On "Intertraction" between Albuminous Substances and Saline Solutions.

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In 1906 I pointed out that hypertonic salt solutions applied to wounds, sinuses and foci of infection increased the discharge from these, supplementing the ordinary mechanical drainage by drawing out from the tissues infected and corrupted lymph.

In the war, and especially in its earlier period when every form of sepsis and gangrene was rife and almost every wound was foul, I again advocated treatment by hypertonic salt solutions. The method was then employed extensively and with good results.

Hereupon followed a detailed study of the action of hypertonic salt solutions upon the wound, and also an examination of their action *in vitro*.* It was in the course of this latter found that when a receptacle containing strong salt solution is connected up with a receptacle containing water by a siphon tube threaded with a wick or filled with water and armed (at the end which dips into the salt solution) with a tight cottonwool plug, water is slowly drawn into the salt solution—the level of this rising and that of the water falling.

A much more rapid and abundant drawing effect—in the form of a down draught of supernatant fluid into a heavier salt solution—was obtained by taking a test-tube, dividing it up into an upper and lower compartment by a plug of cottonwool soaked in a solution of white of egg possessing a specific gravity of 1026 and then filling the lower compartment (through a lateral opening) with a saline solution possessing a specific gravity of 1052, and the upper chamber (which here provides a control) with water. It was found that the egg albumen was under these conditions carried down rapidly and in large quantity into the subjacent heavier salt solution while none found its way into the superjacent water.

That experiment would seem to suggest that the forces of diffusion are at any rate in the case where albuminous substances and saline solutions are brought into conjunction, supplemented by what I should like to call "*forces of intertraction.*"

In the following that hypothesis is subjected to certain further examination.

The method of investigation adopted was to superimpose serum or other albuminous fluids directly upon heavier saline solutions, or upon occasion

* 'Proceedings Royal Institution,' March 9, 1917; and 'Lancet,' June 23, 1917.

lighter salt solutions upon heavier albuminous fluids—adding generally to one or other fluid a trace of colouring matter (eosin) in order to render the course of events more manifest to the eye. The experiments were conducted in capillary tubes, full sized test-tubes and also in a very convenient form of diffusion cell suggested by my colleague, Dr. Alexander Fleming. The diffusion cell just referred to is made by covering a microscopic slide, or larger sheet of glass with a layer of wax of any desired thickness; cutting out a cell of any desired shape; and then bringing down upon the border of wax a companion microscopic slide or glass plate.

The phenomena described below manifest themselves alike in each of the above mentioned types of receptacle. For demonstration and final experimentation the flat cell has of course obvious advantages. When in such a cell serum is allowed to run down gently from a pipette on to the surface of a heavier—*e.g.*, 6 per cent.—saline solution the following train of events occurs.

When the serum impinges upon the surface of the salt solution it indents it, and then takes up by hydrostatic resilience a position on the surface, floating there as a layer which is delimited below by a somewhat undulate outline. Then within a very few seconds—seemingly as a result of whirlpool movements sucking in the wave summits protruding downwards from the under surface of the serum—this specificially lighter fluid is drawn down into the heavier salt solution below. The appearance is then as if pseudopodia or tentacles were being let down into the depths (figs. 1 and 2). Simultaneously with this, as can be seen when we employ a coloured salt solution and an uncoloured serum, the former is carried up into the serum forming there a system of thin ascending streams arranged after the fashion of the teeth of a comb (fig. 2).

This down- and up-streaming process progresses apace and gives, as an intermediate result: a stratum of transported serum upon the floor of the cell; and a layer of transported salt solution ranged at the top of the cell superficially to the original stratum of serum. As a terminal result, we have complete interfusion, manifested to the eye by quite uniform coloration. With a diffusion cell made from microscopic slides (*i.e.*, a cell measuring 1 inch by 3 inches) this is arrived at in something like half-an-hour.

It will be seen that we have here two arresting features: the singular fashion in which the lighter and heavier fluids interpenetrate (we may perhaps speak of this as "*pseudopodial interpenetration*"), and the rapidity with which complete interfusion is achieved.

