globulin neutralized both agents and was approximately 65 percent effective in decreasing the mean symptom score caused by either one. Apparently these agents are antigenic in the human being, and they or related agents have caused rather widespread infections and antibody response. Nonspecific inhibition of the infectious agents is a possibility, but other experiments have discounted this (*3*).

One possible explanation of the lack of resistance against the common cold is that circulating antibody can protect against a systemic infection but is of little importance in the prevention of a local infection of the mucous membranes of the upper respiratory tract. We have shown that the common cold in adults is an afebrile and presumably localized respiratory infection (*1*), whereas children have more systemic symptoms. There is some evidence that local infections with other viruses also might occur in the presence of circulating antibody (*9*).

Prior studies in our laboratory have demonstrated that gamma globulin is consistently found in nasal secretions. It is not clear why the antibody is ineffective in the nose. The concentration of specific antibody might be critical, or inhibitory substances might block the antibody effect. Possibly cellular resistance is independent of antibody. Finally, antigenic variants of the agents that cause common colds might produce recurrent infection.

The demonstration of protective antibodies against the common cold in human gamma globulin is noteworthy with respect to the problem of immunity and immunization against this illness. Further exploration of some of the factors discussed is necessary before it will be known whether it is feasible to protect against common colds by the administration of vaccines.

GEORGE GEE JACKSON
HARRY F. DOWLING
TRUMAN O. ANDERSON
University of Illinois College of Medicine, Chicago, Illinois

References and Notes

1. G. G. Jackson *et al.*, *A.M.A. Arch. Internal Med.* 101, 267 (1958).
2. C. H. Andrewes, *Brit. Med. J.* 1953, No. 9, 206 (1953); Commission on Acute Respiratory Diseases, Armed Forces Epidemiology Board, *J. Clin. Invest.* 26, 957 (1947); A. R. Dochez, G. S. Shibley, K. C. Mills, *J. Exptl. Med.* 52, 701 (1930); G. B. Foster, *J. Infectious Diseases* 21, 452 (1917); W. Kruse, *Münch. med. Wochschr.* 61, 1547 (1914).
3. G. G. Jackson *et al.*, unpublished observations.
4. G. F. Badger *et al.*, *Am. J. Hyg.* 58, 31 (1953).
5. W. H. Price, *Proc. Natl. Acad. Sci. U.S.* 43, 790 (1957).
6. These studies were carried out under the sponsorship of the Commission on Acute Respiratory Diseases of the Armed Forces Epidemiology Board and were supported in part by the Office of the Surgeon General, Department of the Army, and in part by grants from the Lederle Laboratories Division of the American Cyanamid Co. and from Charles Pfizer and Co., Inc. We gratefully acknowledge the assistance of Delores Seketa, Louise Jensen, and Marvin Turck, who participated in conducting the experiments.
7. The solution of pooled human plasma proteins was kindly furnished by Lederle Laboratories Division of the American Cyanamid Co., Pearl River, N. Y., and by Armour Laboratories, Chicago, Ill.
8. Neutralization tests for 2060 and JH viruses were performed by W. J. Mogabgab of Tulane University School of Medicine.
9. J. A. Bell *et al.*, *J. Am. Med. Assoc.* 165, 1366 (1957); M. J. Lipson, F. C. Robbins, W. A. Woods, *J. Clin. Invest.* 35, 722 (1956).

6 March 1958

In vitro Decomposition of 1-Adrenochrome

1-Adrenochrome has been reported to produce psychotomimetic effects following intravenous injection (*1, 2*), and it has also been reported to be markedly elevated in the blood of normal subjects receiving lysergic acid diethylamide (*3*). In view of the reported instability of adrenochrome (*2, 4*) in the solid state and in solution prior to its injection, a study of the variables affecting decomposition was made (*5*).

1-Adrenochrome (*6*) was prepared as follows: To 176.5 mg of synthetic 1-epinephrine in 5 ml of redistilled methanol there was added 0.1 ml of 98-percent formic acid. One gram of freshly prepared silver oxide was added, with stirring. A deep red color developed immediately, and the temperature rose to 42°C. The reaction mixture was stirred for 2 minutes and centrifuged at high speed, and the supernatant solution was allowed to stand at −20°C for ½ hour. The precipitate was filtered and washed with a small volume of cold methanol-ether (1:1) followed by methanol-ether (1:3) and, finally, with ether to remove all traces of methanol. The yield of 1-adrenochrome was 54.3 mg (32.5 percent).

