more accurately the temperature coefficient of photochemical destruction of riboflavin in foods which represent good riboflavin sources.

Samples of fresh skim milk and beaten raw eggs were heated under the conditions described above. The results are listed in Table II.

From Table II it is evident that light destruction of riboflavin in liquid foods proceeds at a rapid rate, although it is somewhat slower than the destruction in clear solutions. The opacity of many foods tends to prevent excessive riboflavin losses during cooking, but large losses in foods such as milk and eggs, which alone contribute approximately 40 per cent. to 50 per cent. of the riboflavin supply in the average American diet, may appreciably aggravate shortages of the vitamin.

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# SCIENTIFIC APPARATUS AND LABORATORY METHODS

## RECLAIMING AGAR FOR BACTERIOLOGICAL USE

WITH the cessation of imports, an acute shortage of agar may be encountered before new sources can be developed. Two proposed substitutes under study in our laboratory, carrageen and sodium alginate, which are also extractives from marine plants, do not appear very satisfactory.

The procedure for reclaiming agar from media herein presented is a modification of the method used by us for several years to purify commercial crude agar. The treatment produces a product which is actually superior to the common commercial agars being used by bacteriologists. Such reclaimed agar has a hardness of approximately 100 compared to 20–50 as determined by our hardness apparatus,[1] has an ash content of 1 per cent. or less compared with an average of 4 per cent. (our findings and those of Whitaker[2]) and a crude protein nitrogen content of 1 to 2 mg per g, which is ½ to ¼ less than commercial samples. Also most of the non-solidifying gum content (30 to 40 per cent.) is removed. The nitrogen content can be still further reduced by treatment with pancreatin, but this has not been considered necessary.

The used culture medium is sterilized, made slightly alkaline to litmus and filtered through cheesecloth and absorbent cotton. It is then cooled slowly until solidified. The layer of sediment is removed and the agar is shredded by passing it through a fine wire screen of at least 16 mesh. After a preliminary washing of several hours in a cheesecloth bag by running water to whiten, the bag is transferred to a container for infusion with tap water with stirring at intervals. The water should contain a residual or added calcium salt. Sodium hypochlorite is added (excess detected by odor) primarily to prevent bacterial growth. The temperature of infusion should not exceed 50° C. The waste filtrate is siphoned off and fresh water added at intervals. After 6 to 8 infusions covering several days the excess water is drained from the agar and traces of hypochlorite and salts are washed out with distilled water. Depending on laboratory facilities, the agar may be either evaporated down on a steam bath or frozen and thawed to remove more water or dried directly in thin layers in an oven at 50° C. to 70° C. and finally at 100° C. When dry, the pieces of agar may be weighed and soaked preliminary to use or may be ground to a powder by aid of a hand mill.

Agar can also be reclaimed from blood-agar and differential media containing indicators and dyes as phenol red, E.M.B. and Endo. Such dye-containing media as Endo's should be processed separately however, and the infusions should be of acid reaction to intensify bleaching. The types of infusion containers preferred are glass or porcelain enamel and the quantity of waste agar should be several liters or more.

At times when a sufficient quantity of waste agar is not available for processing, it may be oven- or sun-dried by pouring in thin layers in pans and stored dry in bottles. This practice of drying and storing of waste agar may be found advisable even in laboratories which appear to have ample reserves of stock agar. For in case no more can be purchased, the waste agar can be reclaimed.

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[1] *Jour. of Bacteriology*, 41: 1, 32, 1941.
[2] *Jour. Am. Pub. Health Asn.*, 1: 632, 1911.

## RECLAMATION OF USED AGAR

SINCE virtually all commercial agar was obtained from Japan, laboratories and other institutions using this product in the preparation of solid culture media are faced with the same serious situation as the various industries preparing rubber products. Until a satisfactory substitute for agar can be obtained, it is obvious that present supplies must be used sparingly and the possibility of reuse should be carefully investigated.

A few reports of reclamation of used agar have appeared in the literature which vary in procedure

from the simple reheating and resolidifying of the medium to methods so complex and expensive as to be impractical.

At this station, where 6,000 to 7,500 grams of agar are used weekly in the preparation of *Brucella* antigen and vaccine and routine bacterial culturing, a procedure for the reclamation of used agar has been developed which has proved extremely satisfactory.

