Biology 40

Experimental Notes

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Experiment II Dehydration method of ~~setting up~~ drying spores. — Perfection of Spray etc.

In preliminary work, Aspergillus Flavus was used since very poor germination results were achieved using it in horse serum with lyophilization.

1 Liter of potato agar was prepared 10/21/48. Formula. 300 grams of potatos were ~~st~~ peeled and sliced and cooked until soft. ~~F~~ with about 400-500 mls tap H_2O. The resulting broth was poured thru cheese cloth. The agar was completed by adding 15g. of agar 15 g. of dextrose and finally bringing up to volume.

The agar was added to 6 Kolle flasks. 10/22/48. 3 of these were inoculated with a heavy spore load of Aspergillus Flavus from a stock culture of Carol Buells. These were allowed to germinate at room temperature. Inoculation done 10/25/48. By 10/28/48. heavy growth had started.

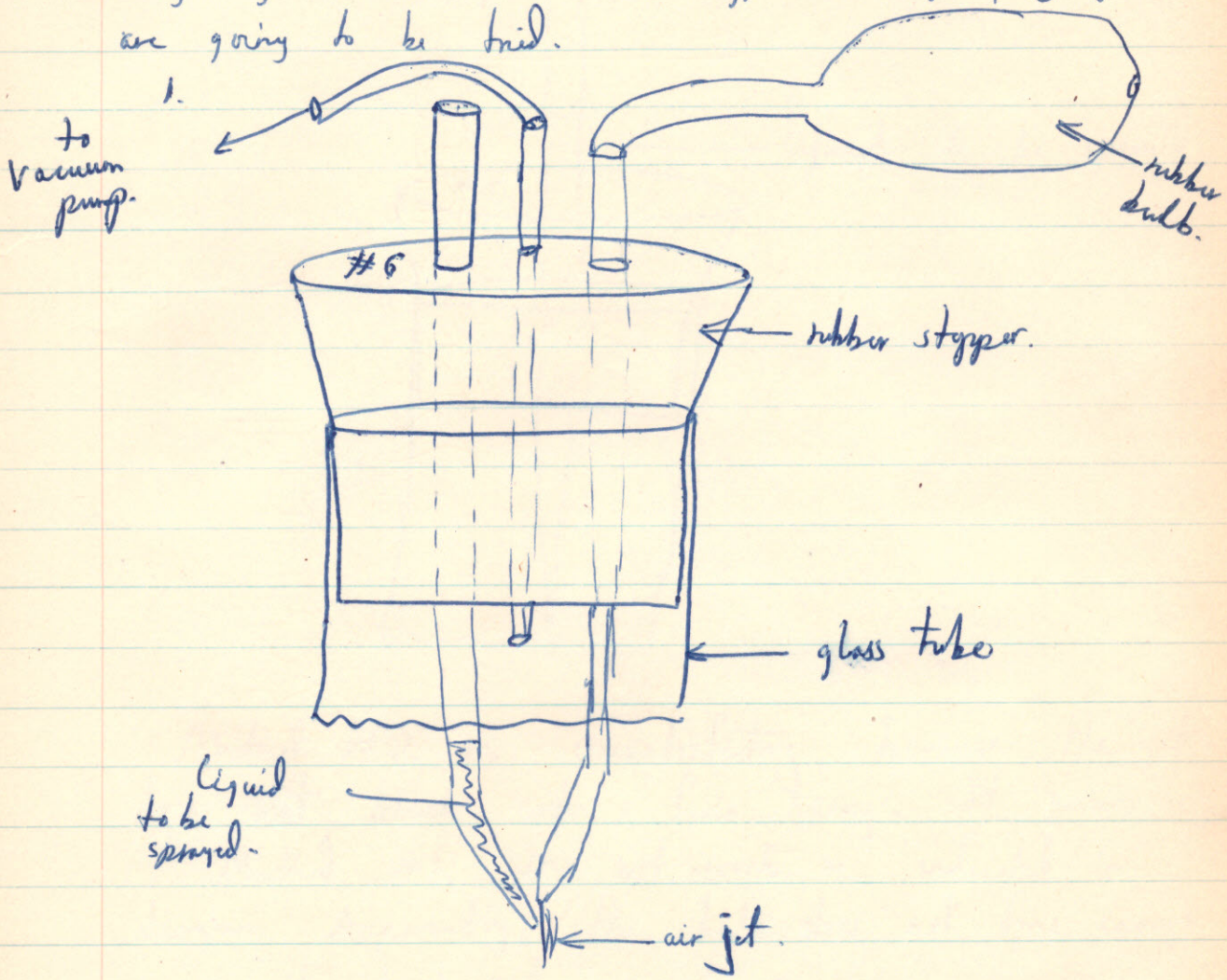
10/28/48. Today. I assembled some of the apparatus for a drying column:

