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A Distilling System for Purer Water

Conventional equipment has been regrouped to produce a continuous supply of sterile, ultrapure water.

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A survey in *Science* (*1*) of the quality of distilled water sampled from several U.S. hospitals sounded an alarm that elicited quick comment (*2*) and reached our notice coincident with a query (*3*) concerning distilled water which we had prepared as a substrate for studies (*4, 5*) of trace contaminants. It occurred to us that our method could be modified to produce distilled water in quantity with a near guarantee of freedom from living organisms or their residues. The separate requirements—for high physical purity and complete sterility—are coextensive, but the emphasis could be slanted as necessary to one or the other need. We describe here the techniques that we used to ensure the physical and biological purity of the water.

Over the years our ideas of what constitutes "pure sample" have changed drastically. In the past the ideal of purity was the meticulously prepared material in the carefully cleaned bottle stored on white paper in the cupboard, from which "standard" samples could be taken from time to time. Now it is almost universally realized that absolute purity is unattainable, that stored samples do not stay pure, and that radiation, the atmosphere, the personnel of the laboratory, and the container itself cumulatively degrade the specimen. A limiting approach to purity is best secured and maintained by continuous repurification so that the material at any moment may be only minutes, hours, or days "old." In the case of water, the advantages of consecutive batch distillation and the disadvantages of long contact with glass were reported by Kohlrausch and Heydweiller (*6*) almost 80 years ago in the course of their efforts to determine the intrinsic conductivity of water. Contamination of distilled water in hospitals seems to have originated less in the stills than in the storage vessels and subsequent means for distribution; the corrective measures to be described here will deal with all three of these potential sources of contamination.

If conventional distilling apparatus is represented by Fig. 1, then Fig 2 epitomizes the rearrangement that we now propose. In our apparatus the still and reservoir are combined so that the distillate will always be covered with steam which also serves to exclude air. Since the equipment is designed to operate without interruption whether there is any demand for water or not, the product water will be recycled whenever the reservoir is full, and this procedure will assure that the quality of the water will be maintained, or even upgraded. The implied requirement, that the contents of the reservoir shall remain at the boiling point at the top, yet acquire ambient temperature toward the bottom for drawoff, has raised an interesting question, namely, how to recycle the water with the approximation to plug flow needed for the efficient replacement of older water by new?

The Prior and Contemporary Art

Two great periods of innovation and development in techniques for the distillation of water spanned the middle of the 19th century and the current third of the 20th century, the earlier phase prompted by shipboard needs and the second inspired by the call for the purest possible water to service cleanroom industries and the drive to convert sea water to fresh water for large-scale use on land. The extended panorama may be reconstructed from the stacks of patent files in the search room of the U.S. Patent Office (*7*).

Early in the development of the more sophisticated approach to water purification, Normandy (*8*) (1858) described a tubular boiler which was fitted with a device for the regulation of the product-residue ratio and aeration facilities to remove impurities. Baird (*9*) (1880) suggested oxygenating the water and deodorizing the distillate with charcoal. In an amusing preamble to his patent (1894) for rendering "foul swamp water" potable, Craney (*10*) mentions distilling, then redistilling with the admission of air, bringing the vapors to near red heat, and partially condensing the water so that the oxidized odors may escape. An engineered version of Craney's scheme was drafted by Witteman (*11*) (1907); the Barnstead patent (*12*) for the conservation of input heat was issued in 1906.

In 1918, Buscher constructed a small electrically heated still (*13*) (Fig. 3), with a long vertical reservoir to provide hot water at the upper faucet and cold water at the base. The hot section was compartmented to lessen the spontaneous mixing which occurs naturally between top and bottom. A fully engineered version, housed in a conventional water cabinet, has been described by Skow (*14*).

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Techniques favored at present include the use of oxygen (*15*) or potassium permanganate for converting organic impurities to carbon dioxide (CO_2) (*16*), vapor recompression (*17*), flash distillation (*18*), other preliminary treatments prior to distillation, and many (*19*) alternatives or accessories to distillation, such as micropore filters and reverse osmosis membranes, which are employed in the hope that the product water will be characterized by high purity and low-to-zero bacterial count. Those who furnish purified water services to householders and industry have long appreciated that there is no one road to pure water. Instead, a wide armamentarium is assembled from which the tools needed in each instance may be selected. Among these are such laboratory refinements as surface scavenging to remove adsorbates, dating back to Agnes Pockels (*20*), and the prolonged leaching and redistillation employed as batch procedures by Kohlrausch and Heydweiller (*6*).

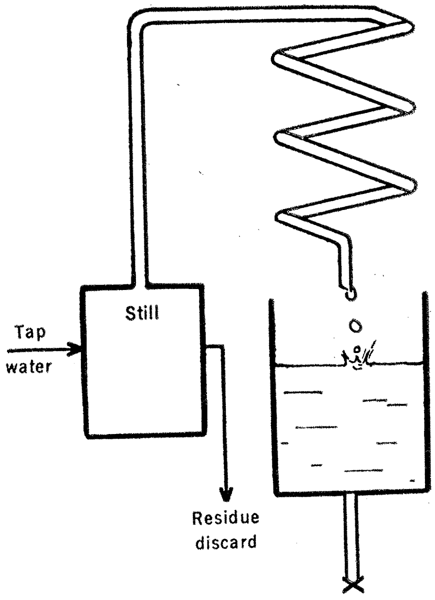
A Prototype Still

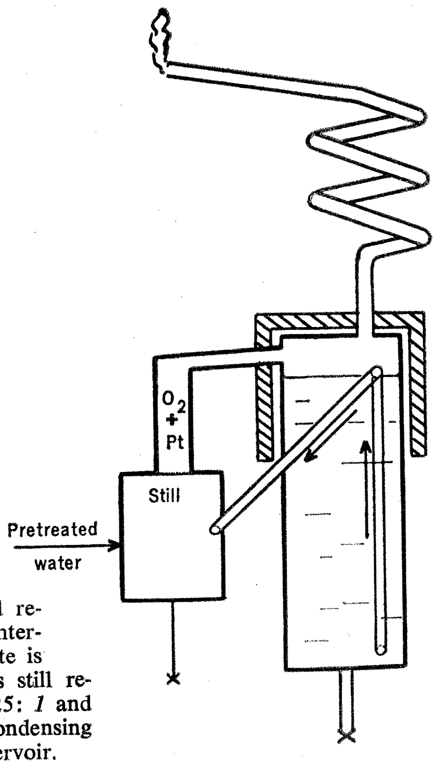
From the profusion of means, we have selected the following components: (i) borosilicate glass vessels, chosen for their availability and ease of fabrication; on the basis of our experience (*21*), we believe that continued leaching of the glass would reduce the contamination of the distillate to acceptable limits; (ii) as the feed water, distillate of Rochester tap water drawn from the service-still of the building, or sometimes tap water sofened to prevent the deposition of solids in the boilers; (iii) distillation with catalyzed oxygenation; (iv) redistillation with the provision that the distillate is kept covered by steam until used (*22*); (v) rejection of some residual steam together with any volatile impurities; and (vi) recycling with partial overflowing of the surface (*21*).

The prototype still (the "polishing" still) would serve to upgrade already purified water. Were we to start with crude city or well water, the boiler would rapidly become clogged with deposit, and this would necessitate cleaning and the interruptions of service we wish to avoid. Instead, we chose to feed the still with commercially distilled water or otherwise softened or deionized water. Two successive boilers were chosen for the prototype still so that residue from the first could be discarded without interrupting the second distillation. Also, the air or oxygen introduced to combust impurities would be eliminated with any oxidized products from the first condenser to ensure that condensate from the second condenser would be substantially free from foreign gases and vapors.

