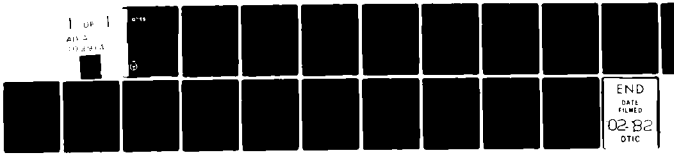
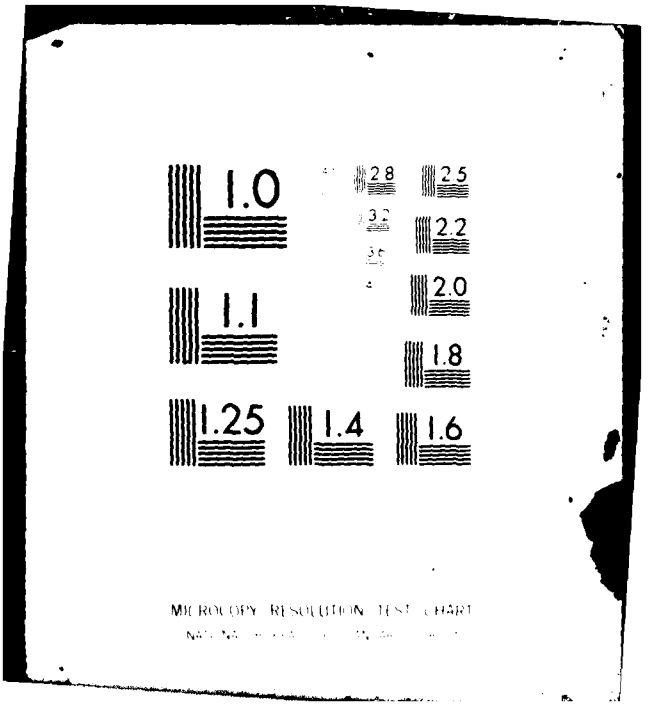
AD-A109 914 DEFENCE RESEARCH ESTABLISHMENT SUFFIELD RALSTON (ALBERTA) F/G 6/5
A TURBIDO-HAEMOLYTIC ASSAY FOR COMPLEMENT,(U)
FEB 81 V L DI NINNO, V K CHENIER
UNCLASSIFIED DRES-MEMO-1009 NL



END
DATE FILMED
02-82
DTIC


1.0
2.8
2.5
3.2
2.2
3.6
4
2.0
1.1
1.8
1.25
1.4
1.6
MICROCOPY RESOLUTION TEST CHART

UNCLASSIFIED

UNLIMITED
DISTRIBUTION

DRES

LEVEL II

(3)

SUFFIELD MEMORANDUM

NO. 1009

A TURBIDO-HAEMOLYTIC ASSAY FOR COMPLEMENT (U)

by

V.L. Di Ninno and V.K. Chenier

PCN 16A10

February 1981

DEFENCE RESEARCH ESTABLISHMENT SUFFIELD : RALSTON : ALBERTA

UNCLASSIFIED

UNLIMITED
DISTRIBUTION

DEFENCE RESEARCH ESTABLISHMENT SUFFIELD
RALSTON ALBERTA

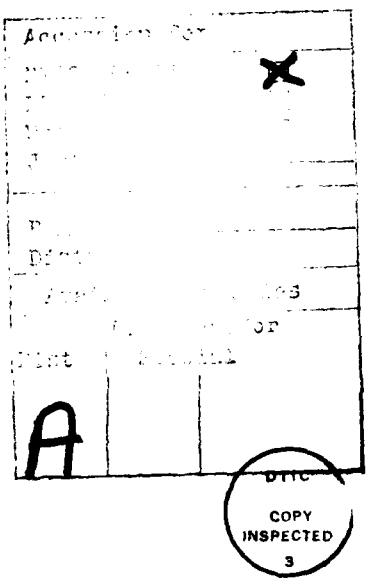
D[illegible] MEMO-1[illegible]

SUFFIELD MEMORANDUM NO. 1009

A TURBIDO-HAEMOLYTIC ASSAY FOR COMPLEMENT (U)

by

V.L. Di Ninno and V.K. Chenier



PCN 16A10

UNCLASSIFIED

403 . 04

UNCLASSIFIED

DEFENCE RESEARCH ESTABLISHMENT SUFFIELD
RALSTON ALBERTA

SUFFIELD MEMORANDUM NO. 1009

A TURBIDO-HAEMOLYTIC ASSAY FOR COMPLEMENT (U)

by

V.L. Di Ninno and V.K. Chenier

ABSTRACT

A method for the haemolytic assay of complement is described. The method is based on the turbidimetric quantitation of sheep red blood cells at a wavelength of 640 nm. This approach allows for the continuous and simultaneous assessment of complement activity and results in a substantial reduction in the time required to perform complement assays as opposed to the conventional haemolytic assays. The turbido-haemolytic assay is used to investigate the anti-complementary activity of κ and λ carrageenans. Lambda carrageenan, the more sulphated polymer, is the more potent inhibitor of complement activation. The application of this technique to the detection and/or identification of microorganisms is presently being investigated.

(U)

UNCLASSIFIED

STATEMENT OF DEFENCE RELEVANCE

Complement fixation has been used extensively, in a clinical diagnostic laboratory setting, to quantitate specific antibodies in sera.

The technique may also be used to quantitate antigen-antibody reactions and, given a specific antibody, may be applied to the identification of infectious agents.

Conventional complement fixation tests may take up to 24 hours to perform and have never been considered as rapid diagnostic techniques. In contrast, the turbido-haemolytic assay described in this report requires only one hour to perform; this includes preparation of reagents, set up and reading results. The instrumentation used in this report allows for the simultaneous assay of five different reactions; there is, however, commercially available instrumentation which could be easily adapted to this assay and which would allow for the simultaneous monitoring of up to 352 reactions.

The turbido-haemolytic technique greatly facilitates the performance of tedious and time-consuming complement fixation tests and brings these tests into the realm of rapid (agent identification) techniques. These are of Defence relevance and interest.

UNCLASSIFIED

DEFENCE RESEARCH ESTABLISHMENT SUFFIELD
RALSTON ALBERTA

SUFFIELD MEMORANDUM NO. 1009

A TURBIDO-HAEMOLYTIC ASSAY FOR COMPLEMENT (U)

by

V.L. Di Ninno and V.K. Chenier

INTRODUCTION

Numerous methods for the titration of haemolytic complement activity have been presented and reviewed (Mayer, 1971). In general, these techniques quantify the dilution of serum (complement) required to lyse a given proportion of sensitized sheep red blood cells (SRBC) under standardized conditions (Mayer, 1971; Williams and Chase, 1977). Haemolysis is quantitated by measuring the amount of hemoglobin released after removal of the unlysed SRBC by centrifugation.

We have identified several areas where the procedure could be significantly improved and in this paper we present a rapid, simple technique for the quantitation of complement.

