

valuable parts of a research paper, the author's own contributions, would thus fare no better than they do today.

In our present indexing journals, many key words are not indexed at all; a paper's title—and even its summary—often can display only a few of the author's ideas. Excellent thoughts, particularly concerning technique, may lie buried deep within an article, lost to the index-reading "public." It is precisely the inventive, busy author who will neglect to publish a significant idea in the form of a separate paper. A citation index, much as it may be worthwhile, would fail to catch and broadcast such an idea.

My suggestion in regard to literature indexing would be to continue and greatly expand the sort of skilled, discriminating indexing that is found in the Armed Forces Medical Library's *Current List of Medical Literature* and in *Chemical Abstracts*, publications that are excellent despite their limited budgets.

The status of the Armed Forces Medical Library should be changed to that of an independent Federal Medical Information Bureau. *Chemical Abstracts* and similar publications should be supported *in part* by the government. Congress should appropriate a truly adequate sum of money to provide these organizations with highly trained indexing personnel (minimal education: M.S. degree).

An impractical dream? All right; but this sort of action, which would conform to the Hoover Commission's recommendation for greater support of basic medical research (*Philadelphia Inquirer*, 1 July 1955) is just what is needed to begin the attack on our massive problem of scientific communication.

Other subsequent efforts in this direction would include the formation of an International Scientific Journal Union (to supervise prompt publication) and the development of departmentalized scientific newspapers as reported by J. A. Behnke [*Science* **120**, 1055 (1954)].

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If the cost of preparing a citation index were comparable to the cost of conventional indexes, this cost would be justified by virtue of the time and money it could save in research. Fortunately, the cost of citation indexes per entry is extremely low because the bulk of the work can be performed by clerks and machines. *Shepard's Citations* adds more than 1 million citations to its cumulations each year. Even though *Shepard's Citations*, Inc., has a large staff of qualified attorneys, their published volumes are not exorbitantly priced. As Schoen-

bach surely knows, the subscription rates for such indexes as the *Bibliography of Agriculture* and the *Current List of Medical Literature* do not reflect their true publication costs. And the government does, in fact, do what Schoenbach wishes it did—support such activities *in part*. If any additional support is forthcoming, it should be from industry and other nongovernmental index users.

Schoenbach implies that a citation index for science is meant as a substitute for the conventional subject indexes rather than an adjunct. This is by no means true. The lawyer may use a digest—that is, a conventional index—as his starting point. Having located an array of references pertinent to his search, he then goes to *Shepard's Citations* for all subsequent citations to the cases in point.

Schoenbach also implies that the *Current List* and *Chemical Abstracts* do keyword indexing—that is, indexing based on titles. This is also incorrect. Each of these publications indexes articles in great depth. However, the number of indexing entries applied has an economic as well as an *intellectual limit*. In a paper I recently presented before the American Chemical Society, "Breaking the subject-index barrier—A citation index for chemical patents," I discussed this all-important "barrier"—the inability of the indexer, no matter how conscientious, to catch the total import of an author's remarks. Furthermore, the author himself is not always aware of the implications of his own discoveries. It is precisely because, as Schoenbach states, "Excellent thoughts, particularly concerning technique, may lie buried deep within an article, lost to the index-reading 'public'" that a citation index is needed. When the use and construction of the citation index is properly understood, then it will become apparent that it can help to "broadcast" these otherwise buried ideas.

When Schoenbach criticizes the limitations of the proposed citation index, he really criticizes present citation practices. There are numerous instances when an author could provide a citation that would establish the necessary association between his new contribution and what has gone before. If it is completely new and unrelated to anything previously published, then the idea will in most cases be caught by the indexer. If neither the author nor the indexer is aware of its significance, some other author will bring it out through a subsequent citation. Through the citation index, one could then use the antecedent article as a new starting point.

I would wholeheartedly support any move to expand the services of the *Current List* through increased financial support from the government or any other interested parties. Hopefully, its expanded services could include a citation

index. Since the conventional subject index and the citation index complement each other in a synergistic fashion, this would, I think, be a great stride forward for science. However, this important problem is in no way related to the merits of the citation index and should receive a more thorough treatment in the pages of *Science* and elsewhere.

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Bactericidal Reaction of Mouse Serum

The lack of bactericidal effect of mouse serum on some gram-negative organisms *in vitro* has been reported (1). The mouse is unique in this respect since the normal serums of other mammals exert a marked bactericidal effect on gram-negative organisms. This bactericidal effect of normal serums results from the concerted action of normal antibody and C' (2).