Another design requirement arose from the anticipated approach to limiting purity. Where, in the stored product water, would the remaining contaminant molecules be likely to accumulate —would they be dissolved in the bulk water, or absorbed to the walls of the vessel, or concentrated at the water-steam interface? The apparatus has thus been designed so that selective attention can be paid to leaching the walls, renewing the stored distillate, and discarding any contaminant gathering on the free surface. If incoming distillate were allowed to flow from a lip or weir at the top of an already filled reservoir, it might flush off the top layer, but it would scarcely displace the water underneath. The situation called for both top and bottom exits from the reservoir, the top path being referred to as overflow and the bottom path as underflow, together with means for selecting the two flows in any proportion. We will show later (in Fig. 13) that discarding between 10 and 25 percent overflow and between 90 and 75 percent underflow occasionally gave water with a higher ohmic resistance (91.5 to 92.5 percent of that of pure water) than could routinely be obtained with 100 percent underflow.

Although preliminary purification of the feed water will remove nearly all the contaminant from a crude water supply, the major purification task is





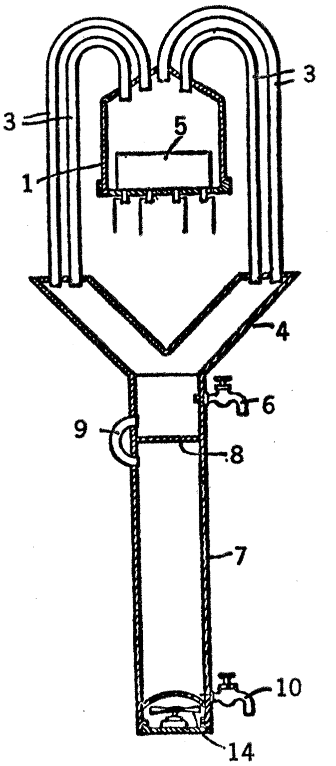


Fig. 1 (left). Conventional still, condenser, and receiver. Fig. 2 (center). Proposed still, counterflow condenser, and sealed receiver; the distillate is covered by steam. Fig. 3 (right). Buscher's still reproduced from figure 2 of U.S. Patent 1,276,025: *1* and *5*, centrally positioned boiler; *3*, cluster of condensing tubes supported by double funnel at top of reservoir.

accomplished in the polishing still. It is this still, the prototype still, that must bridge the far greater span to ultimate purity, as measured in logarithmic decrements of the impurities left in the water.

The apparatus that we used in our original research (*4, 5*) had a reservoir of 200 milliliters, the entering distillate becoming thoroughly mixed with the contents by convection before leaving, thus affording recycling with exponential dilution of the solutes. The prototype still (Fig. 4), now assembled and tested, has a reservoir of 4.5 liters, in which, because of the required temperature gradient, exponential replacement is precluded. With the modification to be described below, 99.9 percent of the contents at any moment is replaced after passage of the next two volumes (9 liters) of distillate through the reservoir during recycling. A photograph of the assembly is shown in Fig. 5.

Replacement Flow through the Reservoir

Under constant daily demand, the contents of the reservoir are quickly replaced, and the behavior during recycling is unimportant. However, when the purpose is to provide very pure water on infrequent occasions, the efficiency of water replacement during recycling is of prime importance.

The operating vessel of the antecedent apparatus was relatively small and heavily jacketed so that no thermal gradients were present except those induced for the experiments. The reservoir of the new still has 22 times the capacity of the antecedent apparatus and is unjacketed throughout most of its length so that the distillate may be withdrawn cold. The smaller vessel filled with new distillate in 83 minutes, and thereafter, by constant mixing and overflowing, half of the contents were displaced each hour. The concentration of solutes present at any moment were thus reduced to about 2^{-24} or to 1 part in 2×10^7 parts in the next 24 hours, values highly conducive to leaching of the glass and reconditioning of the contents after contamination.

Why the vertical reservoir of the prototype still failed at first to provide rapid replacement of distillate by true plug flow can be seen from the photographs assembled in Fig. 6. In the selected example, recirculation was from the base without surface scavenging and, for Fig. 6A, a few drops of concentrated dye were added through the condenser to form a dark layer which floated on the cooler water underneath. The layer persists well demarked through succeeding photographs, becoming diluted rather than drifting bodily downward, and very slowly becoming covered by a layer of colorless water. The displaced dye finally reappears as an equally distinct and segregated layer at the bottom of the reservoir. Many hours later, a ghost of the top dye layer has drifted to the middle of the reservoir whereas much of the bottom layer has been transferred to the purge flask for redistillation. The sequence is even better illustrated if we start with a uniformly dyed charge and admit colorless distillate, which emphasizes the persistent pocketing occurring under these simple conditions. Most of