MATERIALS

Haemolysin was purchased from Flow Laboratories (Mississauga, Ontario), guinea pig complement was purchased from Grand Island Biological Company (Burlington, Ontario, Canada) and Miles Laboratories (Elkhart, Ind., USA). SRBC were obtained from the Institut Armand Frappier (Laval, Quebec) and were used after 1 to 3 weeks aging. Carrageenan, κ and λ fractions, were research preparations supplied by Marine Colloids Inc. (Rockland, Maine, USA).

METHOD AND RESULTS

Instrumentation

A Beckman DU-8 microprocessor-controlled spectrophotometer was used for experimentation and data acquisition. Six samples were monitored continuously and simultaneously throughout the reaction period.

Method

There exists a definite relationship between the number of cells in suspension and the amount of light that passes through it. Turbidity (optical density) has long been exploited to quantitate, very accurately, bacterial cell densities and, likewise, may be used to quantitate red blood cell concentrations. Hirst and Pickels (1942) have described a quantitative spectrophotometric assay for haemagglutination reactions. The turbido-haemolytic assay reported here is based on the turbidimetric quantitation of intact red blood cells during the complement activation process. The assay is conducted at a wavelength of 640 nm. One observes an exponential decrease in turbidity with reaction time since the cells which are lysed no longer contribute to the total light absorption of the red blood cell population (fig. 1). The dynamics of SRBC lysis by activated complement can thus be followed without the necessity of centrifugation as is required for the assay of haemoglobin or Cr^{51} release (Weinrach, 1958; Mayer, 1971; Liske, 1980).

Procedure

The assay consists of mixing 100 µL of a 1% SRBC suspension with 100 µL of a 1/160 dilution of haemolysin (i.e. an excess quantity of haemolysin) and varying amounts of (100 µL to 1 mL) a complement dilution, previously determined to be in the complement-limiting range. The total volume is adjusted to 1.2 mL with Veranol Buffered Saline, pH 7.4 (VBS). All reagents are diluted with VBS and are held and mixed at 0°C (ice bath). The reaction is initiated by heating the cell holder to 37°C.

For the quantitation of haemolysin, complement is used in excess and varying amounts of haemolysin are mixed with 100 µL of a 1% SRBC suspension.

Complement inactivation by carrageenan was quantitated by mixing optimal concentrations of complement and haemolysin with varying amounts of carrageenan and 100 µL of a 1% SRBC suspension. Complement was used at 1/20 dilution (100 µL) and haemolysin at 1/1280 dilution (100 µL). The volume is adjusted to 1.2 mL by the addition of VBS.

In all reactions, a 400 µL aliquot of the 1.2 mL reaction mixture is assayed in Beckman microcuvettes.

RESULTS

Complement Limiting Assays

The relationship between the amount of complement present and the proportion of cells lysed as determined by our technique (fig. 2) follows the typical sigmoidal curve. A more rapid parameter obtained by the turbido-haemolytic assay is the rate of change in O.D. with time. The relationship between the V_{max} expressed as $\Delta A_{640/min}$ and complement concentration is shown in fig. 3 for two complement preparations obtained from different commercial sources. The relationship is seen to follow a sigmoidal curve but is not as steep as the % haemolysis curve in the central region of the curve.

Effect of SRBC Presensitization

Haemolytic assays described to date always utilize presensitized SRBC (Williams and Chase, 1977). This is not the case with the turbido-haemolytic assay described in this paper. Experiments were carried out in parallel with SRBC presensitized with haemolysin in order to determine the effects of SRBC presensitization on the reaction as followed by the turbido-haemolytic assay. As can be seen in fig. 4A and 4B, no significant difference is observed.

Haemolysin-Limiting Assays

The rate of SRBC lysis when increasing amounts of haemolysin are used with complement excess are shown in fig. 5. The relationship between the V_{max} and haemolysin concentration is shown in fig. 6. The total curve is biphasic, the first part showing a linear but steeper slope than the latter part of the haemolysin titration curve (fig. 6 inset).

Effect of Carrageenan on Complement

The turbido-haemolytic assay was used to rapidly evaluate the effects of κ and λ carrageenan on complement activation. The kinetics of complement activation in the presence of varying amounts of κ and λ carrageenans are shown in figs. 7A and 7B, respectively. Lambda carrageenan, the more sulphated polymer, is the more potent inhibitor of complement activation (fig. 8A and 8B).

DISCUSSION

The reaction of haemolysin with SRBC is known to activate the complement cascade. Activation of complement results in the lysis of the red blood cells and subsequent release of haemoglobin into the media. If a spectrophotometer is used to monitor the number of red blood cells in a standardized suspension (turbidimetrically), then one should expect an exponential decrease in turbidity with time. Such a relationship is shown in fig. 1 and can only be obtained if one choses

a wavelength at which haemoglobin, released into the media, does not absorb and interfere with the turbidimetric assay. A wavelength of 640 nm is chosen for this purpose. Setting up the reaction monitoring system as described in this paper serves two functions: a) it allows for the continuous monitoring of the reaction kinetics; and b) it eliminates the need for centrifugation of unlysed SRBC, which is necessary in techniques requiring haemoglobin quantitation, thus resulting in a substantial saving of time.

We have observed the exponential decrease in turbidity even upon the simultaneous mixing of SRBC, haemolysin and complement. This suggests that perhaps presensitization of SRBC was not a necessary step as indicated by the use of sensitized SRBC in haemolytic assays described to date (Williams and Chase, 1977). It can be seen from the results shown in fig. 4 that the same reaction kinetics are obtained whether one uses presensitized SRBC or simply adds an excess amount of haemolysin to the reaction mixture. These results show that, in the turbido-haemolytic assay, one can dispense with SRBC presensitization which again results in a substantial time saving in the assay of complement.

The turbido-haemolytic assay was then used to titre guinea-pig complement. A % haemolysis versus amount of complement curve shows the typical sigmoidal shape. For the complement preparation, obtained from GIBCO, 350 µL of a 1/6400 dilution contained 1 CH_{50} unit (amount of complement required for lyses of 50% of the SRBC in the mixture). This corresponds to a V_{max} of .02 $\Delta A_{640/min}$ (fig. 3). Since a more practical and sensitive part of the the V_{max} curve occurs in the central area, we suggest that when using the V_{max} curve to quantitate complement, that the amount of complement resulting in a V_{max} of 0.1 $\Delta A_{640/min}$ be defined as one unit of complement activity. This value corresponds closely to 1 CH_{100} unit on the % haemolysis curve. The V_{max} curve is more rapidly obtained than the % lysis curve but is less

sensitive than the latter in the central area.

Haemolysin is quantitated by the turbido-haemolytic assay by using an excess of complement in the reaction mixture. The relation between V_{max} and haemolysin concentration consists of a biphasic curve. The first part of the curve is more sensitive to changes in haemolysin concentration than the second part. This is expected as one approaches the saturation of haemolysin receptors (antigenic determinants) on the SRBC.

