fever sufferers in Oberlin at three levels of vitamin intake; 100 mg, 200 mg and 500 mg daily, administered during the ragweed season from August 15 to September 15.

URINARY EXCRETION

In most instances we were able to determine the 24-hour urinary excretion[2] of vitamin C before giving the first dose. Whenever possible we determined the daily excretion again after one week. It is the general opinion that a healthy individual of average weight excretes from 30 to 50 mg of vitamin C daily in the urine.

With ordinary methods of collection we have observed considerable loss by oxidation, so we used the very simple but effective method previously devised by Holmes and Campbell.[3]

The maximum pollen count in Cleveland, thirty-five miles from Oberlin, averaged about 87 for the last half of August and about 80 for the first half of September. "Sneezing begins at 15." Oberlin, away from Lake Erie, has more pollen than Cleveland.

The initial daily vitamin C excretion of twelve patients (including three not charted) averaged only 10 mg, indicating a very low level, due to destruction or inactivation of the vitamin. One excreted 20 mg, three were satisfactory and the others were not measured.

Usually, after a week of treatment the excretion rose to excellent levels, in some instances indicating body saturation. Strangely enough, patients No. 1 and No. 4 showed very good vitamin C levels before starting treatment, yet they were greatly benefited by adequate dosage.

It is evident from the table that only five sufferers made a noticeable gain in health after a week of 100 mg daily dosage, while twelve gained decidedly after a similar period of 200 mg dosage and eight reported remarkable improvement after three or four days at the 500 mg level. One got almost immediate relief after a single dose of 1,000 mg. Apparently there was distinct gain with 88 per cent. of the patients.

TREATMENT

We strongly recommend that pharmaceutical firms prepare 250 mg tablets of vitamin C (or capsules to be emptied on the tongue) in order to lower the cost and to simplify dosage. The patient (after consulting the family physician, as was done in our own recorded experiments) would do well to begin with a daily 250 mg dose and, if no decided improvement results after one week, to try 500 mg daily until satisfactory progress is observed. After that he might get along comfortably on 250 mg or less during the season.

Since excess vitamin C is excreted rapidly in the urine, it is impossible to go beyond body saturation. Rarely are any irritating effects observed, yet one of our patients reported development of a rash.

REDUCING ACIDITY FOR SENSITIVE PATIENTS

Patients objecting to the acidity of ascorbic acid are advised to mix with the vitamin an amount of baking soda nearly equivalent chemically. If the vitamin is visibly crystalline, equal volumes of vitamin and sodium bicarbonate are used; if the vitamin is in a fluffy powder form, about one third that volume of sodium bicarbonate will serve. It is a mistake to mix water solutions to be kept for days, as oxidation occurs rapidly in the neutralized vitamin solution. We proved, by tests on several people, that after keeping a mixture of the dry powders eight hours and then administering there was no apparent loss of the vitamin. Patients with gastric ulcer, usually on a diet low in vitamin C because of difficulty with the roughage of vegetables and the acidity of fruits, may profit by the observation above.

HARRY N. HOLMES
WYVONA ALEXANDER
SEVERANCE CHEMICAL LABORATORY,
OBERLIN COLLEGE

SCIENTIFIC APPARATUS AND LABORATORY METHODS

A SIMPLIFIED PROCEDURE FOR THE CONCENTRATION AND PURIFICATION OF INFLUENZA VIRUS[1]

THE observations of Hirst[2] and McClelland and Hare[3] have clearly demonstrated that influenza virus in chorioallantoic fluid of the chick embryo can be directly adsorbed by the erythrocytes of the embryo. Hirst[4] has also shown that the adsorbed virus can be readily eluted from the red blood cells at temperatures of 22° to 37° C. In addition, these investigators have pointed out[5,6] that the precipitate which forms when

[2] Miss Jean Risinger assisted us with some of the analytical work.

[3] Harry N. Holmes and Kathryn Campbell, *Jour. Lab. Clin. Med.*, 24: 1293, 1939.

[1] These investigations were aided through the Commission on Influenza, Board for the Investigation and Control of Influenza and Other Epidemic Diseases in the Army, Preventive Medicine Division, Office of the Surgeon General, United States Army.

[2] G. K. Hirst, SCIENCE, 73: 335, 1941.

[3] L. McClelland and R. Hare, *Canadian Public Health Jour.*, 32: 530, 1941.

[4] G. K. Hirst, *Jour. Exp. Med.*, 76: 195, 1942.

[5] G. K. Hirst, E. R. Rickard and L. Whitman, *Proc. Soc. Exp. Biol. and Med.*, 50: 129, 1942.

infected fluid is frozen and then thawed contains the bulk of active virus and have employed the procedure for the concentration of influenza virus.

