G. H. EARP-THOMAS.

CONTAINER FOR TRANSPORTING BACTERIAL CULTURES.

APPLICATION FILED JAN. 15, 1909. RENEWED AUG. 31, 1914.

1,137,388. Patented Apr. 27, 1915.

Fig. 1.

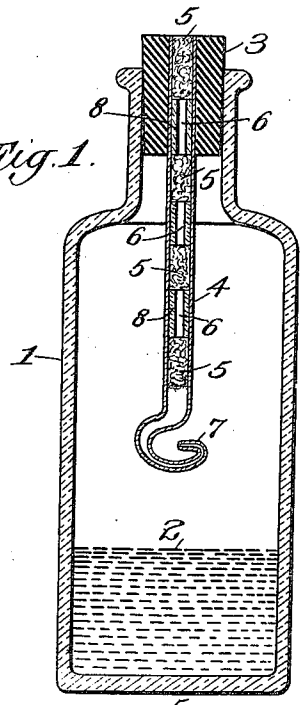


Fig. 2.

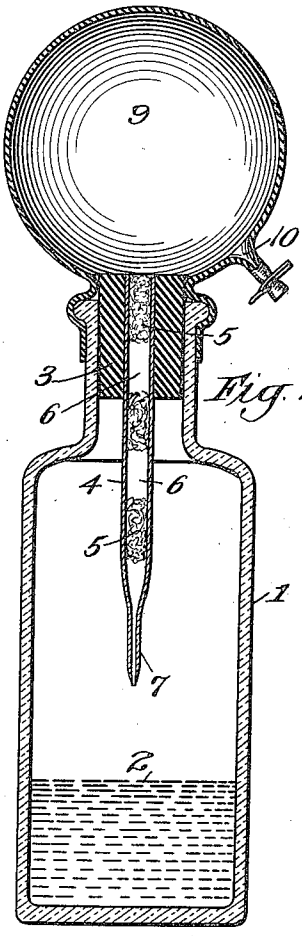


Fig. 3.

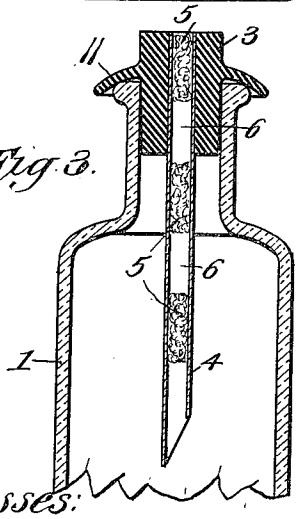
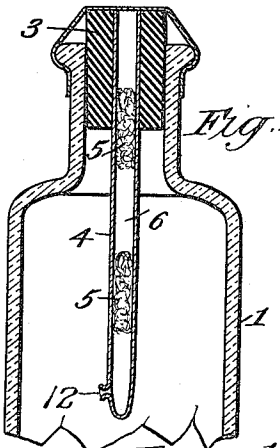


Fig. 4.



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UNITED STATES PATENT OFFICE.

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CONTAINER FOR TRANSPORTING BACTERIAL CULTURES.

1,137,388. Specification of Letters Patent. Patented Apr. 27, 1915.

Application filed January 15, 1909, Serial No. 472,476. Renewed August 31, 1914. Serial No. 859,565.

To all whom it may concern:

Be it known that I, GEORGE H. EARP-THOMAS, subject of the King of Great Britain, residing at Glen Ridge, Essex county, New Jersey, have invented certain new and useful Improvements in Containers for Transporting Bacterial Cultures; and I do hereby declare the following to be a full, clear, and exact description of the invention, such as will enable others skilled in the art to which it appertains to make and use the same.

This invention relates to means for permitting the passage of air or other gas between the interior of closed containers or other vessels and the external air or gas, while at the same time all bacterial and other contaminations are excluded from the interior of the container, and the escape of liquids from or the entrance of liquid into the container is prevented, and loss of moisture from the container by evaporation is reduced to such an extent as to be practically inappreciable.