Davies (1963; 1965) demonstrated that carrageenan could inhibit the activation of complement by inhibiting Cl_q. The carrageenan preparation used by Davies in his experiments was later shown to consist of two distinct structural polymers (McCandless *et al.*, 1973) termed κ and λ carrageenan. We used the turbido-haemolytic assay to rapidly assess the ability (relative potency) of κ and λ carrageenan to inhibit complement activation. The results graphed in figs. 8A and 8B indicate that λ carrageenan, the more sulphated polymer, is more potent than κ carrageenan. Similarly, the turbido-haemolytic assay may be used, with the proper reagents (Mayer, 1971; Williams and Chase, 1977), to measure the haemolytic activity of individual components in isolated form or in serum.

In summary, complement activity is generally measured by assessing the ability of a serum dilution to lyse SRBC sensitized with haemolysin. Haemolytic assays by virtue of the various steps involved are time consuming and tedious. The availability of antibodies to most of the major components of complement has allowed the application of immunochemical techniques to the determination of complement components and these are summarized by Williams and Chase, 1977. These immunochemical assays have received wide usage because of their simplicity. Unfortunately, these assays are independent of and therefore not representative of the biologic function of complement. The haemolytic assays are still the most valuable tools in the evaluation of the complement

system as it represents an assay of complement function and provides an overall measure of the integrity of the classical complement pathway and of the membrane attack mechanism. Of the haemolytic assays, the turbido-haemolytic assay described in these pages, best combines the features of simplicity, speed, sensitivity, convenience and reproducibility.

REFERENCES

Davies, G.E., 1963, Immunology 6, 561.

Davies, G.E., 1965, Immunology 8, 291.

Hirst, G.K. and E.G. Pickels, 1942, J. Immunol. 45, 273.

Liske, R., 1980, J. Immunol. Methods 34, 225.

Mayer, M.M., 1971, in: Experimental Immunochemistry, 2nd ed., eds. E.A. Kabat and M.M. Mayer; Charles C. Thomas publisher (Springfield Illinois) p. 133.

McCandless, E.L., J.S. Craigie and J.A. Walter, 1973, Planta 112, 210.

Weinrach, R.S., M. Lai and D.W. Talmage, 1958, J. Infect. Dis. 102, 60.

Williams, C.A. and M.W. Chase, 1977, in: Methods in Immunology and Immunochemistry, Vol. 4, eds. C.A. Williams and M.W. Chase (Academic Press, N.Y.) p. 133.

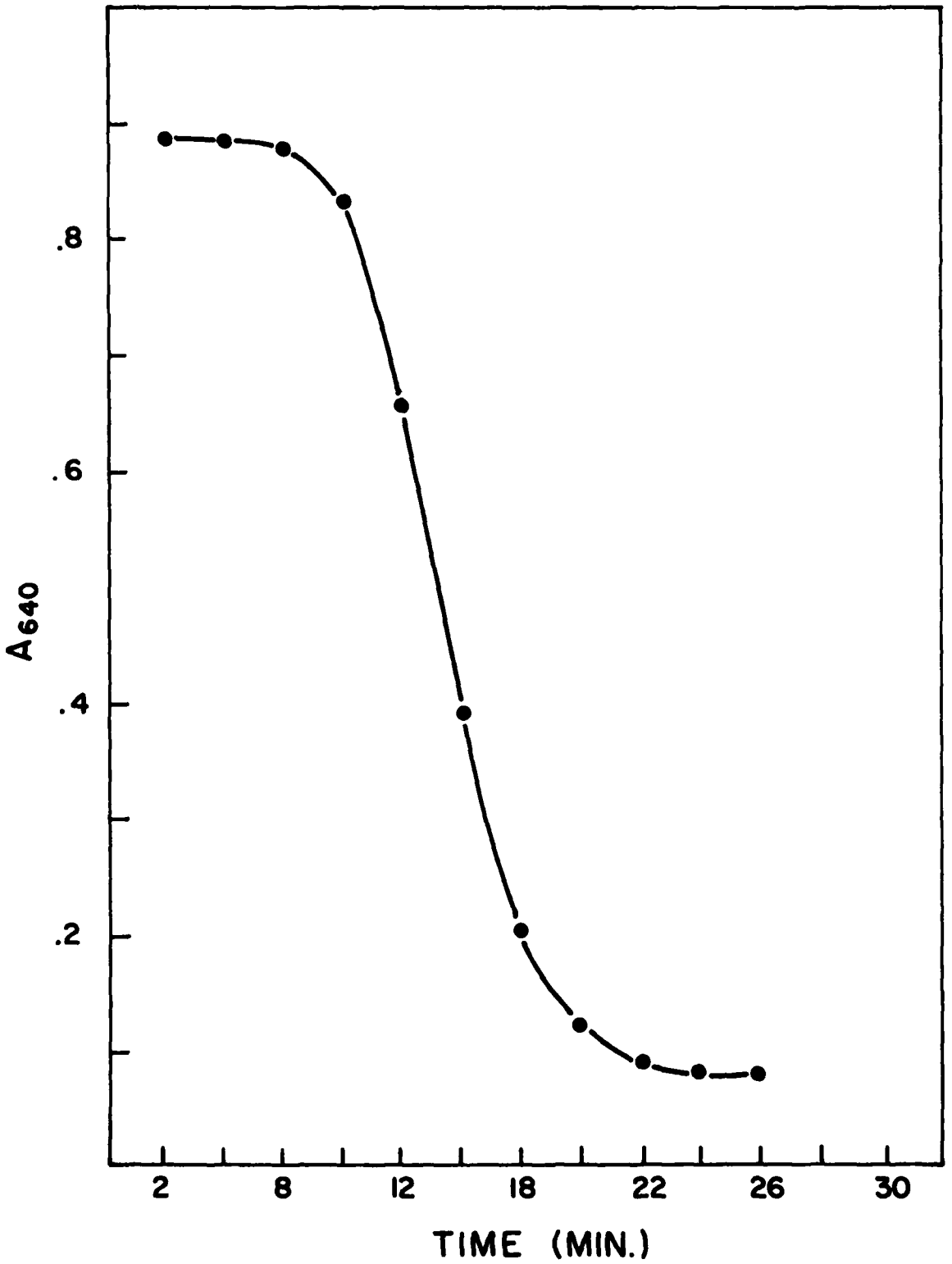


Fig. 1: Lysis of SRBC in the presence of complement and haemolysin as followed by turbidity.

