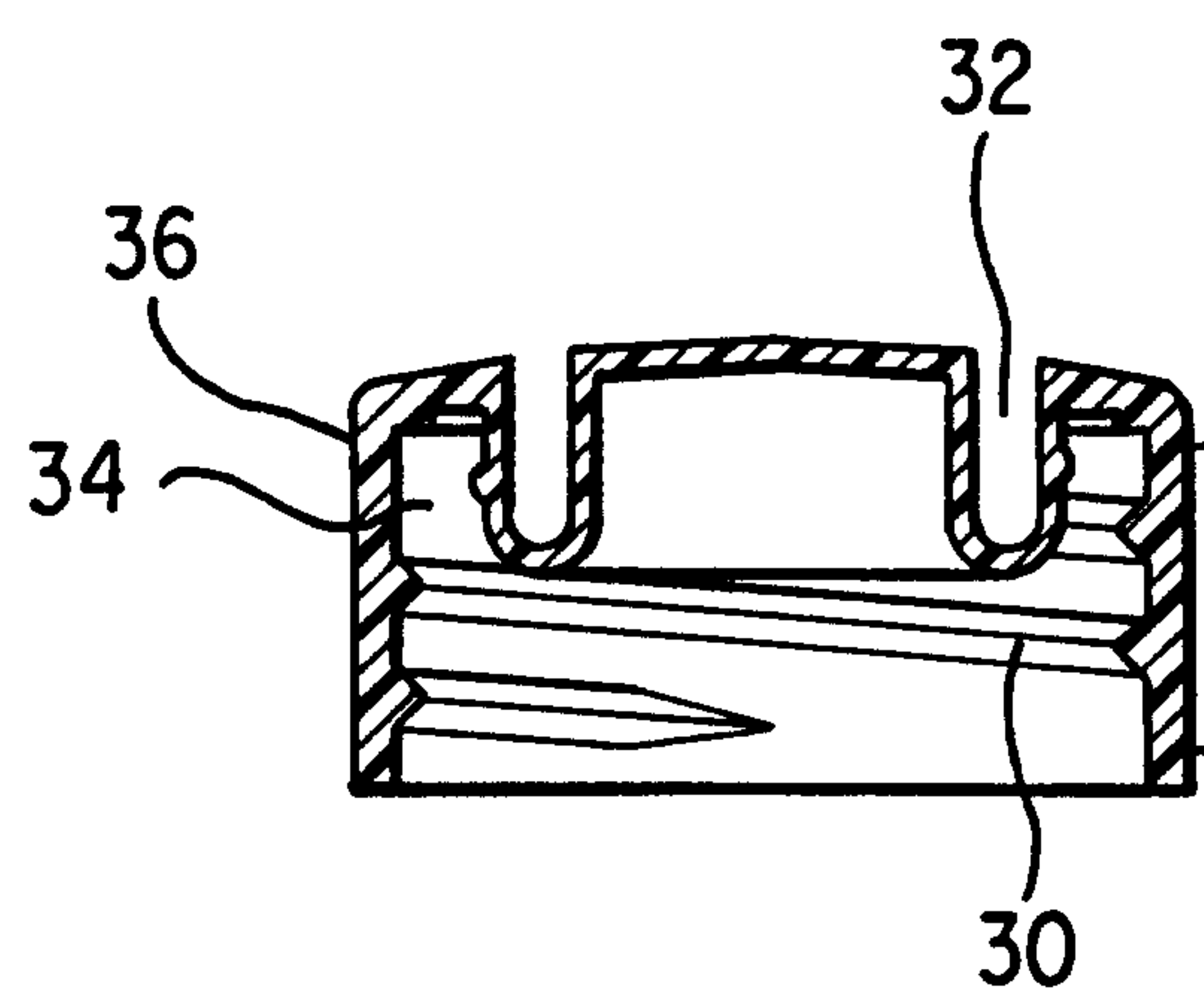


FIG. 8