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Current Comments

Most-Cited Articles of the 1960s. 3. Preclinical Basic Research

Number 5 February 4, 1980

During the past year, we published lists of the most-cited articles of the 1960s in the fields of biochemistry and molecular biology, and the physical sciences.[1,2] We now continue our study of the 1960s scientific literature by examining the most-cited papers in the basic biomedical sciences.

We extracted these lists from the *Science Citation Index®* *(SCI®)* data base covering the years 1961 to 1978. Again, I must emphasize that these studies do not necessarily identify the most "important" papers of the 1960s. The authors of many of these papers would not consider these their most important contributions. But I should add that those papers considered important by authors are usually also well-cited. In any case, to prevent invidious comparisons between articles dealing with very different subject matter, the papers are not ranked by citation frequency.

In spite of the above qualifications, I am sure you will agree that many papers on this list (Fig. 1, which follows this editorial) did indeed have a significant impact upon their respective fields. Moreover, we can say with reasonable confidence that the articles appearing here point to areas of significant interest to researchers in the 1960s and into the 1970s.

Each article was cited at least 521 times between the years 1961 and 1978. Forty-one were cited more than 1,000 times. Fifteen were cited more than 1,500 times. The average paper on this list received 1,271 citations. If we discount the 11,691 citations received by E.S. Reynolds' 1963 paper, the most-cited on this list, then the average drops to 1,157 citations. How difficult is it for a paper to get on this list? Consider that at least two million biomedical papers were published during the 60s.

The 100 articles on this list appeared in 51 journals shown in Table 1. Thirteen were published in the *Journal of Cell Biology*. The *Journal of Biological Chemistry* accounted for eight, the *Journal of Pharmacology and Experimental Therapeutics* contributed five, and *Pharmacological Reviews* published four articles on the list.

All but one of the papers were written in English. The paper by H. Druckrey was written in German and concerns the carcinogenic action of 65 nitrous compounds. It is the most-cited of the five cancer research papers listed.

Twenty-one papers were single-author works. Forty-nine papers had two, 18 had three, and eight had four authors. One paper had five authors, another had six. Two papers had 10 authors.

Sixteen authors have more than one of their papers on the list. E.W. Sutherland authored eight of the papers listed here, R.W. Butcher five. M.J. Karnovsky and L. Hayflick each contributed four, and M.G. Farquhar, D.H. Hubel, G.A. Robison, and T.N. Wiesel each contributed three. Two papers each were contributed by A.H. Conney, A. Dahlström, K. Fuxe, F.C. Greenwood, W.M. Hunter, G. Millonig, G.E. Palade, and T.W. Rall. A total of 204 authors appear on the list.

Sixty-four institutions are represented in this study. They are shown in Table 2,

Table 1: Journals that published the most-cited 1960s articles listed in Figure 1, ranked by the number of articles from each.

	No. of Articles
J. Cell Biol.	13
J. Biol. Chem.	8
J. Pharmacol. Exp. Ther.	5
Pharmacol. Rev.	4
J. Biophys. Biochem. Cy.	3
J. Exp. Med.	3
J. Histochem. Cytochem.	3
J. Immunol.	3
J. Physiol.-London	3
Proc. Nat. Acad. Sci. US	3
Proc. Soc. Exp. Biol. Med.	3
Acta Physiol. Scand.	3
Exp. Cell Res.	2
Immunochemistry	2
J. Ultrastruct. Res.	2
Lancet	2
Nature	2
Science	2
Stain Technol.	2
Adv. Immunol.	1
Am. J. Physiol.	1
Ann. NY Acad. Sci.	1
Annu. Rev. Biochem.	1
Annu. Rev. Physiol.	1
Arch. Biochem. Biophys.	1
Aust. J. Exp. Biol. Med.	1
Bacteriol. Rev.	1
Biochem. J.	1
Biochem. Pharmacol.	1
Biochem.-USA	1
Brain Res.	1
Cancer Res.	1
Circulation	1
Cold Spring Harbor Symp.	1
Diabetes	1
Gut	1
Immunology	1
Int. Arch. Allergy Appl. Immunol.	1
J. Anat.	1
J. Appl. Physiol.	1
J. Gen. Microbiol.	1
J. Lipid Res.	1
J. Neurochem.	1
J. Neurophysiol.	1
Physiol. Plant.	1
Physiol. Rev.	1
Prog. Biophys. Mol. Biol.	1
Radiat. Res.	1
Tex. Rep. Biol. Med.	1
Virology	1
Z. Krebsforsch. Klin. O.	1