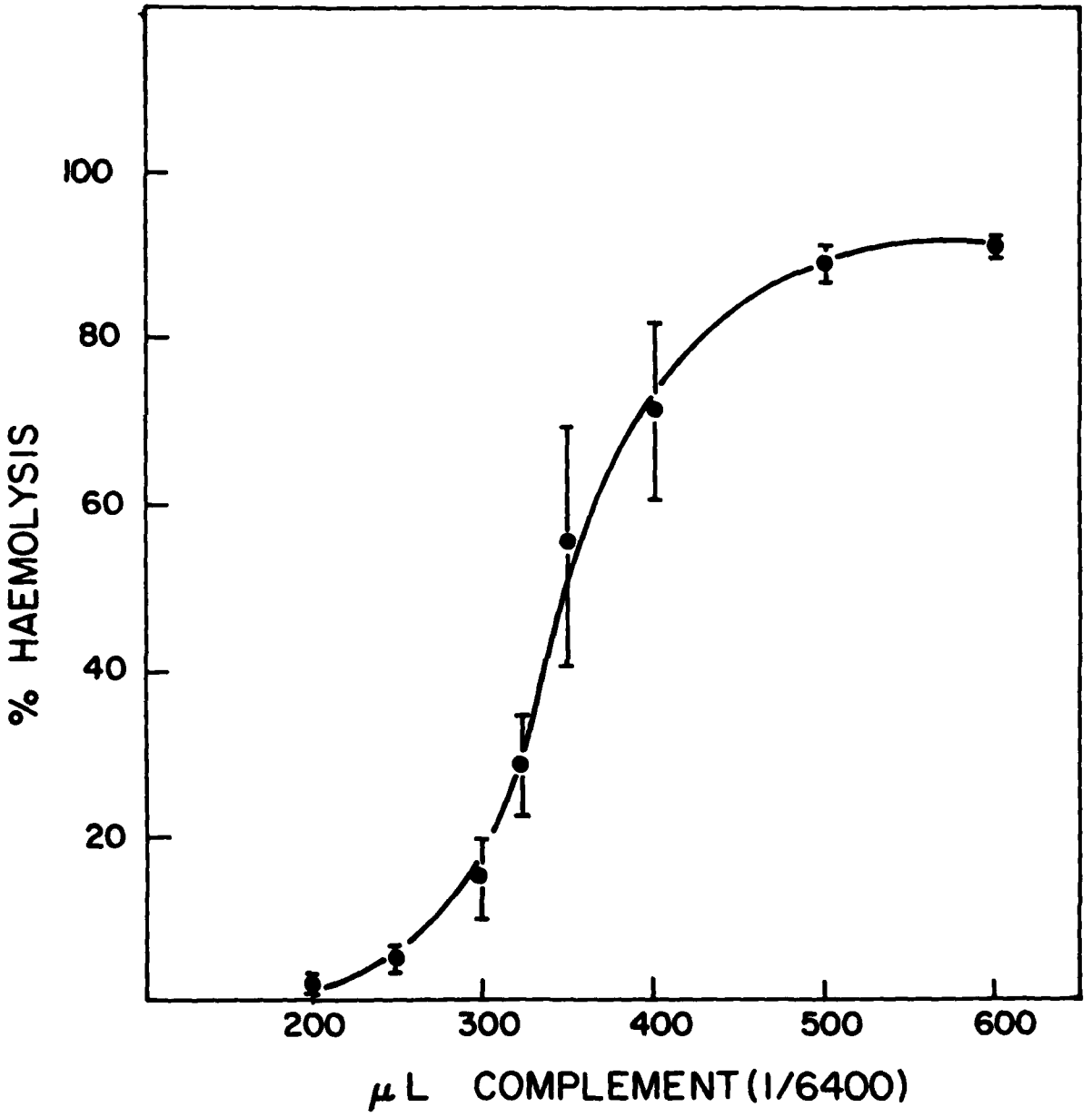


Fig. 2: Percent SRBC lysed by varying amounts of complement in the presences of excess haemolysin. (i.e., error bars represent the S.E. of the mean of 3 independent experiments.)

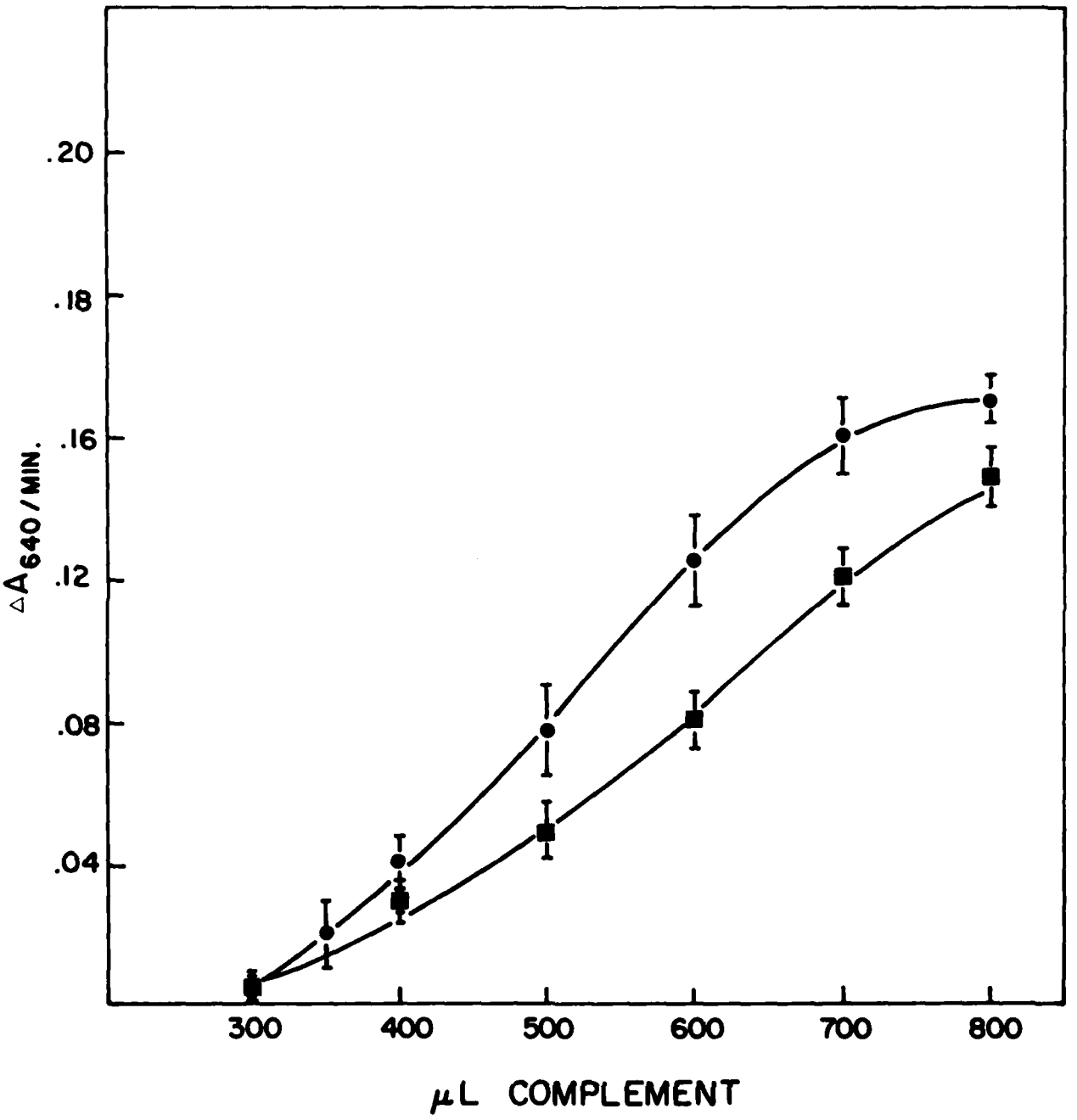


Fig. 3: The effect of varying amounts of GIBCO complement (●—●—●) and Miles complement (■—■—■) on the maximum rate of SRBC lysis.