The singular point about the pseudopodial interpenetration is not so much that a lighter fluid (the serum) is carried down into a heavier one; but that this serum, instead of recoiling to the top, sinks to the bottom, like a heavier

fluid, descending in definite streams to spread itself out upon the floor of the cell. Exactly the same applies to the heavier salt solution. It is not only caught up into the serum, but continues to ascend there in definite streams.

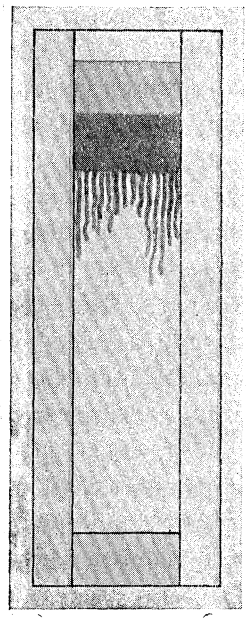


FIG. 1.

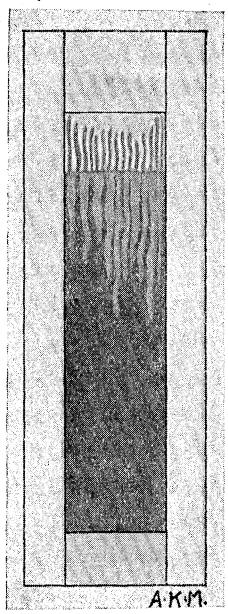


FIG. 2.

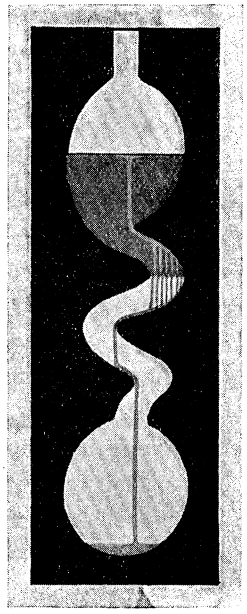


FIG. 3.

FIG. 1.—Flat diffusion cell filled in with 6 per cent. NaCl solution below. Upon this is superposed coloured serum; and again upon this, water. The specifically lighter serum is seen interpenetrating in the form of pseudopodial processes into the heavier subjacent salt solution.

FIG. 2.—Flat diffusion cell filled in below with coloured 6 per cent. NaCl solution. Upon this is superposed uncoloured serum. The subjacent coloured salt solution is interpenetrating into the supernatant coloured serum, and the uncoloured serum into the subjacent coloured salt solution.

FIG. 3.—Flat diffusion cell filled in below with uncoloured 6 per cent. NaCl solution, and above with coloured serum. The ascending rills of uncoloured salt solution here unite to form an ascending stream; and in like manner the descending streams of coloured serum unite to form a cascade.

This process of descending and ascending can be beautifully demonstrated in a specially shaped diffusion cell, which again was suggested by Dr. Fleming. The cell in question has an upper and a lower reservoir (somewhat after the fashion of a Kipp's apparatus), connected by a much narrower channel, which zigzags down after the fashion of a flattened out spiral (fig. 3). The apparatus is charged with a 6 per cent. salt solution as far as the neck of the upper chamber, and then this last is filled with coloured serum. In a cell thus charged the serum descends through the salt solution as a coloured waterfall, forming at

each turn of the channel a separate cascade. At the same time, in the upper reservoir the uncoloured salt solution ascends rectilineally in a thin stream.

It may be adventured as a suggestion that the serum enters into conjunction with salt so as to become heavier, and that the saline solution, parting with its salt to the serum, becomes lighter. There is no question (when operating with unaltered serum) of any falling out of solution of the albuminous substances.

Where, as in the experiments above cited, we set out to obtain evidence of a down draught of serum and an up draught of salt solution, the salt solution employed must contain more than 4 per cent. of salt. For a 4 per cent. solution of salt has a specific gravity equivalent to that of serum (human serum).

When we want to arrange for the reverse effect—that is, for an up draught of serum into a lighter salt solution, or a down draught of this into serum—we are by necessity tied down to the employment of solutions containing less than 4 per cent. of salt. With these weaker solutions dramatic pseudopodial interpenetration and very rapid interfusion are not to be expected, and to achieve effects manifest to the eye, we may best (guiding ourselves here by the principles enunciated by Horace Brown) betake ourselves to diffusion cells of conical section. To achieve a visible up draught of serum we require a cell corresponding in shape to the section of an inverted funnel.