1. A pyrex tube - 3 feet long - about $1\frac{3}{16}$" inside diameter.
2. Stoppers to fit
3. Soft glass tubing.

The problem is to get a method of forcing a fine spray of spore suspension into the tube. so that spores will dry in the slightly evacuated, warmed tube, and fall to the bottom where

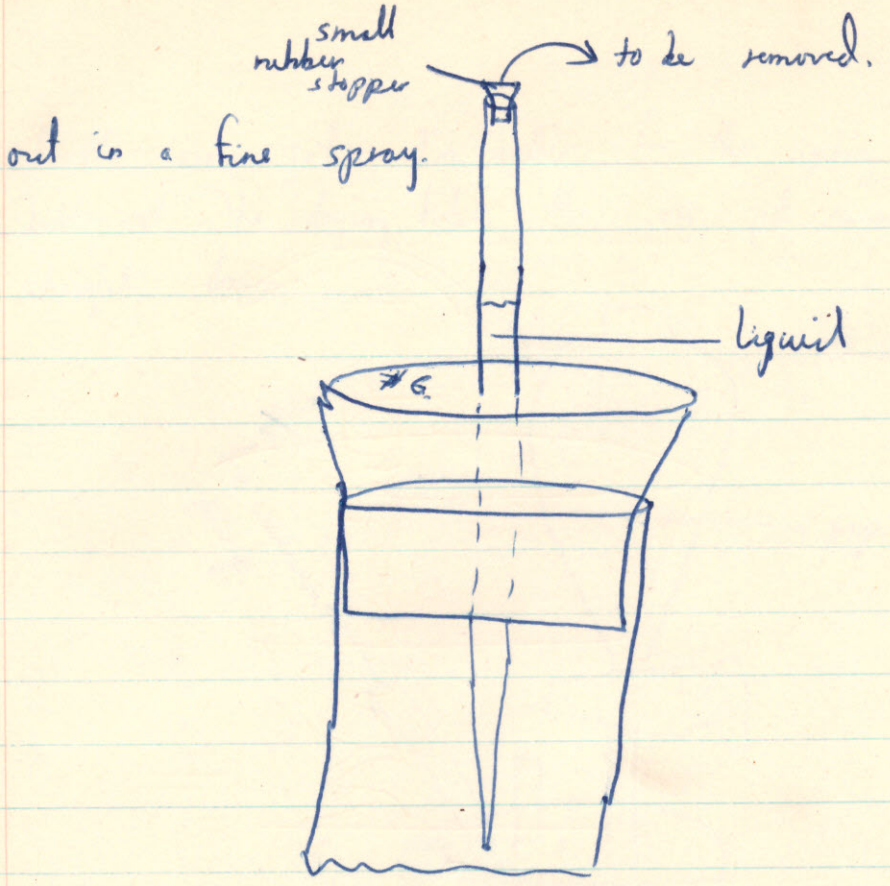
they may be collected. Two types of spray gadgets are going to be tried.

1.



The main trouble with us this method will probably be the difficulty of aligning the fine nozzles and keeping them aligned.

2. The alternate method is to use a single fine nozzled tube which is filled with spore suspension and stoppered. The large tube is then evacuated and the stopper removed. Perhaps the vacuum will force the liquid