The invention may be employed for preserving in an uncontaminated condition various sterilized and other substances to which it is necessary or advisable to supply air or other gases, and is of especial value in preserving such substances during transportation and storing in suitable containers under the conditions of commercial distribution. The invention will also be found adaptable for use in connection with or when applied to containers for various volatile drugs and chemicals.

The invention has been made, however, especially with the idea of providing, and it is the especial object of the invention to provide, means for growing, preserving and distributing cultures of certain micro-organisms which require air or other gases to sustain them in an active, living condition, such as the nodule forming nitrogen-fixing bacteria which infest the roots of clovers, alfalfa, peas, beans and other leguminous plants, and all aerobic or air-requiring bacteria; and the invention relates further, therefore, to the preservation of micro-organisms, and it comprises a transportable or distributing package of micro-organisms, such package comprising a nutritive medium inoculated with the desired organisms and contained in a vessel having means such as above referred to affording communication between the interior of the vessel and the external air or gas and protecting the contents of the vessel from contamination and restricting loss by evaporation and preventing loss of liquid from the vessel, all as more fully hereinafter set forth and as claimed.

The commercial production and distribution of cultures of various bacteria and the like has become desirable and necessary in recent years, but methods and means heretofore suggested to effect such distribution have been unsatisfactory for the reason, among others, that many difficulties attending commercial distribution of bacteria cultures are not met in the laboratory and consequently are not avoidable by the application of ordinary laboratory methods such as it has been attempted to employ hitherto. Cultures of aerobic bacteria, such as the nodule-forming nitrogen-fixing bacteria above referred to, are especially difficult to transport successfully, and the practical necessity or desirability of employing moist culture media, such as liquid or semi-liquid or jelly-like media, has added very largely to the difficulties attendant upon the distribution of such cultures. They must of course be protected against contamination, but if the vessel containing the culture is hermetically sealed then the period for which the culture will remain in good condition will be limited by the amount of air or gas contained in the vessel. To avoid this difficulty, it has been a custom followed to a considerable extent to distribute various aerobic bacteria in containers, usually of glass, having the neck or mouth of the container stoppered with a plug of cotton or similar fiber which serves as an air filter to prevent contamination by organisms carried in the air and dust while permitting the necessary access of air to the bacteria in the container. Such a device serves very well in the laboratory where the container can be kept standing upright and the cotton plug kept dry, but it is not satisfactory under the conditions of commercial transportation where inversion of the container can hardly be prevented. If the plug becomes moist, as is apt to happen when the container is inverted and the culture medium or liquid separated therefrom comes in contact with the cotton, the organisms which have settled on it from the air can readily

grow by simple longitudinal growth and sub-division until they ultimately attain the other side of the cotton plug and fall or are projected into and contaminate the culture within the container.

Another ojection to the use of ordinary cotton-plugged containers for distributing cultures is that the cultures suffer a very considerable loss of moisture from evaporation, which in a comparatively short time changes the character of the medium, not only with respect to its moisture content but with respect to its saline content. The ordinary cotton-plugged container is equally objectionable for gas-producing bacteria, and a hermetically sealed container is not suitable for them because of the pressure which the gases they produce might develop within the container.

In the present invention I have devised a simple, cheap and efficient means for securing all the advantages of both the hermetically sealed and the cotton-plugged culture containers, while avoiding the objectionable features of and difficulties attendant upon both. Such means comprises a gas conduit or tube for connecting the interior of an otherwise hermetically sealed container with the external air or gas, which conduit or tube may be of comparatively small cross section and contains means for substantially preventing passage of microorganisms through said conduit in either direction, such means comprising in the preferred form a plurality of separate air filters therein formed of plugs of cotton, silk fiber, asbestos or other suitable fiber, but preferably of non-absorbent cotton, and preferably spaced away from each other by air spaces, such filters or fiber plugs being properly compacted. With this device, even though an end plug in the tube or conduit should become wet, a plug beyond the wet plug will remain dry and will protect the contents of the container against contamination, while the passage of air or other gas through the conduit will be permitted even if the end plugs are wet. Three or more such spaced air filters or fiber plugs are preferably placed in the conduit, since when at least three such filters are used there will be at least one which will be protected by the filters beyond it from access of moisture from either the interior or the exterior of the container. More than three plugs may be used, however, and, on the other hand, the use of two separated plugs will be found efficient for many purposes, and a single cotton plug may be used as hereinafter explained in connection with certain other features of the invention with good results.

