may be calculated by simple geometry ($V = 4/3\,\pi R^3$) to be 2.7×10^{-13}ml. Since one pneumococcus takes up 4×10^6 antibody molecules, which represents a volume of 8×10^{-13} ml,[5] the addition of antibody would result in a new volume of 10.7×10^{-13} ml. Calculating the radius corresponding to this volume gives a new radius of 0.63×10^{-4} cm, or about 1-1/2 times the original one. These figures are necessarily quite rough, but the order of magnitude is probably approximately correct.

While we have not conducted any experiment to determine the increase in volume of the sensitized pneumococcus, we have found in the literature photographs, which indicate that in Type I Pneumococcus the swelling is probably not more than about two-fold.[2,7] Thus the figure arrived at by simple calculation of antibody volume seems reasonable, and becomes even more cogent, when it is realized that this calculation assumes complete packing of the antibody, leaving no free space between antibody molecules, and since this is certainly unreasonable for physical reasons, the addition of antibody in fact could account for even greater degree of swelling than that calculated above.

The experiments have been of interest in another connection. Since the papain can reactivate neutralized biologic agents, it was thought that it would be a useful tool in isolating viruses in tissues where failure to infect could be due to the presence of neutralizing antibody. We attempted to carry out this in the case of rabbit papilloma, where failure to recover the virus from the papillomae of domestic rabbit was suggested to be possibly due to the presence of antibody but, unfortunately, preliminary experiments showed that this virus[8] is destroyed in a short time by the action of the enzyme so that the experiment could not be carried out satisfactorily.

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DESTRUCTION OF RIBOFLAVIN BY LIGHT

IN the course of conducting riboflavin assays in foodstuffs in association with Professor H. C. Sherman, a few observations were made of the extent of loss of this nutrient in the cooking of certain foods. It seemed noteworthy that large losses, up to 48 per cent., occurred in instances in which the cooking was done in open vessels so that the food was exposed to light during cooking. In view of the present recognition of wide-spread ariboflavinosis in human beings in the United States, an occurrence which is not clearly traceable to specific recent alterations of food habits, it seemed that light destruction at the high temperatures reached in cooking might be of practical importance.

Although György and coworkers[1] and Roscoe[2] noted the phenomenon of photochemical destruction of riboflavin, we are not aware of any study of the temperature coefficient of this photochemical reaction. The effects of temperature and hydrogen ion concentration on the rate of photochemical destruction of riboflavin in water solution are recorded in Table I.

TABLE I

LIGHT DESTRUCTION OF RIBOFLAVIN IN SOLUTION

Time of exposure, minutes (Temperature—100°C.)	Per cent. destruction		
	pH 3	pH 4.5	pH 6.5
5	16	28	42
15	42	62	72
30	60	84	91
45		90	99
60	95	97	> 99
Time of exposure, 1 hour	**Per cent. destruction Temperature**		
	32°	75°	100°
pH 3			95
pH 4.5	16	66	97
pH 6.5	51	> 99	> 99
DARK, 100° for 1 hour	**Per cent. destruction**		
pH 4.5		1	
pH 6.5		0	

These experiments were carried out during early afternoon on sunny days in a well-lighted room, simulating usual kitchen conditions. The illumination was not constant but approximated 60 foot candles on the side of the tubes facing an east window five feet away. The initial concentration of all solutions was 0.1γ/cc. Residual concentrations were determined by the microbiological method of Snell and Strong.[3]

It is apparent from Table I that increases either in alkalinity or temperature accelerate the destruction of riboflavin, at a quite rapid rate. Temperature and pH affect the light reaction rather than other chemical destruction, for when riboflavin solutions are heated in the dark no destruction occurs.

In view of these results it was of interest to test

TABLE II

DESTRUCTION OF RIBOFLAVIN IN MILK AND EGGS

Time of exposure, minutes 100°C.	Per cent. destruction	
	Milk	Eggs
5	26	
15	39	22 (open metal pan)
15		8 (closed pan)
30	48	
45	64	
45 (dark)	5	2 (steamed in autoclave)

[1] P. György, R. Kuhn and T. Wagner-Jauregg, *Natur. Wissenschaft*, 21: 560, 1933.

[2] M. H. Roscoe, *Biochem. Jour.*, 27: 1540, 1933.

[3] E. E. Snell and F. M. Strong, *Ind. Eng. Chem., Anal. Ed.*, 11: 346, 1939.

[7] A. F. Sabin, *Jour. Am. Med. Asn.*, 100: 1584–1586, 1933.

[8] Kindly sent to us by Dr. J. W. Beard.

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