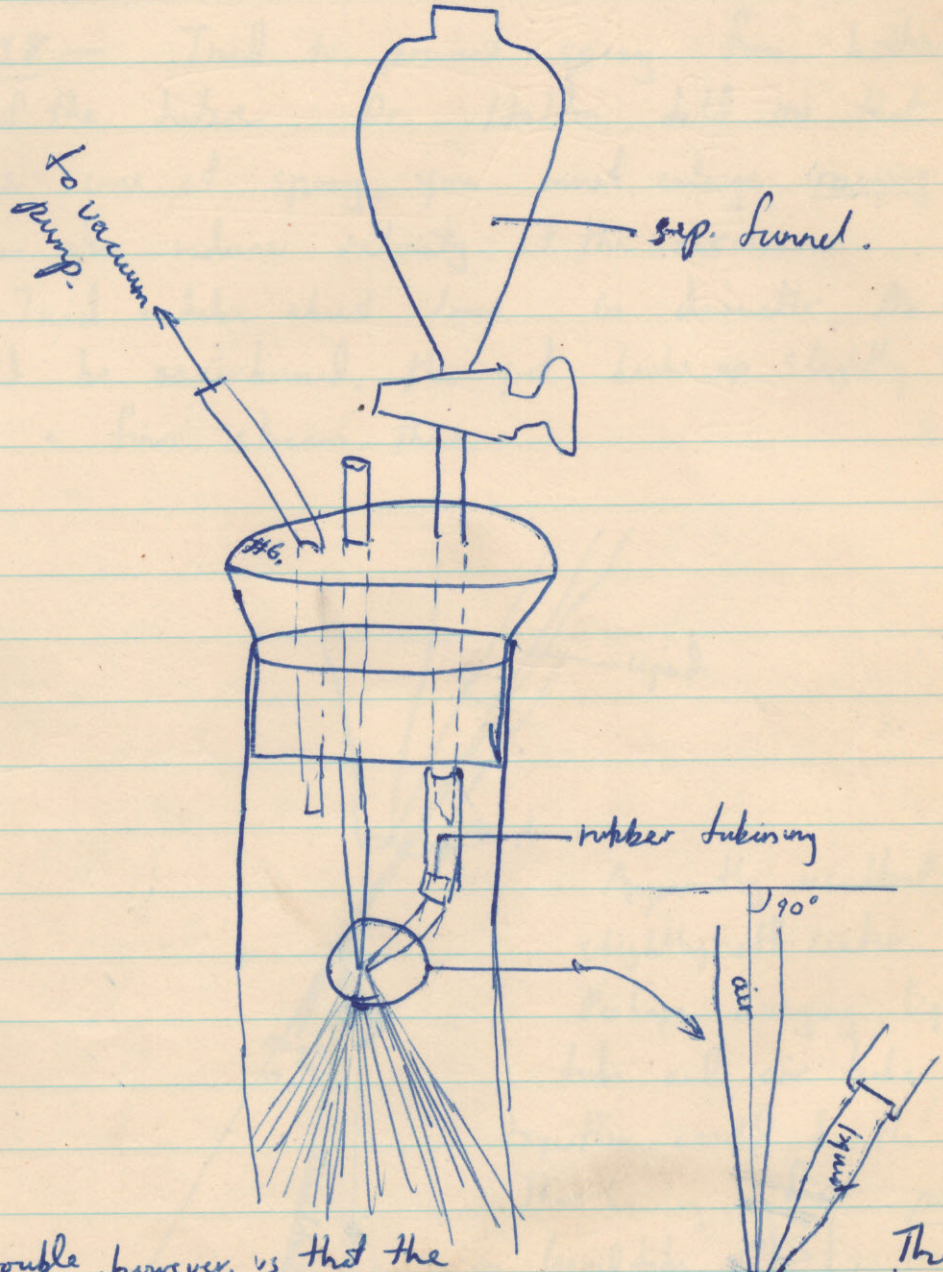


Method #2 is unsatisfactory, because the jet does not break into a fine spray. It remains a jet.

Method #1, does not work as indicated in diagram. Apparently, the bulb does not force enough air in to break jet up into a spray.

~~10~~ 11/1/48. A modification of method 1 was obtained which seems to do the trick. Instead of using a bulb, the air is pulled in in a constant stream, by the vacuum, and the spore suspension (water being used in three preliminary final runs) is placed in a separating funnel. When the stopcock is adjusted so that water comes out dropwise, the air breaks it up nicely into a fine spray. Apparently the direction of the spray is determined by the direction

of the air jet. In order for the spray to reach the bottom of the drying tube, the air jet must be pointed straight down.



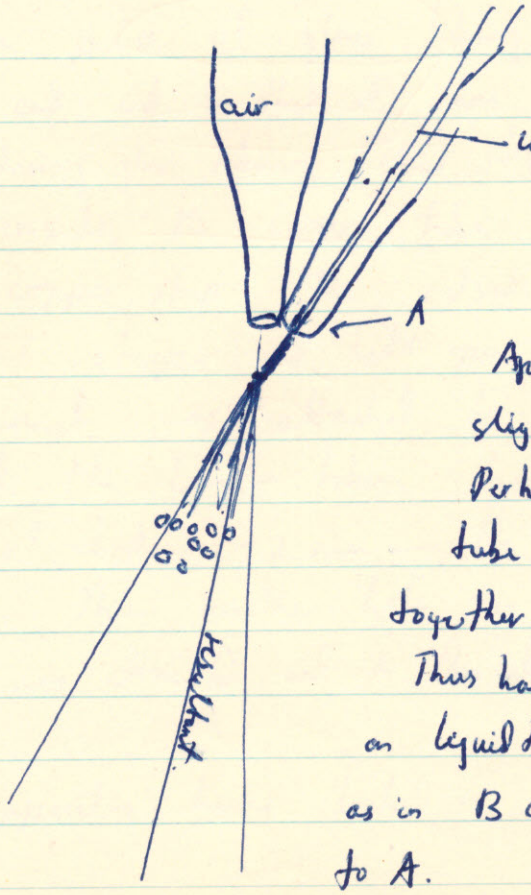
One trouble, however, is that the spray covers too wide an ∠, and impinges on the sides. This might be helped by making the opening of the air jet ~~very~~ a little larger.

The liquid jet's nozzle should be a very tiny.