The conduit containing the separated air filters is preferably arranged so that it extends within the container and is open to the interior of the container at a point removed from any wall thereof, so that with the container only partially filled liquid cannot gain access to the conduit, or cannot gain access in any material amount, whatever the position of the container. The opening from the conduit into the container is preferably also comparatively small, thereby further minimizing loss of moisture by evaporation and lessening the liability of liquid entering the conduit.

A container having a gas conduit provided with a plurality of spaced air filters such as above described and containing a nitrogen-free nutrient medium inoculated with nitrogen-gathering bacteria of the leguminous plants forms a desirable type of culture for commercial distribution, the medium being protected against contamination by foreign organisms and from loss of moisture, while the bacteria remain in an active, virulent condition for long periods of time. The preparation of such inoculated culture media is described in my prior United States Patents 816,850, dated April 3, 1906, and 865,965, dated September 10, 1907.

The legume bacteria require nitrogen and in order to be preserved in an active and virulent condition in a nitrogen-free medium such as the described medium they must be able to obtain nitrogen from the atmosphere within the container. For long preservation of a culture of these bacteria in a nitrogen-free medium, therefore, access of air to the interior of the container is necessary unless the container is made so large relatively to the amount of inoculated medium which it contains that the supply of air within the container will be sufficient for the time for which it is desired to preserve the culture. Practically, therefore, it is necessary to provide for access of air if the culture is to remain in an active, virulent condition for a long period of time.

The present invention provides for such access of air while protecting the contents of the container from contamination and from change due to loss of moisture by evaporation.

The use of a gas conduit providing communication between the atmosphere within a container and the external atmosphere through a restricted opening and a dry cotton plug is also of great value in the preparation and keeping of sterilized medium until wanted for inoculating.

In the accompanying drawings I have shown more or less diagrammatically certain embodiments of the invention.

In said drawing: Figure 1 is a vertical section of a distributing package embodying the invention. Fig. 2 is a similar view showing a slightly modified construction. Fig. 3 is a broken view of a container, showing a stopper constructed in accordance

with the invention and provided with a dust cap, and showing a slightly different form of conduit tube. Fig. 4 is a similar view showing a still further modified form of conduit tube.

Referring to the drawings, and first to Fig. 1, 1 is a container or receptacle carrying a body 2 of nutrient medium containing the desired organisms, as, for example, a body of a jelly-like medium containing nodule-forming nitrogen-fixing bacteria intended for inoculating clover or other legumes. As shown, the receptacle is of glass and is bottle shaped, though it might have other shapes or be of other material, and is provided with the perforated stopper 3 which may be of rubber composition or other suitable material. Through this stopper extends a gas conduit tube 4 ending above or flush with the top of the stopper and, as shown, freely open to the external atmosphere. The conduit tube may be of glass or aluminum or other suitable material. In the tube are a plurality of air filters 5, advantageously three or more, which may be of cotton, silk, flax, asbestos or other suitable fiber. The fiber plugs forming the air filters are preferably spaced apart and separated by distinct air spaces 6 as shown. Much of the advantage of the separate plugs may be secured, however by placing the plugs so close together that the more loosely compacted end portions of contiguous plugs will be in contact, and such a disposition of the fiber plugs might in some cases be employed. The conduit tube as shown extends down to about the middle of the container above normal level of the body of nutrient medium therein. It is formed as shown in this figure with a reduced end 7 bent into a curve. As an aid to placing the plugs of cotton or other fiber forming the air filters in the conduit tube, it is convenient to use short spacing pieces 8 between the several filters or fiber plugs in the tube, such spacing pieces being formed of short lengths of glass tubing, short glass rods of smaller cross section than the interior of the conduit tube, or of pieces of other form. The advantage of having the reduced end 7 of the conduit tube bent around into circular form, as shown in this figure, is that the chance that a drop of liquid even temporarily entering or closing the end of the conduit or tube is thereby very largely decreased, especially when the end of the tube is drawn out or otherwise reduced in size. The advantage of the orifice at the end of the tube being smaller than the passage through the body of the tube is that evaporation through the tube is thereby largely decreased and the chance of entrance of liquid into the tube also considerably lessened.

