group as compared with the checks, together with much additional data which indicate that the disease is not due primarily to organisms, nutritional unbalance, or other factors, lead to the definite conclusion that it is of the nature of a virus disease.

A Quantitative Study of the Complement-Hemolysin Relation

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A precise spectrophotometric method employing the end-point of 50 per cent hemolysis (2) has been used to measure the changes in complement activity that occur when a standard suspension of sheep's erythrocytes is sensitized with graded amounts of a hemolytic antiserum. Complement was titrated without preliminary incubation, and the 50 per cent units were determined by the method of least squares. The latter were then plotted against the corresponding amounts of antiserum to give the curve of the complement-hemolysin relation (5). Analysis of the experimental findings indicated that this curve may be rectified by plotting the ratio x/y, as ordinate, against x, as abscissa, where x represents hemolytic antiserum, and y, the unit of complement. A graph of the results obtained in a
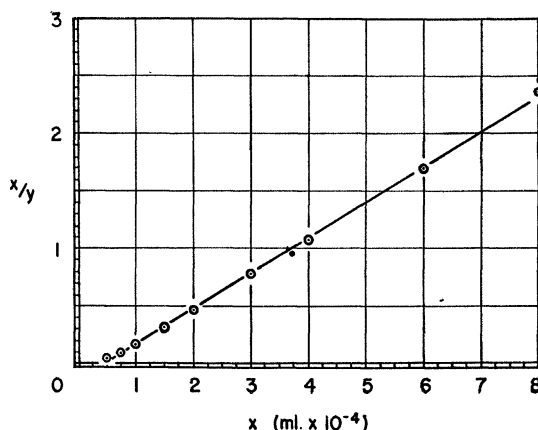

Fig. 1. Graph of x/y vs. x, where x represents hemolytic antiserum; y, the 50 per cent unit of complement.

representative experience is shown in Fig. 1. In fitting the line

$$(x/y) = bx + a \qquad (1)$$

to such data, the method of least squares was used to find values of b, the slope, and a, the intercept on the ordinate axis. For the example given, it was found that b = 0.308 and a = −0.137. To test the applicability of Equation 1 to the experimental points, the ratios x/y were calculated by substituting the predetermined values of a and b in the equation and

[1] With the technical assistance of Helen M. Conway and Willie Mae Bodie.

allowing x to take on those used experimentally. The standard deviation of the observed from the calculated ratios was estimated (3) from

$$s'^2 = \Sigma \, [(x/y)_{observed} - (x/y)_{calculated}]^2_{/N-2}. \qquad (2)$$

The estimate, s' ≅ 0.022, was obtained for the results plotted in Fig. 1.

Solving Equation 1 for y gives

$$y = \frac{x}{bx + a}, \qquad (3)$$

which would express the curve of the complement-hemolysin relation. Vertical and horizontal asymptotes of this curve are demonstrable (1a), respectively, at

$$x = \frac{-a}{b} \text{ and } y = \frac{1}{b}.$$

If the axes are translated to a new origin at the point $(\frac{-a}{b}, \frac{1}{b})$, the equation assumes the standard form for a rectangular hyperbola referred to its asymptotes as axes (1b).

Curves based upon Equation 3 have fitted the experimental points over a wide range of hemolysin quantities but deviate somewhat when low concentrations of the reagent are used.[2]

TABLE 1

Effect of Hemolysin Concentration Upon the Constant, 1/n, in Von Krogh's Alternation Formula

x Hemolytic antiserum (ml. $\times 10^{-4}$)	0.50	0.75	1.0	1.5	2.0	3.0	4.0	6.0	8.0
y 50% unit of complement (ml. $\times 10^{-3}$) (calculated*)	29.41	7.98	5.85	4.62	4.18	3.81	3.65	3.51	3.44
1/n	0.366	0.354	0.254	0.221	0.199	0.175	0.166	0.180	0.189
Complement required for 99% hemolysis (ml. $\times 10^{-3}$) (calculated†)			18.79	12.76	10.43	8.51	7.83	8.03	8.20

* Values of y from Equation 3, where b = 0.308 and a = −0.137.
† From the Von Krogh formula (4).

It is of interest that the highest concentrations employed failed to produce an inhibition of complement activity such as has been ascribed to agglutination of erythrocytes by concentrated antiserum. Such inhibitions have, nevertheless, been observed occasionally in previous experiments, and Equation 3 would not be expected to express the complement-hemolysin relation in the affected range.