Into such a cell we introduce a layer of coloured serum, and then superimpose a 3 per cent. solution of salt, filling in with this the remainder of the contracting cone and also the stalk of the funnel. At the same time, for the purpose of a control, we fill in a second cell, employing here, as our recipient fluid, water instead of salt solution. It will, after the lapse of a few hours, become manifest that the serum is being caught up into the salt solution, and that it is not sensibly diffusing into the water.

The down draught of a lighter salt solution into serum can—taking here again as our guide the principles of Horace Brown—be made manifest to the eye by employing a diffusion cell of triangular shape, disposed with its apex downwards, and filling into the apex of this expanding cone uncoloured serum, and superimposing coloured 3 per cent. salt solution.

Passing on now, the following points may be briefly adverted to:—

Where serum and salt solution are brought into conjunction, the content in albuminous substances plays a very important part in the production of the phenomena described above. When we take a series of capillary tubes, place a fiducial mark upon the upper part of the stems, fill in up to that level with a 6 per cent. salt solution, seal up the distal ends of the tubes, and then with a paraffined hair fine pipette superimpose—in the one tube, upon the

salt solution a coloured serum; and in the others progressive dilutions of this—the drawing effect of the salt becomes progressively less manifest, becoming almost inconspicuous when we reach a 32-fold dilution of the serum.

The concentration of the salt also influences the result. The optimum display of pseudopodial interpenetration and the most rapid interfusion would appear to be obtained when serum is superposed upon 5 per cent. to 8 per cent. sodium chloride solutions. Very concentrated solutions give less striking results, this being presumably due to the greater resistance which these heavier fluids would offer to the down draught of serum.

We have already seen in the experiment with the cottonwool plug which furnished the starting point for these experiments, that solutions of egg albumen react with salt in the same way as serum. This would seem to hold true also of albuminous substances obtained from muscle.

Solutions of commercial peptone give only an indistinct reaction.

All the commoner salts—such as sodium sulphate, potassium chloride, potassium sulphate, and magnesium sulphate—react with serum in apparently the same manner as sodium chloride. The same holds true of solutions of cane sugar, and here again very concentrated solutions give less striking results.

As in the case of diffusion proper, so here temperature exerts a dominating influence. In an experiment conducted by superimposing coloured serum upon 6 per cent. salt solution in diffusion cells made out of pairs of microscopic slides, the time occupied in the descent of the serum to the floor of the cell (a distance of about $2\frac{1}{2}$ inches) was found at a temperature of 45° C. to be forty-two seconds; at 15° C., one minute fifteen seconds; and at 3° C., three minutes.

Before embarking upon any general comment, the data of certain other experiments which have a bearing upon the employment of hypertonic salt solutions in the treatment of foul septic wounds may be briefly put on record. The experiments are as follows:—

Experiment 1.—Two similar capillary pipettes—A and B—are taken. The stems are marked off into divisions of equal length. By the aid of a teat, 6 per cent. salt solution is then aspirated into each—the inflow being arrested when fluid comes level with the fiducial mark in the neck of the stem. The ends of the capillary stems having been sealed, there is now, in pipette A, imposed upon the salt solution a measured volume of a broth culture of staphylococcus mixed with an equal volume of coloured water. In pipette B there is imposed upon the salt solution the same quantity of staphylococcus mixed with an equal volume of coloured serum.

The tubes are now set aside for ten minutes. We then take them in hand,

and in each case cut across the stem at the upper fiducial mark, and then, using for the purpose pipettes drawn out into hair-fine stems, empty out from pipette A and pipette B the contents of the capillary stem—compartment by compartment, planting out as we go upon nutrient agar.

It will be found that in pipette B—that in which infected serum was superimposed upon the salt—the microbes have been carried down to the very bottom of the capillary stem. In pipette A—that in which an infected watery fluid was superimposed—the microbes will have gravitated down only a very short way.