A container open to the atmosphere and provided with a plurality of spaced fiber plugs or air filters, as shown in Fig. 1, is eminently adapted for the transportation of media containing the nitrogen-fixing bacteria of the legumes, since it provides for the necessary supply of air to the interior of the container while protecting the inoculated media from contamination and preventing loss of liquid and largely reducing evaporation from the container, as hereinbefore pointed out. The inoculated nutrient medium within the container may be any medium suitable for the growth of such nitrogen-fixing organisms and may be either liquid or solid. If liquid, its amount should, of course, not be great enough to cause submersion of the orifice of the conduit. If a solid jelly medium be used, the mass, similarly, should not reach the orifice. When the organisms are carried on moistened solids, such as threads, potato fragments and the like, the bulk of the medium is not so important since ordinarily not enough moisture will form to reach the orifice of the conduit no matter what the position of the container.

The structure shown in Fig. 1 may, of course, be employed for transportation of micro-organisms other than the nitrogen-fixing bacteria of the legumes, the medium employed being, of course, in all cases such as is suitable to the organisms to be transported. So also the container shown with its air conduit provided with a plurality of cotton plugs or air filters may be employed for the transportation and preservation of other substances, as hereinbefore pointed out.

In making up the complete transportable package, including the container, the conduit tube with its fiber plugs or air filters, and the inoculated medium, the desired amount of the medium may be placed in the container and the stopper carrying the conduit tube with its air filters then placed securely in the neck of the container. The container with its assembled stopper, conduit tube and medium are then sterilized in any suitable way, as by streaming steam or in the autoclave. It is possible to thus sterilize the assembled parts with the culture medium in the container since the air conduit will prevent the development of pressure within the container by which the stopper might be forced out. After being so sterilized, the interior of the container and the medium therein will be preserved in a sterile condition until inoculated with the desired organisms. Since there is practically no evaporation from the container, the medium may be kept for a much longer time without appreciable loss of moisture than when the ordinary cotton plug stopper is used. If the medium is to be kept uninoculated for a very long period after sterilization, the open end of the conduit tube may be sealed with

a little paraffin wax, which may readily be removed when it is desired to inoculate the medium.

In order to inoculate the medium, it is only necessary to carefully remove the stopper carrying the conduit tube, then inoculate the medium with the ordinary inoculating needle or otherwise, and replace the stopper after carefully flaming it, which may thereafter remain in place until it is desired to make use of the culture. The container will then be kept at a temperature suitable for the development of the organisms with which the medium has been inoculated until the desired growth has resulted, after which it may be kept for a long period of time and transported as may be desired, with the certainty that the organisms and medium within the container will remain free from contaminations, and that the organisms will remain in a living, virulent condition.

In placing the several cotton plugs in the conduit tube, the first plug is pressed toward the inner end of the tube but preferably not into the reduced portion of the tube adjacent to its inner end, and the remaining plugs are then placed successively in the tube and pushed into their respective positions so as to leave a perceptible air space between each successive plug. The object of not having the inner plug extend into the reduced portion of the tube is to lessen the liability of such plug becoming moistened by any liquid which may enter the end of the tube. It may be desirable in some cases to treat one or more of the cotton plugs with a suitable antiseptic, such as corrosive sublimate, 1 to 1000 being a suitable strength, but the first plug at the inner end of the conduit tube should, however, not be so treated as it is not desirable that any antiseptic be allowed to reach the interior of the container.