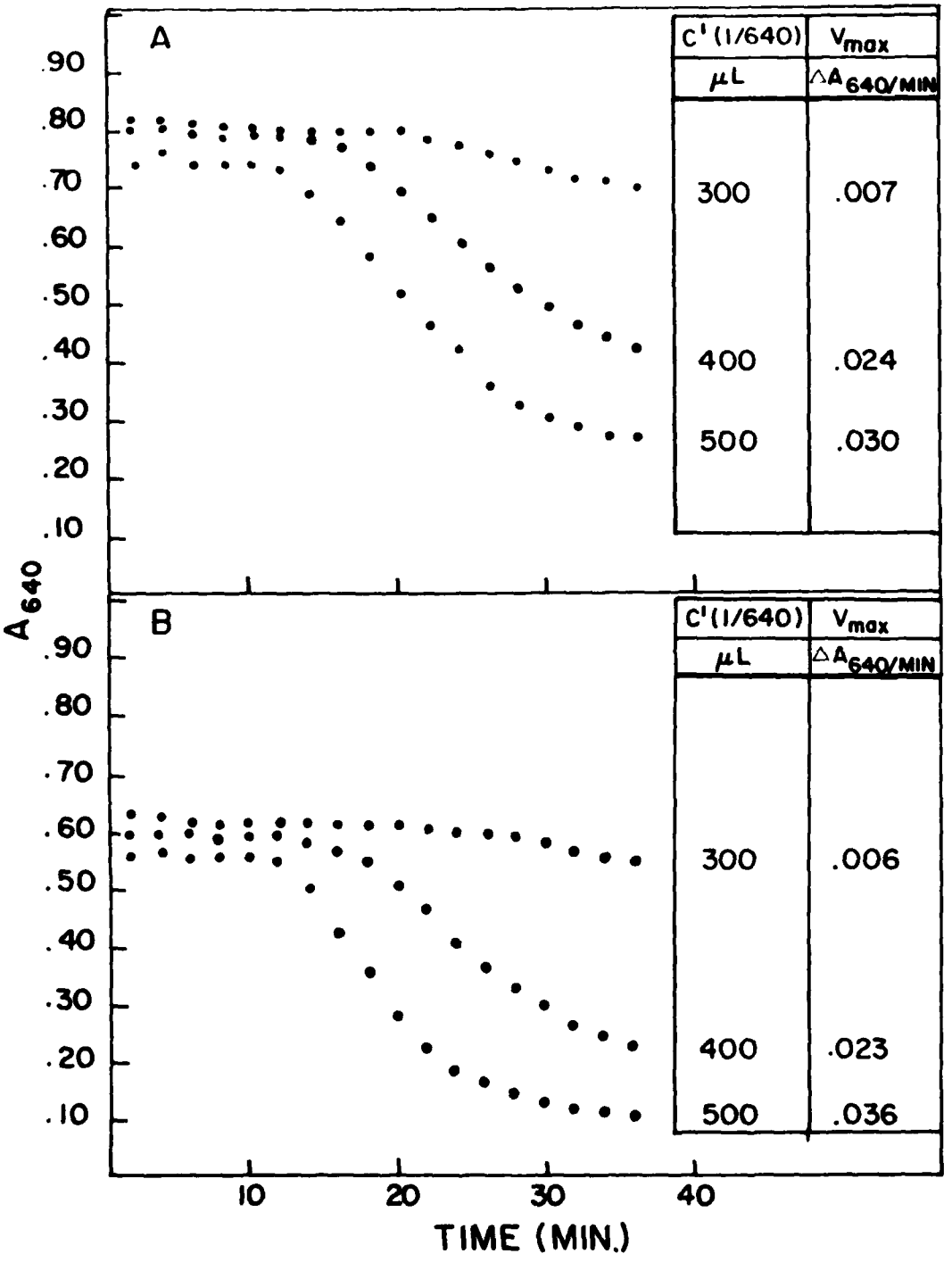


Fig. 4A: Kinetics of SRBC lysis in the presence of excess haemolysin and varying amounts of complement.

Fig. 4B: Kinetics of SRBC lysis utilizing SRBC previously sensitized with haemolysin and varying amounts of complement.

