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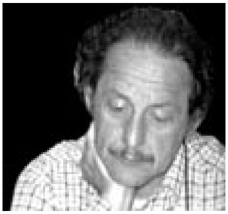
CURRENT COMMENTS



Highly Cited Articles. 39. Biochemistry Papers Published in the 1950s.

Number 25, June 20, 1977

We recently published a list of the 1950s citation classics in the physical and chemical sciences.[1] Here is the list of biochemical articles. The biological, medical, and behavioral classics will follow shortly.

Each article was cited at least 500 times in the 15 year period from 1961 to 1975. Taking the entire universe of published articles into account, only about one in 20,000--or 0.005%--receives so many citations.

In February we published a list of highly cited biochemical articles published in the 1940s.[2] That list of 89 items, like the 1930s life sciences list,[3] which was composed primarily of biochemical articles, reflected one of the basic concerns of biochemistry--the analysis of phosphorus and its physiologic compounds. Some of the 78 highly-cited biochemical articles of the 1950s, listed in Figure 1, continue to reflect the importance of research into phosphorus determination. Phosphorus is basic to the study of DNA, sugars, lipids, and other biochemical substances.

Of the 78 articles in Figure 1, 25 have received over 1,000 citations, and 13 over 2,000. The majority of these papers concern methodology. This should come as no surprise to biochemists themselves, many of whom acknowledge that methods are the backbone of all scientific research. David Gillespie of the National Cancer Institute discussed the process by which a method paper becomes a "classic."[4] He commented that "the distinction between a classic and a quickly outmoded method lies in the ability of the investigators to see the uses to which the method will be put and evaluate particular parameters accordingly and, as importantly, to take heed of the little irregularities that lead to significant improvements."

The importance of methods papers is also emphasized by Karl Piez, Chief of the Laboratory of Biochemistry of the National Institute of Dental Research. His highly-cited 1960 paper[5] modified the methodological procedure of the 1950s paper of Spackman, Stein, and Moore (66). Piez commented that their method was "a highly developed method and one of the most important procedures in modern biochemistry...."[6] Still, Piez was compelled, for the purposes of his own biochemical investigations, to modify the procedure. This illustrates the nature of biochemical advances. Both papers have now achieved very

Figure 1. Highly cited articles in biochemistry published in the 1950s. A = item number. **B** = total citations 1961-1975. **C** = average yearly citations 1961-1975. **D** = citations in 1974. **E** = citations in 1975. Articles are listed alphabetically by first author.

A	B	C	D	E	Bibliographic Data
1.	2791	186	294	281	**Bartlett G R.** Phosphorus assay in column chromatography. *J. Biol. Chem.* **234**:466-68, 1959.
2.	686	46	30	25	**Bennett H S & Luft J H.** Collidine as a basis for buffering fixatives. *J. Biophys. Biochem. Cytol.* **6**:113-17, 1959.
3.	779	52	52	44	**Bertler A, Carlsson A & Rosengren E.** A method for the fluorometric determination of adrenaline and noradrenaline in tissues. *Acta Physiol. Scand.* **44**:273-92, 1958.
4.	1830	122	253	264	**Bligh E G & Dyer W J.** A rapid method of total lipid extraction and purification. *Canad. J. Biochem. Physiol.* **37**:911-17, 1959.
5.	1123	75	42	61	**Boas N F.** Method for the determination of hexosamines in tissues. *J. Biol. Chem.* **204**:553-63, 1953.
6.	506	34	35	28	**Bodanszky M & duVigneaud V.** A method of synthesis of long peptide chains using a synthesis of oxytocin as an example. *J. Amer. Chem. Soc.* **81**:5688-91, 1959.
7.	1203	80	69	73	**Boyer P D.** Spectrophotometric study of the reaction of protein sulfhydryl groups with organic mercurials. *J. Amer. Chem. Soc.* **76**:4331-37, 1954.
8.	918	61	28	24	**Brown J B.** A chemical method for the determination of oestriol, oestrone and oestradiol in human urine. *Biochem. J.* **60**:185-93, 1955.
9.	5037	336	541	506	**Burton K.** A study of the conditions and mechanism of the diphenylamine reaction for the colormetric estimation of deoxyribonucleic acid. *Biochem. J.* **62**:315-23, 1956.
10.	934	62	22	21	**Bush I E.** Methods of paper chromatography of steroids applicable to the study of steroids in mammalian blood and tissues. *Biochem. J.* **50**:370-78, 1952.
11.	564	38	32	16	**Cahn R S, Ingold C K & Prelog V.** A specification of asymmetric configuration in organic chemistry. *Experientia* **12**:81-124, 1956.
12.	506	34	34	26	**Carlsson A & Waldeck S.** A fluorometric method for the determination of dopamine (3-hydroxytyramine). *Acta Physiol. Scand.* **44**:293-98, 1958.
13.	1109	74	34	25	**Caulfield J B.** Effects of varying the vehicle for OsO_4 in tissue fixation. *J. Biophys. Biochem. Cytol.* **3**:827-29, 1957.
14.	550	37	31	53	**Ceriotti G.** A microchemical determination of desoxyribonucleic acid. *J. Biol. Chem.* **198**:297-303, 1952.
15.	721	48	42	47	**Ceriotti G.** Determination of nucleic acids in animal tissues. *J. Biol. Chem.* **214**:59-70, 1955.

