lesions were found, but the mice survived; no inactivation when the mice failed to survive the test period. On this basis the results are summarized in Table I.

TABLE I

INACTIVATION OF INFLUENZA VIRUS BY SECRETIONS OF HUMAN SUBJECTS

Secretions tested	Number of specimens	Degree of inactivation of virus			
		Complete	Almost complete	Partial	None
Common cold and hay fever	33	4	13	8	8
Normal	15	2	7	3	3
Saliva	16	0	1*	1*	14

* Negative when repeated 1 week later.

There was little difference between the results obtained with nasal secretions from patients with common colds and those from normal subjects. Approximately half caused complete or almost complete inactivation of 1,000 lethal doses of virus, while the other half exerted either slight or no inactivation. Saliva was ineffective. Secretions were obtained from six patients during the acute stage of a cold and again in a subsequent normal period. No significant differences were discernible in the results of the two tests. Of five samples of sputum from various patients, two caused partial inactivation; three had no effect.

The same phenomenon has been recently reported by Burnet, Lush and Jackson[3] who studied the action of nasal secretions of normal human subjects upon several viruses, including that of influenza. They noted no differences in the inactivating capacity of different specimens, but conducted their tests with filtered specimens. They state that the agent is destroyed at 100° C. Furthermore, they state that five hours is required for inactivation of virus to occur, and suggest that the agent is an enzyme.

Some characteristics of the inactivating agent have been outlined in the course of the present investigations. It is extremely stable at icebox temperature, remaining for at least six to eight weeks without change in potency. It is ineffective after heating at 70–75° C. for twenty minutes. In some cases, the material can exert its action after dilution, the extent of dilution varying with different samples. The highest effective dilution so far observed has been 1:8. The inactivation of virus by secretions is not the result of bacterial action, since many of the samples are either sterile or yield few colonies on blood agar plates. Furthermore, the bacteria, usually staphylococci, do not appreciably affect the test animals. The agent is not a lysozyme as measured by its action upon a susceptible micrococcus. In a series of tests with specimens exerting various degrees of inactivation upon the virus, the lysozyme content of the samples bore no parallelism to the virus-inactivating capacity but reached an approximate titer of 1:1,000 in each instance. The secretions have not been found to exert a bacteriostatic or bacteriolytic action when tested with smooth or rough pneumococcus, β. hemolytic streptococcus, *streptococcus viridans, staphylococcus aureus* or *albus, M. catarrhalis,* or meningococcus.

In the current study titrations of neutralizing antibody have been conducted in mice with serum from twenty of the patients with common colds and from all the normal subjects. While a sharp correlation between the inactivating effect of the nasal secretions and the antibody titer of the serum was not detected, it was found that the secretion from twelve of seventeen subjects with antibody titers of 1:20 or less gave either slight or no inactivation. The serum of four of the seven patients whose secretions failed to inactivate virus contained no antibodies; the other three had titers of 1:20. On the other hand, the secretions of sixteen of eighteen subjects, whose serum titers were 1:40 or more, completely or almost completely inactivated the test dose of virus. Within these broad limits a relationship is suggested. Furthermore, neutralizing antibodies in the serum are inactivated at the same temperature, 70–75° C., as the agent in the nasal secretions.

These studies are as yet incomplete. They show, nevertheless, that there exists in nasal secretions a substance capable of inactivating relatively large amounts of influenza virus. The inactivating capacity varies widely in different individuals, and in some respects resembles the so-called natural antibodies. It seems highly probable that this phenomenon is of considerable importance in relation to individual susceptibility to epidemic influenza.

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[3] F. M. Burnet, D. Lush and A. V. Jackson, *Brit. Jour. Exp. Path.*, 20: 377, 1939.

CHLOROPHYLL AS THE PROSTHETIC GROUP OF A PROTEIN IN THE GREEN LEAF[1]

IN an earlier publication,[2] it was pointed out that the differences in properties between chlorophyll dissolved in organic solvents and the green pigment as it exists in the leaf can be explained by assuming that the chlorophyll of the leaf is in combination with protein. It is now possible to present further information on the nature of this chlorophyll-protein compound.

The chloroplast material of ground-up spinach and

[1] This material was included in a paper under the same title which was presented at the photosynthesis symposium held at Columbus, Ohio, under the auspices of Section C of the American Association for the Advancement of Science on December 28, 1939.