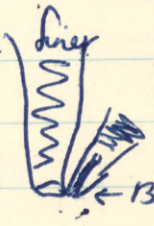
[To draw glass straight, heat and pull vertically, not horizontally]

11/8/48 — Tried to prevent spray from hitting sides of the tube. Dr. Hoffman told me that to reduce cone of spray, you must enlarge opening of air tube or else reduce velocity of the air.

1. Tried a tube about 1mm. in diameter. As far as could be ascertained, the jet broke up slightly but not into a fine stream. Thus.



Apparently resultant is slightly off center. Perhaps bringing liquid tube and air tube closer together, would do the trick. Thus having a fine point on liquid tube as in B compared to A.



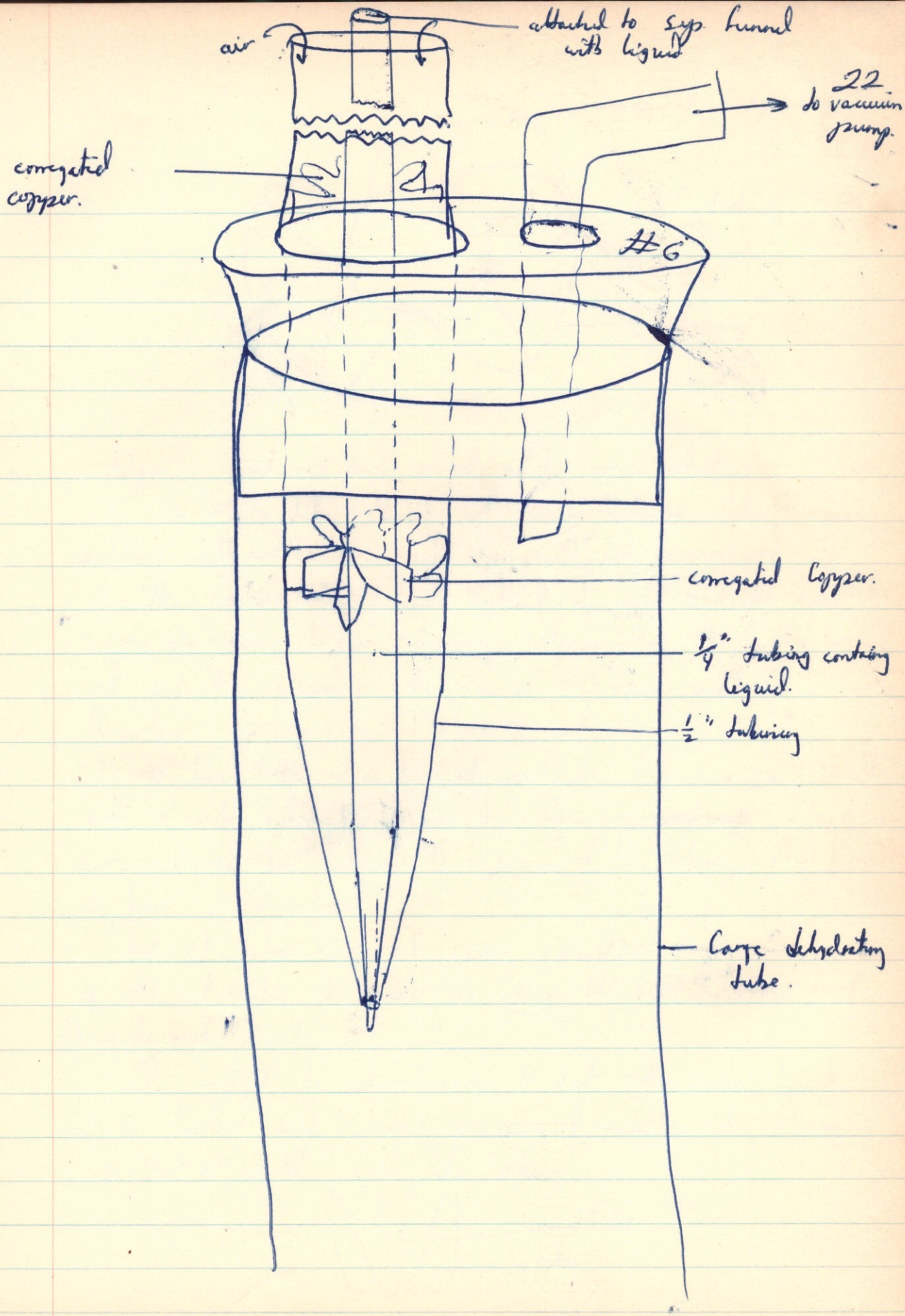
I tried a tube of type B and the result was better. The ~~spray~~ jet was broken up much sooner than when method A was obtained. Using a fairly small opening ~~air~~ jet tube, a nice spray was obtained, but the spray still impinged well up the tube.