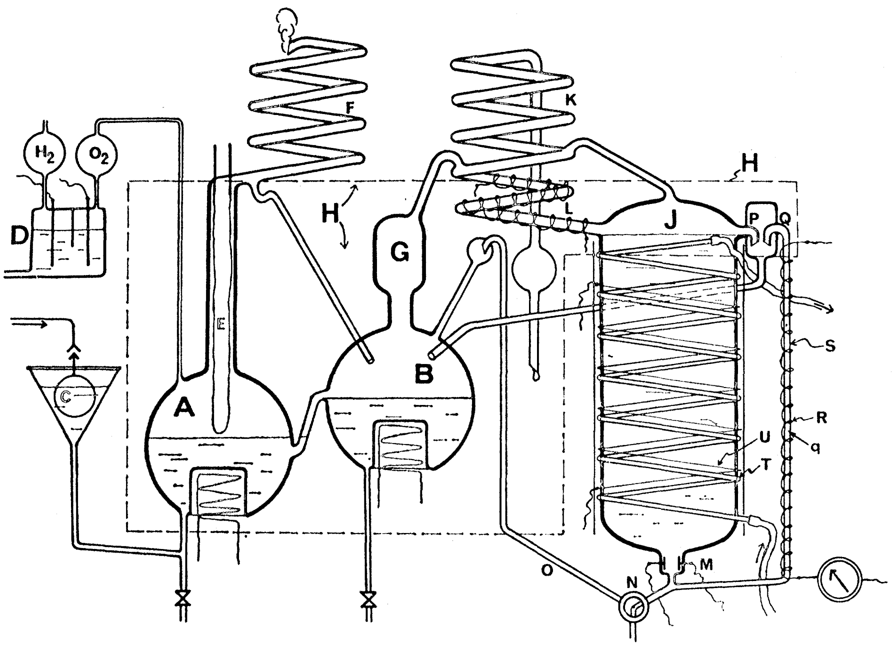


Fig. 4. Prototype still. The water boilers, *A* and *B*, are two 2-liter borosilicate flasks having inverted electric heaters. Source water reaches *A* through the float valve *C*, and the level of water in *B* is maintained by the distillate from *A*. Primary steam from *A* is treated with oxygen (about 0.1 to 1.0 percent by volume) from the electrolytic cell *D* and passes over the glowing platinum wire *E* to the condenser *F*. Radiant heat from the wire dries the wall of the conduit, preventing the creep of distilland. The electrolytic oxygen was replaced (September 1972) by a chemically equivalent mass of air obtained from the laboratory compressed air line and passed through a tube of soda lime. Alternate switching between oxygen and air showed a slight advantage for the use of oxygen alone. The reflux condenser is air-cooled and regulated to reject 1 to 10 percent of the steam at the far exit. Steam from boiler *B* passes through the spray remover *G*, packed with Berle saddles, which is maintained above 100°C within the cabinet *H*. Part of the steam then flows through the reboiler *L*, into the top of the reservoir *J*, together with the distillate; the remainder passes up the condenser *K*, where it is rejoined by purge steam issuing from the top of *J*. The reservoir, also made of borosilicate glass, is 15 centimeters in diameter and 32 centimeters tall (to the water line). A conductivity cell *M* is located at the base in the path of the recycled and drawoff water, the latter controlled by the three-way Teflon stopcock *N* [a substitute arrangement (see Fig. 11) is discussed in the text]. Additional steam from *B* can reach *N* by a third arm *O*. Recycling by overflow is provided by the dropper *P* and by underflow through the companion dropper *Q*, attached to the vertical riser *q*. If dropper *Q* is situated below *P*, the ratio of recycling from the surface and recycling from the base of the reservoir can be adjusted by valve *R*, which returns cooled distillate to the boiler. Alternately *Q* is set a little higher than *P* and heat is applied to the riser by means of the winding *S*. This procedure increases the temperature and buoyancy of the recycled stream, affording, by a twist of the rheostat, any desired proportioning of flows. It is advantageous, although not essential, to employ two steam boilers, as illustrated. However, boiler *B* may be omitted, and the spray remover inserted into the condenser train from *A*. The boilers and appurtenances are housed in a thermally insulated box having observation windows on opposite walls; an auxiliary heater and thermostat maintain the upper half of the box at 103° to 110°C. Suggested improvements would include construction from high-silica glass, particularly the condensers and reservoirs; compaction of the condensers through water cooling; and the addition of thermostatic controls. Our purpose has been less to provide an engineered design of commercial proportions than to demonstrate the utility of a system for providing improved water.

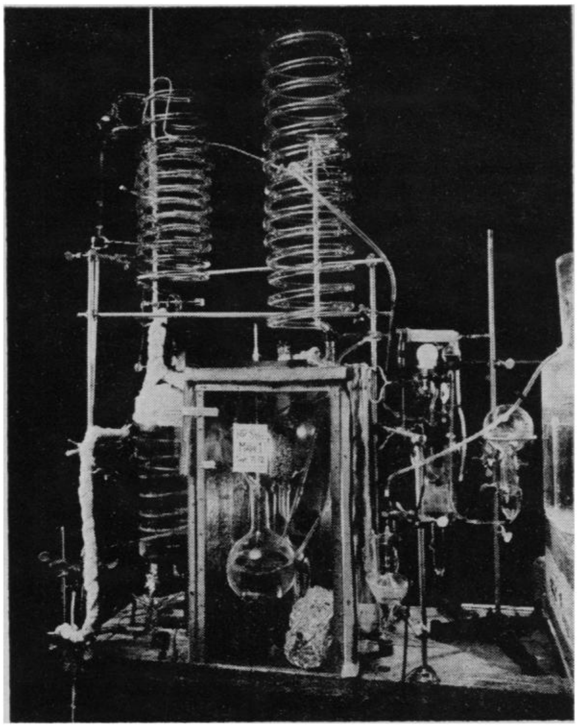


Fig. 5. Photograph of the prototype still. The box houses the purge boiler, with the source boiler behind the purge boiler, the product water condenser is at the upper left, and the source condenser is at the right. At the lower right is the feed water arrangement and, above, the electrolytic cell. The lamp keeps the packed scrubber warm. The distillate reservoir is at the left of the box. The recycling pipe is hidden.

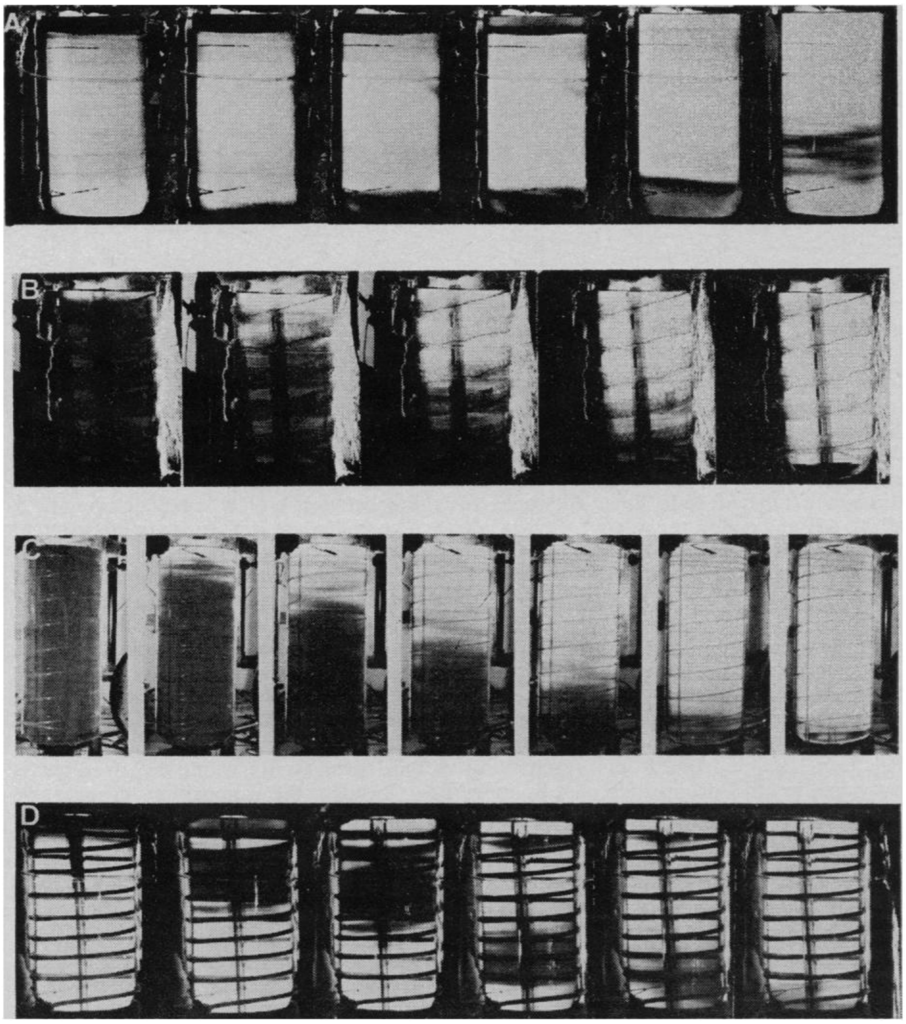


Fig. 6. Photographs of the reservoir, dyed water being replaced by lighter colored water. (A) Natural convection; (B) convection modified by spiral lagging; (C) convection with interleaved heated and cooled spirals; (D) cooled spiral only. [Note: A spiral wire was in place in (A) but not in use. The same disclaimer applies to the center rod in (D).]

the new distillate is thus passing through the container without displacing the contents, a smaller portion being deflected to the central region of slow renewal.

When the flow is viewed through the top of the container, the dye is seen, as suggested in sketch A of Fig. 7 (top), to transfer in a thin sheath or internal "stocking," which slides down the cooled vertical wall to the bottom and then drifts by momentum and replacement of volume up the center core. Photographed from above, the sheath appears darker than the dyed layer, but it is almost transparent when viewed from the side. The pattern of flow is thus the reverse of convection in the usual case of a vessel heated through the bottom or sides.

Sequential flow could be fostered by packing or compartmenting the reservoir, but this would be accomplished at the expense of added structural area and delay in leaching. A packing could also provide harborage for mold or bacterial growth. A remedy which avoids packing consists in periodically interrupting the cooling of the walls or alternately cooling and adding back a portion of the abstracted heat (*23*). Figure 7B represents a fragmentary cross section through the liquid and containing wall, for the case in which the wall has a cooling tube spaced above an electric heater wire or a warming tube. A moiety of dyed liquid is pictured as sliding down the cooled wall, to be interrupted by a reverse stream rising from the wire. The residual momentum drives the sheaths inward to where streaming has been replaced by a fairly even thermocline from top to bottom. The deflected stream will then rise or sink to a region of similar density and then spread horizontally.