[2] E. L. Smith, SCIENCE, 88: 170, 1938.

of Aspidistra has been purified by repeated salt precipitation or by differential centrifugation. Only about half this material is protein, as already found by Menke,[3] and now corroborated by us. To determine the ratio of chlorophyll to protein we estimated the protein chemically by the usual methods for N, and the chlorophyll spectrophotometrically by measuring the height of the absorption band in the red. Although the position of the absorption band in these preparations is different from that of chlorophyll in organic solvents, the same preparation has an identical extinction value in water as protein compound, or in ether or petroleum ether after precipitation of the protein with ten volumes of acetone. We have taken 5.4×10^4 as the value of the extinction coefficient estimated for three parts of chlorophyll *a* and one part of chlorophyll *b* from the values published by Zscheile.[4]

The measurements show a constant relation in the purified chloroplast material of about 16 parts of chlorophyll per 100 parts of protein. This indicates a little over three molecules of chlorophyll per Svedberg protein unit of 17,000 molecular weight. Because of the much smaller absorption at the standard wavelength of chlorophyll *b* compared to chlorophyll *a*, the fraction over 3 may represent an extra chlorophyll *b* molecule. This is in keeping with the observation of Willstätter and Stoll[5] that the leaves of most green plants show a ratio of about 3 to 1 of chlorophylls *a* and *b*. This constant stoichiometric combination of protein and chlorophyll strengthens the conclusion from other evidence that chlorophyll acts as the prosthetic group of a protein.

As stated earlier,[2] the chlorophyll-protein compound can be rendered water-soluble by the action of various detergents such as digitonin (= digitalin), bile salts or sodium desoxycholate. We have studied the action of an additional detergent, sodium dodecyl sulfate (SDS), in some detail. In addition to clarifying completely the green pigment, SDS also quantitatively converts the chlorophyll into phaeophytin, *i.e.*, removes magnesium from the molecule. This conversion, measured spectrophotometrically, proceeds at a rate which is directly proportional to the hydrogen-ion concentration, and takes place even in fairly alkaline solutions (pH 8 to 9). At constant pH, the rate is proportional to the SDS concentration until a maximum rate is achieved. Phaeophytin formation does not occur in 4 per cent. digitonin, or 10 per cent. bile salts at pH 4.5. At this pH, in 0.05 per cent. SDS the reaction is complete in a few minutes. It is therefore apparent that different detergents may have quite different actions on the same compound.

In the presence of SDS, the chlorophyll or phaeophytin (depending on the pH of the solution) remains attached to the protein, since the prosthetic group can not be separated by ultrafiltration, dialysis or by salt precipitations after removal of the SDS. This is confirmed by an ultracentrifugal study[6] of the solutions, which shows in addition that the protein is split into smaller units than in the other detergents. The action of SDS on the chlorophyll-protein compound of spinach differs from its action on the virus of tobacco mosaic disease; in the latter case, Sreenivasaya and Pirie[7] showed not only a splitting of the protein, but also a separation of the prosthetic group (nucleic acid) from the protein.

The fact that the phaeophytin remains attached to the protein indicates that in the smaller units magnesium plays no role in binding chlorophyll to the protein. The magnesium may, however, be concerned in the binding of the larger units, as indicated by the ease with which it is eliminated when the protein is split.

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VITAMIN B_6 AS AN ACCESSORY GROWTH FACTOR FOR STAPHYLOCOCCUS ALBUS[1]

IT has been shown recently by Knight[2] that *Staphylococcus aureus* can be grown on a synthetic amino acid medium provided it contains nicotinic acid and thiamin. Koser, Finkle, Dorfman, Gordon and Saunders,[3] using *Staphylococcus albus*, have reported somewhat similar but less striking results. The present communication reports the result of a study to determine whether or not synthetic vitamin B_6[4] (2-methyl, 3-hydroxy, 4,5-di-[hydroxymethyl] pyridine) has any effect on *Staphylococcus albus*[5] grown in a synthetic amino acid medium.

[3] *Zeits. physiol. Chem.*, 257: 43, 1938.

[4] *Bot. Gaz.*, 95: 529, 1934. When the data of Winterstein and Stein (*Zeits. physiol. Chem.*, 220: 263, 1933) are converted from $\log_e$ to $\log_{10}$ by dividing by 2.303, they are in good agreement with those of Zscheile. The data of Rabinowitch and Weiss (*Proc. Roy. Soc. London*, A, 162: 251, 1937) have somewhat lower values.

[5] "Investigations on Chlorophyll," English translation by F. M. Schertz and A. R. Merz, Washington, D. C., 1928.

[6] E. L. Smith and E. G. Pickels, unpublished.

[7] *Biochem. Jour.*, 32: 1707, 1938.

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[2] B. C. J. G. Knight, *Biochem. Jour.*, 31: 731, 1937.

[3] S. A. Koser, R. D. Finkle, A. Dorfman, M. V. Gordon and F. Saunders, *Jour. Infect. Dis.*, 62: 209, 1938.

[4] Supplied through the courtesy of Merck and Company, Inc., Rahway, N. J.

[5] This culture was furnished through the kindness of Dr. Arnold G. Wedum, University of Cincinnati.