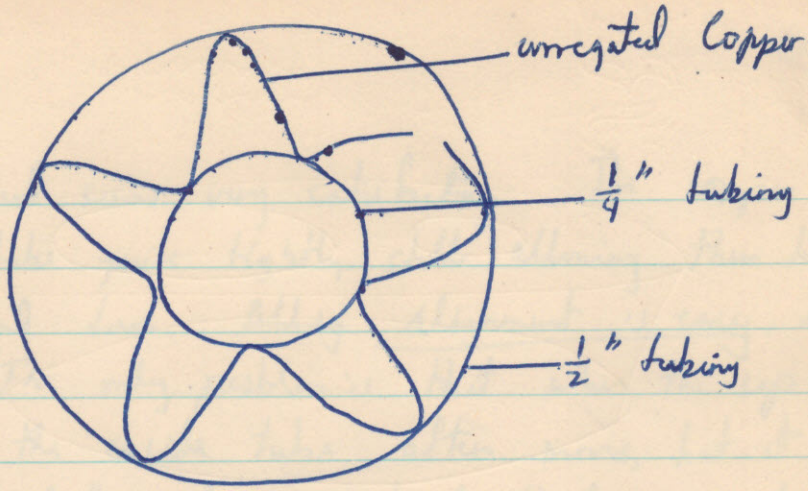
Another problem noted is that the vacuum ~~line~~ tends to pull the spray towards the top of the tube further

11/12/48 – Upon Dr. Westin's suggestion, the following scheme was tried:

A large piece of glass tubing ($\frac{1}{2}$" inside diameter) was obtained, and cut so that it was about 12" long. A piece of ordinary glass tubing (about $\frac{1}{4}$" outside diameter was suspended inside the larger tube by means of a corrugated copper strip. The outside tube was the placed then a #6 stopper, and left open to outside. The small separating funnel was attached to the smaller tubing. Before hand, the larger tubing had been drawn down to a diameter of about 1 - 2 mm. — The inside tube to a very fine opening. Then another hole in the stopper, a short glass tube was attached and to this attached the vacuum pump.

The apparatus hence looked as follows:


air
attached to sep funnel with liquid
22
to vacuum pump.
corrugated copper.
#6
corrugated Copper.
1/4" tubing containing liquid.
1/2" tubing
Large dehydrating tube.



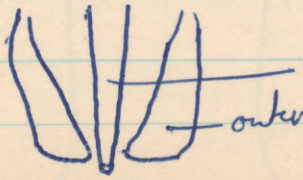
Upon a trial ~~on~~ run several points were noted:

1. The inside tube should not touch outside tube otherwise airflow will not be even and spray will not be very good.
2. The inside tube should extend just beyond outside tube if you are going to have a spray. Apparently, the further beyond it is, the narrower the cone of the spray, until no spray is formed.
3. At first the diameter of outside tube was quite small, and a circle spray was formed. ~~This was undesired~~ The opening was enlarged by cutting off a piece of the tubing. This proves to have 2 advantages.

(a) it makes cone of spray smaller

* (b) it makes it easier to allign the two tubes so that they do not touch.

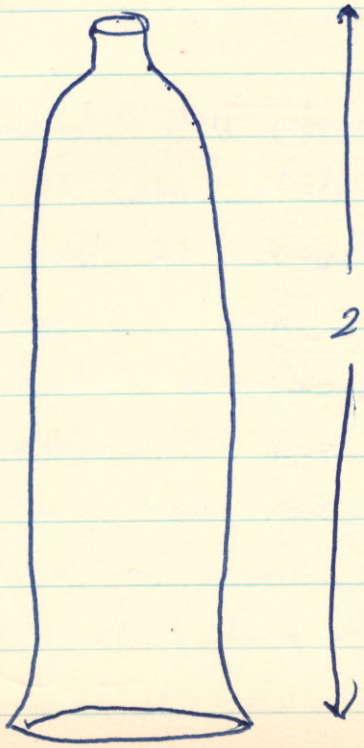
It might be advantageous to cut outer tube back even further, and narrow opening if necessary by flaming. so that it would look like this:



This method seems very satisfactory. The copper strips hold the tube quite tightly, still allowing them to be moved up and down. ~~Abbey~~ alignment is easy, and more permanent. The only problem is that when the sep funnel is attached the inside tube often moves, but it is easily readjusted. And most important of all, this apparatus made a very nice spray.

10/15/48 – Fiddled around some more with above spray method – different opening in two tubes gave varied results. Still haven't decided which opening is best.

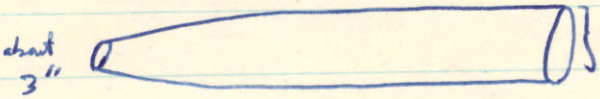
Trouble still is impingement of spray on side walls of tube. Prof Johnston suggested using a large bell jar with a whole in the ~~top~~ top in which to put spray apparatus. Will experiment with this on Thursday.