Any object or binding applied to the wall of the container that will alter the rate of heat transfer through the wall will delay the descent of the convection pattern. Thus, a spiral of thermally insulating tape which leaves gaps of equal width will serve, as illustrated in Figs. 7C and 6B. One of the simplest windings, and among the more efficient, is furnished by the cooled spirals, Fig. 6C, or alternately heated and cooled spirals as at *T-U* of Fig. 4 and Fig. 6D. Here liquid falling from the cooler spiral has the choice of proceeding directly downward or along (around) the spiral to replace material that has already slipped sideways. With a steep lead, the liquid follows the spiral path

Table 1. Cost of water in terms of power consumption; kw, kilowatt; kwh, kilowatt-hour.

Cost	A 4.5-liter still* (0.608 liter/hour)	B 94.5-liter still† (7.9 liter/hour)
Source boiler (kw)	0.500	6.5
Purge boiler‡ (kw)	.450	5.84
Purge reboiler (kw)	.035	0.43
Recycle, reheat (kw)	.025	.33
Reservoir spiral (kw)	.030	.40
Platinum wire (kw)	.140	.42
Box heater (kw)	.130	.40
Electrolysis (5 amperes, 6 volts)	.030	.39
Total power (kw)	1.33	14.7
Total power per liter (kwh)	2.19	1.86
Total power per gallon (kwh)	8.28	7.05
Total power per day (kwh)	32.0	353.0
Cost, at $0.02 per kwh, per day	$0.64	$7.06

* Approximately three refills of the reservoir each 24 hours. † Approximately two refills of the reservoir each 24 hours. ‡ Calculated from the yield of the purge boiler with 5 percent steam rejection; for example, (0.427 watt) × (3.142 British thermal units per watt-hour) × (0.453 kilogram per pound).

exclusively. Here, what had been a vertical convecting path of height ℓ under gravitational force g has been replaced by a path with a length of approximately πdn (turns) and an effective force in the direction of the spiral of $(g\ell)/(\pi dn)$. The time taken for liquid to fall down the sides of the container is thus extended by a function of $(\pi dn)^2$, plus the time taken for the liquid to move inward to regions of appropriate density. Since the liquid is to be cooled during its simulated plug flow downward, any heat added for deflecting purposes must necessarily be less than the heat abstracted. A thermally balanced, steep double spiral causes a partial movement toward the axis, and the descending moiety assumes a wide spiral vortex (Fig. 8A). With a shallow spiral of many turns, stratification is excellent (Fig. 8B), but much of the descending sheath proceeds directly from top to bottom, leaving the rest of the well-ordered sheath to follow, out of turn. The compromise windings shown in Fig. 4 and Fig. 6, C and D, have permitted a replacement of 99.9 percent of the contents with the passage of 2.5 volumes of new distillate, affording a useful approximation to plug flow and a reduction of the initial concentration of contaminant to 10^{-4} of that concentration each 24 hours. However, unsuitable spacing of the thermal deflectors can produce worse pocketing than is observed with natural cooling.

Anyone examining the possibilities of this means for controlling liquid stratification may be astonished at the effects of trifling additions and subtractions of heat. Thus the deflections of flow recorded in Figs. 6 to 8 were produced by additions of 5 to 30 watts of power and 2 to 10 drops per second of cooling water. Merely pressing a thumb to the wall of the reservoir, the thumb being warmer or cooler than the wall, would delay or accelerate, respectively, the transit of dye to produce a transient smudge where the thumb had been. Such imaging is most faithful when the thermal modifier is applied as a narrow horizontal patch. An amusing lecture demonstration consists in varnishing a substantially invisible resistance wire to the wall to outline a fish. The speaker laments, "Oh, where has all my dye gone," and then energizes the wire. The previously invisible dye collects at the warmed area to produce the silhouette of a fish, which, of course, explains everything! Lateral heat transfer has little significance in terrestrial bodies of water which ordinarily occupy shallow basins with slanting, well-insulated edges. For large exposed tanks of water, oil, or liquefied gas, the thermal treatment accorded the side walls could significantly influence the layering of the contents.

Examples of both idealized and experimental dilution curves are presented against linear coordinates in Fig. 9, and the same data with the logarithmic solute concentrations plotted against linear time are presented in Fig. 10. The experimental method was to add dilute ammonium chloride solution at 100°C to the reservoir and to begin adding distilled water when steady-state conditions had been established. Changes in relative concentration have been plotted without correction for the slight increase in equivalent conductance as dilution proceeded, since this can be shown to be negligible at the concentrations used.

The power consumption of the prototype still is listed in Table 1, column A. Comparative estimates are given in column B for a larger still with a reservoir of 94.6 liters (25 gallons) receiving two volumes of distillate each 24

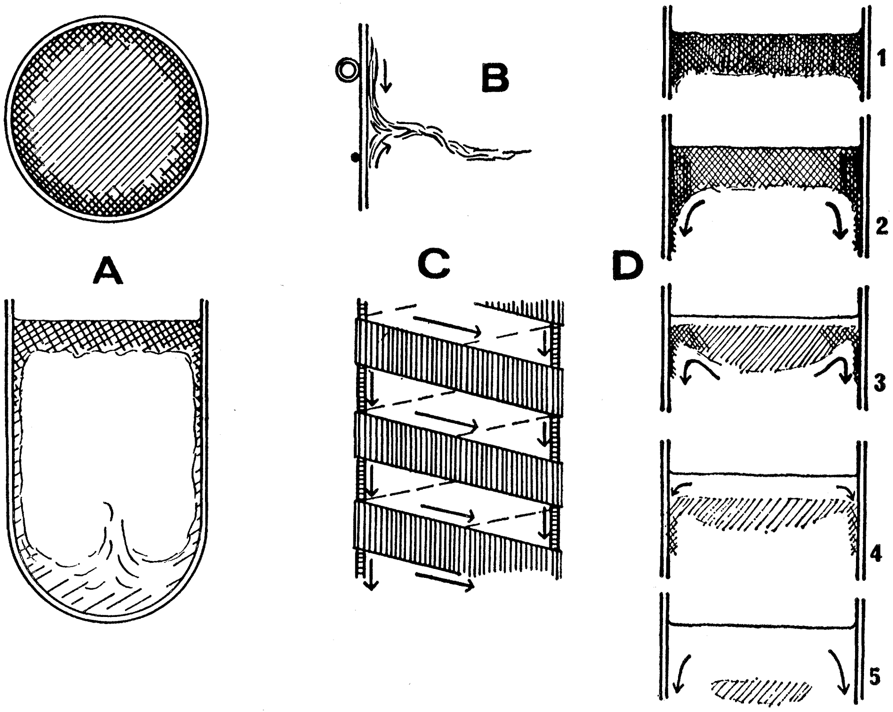


Fig. 7. Convection diagrams: (A) natural pattern; (B) pattern modified by cooling and heating the wall of the reservoir; (C) spiral thermal lagging; (D) pocketing under natural convection.

hours. The power consumed for water recycling in the reservoir over a weekend of 60 hours would be 35.25 kilowatt-hours, leading to a cost of $0.186 per liter ($0.705 per gallon) for ultrapure water ready for use on Monday.

The amount of electricity consumed is 7 to 8 kilowatt-hours per gallon of direct product water and more than 21 kilowatt-hours per gallon for recycled water; the running costs, without amortization, repairs, or attendance are thus about $0.14 to $0.40 per gallon when electricity costs $0.02 per kilowatt-hour. For large-scale use, these amounts would be prohibitive, but they appear feasible as a charge against a patient's bill for intravenous solutions and quite trivial for the demands of more critical researches. The charges would be less for a fully engineered still which could use cheaper steam.