The structure shown in Fig. 2 is similar to that of Fig. 1 save that the curve on the reduced end of the conduit tube is omitted, and that a gas holder 9 is provided, to the interior of which the outer end of the conduit opens, the reduced portion of the conduit serving, in this specific embodiment of the invention, as means to prevent liquid from entering the conduit tube and reaching the plug of fibrous material. Such gas holder may be a simple gas bag provided preferably with an inlet 10 and may be used for holding any gas which it is desired to supply to the organisms or substance within the container. Such a gas holder might also be used for determining the amount of air, nitrogen or other gas absorbed or made use of by certain organisms within the container.

The reduced end portion of the conduit tube may vary in size and length according to the necessities of any particular case. In some cases and more especially for use with liquids, it is desirable to have the end portion of the conduit tube drawn out to such a small size as to form a capillary tube. It is also desirable for use with liquids that the extreme end of the conduit tube be formed with a fine point and a very small orifice, so that a drop of liquid will not be sustained thereon and the possibility of liquid entering the tube will be almost entirely eliminated. The limitation as to the fineness of the point will be found in the capacity of the orifice at the point for the passage of air or other gas, and this limitation will be governed by the air requirements of the vessel dependent upon its size and the necessities of its contents. Such a reduced and pointed end is shown on the conduit tube in Fig. 2. It will be understood, however, that this pointed end with an extremely reduced orifice might be provided on a conduit tube having a curved end portion, as shown in Fig. 1, or on a tube of other desired form, whether the end portion of the conduit tube is drawn out or reduced to form a capillary tube or not.

Fig. 3 shows a further variation in the form of the inner end of the conduit tube, the tube shown in this figure not having a reduced end but the opening of the tube to the interior of the conduit being of the full size of the internal section of the tube. The end of the tube is, however, cut or finished at an angle so that any liquid which may collect on the tube will not be apt to close the orifice at the end of the tube. This figure shows further the stopper as provided with an annular flange or shield 11 which when the stopper is in position in the neck of the container extends over the top of the neck of the container to cover and shield the same from micro-organisms, dust, etc., which might otherwise settle thereon and might possibly enter the neck of the bottle if the stopper were removed. Such a shield carried by the stopper will be found valuable where a container provided with a stopper carrying the air conduit with its plurality of air filters is employed for laboratory or other use which requires the more or less frequent removal and replacing of the stopper.

Fig. 4 shows a still further variation of the conduit tube in which the extreme inner end of the tube is closed and an opening to the interior of the container is formed by heating the side of the tube near its inner end and blowing out a comparatively small opening as shown at 12 in this figure. This figure shows also a cap fastened over the top of the stopper and neck of the receptacle. Such a cap might be for the purpose of protecting the stopper and neck of the bottle, or for holding the stopper in place,

or merely to give a good appearance, and it may be of any suitable material, such as paper parchment, through which air can pass, or it may be suitably constructed or arranged to permit air to have access to the conduit tube. A cap of paper parchment or other suitable material would serve to prevent, under ordinary circumstances at least, water from reaching the first fiber plug in the conduit tube, and in such case a conduit tube with only two filter plugs, as shown in this figure, would make an efficient device. In fact, as the chance of liquid reaching the plug nearest the inner end of the tube is but slight, especially when a solid or semi-solid medium or other substance is in the bottle, a single plug will in many cases answer to prevent the entrance of contaminations. If, however, only one plug were employed, it would be preferable to use a tube having its inner end formed to avoid liquid entering through the orifice and reaching the plug, as for example the curved reduced end shown in Fig. 1, or a capillary tube ending in a fine orifice as shown in Fig. 2.

It is preferable to make the container, stopper and conduit tube of separate parts as shown for economy and ease in assemblage, rather than to make an intergral structure as by sealing a glass conduit tube through one of the walls of the glass container.

A further advantage of the arrangement shown is that the stopper when of proper material such as rubber or a suitable composition acts as a cushion for the conduit tube and avoids danger of breakage of the latter. The whole device might be made of metal, in which case, of course, the objection of frangibility would not obtain as in the case of an integral glass structure.