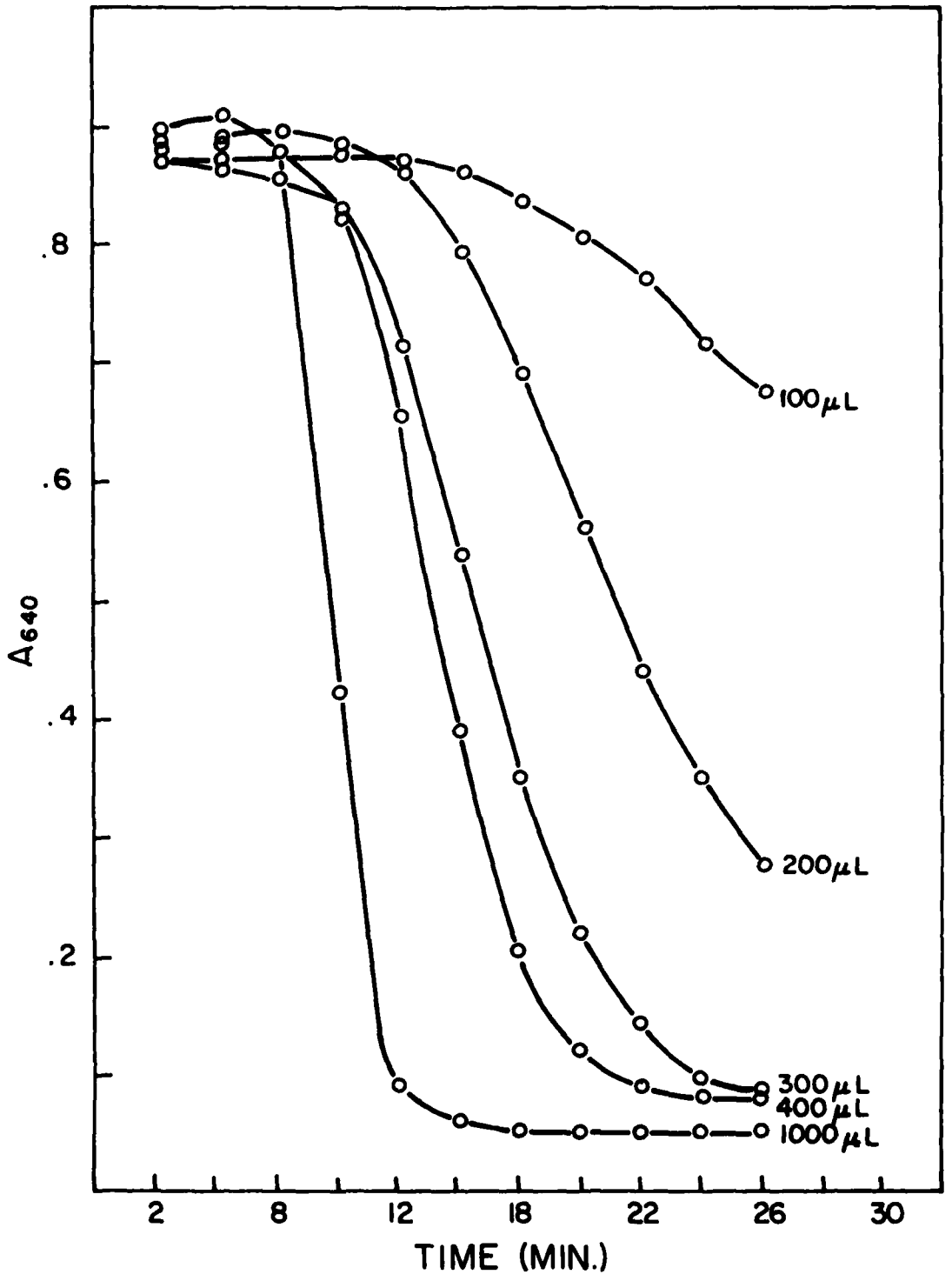


Fig. 5: Lysis of SRBC in the presence of varying amounts of haemolysin and excess complement.

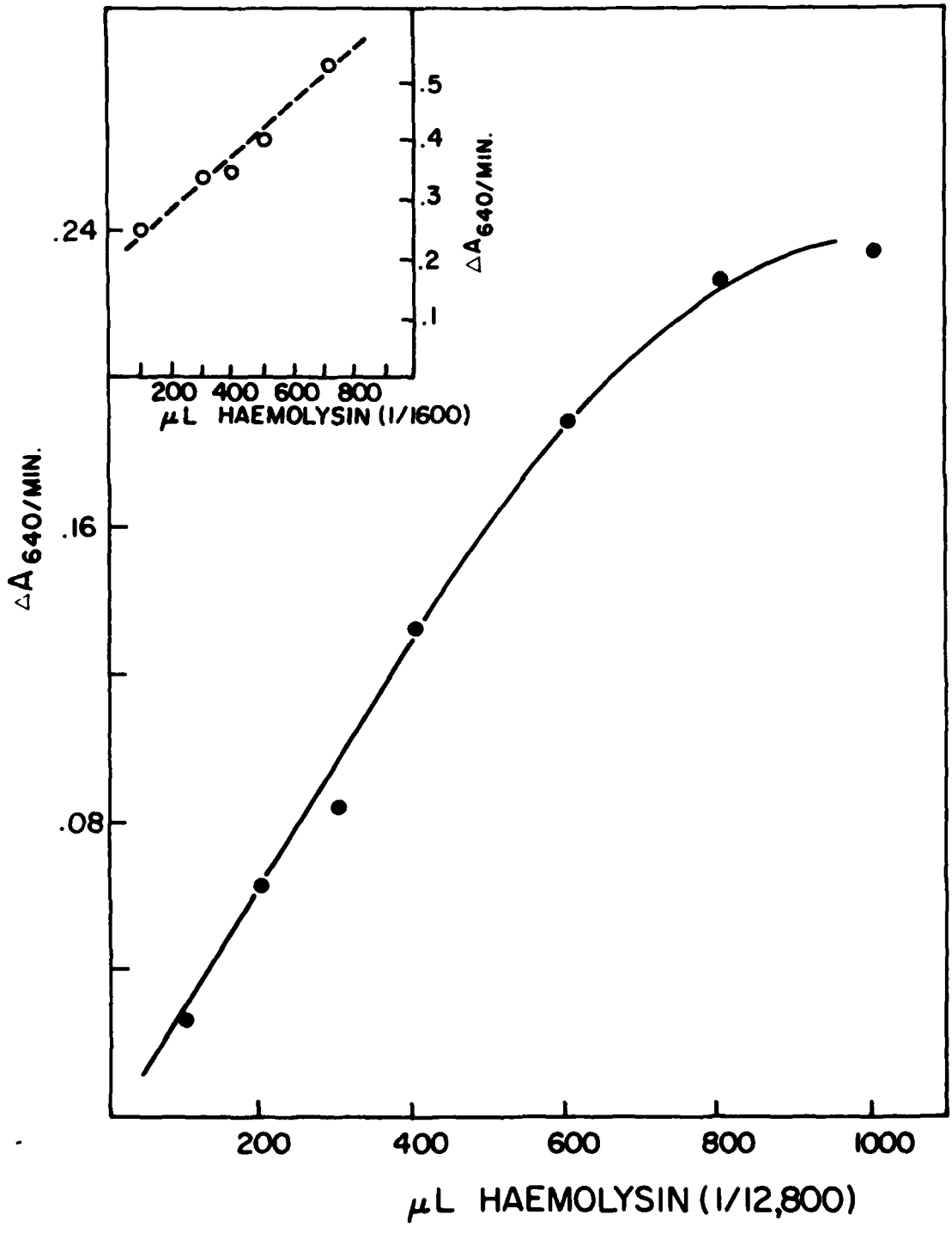


Fig. 6: Effect of varying amounts of haemolysin and excess complement on the maximum rate of SRBC lysis.