Experiment 2.—Two similar capillary pipettes, with stems divided off into segments of equal length, are taken. A coloured mixture, consisting of nine volumes of water mixed with one of staphylococcus culture, is introduced by capillarity into the distal extremity of the one; and a similar quantity of a coloured mixture of nine volumes of serum and one of staphylococcus culture into the other, the inflow being arrested when the fluid reaches the first division mark. Then, in each case, the distal end of the pipette is turned up sufficiently to displace the column of fluid, and to bring it into position a little distally to the antepenultimate fiducial mark. The tips of the tubes are now sealed up in a by-pass flame. Then, by means of a capillary pipette drawn out into a hair-fine extremity, and carried down into the stems of the pipettes A and B to a little short of the point where the bacterial fluid is lodged, we—leaving here a bubble of air—fill in, in the case of the pipette B, a 3 per cent. solution of salt; and, in the case of the pipette A, water.

The stems having been thus filled in, we take in each case a solid-ended hair-fine glass thread (obtained by drawing out in the flame a piece of capillary tube and fusing its end), and thrust it into the stem of the pipette, carrying it down until it enters the bacterial fluid, and pushes this up level with the penultimate fiducial mark. By the aid of the glass thread, the fluid in the upper part of the stem is let down quite gently upon the bacterial fluid—the intervening bubble of air mounting up the while to find escape in the neck of the pipette. We now set aside the pipettes for twenty-fours hours, and then emptying the compartments one by one from above downwards, plant out the contents upon nutrient agar.

The cultures so obtained show that, where salt solution is superposed upon infected serum, the microbes are carried some distance up the stem; while in the case where we have water superposed upon microbes suspended in water, they are confined to the distal end of the stem.

It thus is manifest that we have in an hypertonic solution an agent which is capable of drawing out from the cavities and *cul-de-sacs* of a wound and

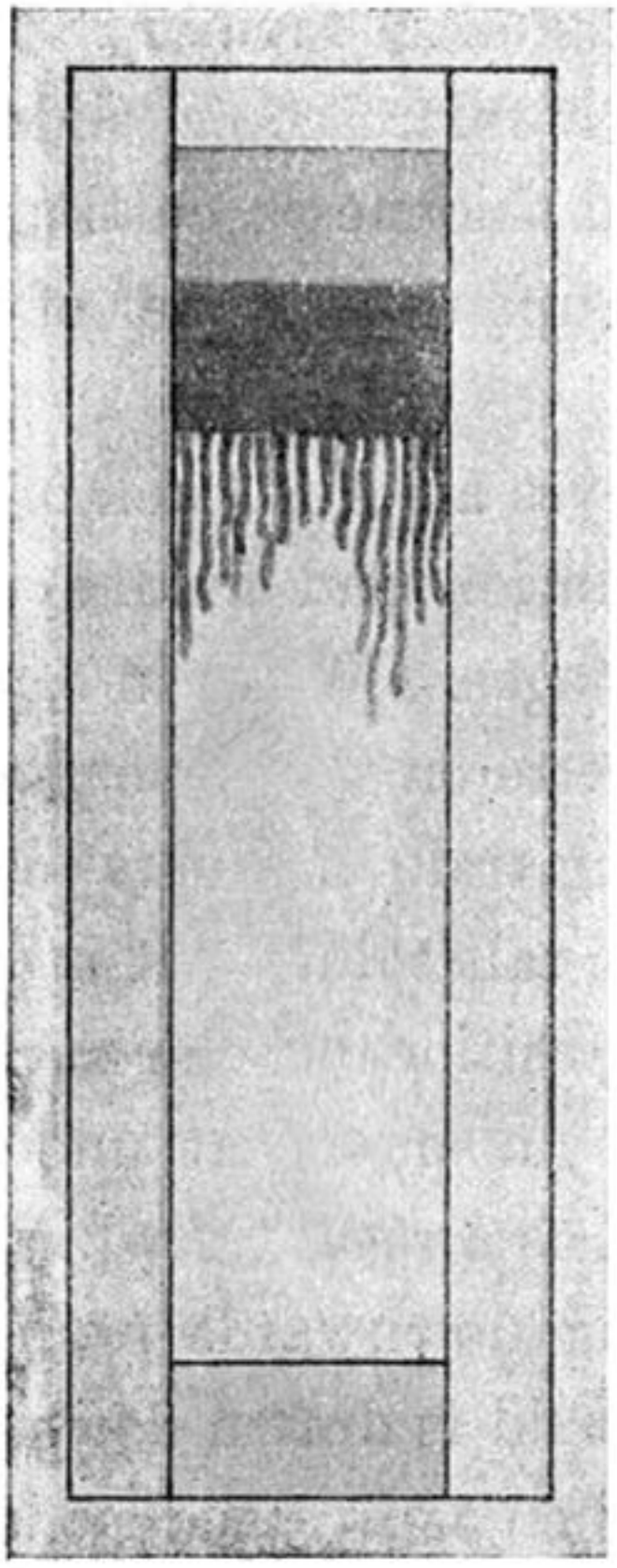
porous tissues, along with the serum lodged there, the microbes which may be suspended in it. And reflection will show that, inasmuch as we have intertraction between serum and salt solutions, our saline solution will also inevitably be carried into cavities and *cul-de-sacs* and porous tissues.