The absorption spectra showed three maxima, at λ-220, λ-300, and λ-480 mμ. The 480-mμ peak was used to follow the decomposition of 1-adrenochrome in 0.9-percent saline (*p*H 7.0), redistilled water (*p*H 7.0), and methanol solutions. It was found that the stability at room temperature (22°C) was greatest in sodium chloride solution and least in methanol solution. The decomposition was approximately 2.5 percent in saline, 5 percent in water, and 10 percent in methanol in 12 hours in the concentration range of 15 to 25 μg/ml and was approximately proportional to the time for at least 48 hours. It also was found that concentration affected the rate of decomposition. Thus, in saline solution, 3.8 mg of 1-adrenochrome per milliliter decomposed to the extent of 34 percent in 12 hours at room temperature, whereas, at 3.4 μg/ml, decomposition was not measurable even after 96 hours. Temperature also affected the decomposition. A solution of 1.9 mg of 1-adrenochrome per milliliter in 0.9-percent sodium chloride solution decomposed to the extent of more than 60 percent in 24 hours at room temperature but only to the extent of 4 percent at 3°C or at −20°C. The *p*H is also an important variable. Zambotti and Moret (*7*) reported that in 0.9-percent saline solution at 37°C in 2 hours there was a loss of 25 percent at *p*H 5.91 and of 90 percent at *p*H 7.38.

The decomposition of 1-adrenochrome in the solid state stored for 3 months at room temperature in an ordinary capped glass vial was also studied. No changes were observed in the infrared spectra obtained in the solid state in a potassium bromide pellet. The spectrum of the 1-adrenochrome synthesized by the method described was identical in all respects with the spectrum of a sample of 1-adrenochrome synthesized by the method of Sobotka and Austin (*8*), which was obtained from the Research Division of Armour and Co.

AARON FELDSTEIN
Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts

References and Notes

1. A. Hoffer, H. Osmond, V. Smythies, *J. Mental Sci.* 100, 29 (1954).
2. H. Osmond, in *Neuropharmacology* (Josiah Macy, Jr. Foundation, New York, 1956), p. 183.
3. A. Hoffer, paper presented at Sci. Conf. Brain Research Foundation, New York, N.Y., 27 Jan. 1958.
4. D. E. Green and D. Richter, *Biochem. J.* 31, 596 (1937); D. J. Ingle, D. A. Shepherd, W. V. Haines, *J. Am. Pharm. Assoc. Sci. Ed.* 37, 375 (1948); F. M. Bacq, *Pharmacol. Revs.* 1, 1 (1949).
5. I wish to acknowledge the technical assistance of Jerry Murphy, precollegiate science summer student, St. Mark's School. This project was completed under grants from the Ford Foundation and the U.S. Public Health Service (B-713).
6. C. L. McCarthy, *Chem. & Ind. (London)* 55, 435 (1946).
7. V. Zambotti and V. Moret, *Arch. sci. biol. (Bologna)* 33, 522 (1949).
8. H. Sobotka and J. Austin, *J. Am. Chem. Soc.* 73, 3077 (1951).

3 February 1958

Cytochemical Demonstration of Masked Lipids

There are several methods for the cytochemical or histochemical *in situ* demonstration of free lipids, or of lipids which give general or special color reactions (*1*). However, the larger fraction of lipids in ordinary cells (excluding fat cells or similar tissues) belongs to the class of, until now, cytochemically nondemonstrable or masked lipids, which should form lipoprotein complexes or complexes of a higher order. For instance, in calf-liver cells it has been possible, by extraction with suitable salt

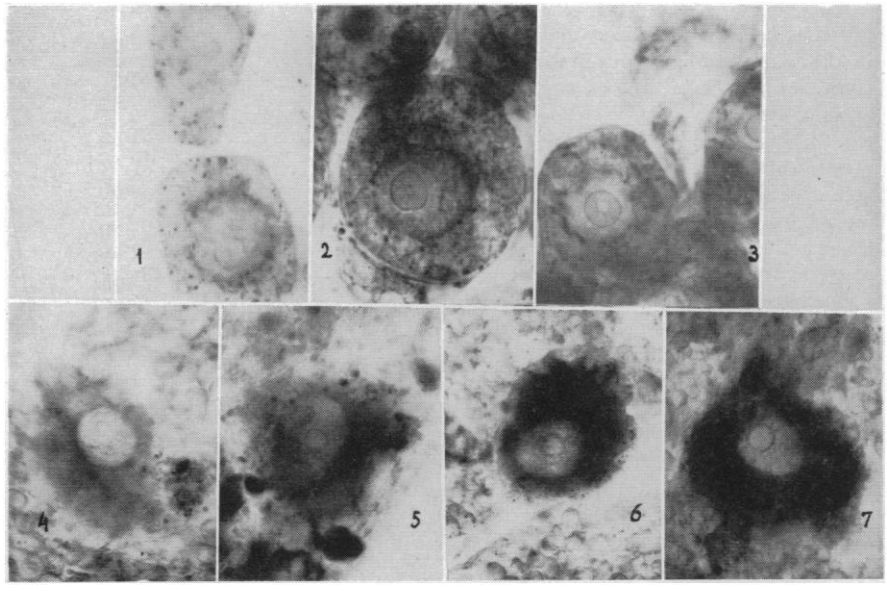
solutions, to prepare "ghost cells" which are almost exclusively composed of lipoproteins and whose aspect, nevertheless, is almost unchanged in comparison with the intact, original cells (*2*).