Figure 1 continued

16.	1984	132	225	225	**Chen P S Jr., Toribara T Y & Warner H.** Microdetermination of phosphorus. *Analyt. Chem.* 28:1756-58, 1956.
17.	1097	73	115	128	**Dixon M.** The determination of enzyme inhibitor constants. *Biochemical J.* 55:170-71, 1953.
18.	2705	180	133	94	**Dole V P.** A relation between non-esterified fatty acids in plasma and the metabolism of glucose. *J. Clin. Invest.* 35:150-54, 1956.
19.	2732	182	367	334	**Dubois M, Gilles K A, Hamilton J K, Rebers P A & Smith F.** Colorimetric method for determination of sugars and related substances. *Analyt. Chem.* 28:350-56, 1956.
20.	2255	150	217	147	**Eagle H.** Amino acid metabolism in mammalian cell cultures. *Science* 130:432-37, 1959.
21.	2216	148	237	262	**Ellman G L.** Tissue sulfhydryl groups. *Arch. Biochem. Biophys.* 82: 70-77, 1959.
22.	557	37	24	28	**Folch J, Ascolli I, Lees M, Meath J A & LeBaron F N.** Preparation of lipide extracts from brain tissue. *J. Biol. Chem.* 191:833, 1951.
23.	7454	497	776	706	**Folch J, Lees M & Sloane-Stanley G H.** A simple method for the isolation and purification of total lipides from animal tissues. *J. Biol. Chem.* 226:497-509, 1957.
24.	612	41	67	43	**Gianetto R & deDuve C.** Tissue fractionation studies. IV. Comparative study of the binding of acid phosphatase, beta-glucorinidase and cathepsin by rat-liver particles. *Biochem. J.* 59:433-38, 1955.
25.	611	41	42	43	**Glock G E & McLean P.** Further studies on the properties and assay of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase of rat liver. *Biochem. J.* 55:400-08, 1953.
26.	1193	80	65	83	**Hirs C H W.** The oxidation of ribonuclease with performic acid. *J. Biol. Chem.* 219:611-21, 1956.
27.	619	41	26	20	**Hirsch J & Anrens E J, Jr.** The separation of complex lipide mixtures by the use of silicic acid chromatography. *J. Biol. Chem.* 233:311-20, 1958.
28.	597	40	46	49	**Hohorst H J, Kreutz F H & Bucher T.** Metabolitgehalte und Metabolitkonzentrationen in der Leber der Ratte (Metabolite content and concentration in rat liver). *Biochem. Zschr.* 332:18-46, 1959.
29.	580	39	22	14	**Hough L, Jones J K N & Wadman W H.** Quantitative analysis of mixtures of sugars by the method of partition chromatography. V. Improved methods for the separation and detection of the sugars and their methylated derivatives on the paper chromatogram. *J. Chem. Soc.* p. 1702-6, 1950.
30.	639	43	27	20	**Huckabee W E.** Relationships of pyruvate and lactate during anaerobic metabolism. I. Effects of infusion of pyruvate or glucose and of hyperventilation. *J. Clin. Invest.* 37:244-54, 1958.