along with the number of authors who appear on our list of 100 papers. The number of papers from each institution is also indicated. Eight of these institutions account for more than half of the 204 authors listed. Thirty-five of the 64 institutions are in the US. Eight institutions are in the UK. Five are in Sweden. Belgium, Canada, and Switzerland each have three institutions on the list. Japan has two. Australia, Denmark, the Federal Republic of Germany, France, and Norway each have one institution.

Three Nobel laureates account for 11 papers—E.W. Sutherland, C. de Duve and G.E. Palade. Eighteen papers on the list have been featured as *Citation Classics* in *Current Contents*®.[3]

We have divided our list of most-cited papers into 11 subject areas: immunology, cell biology, electron microscopy, pharmacology, neurosciences, cancer research, endocrinology, cyclic AMP, tissue culture, and hematology. Papers that did not easily fall into any of these categories were placed under the heading miscellaneous. Articles are alphabetized by first author under each subject area. Since there are only 100 papers we could have created an alphabetic list, but the categorical arrangement seemed more useful. We are aware that many of the biochemically oriented papers could easily have been included in the earlier list of biochemistry papers.[2] There is no one perfect classification for multidisciplinary work.

Fifteen papers on the list were categorized under immunology. Several papers in this group describe techniques for detecting and counting antigens and antibodies.

Cell biology contributed nine papers including R.C. Graham and M.J. Karnovsky's method for studying the absorption of protein in kidney tubules. Several other papers in this group discuss the permeability of various kinds of biological surfaces.

Fifteen electron microscopy papers appear on the list. Four of them deal with lead as a stain for use with electron microscopes. The most-cited paper on the list, by E.S. Reynolds, proposed the use of lead citrate as a stable and reliable stain. Reynolds' paper received 11,691 citations during the 18-year period covered in this study. This makes it the second most-cited paper in the history of science. The paper by J.H. Luft is the seventh most-cited. The

Table 2: Institutional affiliations of authors of the most-cited 1960s articles, ranked by the number of papers. The number of authors from each institution is indicated in parentheses.

National Institutes of Health			13	(24)
National Heart Institute		(9)		
National Cancer Institute		(5)		
National Institute of Arthritis, Metabolism & Digestive Diseases		(2)		
National Institute of Mental Health		(2)		
Lab. Neuropathol. & Neuroanat.		(2)		
Department unspecified		(2)		
National Institute of Allergy & Infectious Diseases		(1)		
National Institute of Neurological Diseases & Blindness		(1)		
Harvard University			10	(15)
Biol. Lab.		(2)		
Medical School		(13)		
Dept. Anatomy	(2)			
Dept. Pathology	(5)			
Dept. Pharmacology	(4)			
Dept. Physiology	(2)			
Rockefeller University, New York, NY			8	(13)
University of California			6	(12)
Berkeley		(4)		
Davis		(1)		
San Francisco Med. Ctr.		(7)		
Vanderbilt University, Nashville, TN			5	(15)
Wistar Institute, Philadelphia, PA			4	(5)
Case Western Reserve, Cleveland, OH			3	(7)
University of Washington, Seattle, WA			3	(7)
Karolinska Institute, Stockholm, Sweden			3	(6)
Washington University School Med., St. Louis, MO			3	(9)
Imperial Cancer Research Fund, London, UK			2	(4)
Massachusetts Institute of Technology, Cambridge, MA			2	(3)
McGill University, Montreal, Canada			2	(3)
Scripps Clinic & Research Foundation, LaJolla, CA			2	(4)
University of Cambridge, Cambridge, UK			2	(6)
University of Göteburg, Sweden			2	(6)
Walter & Eliza Hall Inst. Med. Res., Australia			2	(4)
Max Planck Institut für Immunobiologie, Freiberg-Zahringer, FRG			1	(10)
University of Geneva, Switzerland			1	(6)
University of Oslo, Norway			1	(5)
California Institute of Technology, Pasadena, CA			1	(4)
New York University Sch. of Med., New York, NY			1	(4)
Clin. University St. Pierre, Louvain, Belgium			1	(3)
Duke University, Durham, NC			1	(3)
Merck Institute for Therapeutic Res., West Point, PA			1	(3)
Charles Pfizer & Co., Inc., Groton, CT			1	(2)
Emory University, Atlanta, GA			1	(2)
Institut de Recherches Scientifiques sur le Cancer, Villejuif, France			1	(2)
MRC, Inst. Virology, Glasgow University, Scotland, UK			1	(2)
Swiss Federal Institute of Technology, Zurich, Switzerland			1	(2)
Tufts University Sch. of Med., Boston, MA			1	(2)
University of Chicago, Chicago, IL			1	(2)
University of Florida, Gainesville, FL			1	(2)
University of Liverpool, UK			1	(2)
University of Lund, Sweden			1	(2)
University of Michigan, Ann Arbor, MI			1	(2)
University of Minnesota, Minneapolis, MN			1	(2)
University of Pittsburgh Med. Sch., Pittsburgh, PA			1	(2)
Yale University Sch. of Med., New Haven, CT			1	(2)
University of Stockholm, Wenner-Gren Inst., Sweden			1	(2)
University of Texas, Southwestern Med. Sch., Dallas, TX			1	(2)
University of Tokyo, Japan			1	(2)
University of Wisconsin, Madison, WI			1	(2)
Agricultural Research Council, Cambridge, UK			1	(1)
Burroughs Wellcome & Co., Inc., Wellcome Res. Labs., Tuckahoe, NY			1	(1)
Chester Beatty Res. Inst. Cancer Res., Royal Cancer Hosp., London, UK			1	(1)
Cornell University Med. College, New York, NY			1	(1)
Facultés Universitaires, Notre Dame de la Paix, Namur, Belgium			1	(1)