Certain technical difficulties accompany concentration by precipitation. Care must be taken, while harvesting the fluid, not to rupture the blood vessels since adsorption of virus by red cells results in an appreciable diminution in titer of the fluid. The temperature during the collection of the precipitate must be maintained at or about 0° C. and agitation must be carefully avoided. The procedure for concentration of the Lee strain of Type B virus requires an adjustment in pH. There is a bulk of non-specific material in the precipitate, only part of which is soluble. After drying *in vacuo* the insoluble residue increases.

It appeared that utilization of embryonic red cells for the concentration of virus might eliminate some of these difficulties if satisfactory adsorption and elution could be obtained without loss of activity. This has been possible. The essential features of the procedure adopted are as follows: Into the allantoic sac of hen's eggs, containing embryos in the eleventh or twelfth days of incubation, is inoculated 0.1 cc of a 10^{-3} dilution of infected allantoic fluid in physiological salt solution. Either the PR8 strain[7] of type A or the Lee strain[8] of type B virus has been used. The extra-embryonic fluids are harvested 48 hours later. The shell over the normal air sac is removed, the shell and chorioallantoic membranes and the blood vessels are torn with sterile forceps; the amnion and its main vessel are also torn. The embryo is allowed to bleed into the fluid while the egg is rotated so as to obtain mixing and to prevent the formation of clots. The bloody fluid is removed by aspiration with needle and syringe and collected in a 250 cc centrifuge bottle immersed in an ice-water bath at a temperature of 4° to 6° C. Chilling is important, since it increases the degree of adsorption and limits the elution which occurs rapidly at higher temperatures. The fluid from additional eggs is collected in the same manner and added. The red cells agglutinate while still in the egg and form coarse clumps in the collecting vessel. Since adsorption occurs very rapidly the process reaches its maximum in the time required to harvest the fluid from a few eggs.

When the desired volume of fluid is collected, the red cells, constituting 2 to 2.5 per cent. of the total volume, are separated by centrifugation in a chilled cup for three minutes at 500 to 1,000 r.p.m. The supernatant fluid and any light fibrinous aggregates are poured off and discarded. At this stage the cells are strikingly cohesive and resemble a disc of soft gelatin. The surface of the sediment is gently rinsed with cold (4° C.) 0.85 per cent. sodium chloride solution. No effort is made at this time to break up the agglutinated cells, since it tends to induce hemolysis. The wash fluid remains clear. To the washed, sedimented cells physiological salt solution is added in an amount equal to one tenth the original volume or less, depending upon the degree of concentration desired. The mixture is placed in a water bath at 37° C., agitated gently until the temperature is raised and the suspension is then placed in an incubator at 37° C. for two and one half hours. The clumps of agglutinated cells gradually disperse as the virus is released. The red cells are then centrifuged from the suspension and the supernatant fluid which represents the concentrate is removed. The preparation is usually slightly opalescent with a faint pink tinge.

In this manner approximately tenfold concentration of both the PR8 and Lee strains of virus has been obtained consistently. The infectious and agglutinating titers of the concentrate have remained essentially constant for at least three weeks in the refrigerator. Evidence points to the fact that the major portion of the inert chick protein is eliminated in that no significant precipitate is observed when the material is thawed after freezing with CO_2 ice nor after prolonged standing in the refrigerator. On rehydration after freezing and drying a small amount of finely suspended material remains undissolved.

The results indicate that the simplified procedure of adsorption and elution from embryonic red cells is as effective as precipitation in concentrating influenza virus from infected chorioallantoic fluid and takes advantage of technical features which serve as sources of difficulty in the precipitation process. Experimental results will be presented in detail in a subsequent publication.

THOMAS FRANCIS, JR.
JONAS E. SALK[9]
DEPARTMENT OF EPIDEMIOLOGY AND THE VIRUS LABORATORY, SCHOOL OF PUBLIC HEALTH, UNIVERSITY OF MICHIGAN

[9] Fellow in the Medical Sciences of the National Research Council.

[6] R. Hare, L. McClelland and J. Morgan, *Canadian Public Health Jour.*, 33: 325, 1942.
[7] T. Francis, Jr., SCIENCE, 80: 457, 1934.
[8] T. Francis, Jr., SCIENCE, 92: 405, 1940.

BOOKS RECEIVED

A.S.T.M. Standards on Petroleum Products and Lubricants. Illustrated. Pp. x + 442. $2.25. *A.S.T.M. Standards on Textile Materials.* Illustrated. Pp. xiii + 408. $2.25. American Society for Testing Materials, Philadelphia.

AGNEW, RALPH PALMER. *Differential Equations.* Pp. vii + 341. McGraw-Hill. $3.00.

ARTHUR, PAUL and OTTO M. SMITH. *Semimicro Qualitative Analysis.* Pp. xi + 322. McGraw-Hill. $2.75.

MEAD, MARGARET. *And Keep Your Powder Dry.* Pp. x + 274. William Morrow and Company. $2.50.

ROGERS, J. SPEED, THEODORE H. HUBBELL and C. FRANCIS BYERS. *Man and the Biological World.* Illustrated. Pp. x + 607. McGraw-Hill. $3.50.