Management and Application

For the nonbiological laboratory. The apparatus, substantially as described, is regulated to operate with a steam bleed from the condensers during recycling. When product water is withdrawn, the level in the reservoir falls, the exposed walls serve as an additional condenser, and the steam-air junction passes into the second condenser to a compensatory level. This temporary situation does not affect the quality of distillate, unless the drain is frequent and heavy. That prolonged shortening of the reflux path does affect the quality of the water could indicate that the treatment with oxygen and platinum has been only partially successful at the first pass, or that contaminants from the surroundings, chiefly CO_2, have dissolved in the distillate, or both. The reduction in the quality of the water during batch withdrawal emphasizes the advantages of prolonged recycling past the glowing platinum and full occupancy of the condenser, followed by the rejection of steam at the exit. Further recycling furnishes a tank full of superior water after a rest period, for example, the weekend, with a peak resistance, so far, corresponding to 90 percent of that of pure water, which is equivalent to about 7 parts per billion of potassium chloride.

For the biomedical laboratory. The prototype still is similarly regulated during recycling but the energy input to the second purge boiler, *B* in Fig. 4, must be increased, or an auxiliary heater on the condenser must be energized upon withdrawal of product water from the reservoir, so that bleed steam always issues from that condenser. This precaution is less to ensure uninterrupted removal of volatile impurities than it is to prevent outside air from entering the condenser and contaminating the cooled part of the inside walls. As the steam supply again increases, the advancing margin of distillate dissolves and carries back some of the adsorbate, including organic matter, live or inanimate. Harris and his associates (*5, 24*) have repeatedly shown that the exposure of a small area of the condenser to air for less than 1 hour can influence the ability of the product water to float boules, an indication of surface impurity. The continuance of steam bleed can be ensured by means of a thermostat placed at the exit of the condenser.

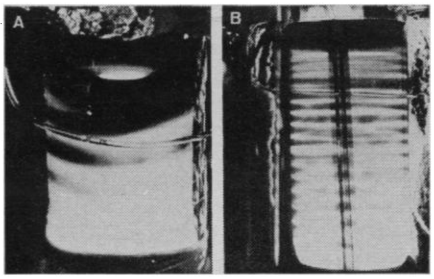


Fig. 8. (A) Voluminous spiral of dyed water induced by a steep cooling spiral; (B) less useful stratification induced by a cooling spiral that is too shallow.

For intravenous, hospital, and other bioinstitutional uses. A large-scale version of the prototype still could be engineered to meet the above requirements. In addition, the distributing pipe or network of pipes leading from the reservoir could be installed with all lateral branches sloping downward, so that the whole system could from time to time be flushed with steam treated with bactericide, if desired. Normal operation of the sterilized network would resume with the shutting off of the terminal faucets flushed with steam.

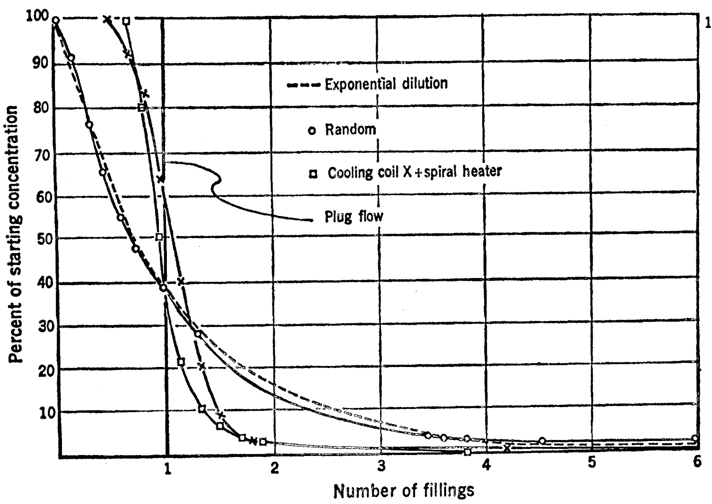
Handling of the product water. The dispensing of very pure water without loss of quality presents its own hazards and difficulties. Even if rigid precautions are taken after withdrawal, the purity of the water has been endangered in the reservoir at the permanent interface with the environment that exists in the exit valve, which affords entry to diffusible impurities and a place of nurture for bacteria. This limitation, as pointed out below, applies to the three-way glass-Teflon stopcock readily flushed by steam in which we trusted, apparently mistakenly. A more successful variant could be an exit valve made of material transparent to bacterial wavelengths and constantly illuminated by a miniature ultraviolet lamp. An interim measure, eventually adopted for the biotesting only, is displayed in Fig. 11.

Redefinition of the Objective

The tone of the introduction to this article may now be sharpened. From the plethora of construction and management details, a philosophy emerges, namely, that water of high purity may be produced by a reshuffling of simple and time-hallowed means. With continued refinement and extension to the chain of distribution and delivery, the onus—to maintain the purity of the water after preparation until the moment of use—could shift to the operator. So exacting can these requirements be that the source of pure water is best made integral with the place of use. The ability of boule-making equipment (*4, 5, 22, 24*) to reveal "new" phenomena in the superheating of water has been due less to the novelty of the experiment or the reagents, which were as old as surface studies with long-chain fatty acids, than to the purity of the host water secured by an attached pot still with facilities for treatment with oxygen plus platinum. As Berg and Acrivos have emphasized (*25*), the physical activities of the water surface and the associated boundary layers can change perceptibly with each decade of surfactant dilution over a wide range. We suspect that in many clever experiments designed to test new concepts in interfacial physics either mere artifacts have been reported or there has been no response at all because, although the experiments have exhibited macroscopic cleanliness of a high order, they have retained unnoticed interfacial soiling. One is reminded of the tidy, spotless-but-lethal conditions that prevailed in surgical wards before the teachings of Lister.

Comparative Chemistry of the System

Until we can research the details of cleanup by oxygen plus platinum, we can only cite the benefits that we have observed as a result of the adoption of



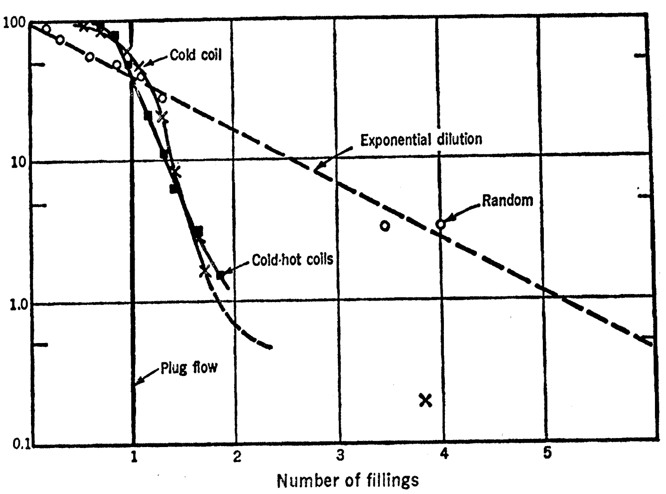


Fig. 9 (left). Curves illustrating the replacement of reservoir water by a constant flow of distillate (linear coordinates). Fig. 10 (right). Curves illustrating the replacement of reservoir water by a constant flow of distillate (logarithmic values of concentration plotted against linear time).

certain techniques. These were as follows: (i) recycling, which affords cumulative treatment; (ii) oxidation, which degrades contaminants to simpler, more volatile forms; and (iii) rejection from the condensers of terminal steam, which carries away the oxidized fractions.