Fysilogisk Inst., Aarhus Univ., Aarhus, Denmark	1	(1)
George Washington University Sch. of Med., Washington, DC	1	(1)
Inst. Histol. & Embryol. Sch. of Med., Geneva, Switzerland	1	(1)*
Karolinska Hospital, Stockholm, Sweden	1	(1)
Keio University Sch. of Med., Tokyo, Japan	1	(1)
New York Hospital, New York, NY	1	(1)*
New York University Res. Serv., Goldwater Mem. Hosp., Roosevelt Island, NY	1	(1)
Oklahoma State University, Stillwater, OK	1	(1)
Ontario Cancer Institute, Toronto, Canada	1	(1)
Radiochemical Center, Bucks., UK	1	(1)
Upjohn Company, Kalamazoo, MI	1	(1)
University College of London, UK	1	(1)
University of Louvain, Louvain, Belgium	1	(1)
University of Pennsylvania Sch. of Med., Philadelphia, PA	1	(1)
University of Texas, Austin, TX	1	(1)
University of Toronto, Canada	1	(1)

*Second affiliation for a single authored paper.

paper by J.H. Venable and R. Coggeshall described a simplified method for preparing what had come to be known as "Reynolds' stain."

There are 10 pharmacology papers on the list. Most-cited among them is A.H. Conney's review of enzyme inducers, molecules that stimulate the production of enzymes.

Eleven papers on the list are from the neurosciences, including three by D.H. Hubel and T.N. Wiesel. Hubel and Wiesel have done extensive research on the physiological basis of vision in cats and monkeys. Their three papers which appear here are related to this work.

Ten endocrinology papers appear here. F.C. Greenwood and W.M. Hunter were authors of two papers that dealt with the radioactive labeling of human growth hormone. Two other papers dealt with diabetes.

Nine papers fall under the heading of cyclic AMP. Cyclic AMP regulates metabolic action within a cell when its level is changed by a hormone. E.W. Sutherland authored eight of the papers in this group. He won the Nobel prize in 1971 for his discovery of cyclic AMP as a mediator in hormone action. G.A. Robison won the 1979 James Murray Luck Award, sponsored by ISI® and *Annual Reviews*, for his numerous reviews on the subject.[4]

Among the six tissue culture papers are two by L. Hayflick which examine the aging process at the cellular level. Hayflick also coauthored another paper in this group which proposed a method for cultivating the Eaton agent, a microorganism implicated in pneumonia.

