Table 1. Means rate of licking, and range, on first contact with the cup and in the first burst of at least 1-second duration.

Session	Rate on initial contact (licks per second)		Rate in first 1-second burst (licks per second)	
	Mean	Range	Mean	Range
		Group 1		
1	7.3	6.3– 9.5	7.2	5.8–8.3
2	8.4	6.3–11.4	7.5	6.6–9.5
3	8.0	7.0– 9.1	7.4	6.0–8.7
4	7.7	6.9– 8.2	7.2	5.9–8.7
		Group 2		
1	8.3	6.5– 9.5	7.0	6.5–7.3
2	7.5	5.3– 9.5	6.6	5.3–7.3
3	8.1	7.1– 9.5	7.2	6.7–7.7
4	7.8	6.9– 8.6	7.8	6.9–8.6

Keehn and Arnold (*2*) have reported a decrement in mean licking rate within sessions of about 1 lick per second in adult rats. Group means for the first and last bursts of licking of at least 1-second duration in session 1 and for the first and last bursts of licking in the first 5 minutes in sessions 2 and 3 indicate that the decrement in licking rate within sessions found in adults is also found in infant rats, even in their first drinking response. For sessions 1, 2, and 3, respectively, the mean initial and terminal rates for group 1 were 7.2 and 6.1; 7.5 and 6.0; and 7.4 and 6.1. The corresponding rates for group 2 were 7.0 and 6.0; 6.6 and 6.0; and 7.2 and 6.1.

Collier (*4*), in making an analysis of rates of licking within bursts in adult rats, found (i) that the initial rate of responding is frequently as high as 9 licks per second, and (ii) that in sustained bursts of licking, high initial rates quickly decrease to a terminal rate between 6 and a little over 8 licks per second, with the mean licking rate typically falling between these values. An analysis of rates of licking within bursts of over 1-second duration in the young rats indicated that by the third session there were animals in both groups whose initial rates of responding were as high as 9.0 licks per second and whose terminal rates within the same burst were as low as 5.2 licks per second. A typical pattern of response within bursts is illustrated by one animal whose initial rate of responding in the first second was 9.0 and whose rate then dropped, over successive seconds, to 6.7, 6.0, 5.7, 5.5, and finally 5.2 licks per second, with a mean for the burst of 6.6 licks per second. Within bursts, decrements of this magnitude were found only in the beginning of the test sessions, and rates higher than 8 licks per second were never found in the middle or terminal sections of bursts sustained for more than several seconds.

The general stability of licking rates suggests that drinking in the rat is probably reflexive. Whether the licking rate is wholly determined genetically and maturationally or whether some learning is involved is a question that needs further investigation. Even when the rat is raised without an opportunity to drink, nursing and grooming may possibly produce learning effects for licking (*5*).

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5. This research was aided by U.S. Public Health Service grants M-3345 and M-3328. The work was completed while one of us (R.W.S.) was a USPHS predoctoral research fellow.

14 August 1961

Complement Fixation by Antibody Fragments

Abstract. Rabbit antibodies (7*S*) degraded by papain into univalent 3.5*S* fragments fail to fix complement when they combine, but do not precipitate, with the homologous antigens. Divalent 5*S* fragments obtained by pepsin digestion (composed of fragment I linked to II, but lacking fragment III) also fail to fix complement although they precipitate with homologous antigens. The amount of specific precipitate formed by the 5*S* antibody fragment is not increased by exposure to complement.

In 1904 Ehrlich and Morgenroth proposed that antibody combines with complement by means of a specific "complementophilic" haptophore (= combining) group, distinct from the combining group for antigen (*1*). In the ensuing years evidence for the existence of a combining group for complement has not been forthcoming (*2*) and Ehrlich's theory has been largely abandoned (*2, 3*). The present report describes experiments suggesting that group(s) essential for complement fixation exist which are distinct from the antigen-combining sites of the antibody molecule.

Rabbit antibodies against crystalline hen egg albumin, crystalline bovine plasma albumin, and type 6 pneumococcal polysaccharide, all predominantly of the 7*S* variety, were broken down to 3.5*S* fragments with papain, cysteine, and sodium ethylenediaminetetraacetate, according to the method of Porter (*4*). The reaction of these nonprecipitating preparations with the homologous antigens was studied by means of the quantitative complement fixation reaction (*5*) over a wide range of antigen concentration, extending from 1/64 to 1024 times the concentration of antigen giving maximal complement fixation with the same dilution of native antibody. No complement fixation was detected after 18 hours' incubation at 4°C either in 10 ml or 3 ml final volume. The same concentration of native antibody fixed from 20 to 40 of 50 50-percent hemolytic units of guinea pig complement. A representative experiment is illustrated in Fig. 1. Essentially similar results have been obtained by Ovary (*6*).

These experiments can be interpreted in at least two ways, which are not mutually exclusive. The first interpretation is: complement is not fixed because aggregation does not occur with the nonprecipitating, univalent antibody fragments obtained with papain digestion (*4, 7*). The formation of a lattice of aggregating antigen-antibody complexes is widely believed to be essential for complement fixation, because antihapten antibodies do not fix complement when they combine but do not precipitate with univalent haptens (although they both precipitate and fix complement with multivalent haptens) (*8*) and because antigen excess inhibits both the formation of a lattice of aggregating antigen-antibody complexes and complement fixation (*9*).