The cleanup of the carbon moieties of the organic compounds (*26*) with oxygen and platinum would follow the general path

$$\text{Oxidation} \xrightarrow{O_2,\ \text{Pt}} \text{aldehyde, ketone, acid} \rightarrow CO_2 \rightarrow \text{out}$$

in varying intermediate sequence. The least preferred of these conversions would be acid formation, the aliphatic acids of lower molecular weight having volatilities similar to that of water. The oxidation of constituents other than carbon and hydrogen, chiefly amino nitrogen and occasionally sulfur and chlorine, by oxygen and platinum should proceed as follows:

$$\vdots N \text{ or } NH_x \rightarrow NO \rightarrow NO_2 \rightarrow \text{out}$$

$$NO, NO_2 \rightarrow HNO_3 \rightarrow \text{delayed, out}$$

$$NO, NO_2 + NH_x \rightarrow N_2 \rightarrow \text{out}$$

$$= S\!< \rightarrow SO_2 \rightarrow SO_3 \rightarrow H_2SO_3 \rightarrow H_2SO_4 \text{ delayed, out}$$

$$-Cl \rightarrow HCl \text{ delayed, out}$$

The destination "delayed, out" implies repeated recycling of the steam with incremental conversion to CO_2 or trapping in the source flask as nonvolatile acids or as sodium salts, or both. Secondary reactions will be as follows:

$$NH_x + NO_y \rightarrow N_2 \rightarrow \text{out}$$

$$SO_3 \rightarrow H_2SO_4 \rightarrow \text{permanent return to source flask}$$

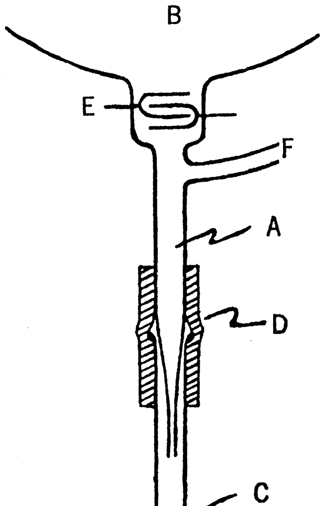


Fig. 11. Means for withdrawing sterile water. The tip of tube *A* at the base of reservoir *B* projects into removable tube *C* which is held in place by elastic silicone tube *D*; *E* is the conductivity cell, and *F* leads to the recycling system. The thermocouple well, not shown, enters behind *E*. Two tubes, *CD*, are provided. When one is serving as closure, the other is available for sterilizing at 200°C for 1 hour. To draw water, the tube *CD* is removed, and the uncovered glass tip (optionally) is flamed while the first volumes of distillate are discarded. To terminate withdrawal, the glass tip is again flamed, and the alternate tube *CD*, taken directly from the oven, is thrust over the tip. The closure, instantly filled with water, slides on easily and is as easily removed later.

If large quantities of ammonia are present in the system, as would be the case after a cleaning operation, and this is countered by increasing the supply of oxygen, the reject steam becomes brown with nitrogen dioxide (NO_2). The NO_2 reacts with any remaining ammonia to hasten the cleanup process. Thus, not all the oxidation reactions are dependent on primary contact with glowing platinum.

A major requirement of the system, of course, is efficient, uninterrupted rejection of internally generated CO_2 and other nonaqueous volatile substances and continuous refusal to admit environmental solutes, particularly CO_2, sulfur dioxide (SO_2), hydrogen sulfide, ureas, and fatty acids (*27*). There are doubtless many ways to keep the entrance barred; the one we have chosen is continuous coverage by steam, always kept moving in the direction from the source still to the drawoff point and beyond.

It is necessary, although perhaps invidious, to compare the techniques of purification described above with the accepted procedures of treating the feed waters with acid or alkaline permanganate or passing fine bubbles of oxygen through water freshly distilled in quartz. The elegant simplicity of the latter, Power's system (*15*), producing water with 77 to 95 percent of the electrical resistance of pure water, commends itself for small batches where a relatively high expenditure of oxygen per volume of water is unimportant, and where dissolved oxygen is not prejudicial to the intended use or is superfluous as in hospital service. The efficacy of our proposed technique should properly be judged in terms of the economy of means and the larger volumes of extremely pure water that it aims to make available.

Quality Testing of the Product Water

A detailed analysis of the product water is beyond the scope of this laboratory, because of the low concentrations of contaminants. Our tests were therefore limited to the following: (i) a measurement of the electrical conductivity of the product water, expressed in absolute units and also as a percentage of that of pure water; (ii) a test for the presence of culturable microorganisms; and (iii) a test for the presence of pyrogens. No attempt was made to assay for suspended solids or dissolved nonionic material. [Carbon dioxide may be readily detected by means of conductivity measurements; see, for example, the papers of Kohlrausch and Heydweiller (*6*).]

All tests were performed on water that was *leaving* the reservoir or on water immediately after it had left the reservoir. The average purity of the water in the reservoir at any moment was inferred from the trends in the external assays.

Electrical conductivity. Two mutually embracing, U-shaped platinum plates were fused into the lower neck of the reservoir to form a conductivity cell having a cell constant of 0.038 reciprocal centimeter. The plates were left unplatinized to prevent contamination due to desorption and also because polarization effects were shown to be negligible at the low conductivities encountered in this work. Temperatures were recorded from a thermocouple probe situated in the cell area; resistance measurements at 364 hertz were made with a conductance bridge (Barnstead PM-70CB). The temperature dependence of the specific conductivity of pure water was calculated from literature values of the dissociation constant and the equivalent conductance of pure water and is plotted in Fig. 12 where it is compared with selected experimental values from the literature (*28*). The specific conductivity of pure water was needed to relate the measured cell resistances, which were necessarily taken at various temperatures between 100°C and the ambient temperature, while the reservoir was drained and allowed to refill. Readings were expressed as a percentage, R_p, of the calculated resistance of pure water at the same temperature (and measured with a cell constant of 0.038 cm^{-1}). To show the sensitivity of R_p to ionic impurities, we have listed in Table 2 the calculated concentrations of potassium chloride that are equivalent to various values of R_p at 25°C.

Table 2. Calculated concentrations of potassium chloride equivalent to various values of the percentage of the theoretical resistance of the product water at 25°C; ppb, parts per billion.

Fraction of the theoretical resistance (%)	Equivalent concentration of KCl (ppb)
1	2700
10	250
20	110
30	63
40	41
50	28
60	18
70	12
80	7
90	3
100	0

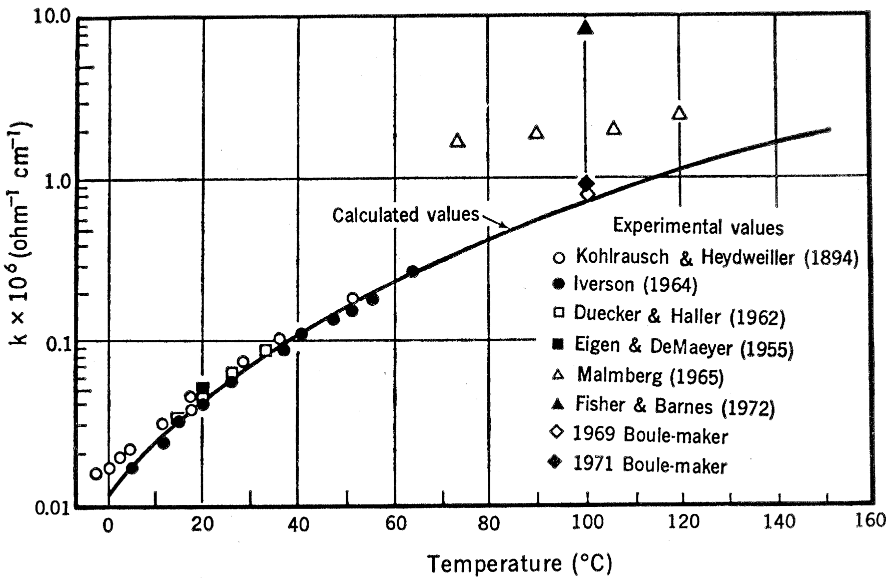


Fig. 12. Temperature dependence of the specific conductivity *k* of pure water and selected experimental values. For further source information, see (*28*).