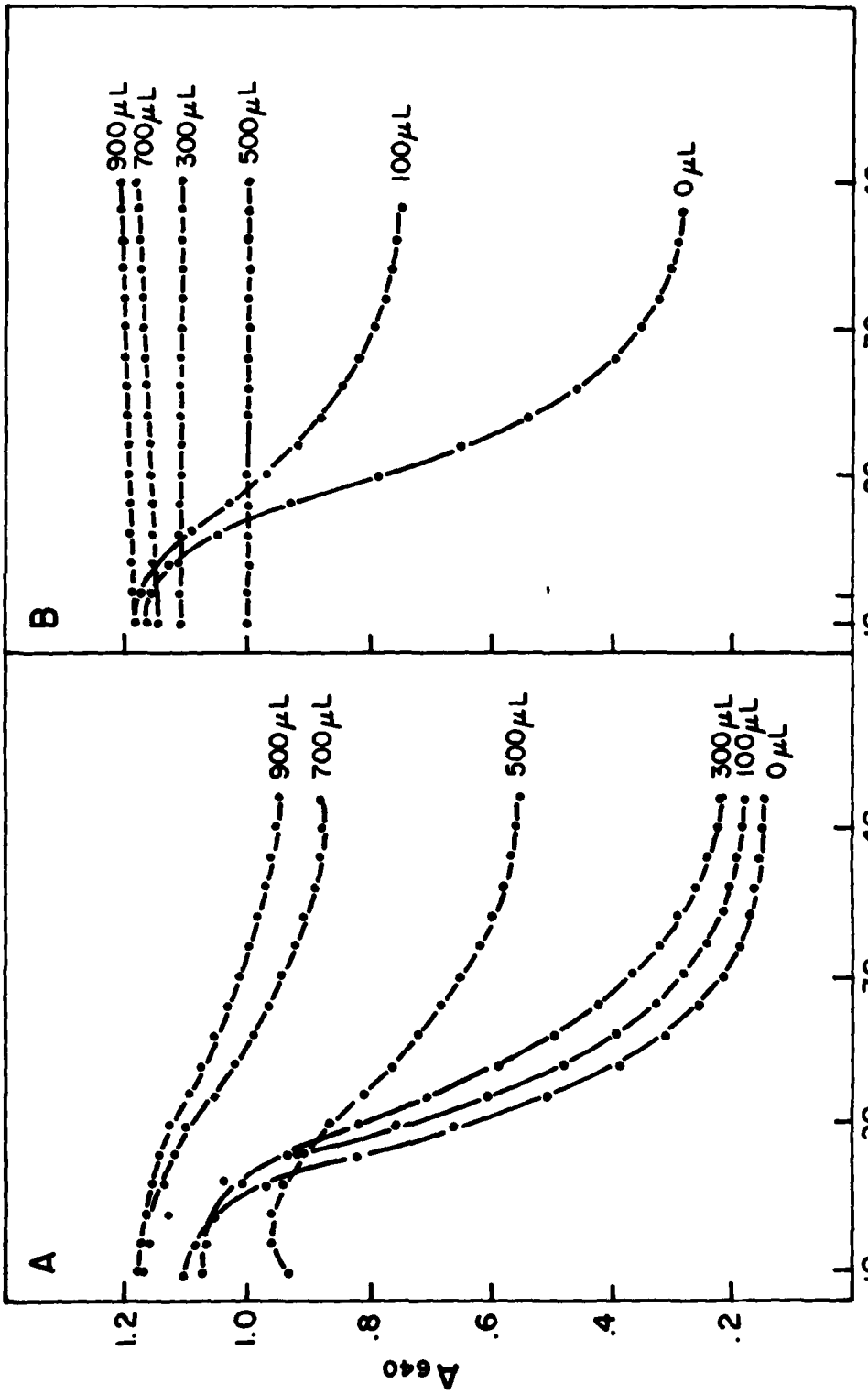


Fig. 7: Dynamics of SRBC lysis in the presence of optimal amounts of haemolysin, complement and varying amounts of:
A. κ carrageenan; B. λ carrageenan.

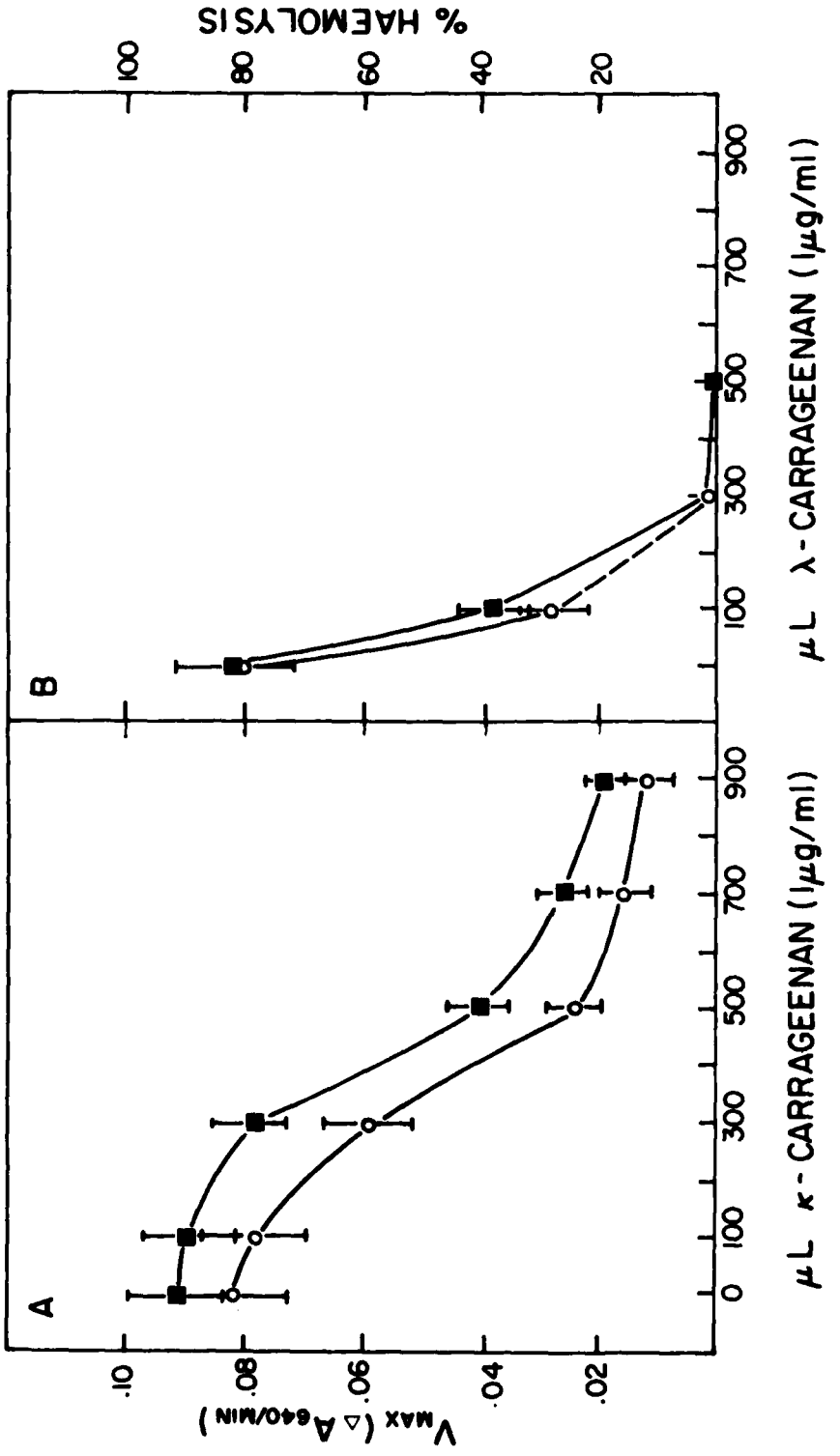


Fig. 8: Effect of A.) κ carrageenan; and B.) λ carrageenan on the proportion of SRBC lysed (■—■) and on the rate of SRBC lysis (○—○) in the presence of optimum concentrations of haemolysin and complement.