The second interpretation is: complement fixation does not occur because the antigen combining groups of the antibody molecules have been cleaved from structure(s) essential for complement fixation. Evidence supporting this interpretation, but not contradicting the

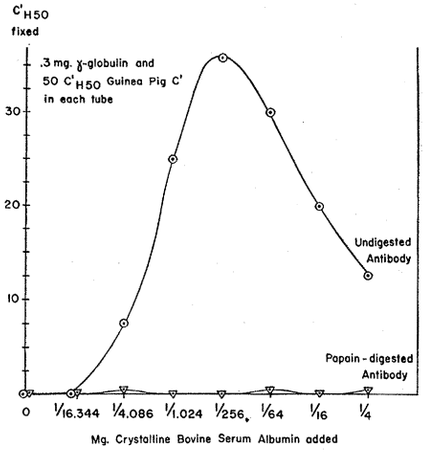

Fig. 1. Quantitative complement fixation at 4°C for 18 hours in 10 ml total volume.

first, was obtained by experiments with 5*S* antibody fragments, which are bivalent and precipitate with the homologous antigens, and comprise fragments I and II, but lack fragment III of Porter. The 5*S* fragments were prepared by means of pepsin digestion, according to the method of Nisonoff (*10*), from the rabbit antibodies previously mentioned.

Quantitative complement fixation tests done over the same wide range of antigen concentration failed to show complement fixation by the 5*S* antibody fragments. No complement fixation was detected even when the concentration of the fragment was increased 5 times over that of native antibody which was able to fix 40 of 50 50-percent hemolytic units. Addition of the chromatographically separated fragment III (*4*) from normal rabbit gamma globulin failed to restore the complement-fixing

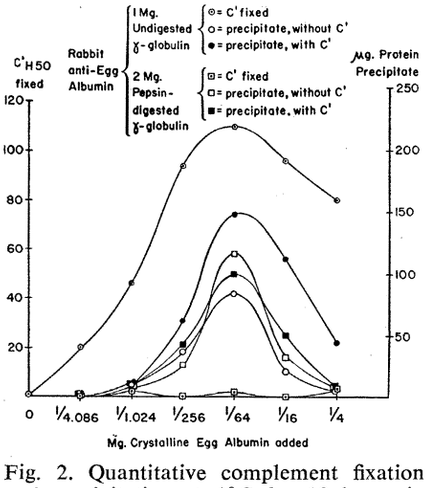

Fig. 2. Quantitative complement fixation and precipitation at 4°C for 18 hours in 3 ml total volume, with and without 200 units of guinea pig complement.

capacity of these 5*S* antibody fragments.

To study more closely the relationship between specific precipitation and complement fixation, the amount of the precipitates and the loss of hemolytic complement in the supernatants were determined in the same test tubes after 18 hours' incubation at 4°C (with 3 ml total volume). Large amounts of complement were used, which allowed determination of complement fixation not only by the decrease of the ability of the supernatants to lyse sensitized red cells, but also by the increase in the amount of precipitates.

A representative experiment is illustrated in Fig. 2. In the absence of complement, the amounts of precipitate obtained with 2 mg of gamma globulin of the 5*S* preparation are greater than those obtained with 1 mg of undigested gamma globulin. However, the undigested antibody fixed up to 110 of 200 50-percent units of complement, but pepsin-digested antibody fragments fixed less than detectable amounts. Moreover, complement consistently increased the specific precipitation of undigested antibody, as expected (*11*), but not the precipitation of the 5*S* fragments. The experiments thus provide two independent indications that the 5*S* fragments do not fix complement, despite specific precipitation with antigen.

It appears, therefore, that the antigen combining fragments I and II, whether separated (as in papain-digested antibody) or united (as in pepsin-digested antibody) are no longer able to fix complement. Whether this results from an alteration in the spatial arrangement of the antigen combining sites of the molecules or from the loss of certain structures in fragment III essential for complement fixation remains to be determined (*12*).

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10 July 1961

International Geophysical Calendar for 1962

Abstract. Coordination of certain types of geophysical observations and analyses throughout the world is accomplished by the advance selection of days and intervals for such work. A committee under the International Council of Scientific Unions has issued the calendar for 1962, together with a brief explanation and examples of how it may be used in planning geophysical programs.

The International Geophysical Calendar 1962 (Fig. 1) (*1*) designates selected days and intervals for special attention for geophysical experiments and analysis during 1962 and is thus a framework for world-wide coordination. It serves mainly the branches of geophysics dealing with the earth's atmosphere in which many phenomena vary significantly during the course of a year. In some experiments, such as the routine recording of variations of the earth's magnetic field, the observational and analysis programs at observatories are normally carried out at a uniform level throughout the year; in these cases the calendar is not needed. However, in many other experiments (for example, rocket experiments), it is not practical or meaningful to carry out the same program on each and every day. Here the calendar can provide a useful mechanism for coordination: experimenters will know that their colleagues in other countries, in other laboratories, and in other disciplines will tend to carry out experiments on the days or intervals marked on the calendar. In this way, results of experiments may later be more easily and usefully compared.

In some scientific fields, international scientific organizations have made specific recommendations for programs to be done on days or intervals marked on