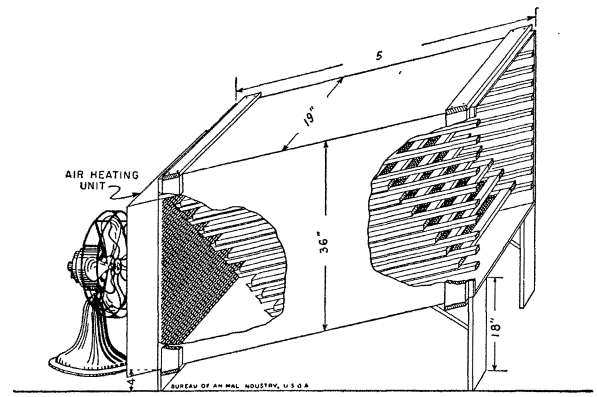
After the removal of the bacterial growth, the medium in flasks and tubes is heated in an autoclave at 15 pounds' pressure for 30 minutes. The melted medium, while still hot, is poured into glass vessels or crocks, preferably larger in diameter at the top than at the bottom. It is allowed to cool slowly in order that solid substances present will gravitate. When the medium has gelled, it is removed from the container and the bottom portion containing the sediment is excised and retreated in like manner until only sediment remains, which is discarded. The recovered medium is forced through a $\frac{1}{8}''$ mesh screen and the particulated material is placed in a 50- to 60-mesh cotton bag. At this station, where large quantities are handled, 100-pound bags are used. The bag is placed on a raised screen base in a tub or large box which has an outlet on the bottom. A rubber hose is inserted in the center of the bag, the open end of the bag tied around the hose, and the other end of hose is attached to a cold water spigot.

A slow stream of water is allowed to flow on the medium. The occasional kneading of the bag will remove air and allow the water to trickle throughout the medium. At first, the wash water is a clear dark brown and gradually becomes lighter. Washing is continued for at least 10 hours, usually overnight, when the agar has become a grayish white. Both wash water and agar at this point are negative to the Biuret test. When the washing is completed, the bag is left on a stand until most of the free water content has drained.

The agar is then gently spread on specially prepared screen trays and placed in a drying cabinet for evaporation.

The cabinet is about $60'' \times 19'' \times 36''$, open at both ends, and made of plyboard. It holds 10 evaporating trays, spaced about 2″ apart and so arranged that air forced through the box is baffled to insure even evaporation. The trays, $18'' \times 36''$, are made of $1'' \times 2''$ wood frame material, strengthened by a median brace. A heavy galvanized iron, $\frac{1}{4}''$ mesh hardware cloth is attached to the frame, over which is placed a 16-mesh wire screening.

Air is forced into the box by a 16″ circulating fan which passes the air primarily through a steam, room-heating element which heats the air to approximately 80° C. After about 12 hours in the evaporating box, the dried agar is only slightly darker than the original material. It is crisp and has the appearance of a flattened sponge sheet, and easily removed from the wire screen in sheets.




DRYING CABINET USED IN RECLAIMING AGAR

FIG. 1.

Media prepared from this reclaimed agar are very slightly, if any, darker than media prepared from U.S.P. agar. It has been tested in other laboratories with more than 30 different microorganisms and the results have been uniformly satisfactory.

In several tests made, it has been estimated that 75 to 80 per cent. of agar treated in the manner described is recovered, which includes the initial loss adherent to flasks in pouring.

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## BOOKS RECEIVED

*American Standard Definitions of Electrical Terms.* Pp. 311. American Institute of Electrical Engineers. $1.00.

BLAIR, THOMAS A. *Weather Elements.* Pp. xvii + 401. Illustrated. Prentice-Hall, Inc. $5.00.

CHANDRASEKHAR, S. *Principles of Stellar Dynamics.* Pp. x + 251. Illustrated. University of Chicago Press. $5.00.

DARRAH, WILLIAM C. *An Introduction to the Plant Sciences.* Pp. xi + 332. Illustrated. John Wiley and Sons, Inc. $2.75.

FERRIS, G. S. *Atlas of the Scale Insects of North America.* Illustrated. Stanford University Press. $6.75.

GROVES, ERNEST R., GLADYS HOAGLAND GROVES and CATHERINE GROVES. *Sex Fulfillment in Marriage.* Pp. xiv + 319. Emerson Books, Inc., New York. $3.00.

KARLING, JOHN S. *Plasmodiophorales.* Pp. ix + 144. 17 plates.

Matematicas y Fisica Teorica. Volume 2. Universidad Nacional de Tucumán.

MUNSHOWER, CARL WALLACE, and JAMES FLETCHER WARDWELL. Basic College Mathematics. Pp. xi + 612. Henry Holt and Company. $3.20.

POFFENBERGER, A. T. *Principles of Applied Psychology.* Pp. xvi + 655. D. Appleton-Century Co. $4.00.

SCHIFF, F. and WILLIAM C. BOYD. *Blood Grouping Technic.* Illustrated. Pp. xiv + 248. Interscience Publishers, Inc. $5.00.

WALD, ABRAHAM. *On the Principles of Statistical Inference.* Pp. 47. University of Notre Dame.