The two hematology papers presented here deal with different topics. J.T. Dodge's paper describes a technique for isolating the membranes of red blood cells for study. The paper by A. Gaarder and colleagues discusses blood clotting.

Eight papers fall under the miscellaneous heading. They range in subject matter from the sensitivity of bone marrow to radiation (J.E. Till & E.A. McCulloch) to E.H. Sonnenblick's study of heart muscle.

We limited our list to 100 papers only because of time, space, and energy requirements. We realize that even a list of several hundred papers would not include every "breakthrough" article published in the 1960s. There were 8,145 papers that were cited over 100 times during this period. This indicates how many separate, individual endeavors contribute to advances in science. And as we have seen in examining lifetime citation rates, even fewer citations represent a respectable impact.[5]

In part four of this series, we will report on the 100 most-cited clinical research papers of the 1960s.

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3. ----------. Introducing *Citation Classics:* the human side of scientific reports. *Current Contents* (1):5-6, 3 January 1977.
4. ----------. The NAS James Murray Luck award for excellence in scientific reviewing: G. Alan Robison received the first award for his work on cyclic AMP. *Current Contents* (18):5-9, 30 April 1979.
5. ----------. Lifetime citation rates. *Current Contents* (2):5-8, 14 January 1979.

Figure 1: 100 most-cited articles of the 1960s in preclinical basic research. Authors' affiliations follow each citation. If an article has appeared as a *Citation Classic*, a reference follows the author affiliation.

Total Citations 1961-1978	Bibliographic Data

IMMUNOLOGY

984 **Avrameas S & Ternynck T.** The cross-linking of proteins with glutaraldehyde and its use for the preparation of immunoadsorbents. *Immunochemistry* 6:53-66, 1969. Lab. de Chim. des Protéines, Institut de Recherches Scientifiques sur le Cancer, Villejuif, France

674 **Cohn Z A & Hirsch J G.** The isolation and properties of the specific cytoplasmic granules of rabbit polymorphonuclear leucocytes. *J. Exp. Med.* 112:983-1004, 1960. Rockefeller Inst., New York, NY

762 **Cunningham A J & Szenberg A.** Further improvements in the plaque technique for detecting single antibody-forming cells. *Immunology* 14:599-600, 1968. Walter & Eliza Hall Inst. Med. Res., Melbourne, Australia

648 **David J R, Al-Askari S, Lawrence H S & Thomas L.** Delayed hypersensitivity *in vitro*. 1. The specificity of inhibition of cell migration by antigens. *J. Immunol.* 93:264-82, 1964. NY Univ. Sch. Med., Dept. Med., New York, NY

554 **Eisen H N & Siskind C W.** Variations in affinities of antibodies during the immune response. *Biochemistry* 3:996-1008, 1964. Washington Univ. Sch. Med., Dept. Microbiol., St. Louis, MO

1226 **Fahey J L & McKelvey E M.** Quantitative determination of serum immunoglobulins in antibody-agar plates. *J. Immunol.* 94:84-90, 1965. NIH, NCI, Immunol. & Med. Br., Metab. Serv., Bethesda, MD

1450 **Jerne N K & Nordin A A.** Plaque formation in agar by single antibody-producing cells. *Science* 140:405, 1963. Univ. Pittsburgh, Med. Sch., Dept. Microbiol., Pittsburgh, PA

655 **Levy H B & Sober H A.** A simple chromatographic method for preparation of gamma globulin. *Proc. Soc. Exp. Biol. Med.* 103:250-2, 1960. NIH, NIAID & NCI, Bethesda, MD

4441 **Mancini G, Carbonara A O & Heremans J F.** Immunochemical quantitation of antigens by single radial immunodiffusion. *Immunochemistry* 2:235-54, 1965. Clin. Univ. St. Pierre, Res. Dept. Internal Pathology, Louvain, Belgium

1145 **McConahey P J & Dixon F J.** A method of trace iodination of proteins for immunologic studies. *Int. Arch. Allergy Appl. Immunol.* 29:185-9, 1966. Dept. Exp. Pathol., Scripps Clinic & Res. Fdn., LaJolla, CA

752 **Miller J F A P.** Immunological function of thymus. *Lancet* 2:748-9, 1961. Chester Beatty Res. Inst. Cancer Res., Roy. Cancer Hosp., London, UK [Citation Classics. *Current Contents* (24):11, 12 June 1978.]