UNCLASSIFIED

Security Classification

DOCUMENT CONTROL DATA - R & D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall document is classified)

1. ORIGINATING ACTIVITY DEFENCE RESEARCH ESTABLISHMENT SUFFIELD	2a. DOCUMENT SECURITY CLASSIFICATION UNCLASSIFIED 2b. GROUP

3. DOCUMENT TITLE

A TURBIDO-HAEMOLYTIC ASSAY FOR COMPLEMENT (U)

4. DESCRIPTIVE NOTES (Type of report and inclusive dates) Suffield Memorandum

5. AUTHOR(S) (Last name, first name, middle initial)

Di Ninno, V.L. and Chenier, V.K.

6. DOCUMENT DATE FEBRUARY 1981	7a. TOTAL NO. OF PAGES 18	7b. NO. OF REFS 8
8a. PROJECT OR GRANT NO. PCN 16A10	9a. ORIGINATOR'S DOCUMENT NUMBER(S) SUFFIELD MEMORANDUM NO. 1009	
8b. CONTRACT NO.	9b. OTHER DOCUMENT NO.(S) (Any other numbers that may be assigned this document)	

10. DISTRIBUTION STATEMENT

UNLIMITED DISTRIBUTION

11. SUPPLEMENTARY NOTES	12. SPONSORING ACTIVITY

13. ABSTRACT

A method for the haemolytic assay of complement is described. The method is based on the turbidimetric quantitation of sheep red blood cells at a wavelength of 640 nm. This approach allows for the continuous and simultaneous assessment of complement activity and results in a substantial reduction in the time required to perform complement assays as opposed to the conventional haemolytic assays. The turbido-haemolytic assay is used to investigate the anti-complementary activity of κ and λ carrageenans. Lambda carrageenan, the more sulphated polymer, is the more potent inhibitor of complement activation. The application of this technique to the detection and/or identification of microorganisms is presently being investigated.

(U)

kappa lambda

DSIS
78-078

KEY WORDS

Complement

Haemolysis

Antigen-Antibody Interaction

Anti-Complementary

INSTRUCTIONS

1. ORIGINATING ACTIVITY: Enter the name and address of the organization issuing the document.

2a. DOCUMENT SECURITY CLASSIFICATION: Enter the overall security classification of the document including special warning terms whenever applicable.

2b. GROUP: Enter security reclassification group number. The three groups are defined in Appendix 'M' of the DRB Security Regulations.

3. DOCUMENT TITLE: Enter the complete document title in all capital letters. Titles in all cases should be unclassified. If a sufficiently descriptive title cannot be selected without classification, show title classification with the usual one-capital-letter abbreviation in parentheses immediately following the title.

4 DESCRIPTIVE NOTES: Enter the category of document, e.g. technical report, technical note or technical letter. If appropriate, enter the type of document, e.g. interim, progress, summary, annual or final. Give the inclusive dates when a specific reporting period is covered.

5. AUTHOR(S): Enter the name(s) of author(s) as shown on or in the document. Enter last name, first name, middle initial. If military, show rank. *The name of the principal author is an absolute minimum requirement.*

6. DOCUMENT DATE: *Enter the date (month, year) of* Establishment approval for publication of the document.

7a. TOTAL NUMBER OF PAGES: *The total page count should* follow normal pagination procedures, i.e., enter the number of pages containing information.

7b. NUMBER OF REFERENCES: Enter the total number of references cited in the document.

8a. PROJECT OR GRANT NUMBER: If appropriate, enter the applicable research and development project or grant number under which the document was written.

8b. CONTRACT NUMBER: If appropriate, enter the applicable number under which the document was written.

9a. ORIGINATOR'S DOCUMENT NUMBER(S): Enter the official document number by which the document will be identified and controlled by the originating activity. This number must be unique to this document.

9b. OTHER DOCUMENT NUMBER(S): If the document has been assigned any other document numbers (either by the originator or by the sponsor), also enter this number(s).

10. DISTRIBUTION STATEMENT: Enter any limitations on further dissemination of the document, other than those imposed by security classification, using standard statements such as:

(1) "Qualified requesters may obtain copies of this document from their defence documentation center."

(2) "Announcement and dissemination of this document is not authorized without prior approval from originating activity."

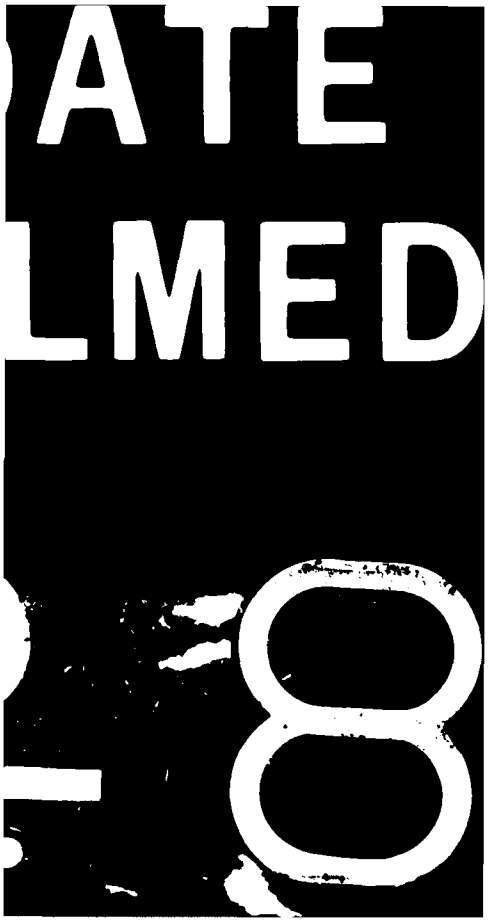
11. SUPPLEMENTARY NOTES: *Use for additional explanatory* notes.

12. SPONSORING ACTIVITY: *Enter the name of the departmental* project office or laboratory sponsoring the research and development. Include address.

13. ABSTRACT: Enter an abstract giving a brief and factual summary of the document, even though it may also appear *elsewhere in the body of the document itself.* It is highly desirable that the abstract of classified documents be unclassified. Each paragraph of the abstract shall end with an indication of the security classification of the information in the paragraph (unless the document itself is unclassified) represented as (TS), (S), (C), (R), or (U).

The length of the abstract should be limited to 20 single-spaced standard typewritten lines; 7½ inches long.

14. KEY WORDS: Key words are technically meaningful terms or short phrases that characterize a document and could be helpful in cataloging the document. Key words should be selected so that no security classification is required. Identifiers, such as equipment model designation, trade name, military project code name, geographic location, may be used as key words but will be followed by an indication of technical context.


ATE
LMED
8