SYNTHETIC RUBBER

Synthetic Rubber from Alcohol. A survey based on the Russian literature by Anselm Talalay and Michel Magat. 298 pp. New York, N. Y.: Interscience Publishers. 1945. $5.00.

THE first part of this book is a description of the Lebedev process which has been developed and used in Russia for the manufacture of butadiene from ethyl alcohol. In contradistinction with the multi-stage Carbide and Carbon process utilized in this country, the Lebedev process is a one-step catalytic process technologically simpler but yielding a large variety of by-products which have to be separated from butadiene before polymerization. Technical data on the rectification of the unconverted ethyl alcohol, on the absorption, desorption and distillation of the crude butadiene, on the separation and utilization of the by-products is given with some details in Chapters I and II.

The second part of the book is a well-integrated exposé of the present theories of polymerization phenomena and of physicochemical measurements of polymer properties. The authors very wisely did not confine themselves to the sodium polycondensation of butadiene, which is, according to the literature, the only process used by the Russians in the manufacture of synthetic rubber from butadiene; they present one of the best general treatments of the various polymerization techniques to be found in the literature. They have classified the abundant physicochemical data of the Russian investigators and frequently interpreted them in the light of recent theoretical concepts. They have also compared, when data were available, physical characteristics of sodium polybutadienes with that of emulsion polybutadienes and natural rubbers.

If the sole purpose of the authors was to make available, in a comprehensive form, the abundant literature published in Russia on the subject, they have fully succeeded in their task. As such, the book will be found very helpful to those interested in the field of polymerization.

From a general point of view this book is a war casualty of censorship and withholding of information. Examination of the bibliography shows how very little information has been published in Russian journals since 1936. It is the feeling of the reader that for one reason or another much pertinent scientific information has been omitted, thus rendering the treatment sometimes incomplete and occasionally scientifically meaningless. As a flagrant example, catalysts used in the Lebedev process are identified as a mixture of catalysts A and B with c and d as promoters. There is no indication as to what is the specific identity of catalysts A, B, c and d; whether the catalyst mixtures were identified by A, B, c, d in the Russian literature is not clarified, surprisingly enough considering how much other detailed information is given. The yield of butadiene, the nature of the by-products, the actual operational conditions are an intimate function of the specific nature of the catalysts used. Many graphs, tables and considerations in Chapter I lose most of their meaning, since they are not related to specific catalysts. The publishers must undoubtedly realize that the withholding of such information markedly decreases the value and therefore the saleability of the book.

To summarize, the quality of the book is uneven; Chapter III on polymerizations is by far the best; the book as a whole is an excellent survey of the Russian literature on the subject.

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SPECIAL ARTICLES

THE MECHANISM OF THE VIRUCIDAL ACTION OF ASCORBIC ACID[1]

THE virucidal activity of ascorbic acid has been reported against vaccina virus[2] and poliomyelitis.[3] Knight and Miller[4] have recently demonstrated the virucidal activity of this compound against influenza A virus. We have confirmed the virucidal action of ascorbic acid against influenza A virus and will report our results indicating the mechanism of action of this compound.

Barron, DeMeio and Klemperer[5] have shown that the oxidation of ascorbic acid in air is catalyzed by Cu and they postulated that H_2O_2 is formed during the reaction. In the presence of 0.0002 mM of $CuCl_2$, pH 6.95, the half oxidation of ascorbic acid occurred in 10.3 minutes. These workers did not demonstrate the actual presence of H_2O_2, which they indicated was difficult because of its rapid decomposition into water and oxygen. Lyman, Schultze and King[6] have reported that their qualitative tests indicated the presence of H_2O_2 during the Cu catalyzed oxidation of ascorbic acid in air.

If H_2O_2 is the active virucidal agent, then catalase, which acts specifically upon H_2O_2, should completely abolish the activity of ascorbic acid. It should also be possible to show that H_2O_2 itself, in concentrations

[1] This investigation has been aided by a grant from the Josiah Macy Jr. Foundation.

[2] I. J. Kligler and H. Bernkopf, *Nature*, 139: 965, 1937.

[3] C. W. Jungeblut, *Jour. Exp. Med.*, 62: 517, 1935.

[4] C. A. Knight and W. M. Stanley, *Jour. Exp. Med.*, 79: 291, 1944.

[5] E. S. G. Barron, R. H. DeMeio and F. Klemperer, *Jour. Biol. Chem.*, 112: 625, 1936.

[6] C. H. Lyman, M. O. Schultze and C. S. King, *Jour. Biol. Chem.*, 118: 757, 1937.

approximating that theoretically produced by the oxidation of ascorbic acid at pH 7, inactivates influenza A virus.

MATERIALS AND METHODS

Ascorbic acid: l-ascorbic acid (Eastman) was freshly prepared before each titration. Solutions were adjusted to pH 7 and diluted in M/10 phosphate buffer.