This being so, it would seem possible, by mixing an antiseptic with salt solution, to convey this also into corners and recesses. But clearly, when thus carried in, the bactericidal efficacy of the antiseptic would depend upon whether it was or was not quenched by the albuminous substances with which it there comes in contact.

Comment.—The body of observations set out above would appear to invite to a re-examination of the doctrine that in *diffusion*—or as it would seem more proper to call it *interfusion*—we are dealing in every case with a perfectly passive recipient fluid and with a disbursing fluid which has a monopoly of activity. In other words, the data here obtained would seem to invite some review of the doctrine that when a solute passes out from its menstruum into an adjoining fluid territory, or a diluting fluid is carried into a concentrated solution, dispersive forces resident in the solute are the only forces which come into operation.

While that doctrine ostensibly holds the field there is to be noted that in German text-books the term *Adhäsion*, and in French text-books the term *appel* are still employed in explanation of the passage of water through a dialysing membrane into salt solution. In view of the observations here set out it may perhaps be legitimate to put forward for consideration whether the term *Adhäsion* (which would have as its English equivalent "binding or conjoining force") and the term *appel* (which might perhaps be translated into the invocation "come hither") are simply a figure of speech, a figure behind which there lurks nothing substantial and objective.

And if it be permissible to generalise from the case of what happens when albuminous substances and saline solutions are brought into conjunction, it may be suggested that it would be appropriate explicitly to recognise the existence of *tractor* or *drawing forces*, and more generally of *intertraction* as an agency which may co-operate with diffusion and assist in bringing about interfusion.



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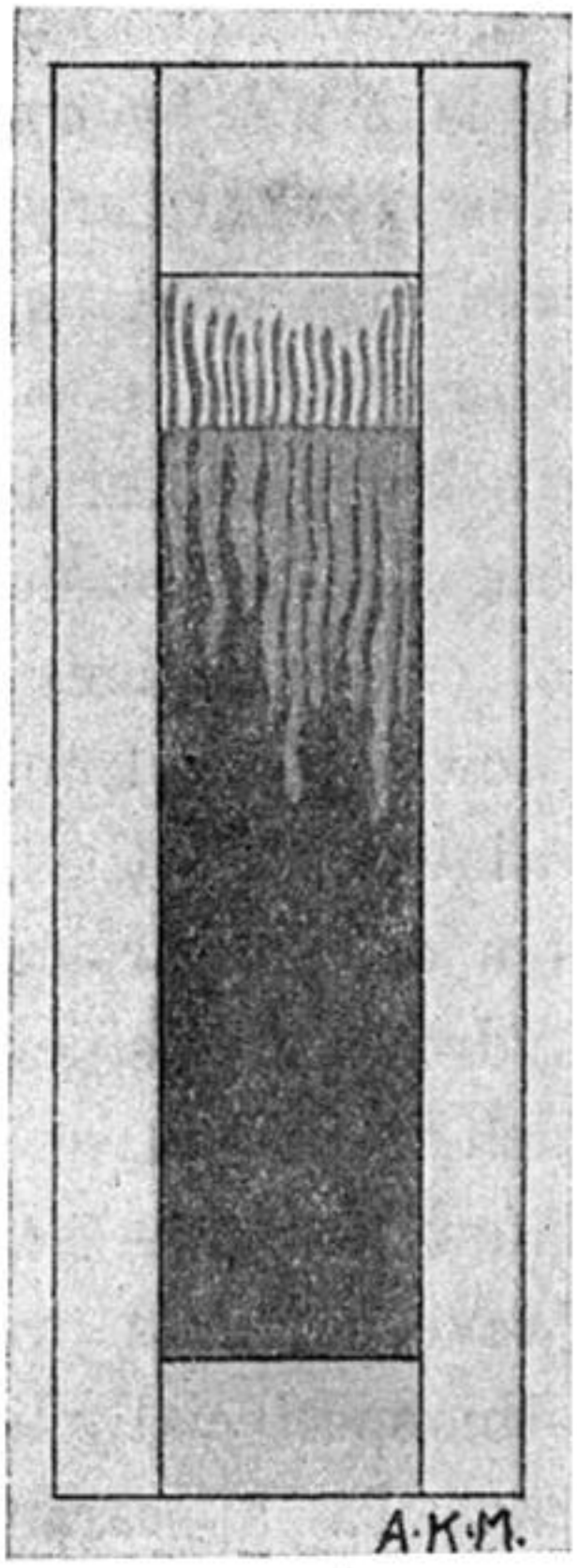