Figure 1 continued

31.	768	51	57	60	**Huggett A S G & Nixon D A.** Use of glucose oxidase, peroxidase and o-dianisidine in determination of blood and urinary glucose. *Lancet* 2:368-70, 1957.
32.	767	51	27	20	**Hurlbert R E, Schmitz H, Brumm A & Patter V R.** Nucleotide metabolism. II. Chromatographic separation of acid-soluble nucleotides. *J. Biol. Chem.* **209**:23-39, 1954.
33.	619	41	29	33	**Katz A M, Dreyer W J & Anfinsen C B.** Peptide separation by two-dimensional chromatography and electrophoresis. *J. Biol. Chem.* **234**:2897-2900, 1959.
34.	896	60	92	90	**Kauzmann W.** Some factors in the interpretation of protein denaturation. *Adv. Protein Chem.* 14:1-63, 1959.
35.	766	51	30	29	**Kay E R M, Simmons N S & Dounce A L.** An improved preparation of sodium desoxyribonucleate. *J. Amer. Chem. Soc.* **74**:1724-26, 1952.
36.	776	52	52	57	**Kellenberger E, Ryter A & Sechaud J.** Electron microscope study of DNA-containing plasms. II. Vegetative and mature phase DNA as compared with normal bacterial nucleoids in different physiological states. *J. Biophys. Biochem. Cytol.* 4:671-78, 1958.
37.	672	45	20	20	**Kirby K S.** A new method for the isolation of ribonucleic acids from mammalian tissues. *Biochem. J.* **64**: 405-8, 1956
38.	805	54	102	136	**Layne E.** Spectrophotometric and turbidimetric methods for measuring proteins. I. Turbidimetric methods. *Methods Enzym.* 3:447-49, 1957.
39.	50016	3334	7075	6842	**Lowry O H, Rosebrough N J, Farr A L & Randall R J.** Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**:256-65, 1951.
40.	712	47	24	27	**Markert C L & Mollner F.** Multiple forms of enzymes; tissue, ontogenetic, and species specific patterns. *P. Nat. Acad. Sci. USA* **45**:753-63, 1959.
41.	585	39	21	14	**Markham R & Smith J D.** The structure of nucleic acids. I. Cyclic nucleotides produced by ribonuclease and by alkaline hydrolysis. *Biochem. J.* **52**:552-57, 1952.
42.	540	36	11	10	**Marshall J D, Eveland W C & Smith C W.** Superiority of fluorescein isothiocyanate (Riggs) for fluorescent-antibody technic with a modification of its application. *P. Soc. Exp. Biol. Med.* **98**:898-900, 1958.
43.	513	34	62	67	**McFarlane A S.** Efficient trace-labelling of proteins with iodine. *Nature* **182**:53, 1958.
44.	579	39	21	21	**Meselson M, Stahl F W & Vinograd J.** Equilibrium sedimentation of macromolecules in density gradients. *P. Nat. Acad. Sci. USA* **43**:581-83, 1957.
45.	519	35	91	69	**Miller G L.** Protein determination for large numbers of samples. *Analyt. Chemistry* **31**:964, 1959.
46.	665	44	20	16	**Moffitt W & Yang J.** The optical rotary dispersion of simple polypeptides. *P. Nat. Acad. Sci. USA* **42**:596-602, 1956.

Figure 1 continued

47.	1368	91	74	57	**Moore S & Stein W H.** A modified ninhydrin reagent for the photometric determination of amino acids and related compounds. *J. Biol. Chem.* 211:907-13, 1954.
48.	1475	98	55	65	**Moore S, Spackman D H & Stein W H.** Chromatography of amino acids on sulfonated polystyrene resins. *Analyt. Chem.* 30:1185-90, 1958.
49.	1041	69	61	47	**Nachlas M M, Tsou K, DeSousa E, Cheng C & Seligman A M.** Cytochemical demonstration of succinic dehydrogenase by the use of a new p-nitropheneyl substituted diterzole. *J. Histochem. Cytochem.* 5:420-36, 1957.
50.	706	47	124	106	**Nash T.** The colorimetric estimation of formaldehyde by means of the Hantzch reaction. *Biochem. J.* 55:416-21, 1953.
51.	570	38	16	16	**Neuman R E & Logan M A.** The determination of hydroxyproline. *J. Biol. Chem.* 184:229-306, 1950.
52.	664	44	26	16	**Ogur M & Rosen G.** The nucleic acids of plant tissues. I. The extraction and estimation of desoxypentose nucleic acid and pentose nucleic acid. *Arch. Biochem. Biophys* 25:262-76, 1950.
53.	941	63	25	14	**Peterson E A & Sober H A.** Chromatography of proteins. I. Cellulose ion-exchange adsorbents. *J. Amer. Chem. Soc.* 78:751-55, 1956.
54.	564	38	12	12	**Peterson R E, Karrer A & Guerra S L.** Evaluation of Silber-Porter procedure for determination of plasma hydrocortisone. *Analyt. Chem.* 29:144-49, 1957.
55.	1242	83	85	82	**Porter R R.** The hydrolysis of rabbit gamma-globulin and antibodies with crystalline papain. *Biochem. J.* 73:119-26, 1959.
56.	728	49	52	61	**Reissig J L, Strominger J L & Leloir L F.** A modified colorimetric method for the estimation of N-acetylamino sugars. *J. Biol. Chem.* 217:959-66, 1955.
57.	604	40	36	36	**Rondle C J M & Morgan W T J.** The determination of glucosamine and galactosamine. *Biochem. J.* 61:586-89, 1955.
58.	845	56	52	41	**Rosen H.** A modified ninhydrin colorimetric analysis for amino acids. *Arch. Biochem. Biophys.* 67:10-15, 1957.
59.	694	46	59	63	**Schwert G W & Takenaka Y.** A spectrophotometric determination of trypsin and chymotrypsin. *Biochim. Biophys. Acta.* 16:570-75, 1955.
60.	733	49	41	51	**Seifter S, Dayton S, Novic B & Muntwyler E.** The estimation of glycogen with the anthrone reagent. *Arch. Biochem. Biophys.* 25:191-200, 1950.
61.	647	43	38	27	**Sheehan J C & Hess G P.** A new method for forming peptide bonds. *J. Amer. Chem. Soc.* 77:1067-68, 1955.
62.	586	39	52	37	**Simon E J & Shemin D.** The preparation of S-succinyl coenzyme A. *J. Amer. Chem. Soc.* 75:2520, 1953.