Hydrogen peroxide: Merck, Superoxol (30 per cent. H_2O_2) diluted in M/10 phosphate buffer.

Catalase: Blood (rabbit) catalase, water clear[7] was kindly supplied by Dr. M. G. Sevag of this department.

Virus: The PR8 strain of influenza A virus was grown in the allantoic cavity of 10-day-old chick embryos. The test virus consisted of allantoic fluid diluted in buffered saline pH 7, and containing either 100 or 10 MLD of virus. Tests for viral activity were performed by instilling 0.05 ml of fluid intranasally into lightly etherized mice. The mice were observed for 10 days and death with typical lobar consolidation was recorded as D_6D_8, etc., *i.e.,* death occurred on the sixth or eighth day following inoculation. Mice surviving 10 days were killed and the extent of lung lesions reported as 0 (normal), 1^+, 2^+, 3^+, 4^+.

EXPERIMENTAL

The virucidal action of ascorbic acid and its neutralization by catalase: One ml of a 0.05M solution of ascorbic acid was added to 1 ml of influenza A virus solution containing 100 or 10 MLD; 0.5 ml of buffered saline was added to the solution to make a total volume of 2.5 ml. After remaining at room temperature for 10 minutes, 0.05 ml of the solution was inoculated into mice. The mice were observed for 10 days and the presence of virus reported as indicated under Methods. A 0.05 M solution of ascorbic acid is close to the minimal virucidal concentration since it caused only partial inactivation of a test solution containing 100 MLD of virus with complete inactivation occurring only in a solution containing 10 MLD (Table 1).

TABLE 1

THE VIRUCIDAL ACTION OF ASCORBIC ACID UPON INFLUENZA A VIRUS AND ITS NEUTRALIZATION BY CATALASE

Action of Ascorbic Acid	Neutralization by Catalase	Virus Control	Catalase Control	Catalase and Ascorbic Acid Control	Catalase and Virus Control
1 ml of 0.05M ascorbic acid, 1 ml of influenza A virus, 100 MLD (A) and 10 MLD (B). 0.5 ml buffered saline	1 ml of influenza A virus of 100 MLD (A) and 10 MLD (B). 0.5 ml catalase (1:10). 1 ml 0.05M ascorbic acid.	1 ml influenza A virus 100 MLD (A) and 10 MLD (B) 1.5 ml buffered saline.	0.5 ml catalase (1:10). 2 ml buffered saline.	1 ml catalase (1:10). 1 ml 0.05M ascorbic acid. 0.5 ml buffered saline.	1 ml virus 100 MLD. 1 ml catalase (1:10). 0.5 ml buffered saline.
A. (100 MLD of virus)					
D_7, D_{10}, 3+, 3+, 2+, 2+, 1+, 1+, 0, 0	D_6, D_6, D_6, D_6, D_7, D_7, D_8, D_8	D_6, D_6, D_7, D_8, D_8	0, 0, 0, 0	0, 0, 0, 0	D_7, D_7, D_7, D_9
B. (10 MLD of virus)					
0, 0, 0, 0, 0, 0, 0, 0	D_7, D_7, D_7, D_7, D_8, D_9, 2+	D_7, D_7, D_8, D_8, 3+			
Substituting 0.1% H_2O_2 for 0.05M ascorbic acid					
0, 0, 0, 0, 0	D_7, D_7, D_7, D_8, D_8				

In order to determine the inhibitory action of catalase 1 ml of virus was added to 0.5 ml of catalase diluted 1:10; 1 ml of 0.05 M ascorbic acid was then added and after 10 minutes at room temperature 0.05 ml of the solution was inoculated into mice. The results in Table 1 show that catalase completely neutralized the action of ascorbic acid.

Action of hydrogen peroxide upon influenza A virus: Hoagland, Ward, Smadel and Rivers[8] have shown that Cu is present in high concentration in their purified preparations of vaccinia virus. Though quantitative data are not available on the presence of Cu in influenza A virus it is reasonable to assume that Cu is present, since it is a normal constituent of living cells and exists in relatively high concentration in the chick embryo, the host for the growth of our strain of influenza virus. Assuming the presence of Cu in amounts as small as 0.0002 mM then at pH 7 half oxidation of ascorbic acid occurs in 10.3 minutes (4). At room temperature the theoretical yield of H_2O_2 from 0.05 M ascorbic acid would be 0.085 per cent.; in the presence of 0.01 mM of Cu the H_2O_2 formed would be approximately 0.14 per cent.

We have found the minimal concentration of H_2O_2 causing complete inactivation of 10 MLD of influenza A virus is 0.1 per cent. (Table 1). Therefore the virucidal activity of a 0.1 per cent. solution of H_2O_2 approximates the virucidal activity of the H_2O_2 re-

[7] M. G. Sevag and L. Maiweg, *Biochem. Z.*, 288: 41, 1936.