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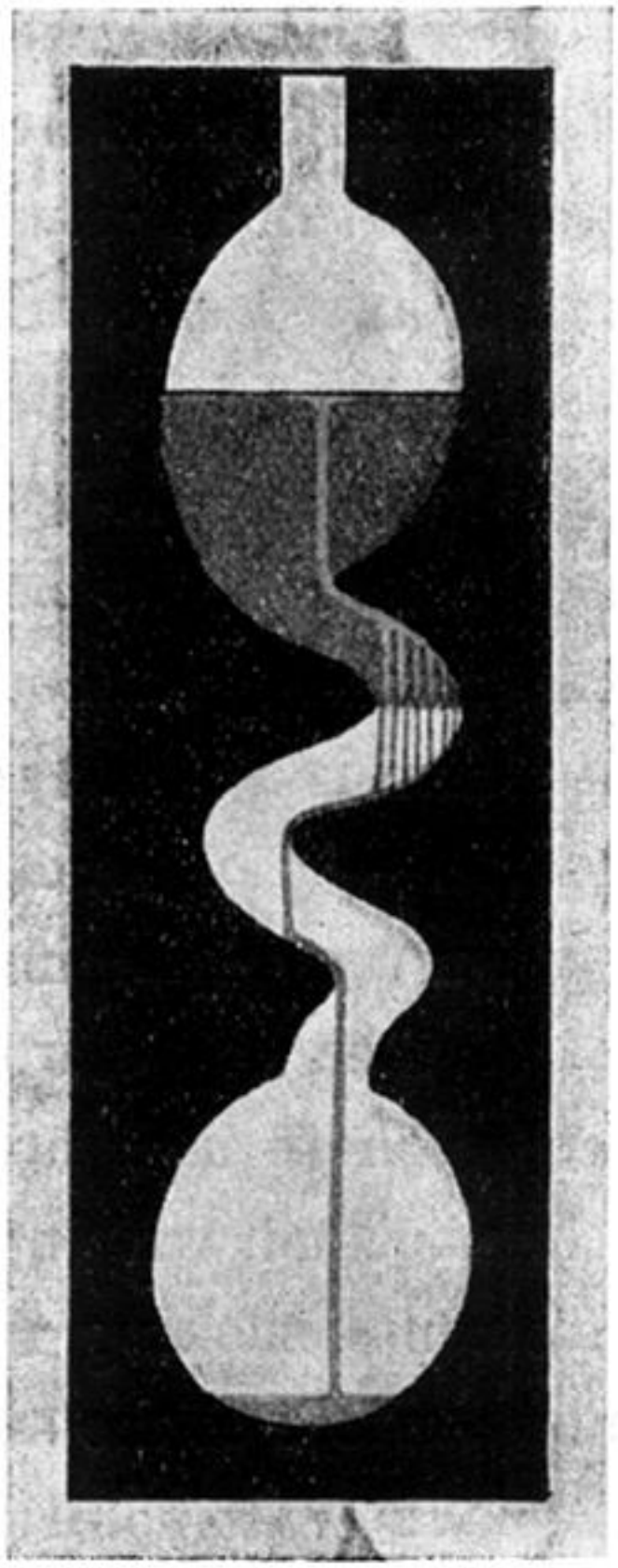

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