Figure 1 continued

63.	752	50	38	31	**Skou J C.** The influence of some cations on an adenosine triphosphatase from peripheral nerves. *Biochim. Biophys. Acta* **23**:394-401, 1957.
64.	2041	136	49	56	**Smithies O.** Zone electrophoresis in starch gels; group variations in the serum proteins of normal human adults. *Biochem. J.* **61**:629-41, 1955.
65.	1439	96	99	81	**Somogyi M.** Notes on sugar determination. *J. Biol. Chem.* **195**:19-23, 1952.
66.	5727	382	450	438	**Spackman D H, Stein W H & Moore S.** Automatic recording apparatus for use in the chromatography of amino acids. *Analyt. Chem.* **30**:1190-1206, 1958.
67.	562	37	55	52	**Spizizen J.** Transformation of biochemically deficient strains of *Bacillus subtilis* by deoxyribonucleate. *P. Nat. Acad. Sci. USA* **44**:1072-78, 1958.
68.	701	47	63	65	**Svennerholm L.** Quantitative estimation of sialic acids. II. A colorimetric resorcinol-hydrochloric acid method. *Biochim. Biophys. Acta* **74**:604-11, 1957.
69.	561	37	54	57	**Taussky H H & Schorr E.** A microcolorimetric method for the determination of inorganic phosphorus. *J. Biol. Chem.* **202**:675-85, 1953.
70.	725	48	41	36	**Tiselius A, Hjerten S & Levin O.** Protein chromatography on calcium phosphate columns. *Arch. Biochem. Biophys.* **65**:132-55, 1956.
71.	2244	150	185	140	**Trevelyan W E, Procter D P & Harrison J S.** Detection of sugars on paper chromatograms. *Nature* **166**:444-45, 1950.
72.	823	55	114	91	**Vogel H J.** Acetylornithinase of *Escherichia coli*; partial purification and some properties. *J. Biol. Chem.* **218**:97-106, 1956.
73.	2656	177	282	293	**Warren L.** The thiobarbituric acid assay of sialic acids. *J. Biol. Chem.* **234**:1971-75, 1959.
74.	552	37	19	16	**Watson J D & Crick F H C.** A structure for deoxyribose nucleic acid. *Nature* **171**:737-38, 1953.
75.	2509	137	214	181	**Watson M L.** Staining of tissue sections for electron microscopy with heavy metals. *J. Biophys. Biochem. Cytol.* **4**:475-79, 1958.
76.	767	51	18	10	**Wyatt G R.** The purine and pyridimine composition of deoxypentose nucleic acids. *Biochem. J.* **48**:584-90, 1951.
77.	541	36	15	11	**Yarmolinsky M B & dela Haba G L.** Inhibition by puromycin of amino acid incorporation into protein. *P. Nat. Acad. Sci. USA* **45**:1721-29, 1959.
78.	741	49	45	47	**Yemm E W & Cocking E C.** The determination of amino acids with ninhydrin. *Analyst* **80**:209-13, 1955.

high citation rates.