The purity of the water in boule-making devices (*4, 5, 22, 24*) that have been operating continuously for up to 3 years has improved to a point where the electrical conductivity at 99°C reaches $(0.82 \pm 0.03) \times 10^{-6}$ ohm^{-1} cm^{-1}, which approaches the calculated value of 0.753×10^{-6} ohm^{-1} cm^{-1} at that temperature. A later boule-maker (*29*) in operation for only 1 year, has given an electrical conductivity of $(0.92 \pm 0.05) \times 10^{-6}$ ohm^{-1} cm^{-1} at 99°C. Superheating measurements on the older apparatus suggest that the volatile surfactant concentration of the product water is less than 1.5×10^{-11} mole per liter (*4, 5, 29*).

The prototype still has been in operation for 10 months, a period which includes early interruptions and alterations. After conditions had been standardized, the electrical resistance of the product water rose from 30 to 90 percent of the electrical resistance of pure water. Later trends in the quality of distillate, to a highest value of 91.5 percent of the electrical resistance of pure water, are shown in Fig. 13, which spans the period from August 1972 to January 1973.

In order to simulate operating conditions, half the contents of the container were withdrawn daily for a week, and on another occasion for a week, with little alteration of conductivity being observed from day to day. Further conductivity measurements made during bacteriological sampling confirmed that the water quality was essentially uniform throughout the container and that pockets of more conductive water (that is, poorer quality) were absent.

Nonvolatile solutes. Inherent in the method is provision for the continuous leaching of the apparatus. Thus, the conductivity of the water in the reservoir, sampled from the base, increases about 20 percent per day if the water is not continuously renewed by withdrawal or recycling from the bottom.

As mentioned above, such recycling is termed underflow, in contrast to simple overflow from the top of the reservoir which displaces very little of the cooler water resting beneath. When recycling is shared between underflow and overflow, the conductivity of the stored water rises and, if distillation is stopped, permitting the admission of air, the conductivity increases manyfold, indicating a precipitous decrease in purity.

Gases and vapors. Even with the precautions described here, the still is sensitive to the outside air and volatile contaminants in the source water; hence, it is necessary to use pretreated feed water. Either interrupting the supply of oxygen to the source flask or switching off the glowing platinum wire caused a delayed increase in the percentage of ionic impurities, as illustrated in Fig. 14. The delayed response was caused partly by the fact that the resistance was monitored at the point of outflow of the water, partly by the necessity that all the recycled water find its way to the source flask for further passage with oxygen over the glowing platinum, and partly by the poor contact afforded by a single pass of the steam over the platinum wire. Efforts to improve the composite situation will thus be a priority task in the future. Note that treatment with oxygen plus platinum should not be started in the early stages of cleanup after formaldehyde sterilization; acids less volatile than formaldehyde are formed before final combustion to CO_2.

Carry-over. In an early construction, a spiral spray remover had been inserted between the purge (second) still and the condenser. When sodium chloride was added to the boiler (to a concentration of $1.0M$), the relative resistance of the distillate fell from between 32 and 37 percent to between 2 and 8 percent. The spiral was replaced by a vessel, *G* in Fig. 4, packed with Berle saddles, the unit then being kept above 120°C to intercept surface creep as well as particulate carry-over. The experiment with sodium chloride was not repeated; purity values here reported are based on the packed construction.

The feed water used was routinely supplied by the service-still in the photographic department of the Rochester Institute of Technology. When the feed was changed to tap water, 180 parts per million of total dissolved solids, filtered and passed through a small deionizer (ILLCO-Way Universal), the percentage of ionic impurities in the distillate remained unchanged.

Bacteriological tests. The following uniform procedure was used to test for the sterility of the product water on seven occasions over a 5-month period. On every occasion, 40 samples (each 1 ml) of product water were placed in tubes, each containing 10 ml of trypticase soy broth (Baltimore Biological Laboratory). For samples developing turbidity, the broth was streaked over trypticase soy agar in plates. Tubes and plates were incubated at 37°C for 1 week. Colonies were visually and microscopically inspected, but no effort was made to identify the species of bacteria that were isolated. Before collecting the samples of product water, the outlet tube from the three-way stopcock, *N* in Fig. 4, was thoroughly flamed, while steam was allowed to issue for 5 minutes. Next, the outlet tube was flushed with about 250 ml of product water which was discarded. Forty 1-ml samples were collected from a steady stream. Inoculating the 40 tubes required about 4 minutes. During this period, ten tubes were left open, exposed to the room air near the outlet

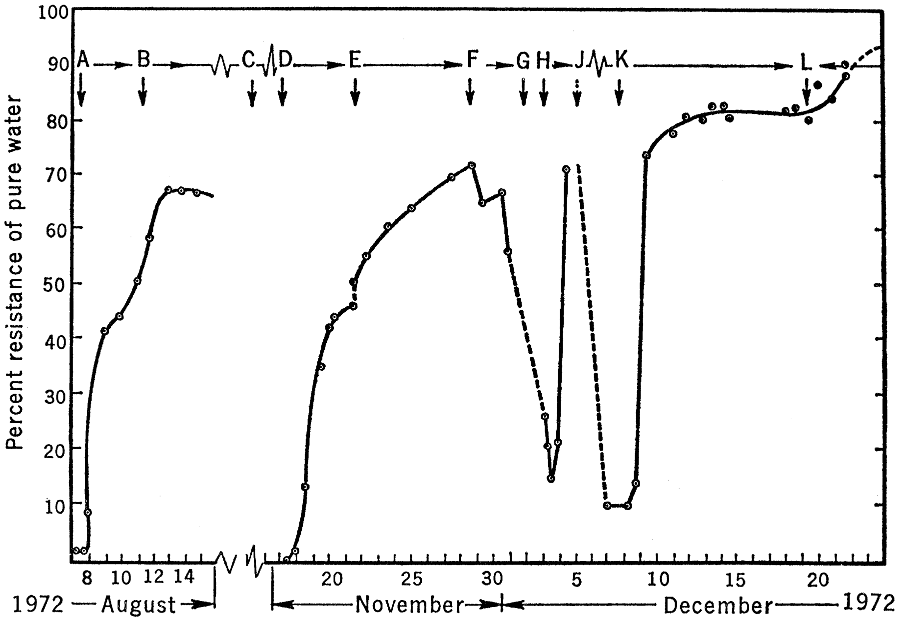


Fig. 13. History of distillate quality after restarting the still. *A*, The still is started after a month's inactivity. *B*, Feed water is changed from laboratory distilled water to deionized tap water. *C*, Conductivity cell is replaced and calibrated. *D*, Still is steamed and restarted. Feed water is distilled water; distillate is recycled. *E*, Thermocouple attached to the conductivity cell is recalibrated. *F*, Recycling is interrupted, and product water withdrawn. *G*, Power failure over the weekend; still remains idle for about 2 days. *H*, Still is restarted. *J*, Second power failure (repairs in building). Cold distillate in the reservoir is open to the air. *K*, Still is restarted, recycling with underflow. *L*, Recycling changed to 70 percent underflow, 30 percent overflow. Still has run uninterruptedly until 5 January 1973, with the product water 91.5 percent free of ionic impurities.

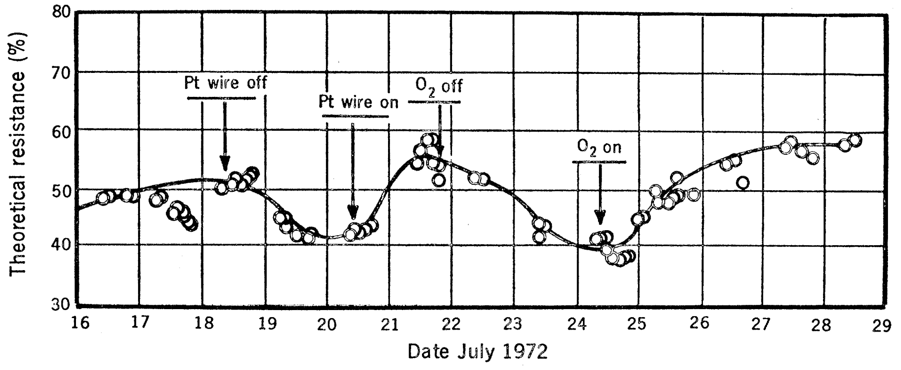


Fig. 14. Fluctuations in the quality of the distillate with and without an interruption in the supply of oxygen and with the platinum wire switched on and off.