Another advantage of the arrangement shown is that the stopper and conduit tube may be used for different containers or vessels, just as an ordinary rubber or cork stopper may be used. The conduit tube might, of course enter the vessel at any point and might for some purposes be arranged to extend partly or mostly outside of the container or vessel, but the arrangement shown, in which the opening from the conduit into the interior of the container is located near the center of the container, is preferable for most purposes and especially where the container is to be used for transportation or is otherwise liable to inversion.

What is claimed is:

1. A container comprising a closed receptacle and a conduit communicating with the atmosphere within the receptacle and containing three or more separate plugs of fibrous material.

2. A container comprising a closed receptacle and a conduit communicating with the atmosphere within the receptacle at a point removed from the walls thereof, said conduit containing a plurality of separate plugs of fibrous material and the inner end of said conduit being bent in circular form and ending at such point of communication.

3. A container comprising a closed receptacle, a conduit tube connecting the atmosphere within the receptacle with the atmosphere external to the receptacle and having its inner end reduced and curved circularly and ending and open to the interior of the receptacle at a point removed from the walls of the receptacle, and a plug of fibrous material in the conduit tube.

4. A container comprising a receptacle, a stopper for closing the mouth of the receptacle, a conduit tube extending through the stopper and open to the interior of the receptacle at a point removed from the walls thereof, a plug of fibrous material in the conduit tube, and means for preventing liquid entering the conduit tube and reaching the plug of fibrous material.

5. A container adapted for transporting and preserving aerobic bacterial cultures comprising a receptacle, a stopper for closing the mouth of the receptacle, a conduit tube traversable by air extending through the stopper and open to the interior of the receptacle at a point removed from the walls thereof, said conduit tube containing a plurality of separate plugs of fibrous material and having a reduced end through which connection is established with the interior of the receptacle at said point removed from the walls of the receptacle.

6. A container adapted for transporting and preserving aerobic bacterial cultures comprising a receptacle, a stopper of flexible and elastic material for closing the mouth of the receptacle, and a conduit tube of glass open to the exterior of the receptacle and extending through the stopper and open to the interior of the receptacle at a point removed from the walls thereof, said conduit tube containing a plurality of separate plugs of fibrous material.

7. A container adapted for transporting and preserving aerobic bacterial cultures comprising a receptacle, a stopper for closing the mouth of the receptacle, and a conduit tube extending through the stopper and open to the interior of the receptacle at a point removed from the walls thereof and connecting the atmosphere within the receptacle with atmosphere external thereto, said conduit tube being provided with means for intercepting micro-organisms passing therethrough and preventing such micro-organisms from entering the receptacle.

8. A container adapted for transporting and preserving aerobic bacterial cultures comprising a receptacle, a stopper for clos-

ing the mouth of the receptacle, a conduit tube extending through the stopper and open to the interior of the receptacle at a point removed from the walls thereof and connecting the atmosphere within the receptacle with atmosphere external thereto, said conduit tube being provided with means for intercepting micro-organisms passing therethrough and preventing such micro-organisms from entering the receptacle, and means for preventing the entrance of liquid from the receptacle into said conduit tube.

9. A container comprising a receptacle, a stopper for closing the mouth of the receptacle, and a conduit tube extending through the stopper and open to the interior of the receptacle at a point removed from the walls thereof and connecting the atmosphere within the receptacle with atmosphere external thereto, said conduit tube being provided with means for intercepting micro-organisms passing therethrough and having its inner end reduced and bent in such manner as to hinder entry of liquid into the conduit tube.

10. A container adapted for transporting and preserving aerobic bacterial cultures which comprises the combination, with a closed receptacle, of a gas conduit affording communication between the exterior of said receptacle and the interior thereof at a point removed from the receptacle walls, and means in said conduit at a location within the receptacle for preventing passage of micro-organisms through said conduit.