Because of the widespread use of the mouse in immunological investigations, it was significant to determine whether the lack of bactericidal action of normal mouse serum results from a lack of normal antibody or bactericidal C' or both. Most studies of the action of C' have used a standard hemolytic system of rabbit antiserum against sheep erythrocytes. Mouse C' is practically lacking in hemolytic activity in the standard system, although some reaction may be elicited under particularly sensitive conditions (3). Guinea pig C' is extremely active in this system. Bovine serum is without activity, but it is among the most potent sources of C' in the bactericidal system against *Brucella* organisms (4) and *Salmonella typhosa* (5). The failure of mouse serum to exert a bactericidal effect could not be attributed arbitrarily, therefore, to a low level of hemolytic C' since no simple association exists between hemolytic and bactericidal C'.

The turbidimetric growth assay technique was used for determining bactericidal reactivity with *S. typhosa* 0901 (5). The assay technique consists of two phases: (i) a reaction period of 60 minutes during which the organisms are exposed to the inhibitory action of antibody and C' in the presence of an optimum concentration of Mg ion (5) that is incorporated in the saline diluent; (ii) the relative numbers of surviving organisms are then estimated by subculture and optical density determinations in a photoelectric colorimeter. Assays of hemolytic C' were performed with the standard hemolytic system (6), C' was fractionated by the dialysis method, C'3 was in-

activated by zymosan (7), and C'4 by ammonium hydroxide (8).

Several pools of fresh mouse serums were tested alone, and in amounts up to 0.5 ml failed to show detectable bactericidal action. Fresh mouse serum was combined with heat-inactivated (56°C for 30 minutes) normal and immune human and rabbit serums, but no bactericidal effect was observed. These results indicate a deficiency of bactericidal C' in mouse serum. The lack of hemolytic C' in mouse serum is thus paralleled by a lack of bactericidal C'.

To determine the particular C' component that is lacking in mouse serum, M (9) and E (10) and C' lacking C'3 and C'4 were prepared from human and guinea pig serum pools. Fresh mouse serum was added to these fractions, and the reinforced serum was tested for its hemolytic and bactericidal activity in the presence of excess antiserum. Mouse serum proved capable of activating guinea pig E and guinea pig C' lacking C'4 to a marked extent in the hemolytic reaction (Table 1). Mouse serum thus appears to be able to furnish components C'1 and C'4. Its inability to activate guinea pig M and guinea pig C' lacking C'3 to any appreciable extent suggests a deficiency of C'2 and C'3. Mouse serum did not activate similar human serum complement fractions. In the bactericidal reaction, mouse serum was able also to activate guinea pig E and guinea pig C' lacking C'4 (Table 2). The combined guinea pig fractions did not function as an effective C', probably because of loss of activity resulting from the fractionation procedure and the relatively large volumes of C' that are required for the bactericidal reaction. Human C' fractions were not tested.

These results suggest a relative deficiency of C'2 and C'3 in mouse serum in both the hemolytic and bactericidal reactions. Hemolytic experiments alone

Table 2. Bactericidal action of mouse serum and guinea pig C' components with excess of inactivated rabbit antiserum against *S. typhosa* 0901. Saline diluent was added to bring all tubes to equal volume. Controls with each fraction, separately, and lacking mouse serum were negative.

Tube	Rabbit anti-serum, 1/400 (ml)	Guinea pig				Mouse serum (ml)	Culture (ml)	Kill (%)
		M (ml)	E (ml)	Serum lacking C'3 (ml)	Serum lacking C'4 (ml)			
1	0.1	0.1	0.1	0	0	0	0.3	0
2	0.1	0	0	0.1	0.1	0	0.3	0
3	0.1	0	0.1	0	0.1	0	0.3	0
4	0.1	0.1	0	0.1	0	0	0.3	0
5	0.1	0.1	0	0	0	0.2	0.3	0
6	0.1	0	0.1	0	0	0.2	0.3	75
7	0.1	0	0	0.1	0	0.2	0.3	0
8	0.1	0	0	0	0.1	0.2	0.3	75
9	0.1	0	0	0	0	0.2	0.3	0
10	0.1	0	0	0	0	0	0.3	0
11	0	0	0	0	0	0	0.3	0

have shown previously that mouse serum is lacking in C'2 (11). Our findings are in agreement, but they also indicate a relative lack of C'3.

The next experiments were performed to determine whether mouse serum possessed normal bactericidal antibody despite its lack of bactericidal C'. C' was prepared by absorbing guinea pig serum with heat-killed organisms to remove normal antibody. The combined effect of this absorbed C' and mouse serum was determined. Definite bactericidal action resulted. The logarithm of the amounts of mouse serum plotted against the probability of the percentage of killed organisms yielded a straight line. This relationship has been observed in titrating other normal serums and antisera. Two pools of unheated mouse serums each required about 0.3 ml to kill 50 percent of a test dose of about 2×10^7 organisms. A three to four-fold loss of activity occurred as a result of heat inactivation (56°C for 30 minutes) of the mouse serum. Such ther-

mal instability is characteristic of normal antibody.