Table 3. Summary of bacteriological tests.

Date of test (1972)	Description	Temperature range of water sample (°C)	Ionic purity rating (%)	Number of tubes receiving distillate turning turbid ($N = 40$)	Types of colonies* on agar
2 May	First test	42.5° to 49.5°	74.2 to 81.9	4	Porcelain-white transparent
9 May	Container disinfected with 10% formaldehyde; sample tested after a waiting period of 4 days	37.0° to 40.5°	70.6 to 76.7	0	
19 May	Sample tested after the container had been under recycling procedure for 10 days	26.0° to 28.5°	40.7 to 48.6	0	
26 May	Simulated operating conditions; sample tested after 2 liters had been drained out daily for 7 days	35.5° to 38.0°	22.7 to 22.8	3	Waxy opaque
22 June	Container "steamed out" with formaldehyde; sample tested 9 days later	50.5° to 58.0°	70.8 to 73.2	1	Two tubes exposed to air were pale lemon yellow and turbid
17 July	Simulated operating conditions; sample tested after 2 liters had been drained out daily for 7 days; stopcock swabbed	45.5° to 52.5°	53.5 to 58.2	2	Two tubes exposed to air were porcelain-white and one tube was golden yellow
22 September	Stopcock replaced by glass-tip device shown in Fig. 11; samples assayed after 5 days of bulk withdrawals	51.5° to 56.5°	54.0 to 57.0	0	Sterile

* These colonies were obtained from product water samples.

tube. This test was carried out to ascertain the likelihood of bacteria from the air falling into the opened tubes while the product water was being sampled and to afford a comparison of such bacteria with others that might be obtained. Next, 1-ml samples of supply water, which was collected with a sterile 1-ml pipette from funnel *C* in Fig. 4, were added to each of five tubes. Five additional tubes were left unopened in order to test the medium for sterility (controls).

The tests are listed in Table 3. The first test was carried out on the assembly without any chemical cleaning or sterilization to see whether the recycling at 100°C, which had led to low-conductivity water, would also destroy any microorganisms originally associated with the materials of construction. The second test was made 4 days after the entire apparatus had been fumigated with 10 percent formaldehyde, and then thoroughly steamed. Two tests (May 26 and July 17) were made after daily bulk withdrawal of water, as would be the case in routine use.

Test results recorded in Table 3 indicate a very low concentration of bacteria in the product water from the still. Among 304 samples tested, 292 were sterile and 12 contained bacteria which grew in the broth by enrichment. All of these bacteria were catalase-positive cocci, never rods. On the contrary, the supply water in funnel *C* in Fig. 4 contained an apparently pure culture of small, motile rod-shaped organisms which formed yellowish colonies on agar plates. Since this bacterium was not found in the product water, we suspected that the cocci isolated from the still were actually bacteria from the room air. The three-way stopcock appeared to be a likely source of such bacteria, because a portion of the cock is necessarily exposed to the room air. Two types of catalase-positive cocci were isolated from swabbed surfaces of the stopcock: a golden type not seen in earlier tests and a porcelain-white type isolated several times earlier.

Another test was conducted after the three-way stopcock had been replaced by the expedient shown in Fig. 11, and the reservoir was subjected to the routine of bulk drawoff for 7 days before any test samples were collected. The procedure was the same as before: after flushing through 250 ml of product water, 40 samples (each 1 ml) were collected in broth. The results, no bacteria in any of the samples, indicated that the water from the reservoir was sterile.

Out of all the test tubes (64) exposed to room air during the sampling periods, two exhibited turbidity and a vile aroma, reminiscent of the characteristics of fecal bacteria in broth. When the contents had been streaked out, a mixture of bacteria was found. One of the broth tubes gassed strongly. Such results had never been observed from the product water samples.

Pyrogens. Standard assays for pyrogens were carried out by Dr. Grace Brown (*30*). Samples of product water were taken on three successive days, one coincidental with the sample taken on 17 July (Table 3). The samples were passed from the reservoir into 200-ml Pyrex flasks with drawn-down necks. The flasks had been washed and then heated to 250°C and maintained at that temperature for 2 hours. The necks were flame-sealed, and the units were mailed to Dr. Brown, who reported that no rabbits or dogs tested developed a fever.

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31. We thank Dr. H. K. Cammenga, who gave us valuable information on the specific conductivity of water; Dr. W. Kayser, who refined the method of spiralizing plug flow and provided three photographs for Figs. 7 and 8; and A. Roggen, who assisted in the construction of the still and, with G. Mosholder, helped in the preparation of the manuscript. The work was supported by the Office of Saline Water under grants 14-30-2572 and 14-30-2964.

Enzymatic Interconversion of Active and Inactive Forms of Enzymes

A new mechanism of metabolic control has become evident.

Harold L. Segal

Every student of biochemistry has seen, and perhaps even looked at, two-dimensional metabolic maps that outline the myriad pathways of biodegradation and biosynthesis. He understands that for each arrow on the map pointing from one metabolite to another, one step modified, there exists a corresponding enzyme, which in principle can be named by inspection of the reaction. The virtual completion of the basic framework of such maps in all its detail has been a triumph of biochemistry, largely over the last quarter century, although occasionally a new addition not fully anticipated is still made.

Recently new kinds of arrows have begun to appear on such maps indicating that certain metabolites are also modulators of reactions in which they are not reactants or products. This new order of complexity, encompassed in the concept of modulator control, while certainly far from completely worked out, adds greatly to the reality that metabolic maps are intended to convey. The inclusion of this aspect of metabolic control requires the addition of essentially no new elements to the matrix. The same enzymes and the same metabolites and cofactors which comprise the pathways of metabolism themselves are the units which effect modulator control. It is only necessary to enlarge the concept of enzyme action to include in some cases modulator binding at sites distinct from the reaction sites. The map, however, is still two dimensional.

The experiments of the Coris and their co-workers 25 to 30 years ago, revealed that one of the enzymes present on metabolic maps, glycogen phosphorylase, exists in two forms, called *a* and *b* (*1*). With the discovery that these forms, which differ from one another covalently by the presence (*a* form) or absence (*b* form) of esterified phosphate groups, could be interconverted enzymatically, a new dimension of enzymology emerged. Phosphorylase *a* phosphatase, which catalyzes the *a* to *b* conversion, and phosphorylase *b* kinase, which catalyzes the *b* to *a* conversion, are of a different sort functionally from the enzymes that interconvert metabolites (metabolic enzymes). They are enzymes whose substrates are enzymes. The inference is that the interconversion of forms of metabolic enzymes has a control function, and there is substantial evidence to support this view.

In addition to the opportunities such systems provide for metabolic control, there are some genetic and developmental consequences as well in the possibilities for multigenic control of enzyme expression. Where an "activating enzyme" is required for the conversion of a metabolic enzyme, or other physiologically active protein, to its functional state, a failure of appearance of activity may not denote a lack of a functional structural gene for the protein in question, or even the absence of the protein itself. What may be lacking is the "activating enzyme." One wonders how often, in the catalogs that relate appearance of functional enzyme activity with embryological development, it is gene expression for an "activating enzyme" that is manifested rather than for the functional protein per se. Analogous questions can be

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