[8] C. L. Hoagland, S. M. Ward, J. E. Smadel and T. M. Rivers, *Jour. Exp. Med.*, 74: 69, 1941.

sulting from the Cu catalyzed oxidation of 0.05 M ascorbic acid.

DISCUSSION

The role of Cu in the virucidal action of ascorbic acid is still not clearly defined, though our preliminary observations have indicated that KCN inhibits the virucidal action of ascorbic acid presumably by combining with Cu to form a stable compound.

It is of interest to note that the bactericidal activity of Penicillin B has also been reported as being due to the production of H_2O_2. The Penicillin B was found to be a glucose oxidase producing H_2O_2 in the presence of glucose and oxygen.[9]

SUMMARY

The theoretical yield of H_2O_2 formed during the oxidation of a virucidal solution of ascorbic acid approximates the virucidal action of an equivalent amount of H_2O_2. Both the action of ascorbic acid and H_2O_2 are completely neutralized by catalase. The action of ascorbic acid against influence A virus may therefore be explained as being due to the H_2O_2 formed during the Cu catalyzed oxidation of ascorbic acid.

The observed *in vitro* virucidal activity of ascorbic acid obviously can not be utilized therapeutically because of the presence of catalase in body tissues.

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BLOOD, URINE AND FECAL LEVELS OF STREPTOMYCIN IN THE TREATMENT OF HUMAN INFECTIONS OF E. TYPHOSA[1,2,3]

THE clinical studies of the effect of streptomycin on the course of typhoid infections in humans will be reported by Reimann, Elias and Price.[4]

The potency assay of streptomycin depends on a cylinder-plate technique, similar to the method used in penicillin assay, in which a strain of *B. subtilis* was substituted for *S. aureus*.[5] This procedure is also like that used in the assay of streptothricin.[6]

During this investigation, patients were given streptomycin in dosages ranging from one million to four million units daily. The drug was given by the intramuscular or intravenous routes and, in one patient, by the oral route which, on discontinuance, was followed by injection, using the intravenous drip method. Intramuscular and oral doses were given at three-hour intervals.

Blood and serum samples were usually collected after the first dose at intervals of 1, 2, 3, 6, 12, 24, 36, 48, etc., hours of treatment. Samples were also drawn at short intervals on discontinuance of dosage. Urine samples were collected at a number of short intervals following the initial dose and again on discontinuance of dosage. In the interim, 24-hour urine collections were made to determine the amounts of streptomycin present. In two cases fecal samples were assayed, when possible, on the basis of 24-hour collections.

INTRAMUSCULAR AND INTRAVENOUS DOSAGE

These routes of administration produced similar blood and serum levels in direct proportion to dosage. Four million units daily, given intravenously, resulted in whole blood levels with a mean of 12 units per ml and a mean of 28 units per ml of serum. Peak levels were reached within a few hours after the start and disappeared within 24 hours after cessation of dosage.

With an intramuscular administration of 20 million units over six days, 44 per cent. of the streptomycin was recovered in the urine. Following the intravenous dosage of 28 million units over 7 days, 70 per cent. of the streptomycin was accounted for in the urine. Urinary excretion appeared within 1½ hours, with only traces remaining 72 hours after cessation of dosage.

Fecal levels were not determined with intramuscular dosage, nor were total recoveries determined by intravenous administration. However, four million units daily, by the intravenous route, produced from 100 to 130 units per gram of feces.

ORAL DOSAGE FOLLOWED BY INTRAVENOUS ADMINISTRATION

Four million units daily, by oral ingestion, produced no demonstrable blood level. Approximately 1 per cent. appeared in the urine, while at least 64 per cent. was eliminated in the stools over the period of oral dosage, with as high as 21,700 units per gram of fresh feces.

When oral administration was suddenly changed to intravenous injection, streptomycin promptly appeared in the blood and serum at levels comparable

[9] O. Schales, *Arch. Biochem.*, 2: 487, 1943.

[1] The method for the assay of streptomycin in citrated blood, hemolysed with saponin, and urine was obtained through the courtesy of Dr. J. M. Carlisle and Dr. D. F. Robertson, of the Merck Institute for Therapeutic Research, Rahway, N. J.

[2] This work was conducted in the laboratory of Dr. H. A. Reimann in Jefferson Medical College, Philadelphia, Pa.

[3] Detailed methods of procedure will be presented in a subsequent publication.

[4] H. A. Reimann, W. F. Elias and A. H. Price, *Jour. Am. Med. Asn.*, in press.

[5] H. J. Robinson and D. G. Smith, *Jour. Pharmacol. and Exp. Therap.*, in press.

[6] J. W. Foster and H. B. Woodruff, *Jour. Bact.*, 45: 408, 1943.