1126 **Mishell R I & Dutton R W.** Immunization of dissociated spleen cell cultures from normal mice. *J. Exp. Med.* 126:423-42, 1967. Scripps Clinic & Res. Found., Dept. Exp. Pathol., LaJolla, CA

636 **Perlmann P & Holm G.** Cytotoxic effects of lymphoid cells *in vitro*. *Adv. Immunol.* 11:117-93, 1969. Univ. Stockholm, Wenner-Gren Inst., Dept. Immunol., Stockholm, Sweden

1653 **Sever J L.** Application of a microtechnique to viral serological investigations. *J. Immunol.* 88:320-9, 1962. NIH, NINDB, Perinatal Res. Branch, Bethesda, MD

522 **Watanabe T.** Infective heredity of multiple drug resistance in bacteria. *Bacteriol. Rev.* 27:87-115, 1963. Keio Univ. Sch. Med., Dept. Bacteriol., Tokyo, Japan

CELL BIOLOGY

535 **Borisy G G & Taylor E W.** The mechanism of action of colchicine: binding of colchicine-^{3}H to cellular protein. *J. Cell Biol.* 34:525-33, 1967. Univ. Chicago, Dept. Biophysics, Chicago, IL

1184 **de Duve C & Wattiaux R.** Functions of lysosomes. *Annu. Rev. Physiol.* 28:435-92, 1966. Rockefeller Univ., New York, NY & Univ. Louvain, Louvain, Facultés Universitaires, Notre Dame de la Paix, Namur, Belgium

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1315 **Farquhar M G & Palade G E.** Junctional complexes in various epithelia. *J. Cell Biol.* 17:375-412, 1963. Rockefeller Inst., New York, NY

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ELECTRON MICROSCOPY

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2421 **Karnovsky M J.** A formaldehyde-glutaraldehyde fixative of high osmolality for use in electron microscopy. *J. Cell Biol.* 27:137A-8A, 1965. Harvard Med. Sch., Dept. Pathol., Boston, MA

1439 **Karnovsky M J.** Simple methods for "staining with lead" at high pH in electron microscopy. *J. Biophys. Biochem. Cytol.* 11:729-32, 1961. Harvard Med. Sch., Dept. Pathol., Boston, MA & Inst. Histol. Embryol., Sch. Med., Geneva, Switzerland

663 **Kopriwa B M & LeBlond C P.** Improvements in the coating technique of radioautography. *J. Histochem. Cytochem.* 10:269-84, 1962. McGill Univ., Dept. Anatomy, Montreal, Canada

8345 **Luft J H.** Improvements in epoxy resin embedding methods. *J. Biophys. Biochem. Cytol.* 9:409-14, 1961. Univ. Washington, Dept. Anat., Seattle, WA
[Citation Classics. *Current Contents* (20):8, 16 May 1977.]

1706 **Millonig G.** Advantages of a phosphate buffer for OsO_4 solutions in fixation. *J. Appl. Physiol.* 32:1637, 1961. Rockefeller Inst., New York, NY

1202 **Millonig G.** A modified procedure for lead staining of thin sections. *J. Biophys. Biochem. Cytol.* 11:736-9, 1961. Rockefeller Inst., New York, NY

1130 **Mollenhauer H H.** Plastic embedding mixtures for use in electron microscopy. *Stain Technol.* 39:111-4, 1964. Univ. Texas, Electron Microscope Lab., Austin, TX

890 **Moor H & Muhlethaler K.** Fine structure in frozen-etched yeast cells. *J. Cell Biol.* 17:609-28, 1963. Swiss Federal Inst. Technol., Dept. Gen. Botany, Lab. Electron Microscopy, Zurich, Switzerland

11691 **Reynolds E S.** The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *J. Cell Biol.* 17:208-13, 1963. Harvard Med. Sch., Dept. Anat., Boston, MA

1377 **Richardson K C, Jarett L & Finke E H.** Embedding in epoxy resins for ultrathin sectioning in electron microscopy. *Stain Technol.* 35:313-23, 1960. Washington Univ. Sch. Med., Dept. Anat., St. Louis, MO

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