11. A stopper for containers or receptacles comprising a stopper body, and a conduit tube extending therethrough and prolonged for a distance beyond the inner end of said stopper body, said conduit tube containing three or more separate plugs of fibrous material.

12. A stopper for containers or receptacles comprising a stopper body of rubber or other suitable flexible and elastic material, and a conduit tube extending through the stopper body and prolonged for a distance beyond the inner end of said stopper body, said conduit tube containing a plurality of separate plugs of fibrous material and having its end which is intended to extend into the container or receptacle reduced and bent into a curve to hinder entry of liquid into the conduit tube.

13. A stopper for containers or receptacles adapted for transporting and preserving aerobic bacterial cultures comprising a stopper body of rubber or other suitable flexible and elastic material, and a conduit tube of glass extending therethrough and prolonged for a distance beyond the inner end of the stopper body, said conduit tube being traversable by air and containing a plurality of separate plugs of fibrous material.

14. A stopper for containers or receptacles adapted for transporting and preserving aerobic bacterial cultures comprising a stopper body of rubber or other suitable flexible and elastic material, a conduit tube traversable by air extending therethrough and prolonged for a distance beyond the inner end of said stopper body and containing a plug of fibrous material, and means for preventing liquid entering the conduit tube and reaching the plug of fibrous material.

15. A stopper for containers or receptacles adapted for transporting and preserving aerobic bacterial cultures comprising a stopper body of rubber or other suitable flexible and elastic material, and a conduit tube traversable extending therethrough and prolonged for a distance beyond the inner end of the stopper body, said conduit tube being provided with means for intercepting micro-organisms passing through the tube and for retaining the same within the tube.

16. A stopper for containers or receptacles comprising a stopper body of rubber or other suitable flexible and elastic material, and a conduit tube extending therethrough and prolonged for a distance beyond the inner end of the stopper body, said conduit tube being provided with means for intercepting micro-organisms passing through the tube and for retaining the same within the tube and having its end beyond the inner end of the stopper body reduced and bent into a curve approximating nearly a complete circle.

17. A container adapted for transporting and preserving aerobic bacterial cultures which comprises the combination, with a closed receptacle, of a conduit tube supported in such manner as to be located substantially wholly within said receptacle and affording an air passage extending from the exterior of the receptacle to the interior thereof at a point removed from the receptacle walls, and separate plugs of fibrous material disposed in said tube to prevent passage of micro-organisms therethrough.

18. A container adapted for transporting and preserving aerobic bacterial cultures which comprises the combination, with a closed receptacle, of a conduit tube supported in such manner as to be located substantially wholly within said receptacle and affording an air passage extending from the exterior of the receptacle to the interior thereof at a point removed from the receptacle walls, and means in said tube to prevent passage of micro-organisms therethrough, provision being also made to hinder entry of liquid from the receptacle into said tube.

19. A gas filtering device formed of a conduit tube containing a plurality of separate plugs of fibrous material and having one end reduced and bent into a curve to hinder entry of liquid into said conduit tube.

20. A bacterial distributing package com-

prising a closed receptacle, a moist culture medium carrying a culture of micro-organisms contained in the receptacle, and a gas conduit communicating with the atmosphere within the receptacle at a point removed from the walls thereof, said conduit being provided with means for intercepting micro-organisms passing therethrough, and said culture medium being in amount insufficient to submerge the orifice of the conduit in any position of the receptacle.

21. A bacterial distributing package comprising a closed receptacle, a moist culture medium carrying a culture of nitrogen-fixing organisms contained in the receptacle, and a conduit leading to the interior of the receptacle and having an orifice located at a point removed from the walls of the receptacle to supply air to the atmosphere within the receptacle, said orifice being of small size compared to the size of the body of the conduit, and said conduit containing fibrous material forming a gas filter, and said culture medium being in amount insufficient to submerge the orifice of the conduit in any position of the receptacle.

In testimony whereof I have hereunto affixed my signature in the presence of two witnesses.

GEORGE H. EARP-THOMAS.

Witnesses:
HARRY S. CONNOLLY,
A. L. KENT.