The data were also examined with regard to the effect of

[2] As a result of consultations with W. R. Thompson, of the Division of Laboratories and Research, New York State Department of Health, it appears that a better fit may be obtained with the more general form of rectangular hyperbola, $(x - x_0)(y - y_0) = k$, where x_0, y_0, and k are parametric constants. The application of this formula will be discussed in a more comprehensive report.

hemolysin concentration upon the slope of the hemolytic curve. The parametric constant, $1/n$, in Von Krogh's alternation formula (4) was taken as the index of slope, and its values were determined, by the method of least squares, at the same time as those of the 50 per cent units of complement (2). It was observed that $1/n$ approaches a minimum in a central range of hemolysin concentration and increases progressively with the use of greater or lesser amounts of the reagent. Table 1 gives the values obtained in the experience illustrated by Fig. 1. More pronounced increases of the slope parameter were observed in the presence of concentrated hemolysin when antisera of higher titer were tested.

In an attempt to estimate the influence of this variable in determining the least quantity of complement which may safely be used in complement-fixation tests, the amounts of complement which would be required for 99 per cent hemolysis were calculated from the Von Krogh formula by substituting the predetermined values of $1/n$ and the 50 per cent units of complement. Estimates obtained in this manner are included in Table 1. In general, the concentration of hemolysin requiring a minimum amount of complement for 99 per cent hemolysis corresponded closely with that exhibiting a minimum value of $1/n$. It is noteworthy that both minima were observed in the range of hemolysin concentration which has been taken as optimal for the complement-fixation reaction (5).

The application of the foregoing criteria in the standardization of hemolysin is being studied. Equation 3 would permit the objective definition of an optimal concentration for complement-fixation tests.

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Reduction in the Vital Capacity of Asthmatic Subjects Following Exposure to Aerosolized Pollen Extracts

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The difficulty of determining the allergic factors responsible for asthmatic attacks is well recognized. Skin tests are frequently of little value for two reasons: first, because intense reactions in the skin may be produced by extracts of pollens or other substances which apparently are not factors in the production of the asthmatic attacks; and second, because many individuals with asthma do not show skin reactions to allergenic extracts.

A new approach to the study of asthma and the problem of determining the specific causes of asthmatic attacks was suggested by the demonstration that in the great majority of asthmatic subjects a reduction in vital capacity follows the parenteral administration of histamine or mecholyl (1). In the study to be described here, these changes in vital capacity were observed to follow inhalation of one or more extracts of birch, oak, grass, and ragweed pollens and certain other substances in 6 or 7 asthmatic subjects studied. Exposure to the aerosol was obtained by 15 or less deep inhalations of aerosolized extract from a small hand nebulizer attached to a tube with oxygen flowing at a rate of 6 l./minute. The nebulizer was held in front of the open mouth of the subject during the inhalations. Aerosols of the extracting fluid used in the preparation of the allergenic extracts failed to produce changes in vital capacity. Reductions to as little as 60 per cent of the control values have been produced by the pollen extracts, and in no instance has there been any associated reaction other than cough, a tight feeling in the chest, and symptoms of hay fever. These sensations were, according to statements made by the patients, identical with spontaneously occurring attacks of hay fever or asthma. Physical signs of asthma appeared with the more marked reductions in vital capacity but were never striking with the dosages used.

In those instances in which a reduction in the vital capacity followed inhalation of an aerosolized extract, the maximum decrease usually occurred between 6 and 10 minutes after exposure. In all patients but one the vital capacity returned to the control value within 50 minutes.

We have no reason to doubt the reliability of this method of testing for specific factors in asthma. In the patients studied to date, substances which produced a fall in vital capacity have always been of clinical significance as judged by the patients' histories and skin tests. Nevertheless, sufficient data to determine the value of the test in diagnosis are not available at the present time. Substances which were suspected of being clinically important have not always produced a fall in vital capacity. Furthermore, the method is time consuming, requires the cooperation of the patient, and is hampered at the present time by the lack of any accurate means of measuring the intensity of the exposure to the aerosol. Although cough and severe symptoms of hay fever may follow exposure to an aerosolized extract and may interfere with the measurement of the vital capacity, this has not been frequent in our experience, even when a marked decrease in vital capacity occurred. As such symptoms are clearly allergic in origin, their appearance may be helpful in diagnosis.

In spite of the disadvantages mentioned above the method appears to be very promising for the following reasons: (1) Reductions in vital capacity can be safely induced in the laboratory in asthmatic subjects by substances known to be capable of producing asthmatic attacks; (2) the lung, the chief site of the disease process, serves as the test organ; (3) the effect of various drugs in preventing or modifying changes in vital capacity following exposure to aerosolized extracts can be readily studied; and (4) the method affords an objective test in many instances because a considerable decrease in the vital capacity may occur without awareness on the part of the subject that a reaction to the inhaled extract is taking place.

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