On the other hand, the article by Watson and Crick (74) may be indicative of what is called the "obliteration phenomenon."[7] Many important scientific discoveries are quickly incorporated into the common wisdom of the field. Authors no longer feel compelled to cite the original discovery. Compared to the citation rates for other classics on the list, and considering the enormous amount of research in molecular biology, the Watson and Crick paper will probably soon become obliterated. In the period 1961-1975, it received only 552 citations--a yearly average of 37. In 1975 it was cited "only" 16 times. Few other papers in Figure 1 have registered such sharp decreases.

Many of the papers on this list are citation superstars--Lowry (39) with 50,016 citations; Folch (23) with 7,454; Burton (9) with 5,037; and Bartlett (1) with 2,791. All of these have 1974 and 1975 citation rates well above their yearly averages, indicating that activity in these fields has increased in recent years.

Six authors of these 1950s articles also appeared on our 1940s list with method papers: Lowry (39), Markham and Smith (41), Moore and Stein (47, 48, and 66), and Taussky (69). Those by Markham and Smith, and Moore and Stein, concerned chromatographic techniques.

Eleven of these articles have been authored by 11 Nobel laureates. Watson of the U.S. and Crick of England (74) shared the Nobel Prize for medicine in 1962. Tiselius of Sweden (70) received the 1948 Prize in chemistry for his discovery and isolation of mouse paralysis virus. In 1972 Porter of Britain (55) received the medical Prize for research on the chemical structure and nature of antibodies. The 1970 Nobel Prize for chemistry was awarded to Leloir of Argentina (56) for his discovery of sugar nucleotides and their role in the biosynthesis of carbohydrates. For his research into the inner workings of living cells, deDuve of the U.S. (24) received the 1974 Nobel Prize for medicine. The 1975 Prize for chemistry was awarded to Prelog of Switzerland (11), a native of Yugoslavia, for his research on the structure of bio-

Figure 2. Journals that published the highly cited 1950s articles listed in Figure 1, according to number of articles. A = number of articles. (Present titles of some journals are given in parenthesis.)

A	Journals
18	J. Biol. Chemistry
14	Biochemical Journal
6	Analytical Chemistry
6	J. Amer. Chem. Soc.
5	Arch. Biochem. Biophys.
5	Proc. Nat. Acad. Sci. USA
4	J. Biophys. Biochem. (J. Cell Bio.)
3	Biochim. Biophys. Acta
3	Nature
2	J. Clin. Invest.
1	Acta Physiol. Scand.
1	Adv. Protein Chem.
1	Analyst
1	Biochem. Zschr. (Eur. J. Biochemistry)
1	Canadian J. Biochem. Physiol.
1	Experientia
1	J. Chem. Soc.
1	J. Histochem. Cytochem.
1	Lancet
1	Methods Enzymol.
1	Proc. Soc. Exp. Biol. Med.
1	Science

logical molecules. In 1955 duVigneaud of the U.S. (6) received the chemistry Prize for his work on pituitary hormones. Three other Americans, Anfinsen (33), Moore and Stein (47, 48, and 66) shared the 1972 chemistry Prize for their pioneering studies on enzymes.

Figure 2 lists the 22 journals that published these articles. Just two journals published 32 of the 78 articles. The *Journal of Biological Chemistry* published 18 articles, and the *Biochemical Journal* 14. *Analytical Chemistry* and the *Journal of the American Chemical Society* published 6 each. All but 3 of the articles authored by Nobelists appeared in these four journals, which accounted for 56% (44 articles) of the papers in Figure 1.

The great majority of these 78 articles were published in the latter half of the 1950s. From 1950-1954, 23 articles were published. The remaining 55 articles appeared from 1955-1959, with increasing frequency by the years. In the late 1940s and early 1950s there was a tremendous amount of research done on the structural elements of DNA. Both Watson[8] and Crick[9] have acknowledged that at the time of their own efforts numerous other investigators were active. The success and impact of their work helps explain the flurry of activity in biochemistry in the mid to late 1950s--with a little monetary help from our friends in Washington and elsewhere.

Much of the methodological groundwork for the 1950s biochemical research--such as chromatography and its application to nucleic acids--had been laid in previous decades. In the same way, these classic papers of the 1950s helped lay the foundation for biochemical research today. In particular, the discovery of DNA and its capacity for carrying genetic material has led science to a more complete understanding of the mysteries of life.

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6. **Piez K A.** Personal communication, 18 January 1977.
7. **Garfield E.** The 'obliteration phenomenon' in science, and the advantage of being obliterated. *Current Contents* No. 51/52, 22 December 1975, p. 5-7.
8. **Watson J D.** *The double helix: being a personal account of the discovery of the structure of DNA.* New York: Atheneum, 1968.
9. **Olby R.** *The path to the double helix.* Seattle: University of Washington Press, 1974.