The lack of bactericidal action of mouse serum may be attributed, therefore, to a lack of complementary activity notwithstanding the presence of normal antibody. The complement deficiencies are in C'2 and C'3 components. However, C'1 and C'4 appear to be present and effective in supplementing guinea pig fractions that lack these components in both the hemolytic and bactericidal reactions. Normal bactericidal antibody, as found in other animals, is present in the mouse.

The C' deficiency of mouse serum raises doubts concerning the validity of protection tests in mice for gaging the immunizing action of vaccines of gram-negative organisms. Nevertheless, protection tests giving a positive result are qualitatively valid. The role of serum bactericidal components in immunity to infection with gram-negative organisms is not clear. Whatever part it does play would seem to be inadequately assessed in the mouse.

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Table 1. Hemolytic action of mouse serum and guinea pig C' components on sheep erythrocytes optimally sensitized with rabbit anti-sheep hemolysin. Saline diluent was added to bring all tubes to equal volume. Controls with each fraction, separately, and lacking mouse serum were negative.

Tube	Guinea pig				Mouse serum (ml)	Sensitized rbc (1½%) (ml)	Hemolysis (%)
	M (ml)	E (ml)	Serum lacking C'3 (ml)	Serum lacking C'4 (ml)			
1	0.1	0.1	0	0	0	1.0	80
2	0	0	0.1	0.1	0	1.0	100
3	0	0.1	0	0.1	0	1.0	100
4	0.1	0	0.1	0	0	1.0	90
5	0.1	0	0	0	0.5	1.0	0
6	0	0.1	0	0	0.5	1.0	50
7	0	0	0.1	0	0.5	1.0	15
8	0	0	0	0.1	0.5	1.0	70
9	0	0	0	0	0.5	1.0	0
10	0	0	0	0	0	1.0	0

References and Notes

1. S. Marcus, D. W. Esplin, D. M. Donaldson, *Science* 119, 877 (1954).
2. Complement is generally designated by the symbol C', and its four components are designated by the symbols, C'1, C'2, C'3, and C'4.
3. R. B. McGhee, *Proc. Soc. Exptl. Biol. Med.* 80, 419 (1952).
4. M. R. Irwin and D. T. Berman, in *Brucellosis* (AAAS, Washington, D.C., 1950), p. 86.
5. L. H. Muschel and H. P. Treffers, *J. Immunol.*, in press.
6. L. H. Muschel and K. M. Lowe, *J. Lab. Clin. Med.* 46, 147 (1955).
7. Zymosan was kindly supplied by Fleischmann

Laboratories, Standard Brands, Inc., Stamford, Conn.

8. E. A. Kabat and M. M. Mayer, *Experimental Immunochimistry* (Thomas, Springfield, Ill., 1948), p. 122.
9. M is the symbol for the midpiece C' fraction. It contains C'1, C'3, and a little C'4.
10. E is the symbol for the endpiece C' fraction. It contains C'2, some C'3, and much C'4.
11. G. C. Brown, *J. Immunol.* 46, 319 (1943).

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Detection of Staphylococcus Enterotoxin by Infrared Spectrophotometry

This communication describes briefly the results of an infrared spectrophotometric examination of boiled and lyophilized preparations that were obtained from cultures of enterotoxigenic and non-enterotoxigenic staphylococci (*Micrococcus pyogenes* var. *aureus*) in accordance with the "cold-ethanol" method developed by Thatcher and Matheson (1). The products were known to contain variable amounts of α -, β - and δ -hemolysins and differed widely in enterotoxigenic activity.

Exploratory experiments were carried

out on specimens mounted between two silver chloride disks in a specially constructed demountable microcell. A drop of the aqueous concentrate was placed on one of the disks, a stream of dry nitrogen was passed over it to remove excess solvent, and the specimen was thoroughly dried over phosphorus pentoxide. Then its absorption was measured in a Perkin-Elmer double-beam recording infrared spectrophotometer. Remarkable contour similarity was noted to exist among the various spectral curves that were obtained by this procedure, and it soon became apparent that only a quantitative evaluation of the absorption characteristics of the different preparations might prove to be of diagnostic value.