We present here a method for the demonstration of masked lipids *in situ* which has given us good results in the study of these compounds in the cell. The method may be quantized in the future by coupling it with lipid extraction and with determination of concentration by optical means. The latter procedure alone—that is, mass determination by absorption (soft x-rays) or interferometry and refractometry, coupled with extraction—is capable of giving an idea of the quantity of total lipids or of certain classes of these compounds, but it suffers not only from the errors of unspecific and incomplete extraction but also from those derived from differences in the optical properties of the protoplasm before and after extraction. As a result of such errors, these procedures do not generally allow of a fine location of the compounds in the cell structures (for instance, within the nucleolus), while valid cytochemical *in situ* methods, of the specific-coloration or colored-end-product type, necessarily give such details. Moreover, cytochemical *in situ* methods generally are much more sensitive than mass determinations.

The method is based essentially on the finding of a specific coloration for lipids, after suitable fixation, coupled with an incipient separation of the protein from the lipid in the complexes by means of reagents which mildly attack the protein and not the lipid. Concentrations of these attacking or demasking reagents are employed which give a separation of lipids sufficient for their taking up of the specific dye but which do not dissolve the protein and thus distort the microscopic structure of the cell. Finer location and control of the cell structures in the course of the reaction would be possible if an electron microscope and suitable lipid "electron dyes" were used.

The results are appraised by comparing the aspects of cells colored with and without the demasking reagent and by extracting the lipids and coloring afterwards.

Fixation and coloration may be carried out apart, but it was found to be more convenient to perform both simultaneously, and together with the demasking. A layer of 10 ml of absolute alcohol saturated with Sudan black is carefully poured into a graduated cylinder, over an equal volume of Baker's (*3*) fixative-preserving solution for lipids [consisting of a mixture of 10 ml each of 40-percent formalin, 10-percent calcium chloride (anhydrous), and 10-percent cadmium chloride, and 70 ml of distilled water]. These are mixed at once by shaking and



Results obtained by means of fixation, coloration, and demasking, with Sudan black. Figs. 1, 2, and 3: root tips of *Vicia faba*; Figs. 4, 5, 6, and 7: oocytes of the snail *Cepaea nemoralis*. Figs. 1 and 4 show results when no demasking reagent was used; Figs. 2 and 5 show results when barium hydroxide was used as the demasking reagent; Figs. 3 and 7 show results when urea was used; and Fig. 6 shows results when a mixture of barium hydroxide and urea was used. Differences in coloration of the cytoplasm and the nucleus, especially apparent in the nucleolus in *Vicia,* are seen on comparing Figs. 1 and 4 with those that show results of using preparations that contained a demasking reagent. (Figs. 1, 2, and 3, about × 890; Figs. 4, 6, and 7, about × 200; Fig. 5, about × 310.)

are filtered through rapid-filtering filter paper, or simply through cotton wool. The demasking compound is now added to an aliquot of the filtrate, and this is immediately used (otherwise the Sudan black colloidal solution may settle). Coloration, carried out in small stoppered test tubes, is obtained in about 18 to 48 hours, a mean time of 24 to 36 hours at 20°C generally giving good preparations.

It should be remarked that the pieces to be fixed should consist of a small number of cell layers, preferably sections (obtained free-hand or with the freezing microtome); otherwise uneven penetration of the colloidal dye results.

We found that barium hydroxide (obtained from the solid form that is sold in small lumps) and urea give good results. Other compounds or reagents which denaturate or slightly attack proteins may also be employed. To 1 ml of the filtrate is added about 25 mg of solid, freshly powdered barium hydroxide, or 50 to 200 mg of crystallized urea. Several concentrations and the mixture of both compounds were tried, and it was found that, according to the tissues, urea should be added in lower or higher concentration—lower for animal tissue, higher for plant tissue; the mean concentration here recommended for general purposes is 0.1 g/ml.

Parallel colorations were made on similar sections (i) after a thorough lipid extraction (which may be a selective extraction, only for certain classes of lipids) and (ii) with and without the demasking compound having been added to the fixing-coloring filtrate. Extraction errors excepted, the differences between preparations with and without extraction give the total lipids. Differences between preparations to which the demasking reagent has been added and those to which it has not been added give the masked lipids; it is to be noted, however, that the 50-percent alcohol and the cadmium salt in the fixing-coloring mixture also effect some demasking, though only a mild one. Nonspecific colorations, or impregnations, produced as a result of the long coloration time are appraised by comparing the sections which underwent extraction with those which did not undergo extraction or demasking.

Figures 1 to 7 give an idea of the results of this method; they show some aspects of plant and animal cells stained both with and without the action of demasking reagents. Differences are conspicuous in the nucleus and in the cytoplasm.

J. A. SERRA
Institute of Zoology, Faculty of Science, Lisbon, Portugal

References

1. L. Lison, *Histochimie et cytochimie animales* (Gauthier-Villars, Paris, ed. 2, 1953), chap. 16, summary.
2. N. C. Bruemmer and L. E. Thomas, *Exptl. Cell Research* 13, 103 (1957).
3. J. A. Baker, *Quart. J. Microscop. Sci.* 87, 441 (1946).
4. A more detailed account and a discussion of the method and its variants is in preparation.

24 January 1958