11/18/48 — Set up apparatus on Bell jar. I put a piece of tubing over the air intake and clamped it so as to better procure a vacuum. But when evacuation had proceeded for a bout two minutes, the glass plate on which the bell jar had been sealed with vaseline, blew in (because of the vacuum), breaking the flange off the bell jar, and smashing the spray apparatus to bits.

11/19/48 — Hunted around for other bell jars — unsuccessful.

11/21/48. Took the broken bell jar, another bell jar from the stock room, and a large 3' section of tubing drawn narrow at one end, down to Macallister-Bicknell to be cut and ground above the cracks which these vessels had.



11/23/48 — Received glass ~~where~~ ware from M. Bicknell. Further experimental with spray, obtaining whole milk. The set up was the same as on page 22 except that bell jar was obtained instead of glass tubing. I also ~~attempt~~ heated the jar with an electric heater given to me by Prof. Weston. The run was unsuccessful, since no vacuum was built up because air kept going in air intake tube. As a result no vacuum was produced. What is necessary is some method of building ~~of~~ up a vacuum and then introducing in a spurt, a spray of milk (or spore load).

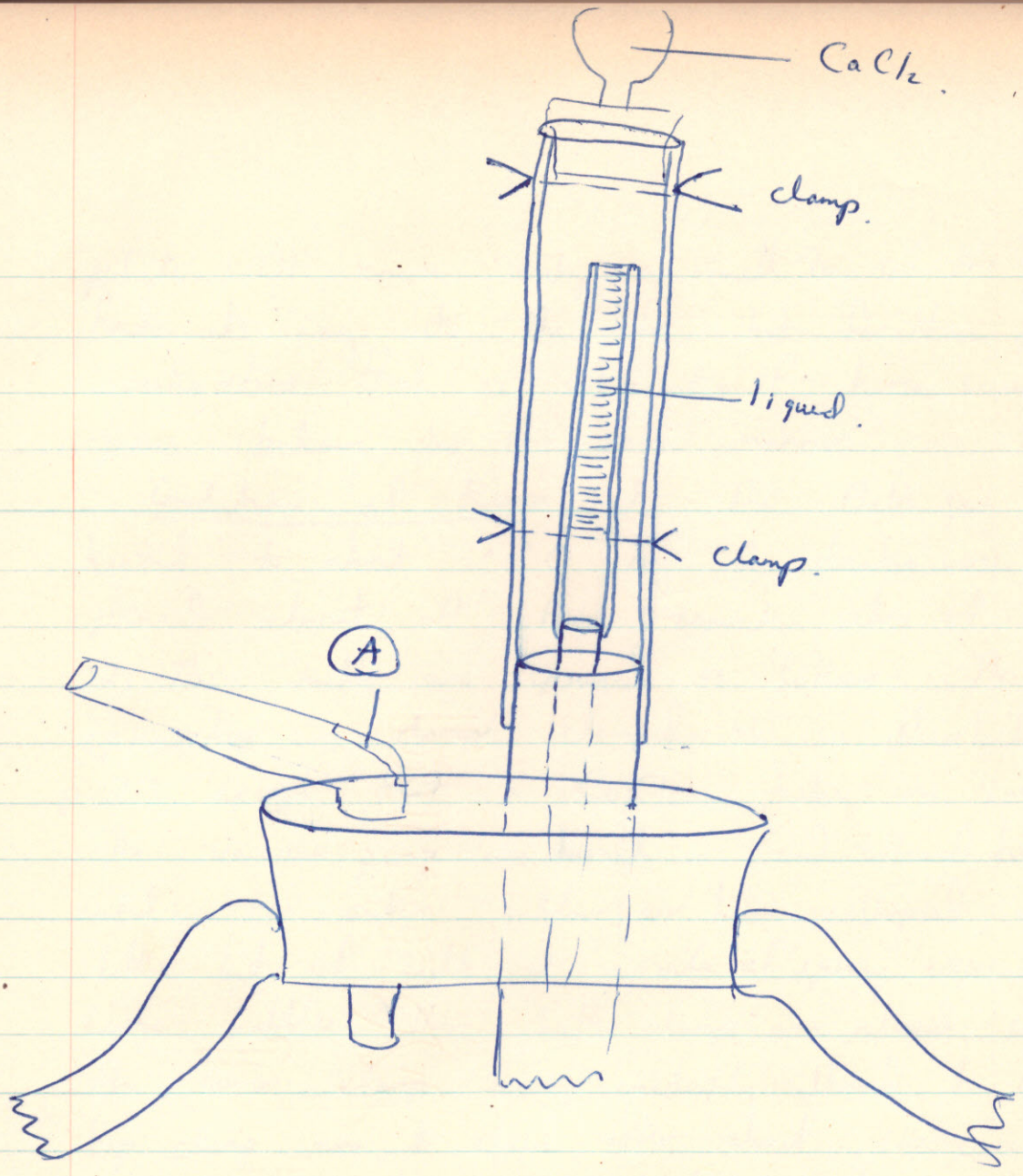
11/29/48 - The following set up was tried. ~~The liquid tube (which was drawn out to a very fine point) was filled with milk.~~ About six inch sections of thin walled tubing were placed over respective liquid and air tubes. A strong spring clamps was applied to both tubes at their base. The liquid tube was then filled*, and the air tube was clamped shut above the liquid tube. Finally a $CaCl_2$ tube was fitted on to the air tube in an attempt to dry the incoming air. The Vacuum pump was then turned on and pumping allowed to proceed until air tube collapsed indicating a vacuum. Both clamps were then released simultaneously, and an excellent spray resulted, as the incoming air rushed in. Preliminary runs seemed encouraging altho the milk failed to dry.