Accordingly, the specimens were finely powdered in a mechanical grinder until they would pass through a 250-mesh sieve (U.S. Standard Sieve Series No. 230). An accurately weighed portion of 5 mg was then mixed intimately with 995 mg of ACS reagent grade potassium bromide that had been prepared similarly and dried overnight at 125°C. A 200-mg aliquot of the mixture was subjected in a

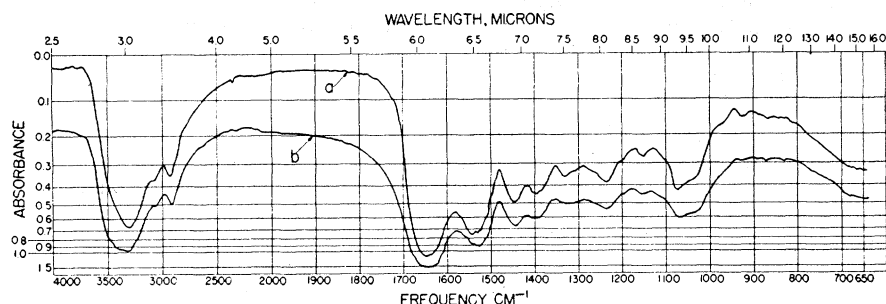


Fig. 1. Infrared absorption curves of 12069 α Dolman preparation measured by the silver chloride technique (a), and the potassium bromide technique (b).

Table 1. Correlation between enterotoxigenicity and infrared absorption of a specific fraction* of the filtrates of seven strains of *M. pyogenes* var. *aureus*.

Strain	Enterotoxigenicity (cat test)			Area under curve from 1100 to 1000 cm^{-1} (cm^2)
	Material injected [†] (mg)	Cats injected (No.)	Cats vomiting in 2 hr (No.)	
L16	2	4	4	15.8
J-32A	2	4	3	18.5
S6	2	4	3	23.7
	5	1	1	
12069 α	2	4	4	23.8
Control media	2-5	4	0	32.4
31	2-5	4	0	30.4
7	2	4	0	30.7
224 \ddagger	2	4	0	33.8
	5	1	0	

* Water-soluble precipitate obtained by the "cold-ethanol" procedure of Thatcher, Matheson, and Simon (1).

[†] All material dissolved in 2 ml of 0.95-percent saline prior to injection.

[‡] This strain is usually weakly enterotoxigenic but was found to contain no enterotoxin in this particular preparation.

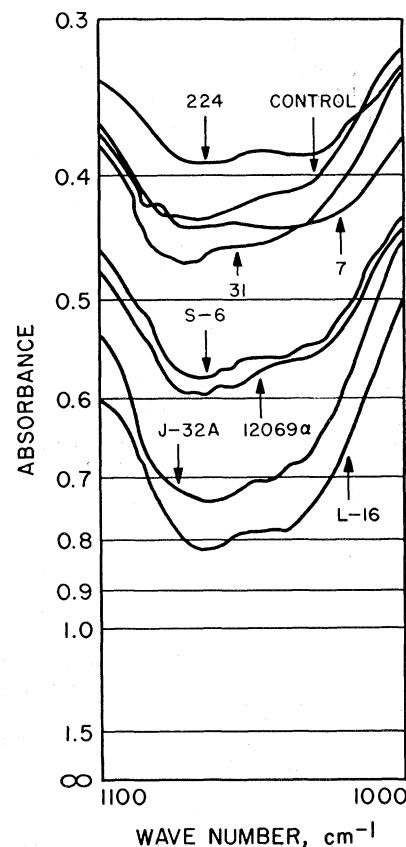


Fig. 2. Infrared absorption of seven strains of *M. pyogenes* var. *aureus* throughout the 1100-1000 cm^{-1} region.

vacuum for about 5 minutes to a pressure of 10,000 lb/in.² The absorbancy of the clear disk thus produced was measured over the frequency range extending from 4000 to 650 cm^{-1} . A potassium bromide disk prepared under comparable conditions was placed in the path of the reference beam to compensate for absorption by the reagent. After each experiment, the disks were weighed accurately in order to determine the amount of specimen per sample. The variations between different disks never exceeded 1 percent.

Figure 1 shows two spectral curves obtained on one of the preparations (12069 α Dolman) using both the silver chloride and potassium bromide techniques. Strong N—H and characteristic C—H stretching vibrations are observed at 3400 cm^{-1} and 2900 cm^{-1} , respectively. The marked absorptions noted at 1650 and 1540 cm^{-1} are indicative of the presence of polypeptide bonds, while the characteristic band occurring at 1065 cm^{-1} may be considered to be associated with ester linkages such as —C—O or C—O—P that are found in phospholipids.

Because all samples were treated identically by the pressed potassium bromide technique, their absorbancies could be compared by accurately measuring cor-