dry form both acid and salt keep only *in vacuo*. This procedure has given uniform results and a yield of over 50 per cent. of the original potency.

A considerable increase in stability of the solutions was obtained by acetylation or benzoylation of the ammonium salt. The free acids of the acyl derivatives form fine needles which have about the same *in vitro* activity as the mother substances.

The analysis of penicillin best fits the formula $C_{14}H_{19}NO_6$ or $C_{14}H_{17}NO_5+H_2O$. The Oxford authors have stated that their preparations are nitrogen free. All our highly active and pure preparations, including the acyl derivatives, analyze for one N atom. Chromatographic adsorption or treatment with charcoal did not lower the N content. Penicillin is strongly dextrorotatory and has an absorption maximum at 2750 A°.[7]

Biologically our preparations are inactive against *E. coli*. The minimal concentration showing activity against 2 to 3 million hemolytic streptococci per cc is at a dilution of 1:32 million. This corresponds to about 240 Oxford units per mg. The Oxford standard has an activity of 42 units per mg.

KARL MEYER
ELEANOR CHAFFEE
GLADYS L. HOBBY
MARTIN H. DAWSON
ERWIN SCHWENK
G. FLEISCHER

THE REVERSAL OF PNEUMOCOCCUS QUELLUNG BY DIGESTION OF THE ANTIBODY WITH PAPAIN

IN a recent study[1] we have been able to remove the antibody by means of digestion with the proteolytic enzyme papain and recover several neutralized biologic agents in the active state. Among the agents studied was Pneumococcus, Type I, and it was found that it regained its virulence for the mouse (even after gross overneutralization) when the suspension of neutralized pneumococci was treated with papain.

Since there is no clear evidence as to the nature of the Quellung phenomenon, we thought it worth while to investigate whether the reactivation of the pneumococci was accompanied by reversal of the Quellung as a result of the action of papain, since such a result would indicate that the Quellung merely represents the addition of antibody with no further mechanism needing to be postulated. In fact, Etinger-Tulzynska,[2] on the basis of experiments wherein the Quellung reaction was reversed by means of heat treatment, postulated that the Quellung phenomenon was merely a visualization of the capsule that was already there. Nungester and Kempf[3] have likewise claimed that there is a reversal of the Quellung upon the addition of specific soluble substance, which in fact may represent the removal of antibody, although this involves assuming that the antibody was "pulled" off the pneumococcus by the soluble polysaccharide.

Our investigations were carried out in the following manner: Equal volumes of twenty-four hour tryptose broth culture of Pneumococcus Type I and undiluted antibody (rabbit serum) were mixed and a small portion was immediately removed and examined under the microscope for Quellung, which was plainly evident in a few minutes. The remainder of the mixture was incubated at 37° C. for thirty minutes, after which time a small portion of activated papain was added to half of the quelled pneumococcus suspension, and as a control papain, which had not been activated, was added to the other half. It was noted that, in the presence of the active papain, in a few minutes the clumps of agglutinated pneumococci were dispersed and went into a homogeneous suspension. Examination of the digested mixtures after about thirty minutes' incubation indicated a complete loss of the Quellung, while the control mixture with inactive papain remained agglutinated and swollen.

In order to ascertain whether the de-quelled pneumococci were capable of being re-quelled, after the digestion had taken place, the bacteria were spun down in the angle centrifuge and washed five times with saline in order to remove the enzyme. More serum was then added and Quellung occurred almost immediately. Thus, the cycle has been completed; it has been possible to reverse the Quellung by means of the removal of antibody and to re-quell the same pneumococci by the addition of more antiserum.

This experiment indicates that the Quellung merely represents the addition of antibody, an explanation which has been suggested.[4] We can bolster this theory by pointing out that from the quantitative point of view it is possible for the antibody to account for the increase in size, simply by its own volume. This is possible since we now have knowledge of the amount of antibody which the very same culture pneumococcus takes up.[5]

White[6] gives the size of the pneumococcus as from 0.5–1.2 μ. Taking as an average a diameter of 0.8 μ, or a radius of 0.4×10^{-4} cm, the volume of a sphere

[7] We thank Dr. H. Darby for the spectrographic analysis.

[1] G. Kalmanson and J. Bronfenbrenner, *Federation Proceedings*, 1: 2, 179, 1942.

[2] R. Etinger-Tulzynska, *Ztschr. f. Hyg. u. Infectsionkr.*, 114: 769–789, 1933.

[3] W. J. Nungester and A. H. Kempf, *Proc. Soc. Exp. Biol. and Med.*, 43: 705–706, 1940.

[4] A. D. Hershey, *Jour. Immunol.*, 42: 485–513, 1941.

[5] A. D. Hershey, *Jour. Immunol.*, 39: 383–396, 1940.

[6] B. White, "The Biology of the Pneumococcus," p. 31. Oxford University Press. 1938.

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.

G. M. KALMANSON
J. BRONFENBRENNER
WASHINGTON UNIVERSITY SCHOOL OF MEDICINE,
ST. LOUIS

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.