Points of note = 1. If only one clamp was used, or if the liquid tube extended above the air tube and another clamp used near tip, a tight seal was not formed and a very poor spray was formed.

2. The thin liquid tube is inclined to block up from the use of whole milk. Up to now, I haven't been able to obtain any skimmed milk.

3. Apparently, the first breakage was the result of air getting under plate and pushing upward smashing plate. A heavier plate seems to be more desirable.

* By utilizing a 1/2" piece of tubing drawn down to form a funnel. ~~which can~~ The small end was placed in liquid tube and milk added thru it.



12/2/98- I obtained some skimmed milk, also a heavy glass plate to put under the bell jar. I made a run as on 11/29, but obtaining skimmed milk instead of whole milk.

** Result - Dry, powdered milk appeared in the vacuum tube at point marked (A). This result which was wholly unexpected may prove very fortunate, as the spores will be much easier to collect from here than they could from the glass

plate. Of course only a small % of the liquid finds its way into the tube, but for these preliminary investigations that is sufficient - if horse serum and sprays behave in the same manner.

Conditions of Runs 1. The Bell jar was heated to about 60°C by 2 electric coil heaters placed about 4" from opposite sides of the bell jar. 2. The milk was sprayed as follows: The spraying apparatus was ~~dampt~~ clamped as in illustration on p. 27, except that a $CaCl_2$ tube was not used. The vacuum pump was turned on and allowed to pump until the outer rubber air tube collapsed, and the little bit of milk at nozzle of spray, expanded into large bubbles. Then both clamps were released simultaneously, the lower clamp being immediately allowed to close. The top clamp was ~~$$~~ closed after about 1-2 seconds. The ~~liquid~~ milk which had dropped into the glass tube was sprayed out by ~~opening~~ opening the top clamp for about a second at 10 second intervals. When the tube was empty, the procedure was repeated.

The entire run was repeated 3 times. In the ~~last two times~~ (and others) First run $CaCl_2$ was placed in a petri dish at bottom of bell jar to absorb any liquid milk that might drop. But in the following runs, no $CaCl_2$ was used, just to make sure that the

white powder was milk. Both the 2nd. and third runs gave positive results. Some solid also appeared to be deposited on the plate, but it was messed up by liquid milk on which had failed to dry falling on the plate.

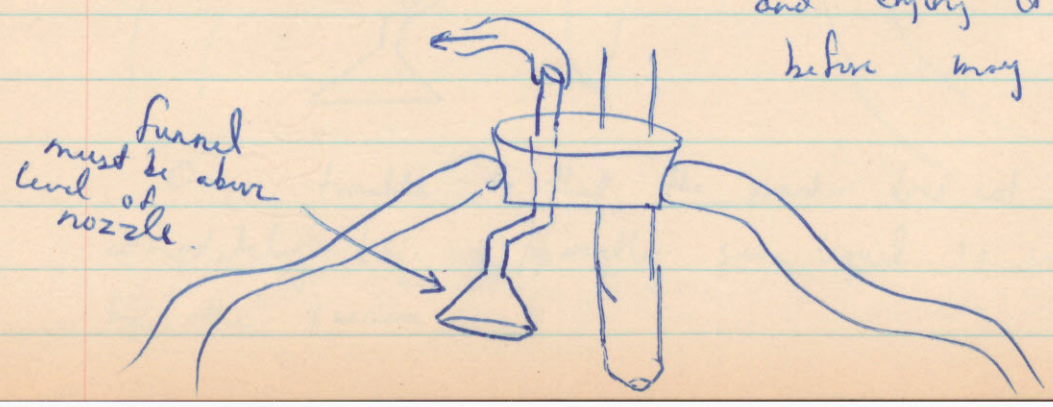
To make sure that the dry powder was milk, I
1. put a little on a spatula, and heated in a flame. The powder turned black and smelt like burnt proteins.
2. When powder was dissolved in H_2O, a blue-white milk color formed. When $CaCl_2$ is mixed with H_2O, it forms a clear solution.

~~Things to improve~~:
1. ~~The tube of~~ fos.

Explanation: I imagine that the milk that dry's almost instantaneously is pulled up by the vacuum into the tube.

Things To improve: The tube of fois such a small area to the ~~spoon~~ milk, that only a few probably enter it. This might be improved by stabilizing a funnel as a vacuum tube.

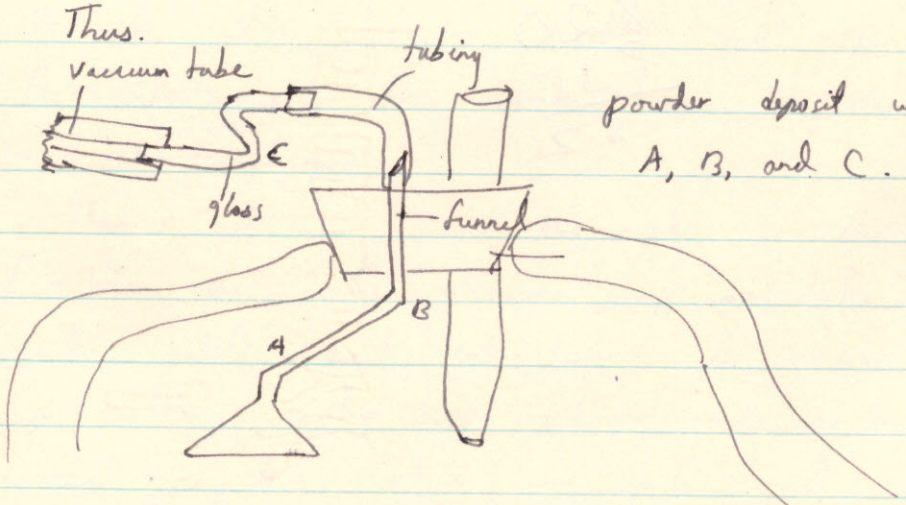
Thus. This will be tried 12/3/48. Heating the air and drying it as suggested before may improve the apparatus



12/3/48 - A funnel was bent as indicated in the diagram on page 29, and ~~the~~ ~~run~~ a run was made as on 12/2. The results were a slight improvement as some powdered milk did deposit - somewhat more than occured the preceding day. One trouble is that the milk did not seem to be completely dry, but otherwise the method seems satisfactory.

* 12/9/48 - A run was made utalizing horseserum instead of powdered milk. - Otherwise ~~ex~~ everything was the same. The results were even more satisfactory than with skimmed milk. A dry wide powder was deposited in the funnel., and trap (see below)

An innovation not mentioned before was the placing of a glass tube between the funnel and the vacuum hose. This tube was bent to catch dried powder and it worked quite well.



powder deposit was heaviest at A, B, and C.

One trouble is that the powder does not seem to be completely dry. Apparently some liquid is sucked up by the vacuum.

This trap method, however works very well, because it facilitates collecting the powder. Also it may be possible to simply seal off the ends of the tube in order to store the spore - horse-serum powder.

12/10/48 - The probable reason for some liquid being sucked up into the funnel is that the mouth of the funnel is too close to the opening of the air jet. Hence, today, I made a longer ~~air~~ air jet tube, so that the opening of the air jet tube was about 1½" below the mouth of the funnel.

* The run was made with a spore suspension in horse serum was was prepared as follows:

~~I~~ A heavy spore load ~~was~~ of Aspergillus flavus which has been growing since 10/22/48, was transferred to a small serum tube. 1-2 cc. of 0.1% Aerosol wetting agent solution was added, and the mixture stirred well with the transfer needle until spores were well dispersed. The contents of the serum tube were then added to 5-10 cc. of Normal horse serum.

~~The~~ A run was then made in the usual manner. [When the ~~the~~ vacuum pump was first turned on, some liquid leaked back into trap. The could be prevented by putting another trap between the spore trap and the vacuum pump to catch any such liquid]. As before, a white powder deposited in the funnel and in the trap. ~~The~~ After using up ~~the~~ about 5 cc, the vacuum was turned off, the trap tube was broken at the bend by sliding

a triangular file. The first thing noticed was that the powder was much dryer than has previously been the case. This is probably a result of ~~heating~~ having the funnel appreciably above the opening of the air tube.

Slides were made of both the original spore - horse serum mixture and the ~~for~~ dried horse-serum. With the latter the technique was as follows: 1 drop of distilled H_2O was added to the slide. The dried powder was tapped onto the drop and stirred until a suspension was formed. Examination under the scope showed that the original suspension was too thin for there ~~was~~ were few spores present in the dried material.

The slide ~~and~~ holding the dried - horseserum - spore mixture was placed in a moist chamber to see if any germination would occur ~~in~~ during the following 24 hours.

The next & go experiment will involve careful controlled tests to determine the % of germination of Aspergillus flavus after it has been put thru this process.

[Note: Heaters are placed on opposite sides of the bell jar, about 6" from it. This gives a temp of 53° - 57° C inside the bell jar.