TRANSLATION NO. 942

DATE: Oct. 1963

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

ON THE QUESTION OF THE REDUCTION OF 2, 3, 5-TRIPHENYL-TETRAZOLIUMCHLORIDE BY MICROORGANISMS BELONGING TO DIFFERENT FAMILIES

[Following is a translation of an article by A. Hirsch, C. Cattaneo and M. Morellini, of the C. Forlanini Institute of the I.N.P.S. Tuberculosis Clinic of the University of Rome (Director: Prof. A. Onodei Zorini), in the German-language periodical Zeitschrift fur Hygiene Infektionskrankheiten (Journal for Hygiene of Infectious Diseases), Vol CXLV, 1958, pages 355-366.]

The behavior of a living creature toward its environment is characterized by its metabolic capabilities and requirements in accordance with the modern, predominantly functional approach of biochemical knowledge in microbiology. These metabolic-dissimilatory processes form the basis of the concerted or opposing action of cells and cell systems and they are, therefore, also the cause of pathological processes when they meet a suitable micro and microorganism. From the physical-chemical point of view in this connection it is always a question of an exchange of energy, in which the release of energy or electrons between cell and environment is controlled by its redox potential (cf. Baldwin, Neilands-Stumpf). After pertinent studies in our specialty of Mycobacteria had unmistakably permitted a differentiation of the various species and strains on the basis of their different redox potential and had led to a metabolic-functional classification in strains with parasitic or saprophytic heterotrophy (Hirsch-Cattaneo (1)), we attempted, in appropriate additional pursuit of this problem, to find eventual means of differentiation also in the narrow field of the biological reduction processes. We chose these reactions, since they are the basis of every life-process and they also occur naturally depending on the redox potential of the system and can be measured in a relatively simple and exact manner. The credit goes to Kuhn-Jerchal (1941) for having had recourse to tetrazolium salts, which had already been known in chemistry for a long time, for measuring physiological reduction processes, and for having thus opened the way to this direction of research. These salts show the unusual peculiarity of having a colorless, waterless oxidation substance and a strongly colored, insoluble reduction substance. From the chemical point

of view it is a question here of the formation of red formazan [unable to identify in any of the standard works on microbiology] in which 2, 3, 5-triphenyltetrazoliumchloride (TTC) and neotetrazoliumchloride (NTC) are used mainly as initial products (cf. Jambor, Mattson-Jensen-Dutcher, Smith). We chose TTC, because with it, as with the other monotetrazolium salts, reduction occurs smoothly on one level, while with NTC by-products are formed that apparently have a strongly toxic effect on certain cells and also may affect a quantitative measurement (Burtner-Bahn-Longley). It can also be established from the basic reaction that these processes depend on the redox potential of the reducing system, that TTC does not act as a redox indicator but rather as a simple hydrogen acceptor, since, on the one hand, the formation of formazane is considered as irreversible for biological conditions, on the other hand, it is only a question of uniting with hydrogen in the reaction, which comes from another system, at a certain point in the course of the complex dehydrogenation process, consequently of the metabolic migration of hydrogen, naturally at the level on which it corresponds to the redox potential of TTC.

From the practical point of view, the reduction of the tetrazolium salts is, accordingly, to be interpreted as the expression of a single, although essential, partial factor of the dissimilatory metabolic capabilities of a cell or of a group of cells and is, therefore, directly linked to the life of the cell or to the activated, specific enzymes present in it. The various, and at a superficial glance, contradictory appearing opinions of writers on the subject are explained by the very complex processes. However, it is principally a question only of differences in interpretation, if the too narrowly limited initial points of view do not make a more highly placed opinion possible. Thus part of the researchers see in the TTC reaction the vital staining or the expression of the viability and germinative ability of the cells; others, on the other hand, take a considerably broader view, including various metabolic processes, while still others, in view of the toxic effect of the tetrazolium salts or of the formazan that is produced, have recourse to inhibition experiments.

Now, the purpose of the present study was, in further pursuit of the experiments which have been performed (Hirsch-Cattaneo (2, 3) with the family of Mycobacteriaceae for introducing a TTC test for differentiating the various species and strains on the basis of their fundamental metabolic differences, to conduct research on the extent to which these experiments can be generalized, that is, whether it is a question here of a phenomenon that is generally valid for microorganisms. Therefore it was primarily necessary to make certain whether connections between the TTC reaction and virulence and the pathogenetic capability of a strain, closely linked to these connections, could be detected in other families.

Material and Our Own Studies

In order to have the greatest possible survey of the entire field

of the predominantly pathogenic microorganisms, we extended our experiments on the TTC reduction ability to a total of 95 different, classified strains. They belong to 11 families of the class Schizomycetes (order: Pseudomonadales, Eubacteriales, Actinomycetales) and for purposes of comparison to one family of the class Deuteromycetes (order: Thallosporales. They come from the collection of the Istituto Superiore di Sanita (Graduate School of Health), the Istituto di Microbiologia dell'Universita di Roma (Microbiology Institute of the University of Rome) and the Instituto Carlo Forlanini (Carlo Forlanini Institute). Growth took place on solid or liquid culture media, according to the conditions of the individual strains, at a temperature of 37° C. The entire list of the strains studied is in the tables, in which the classification of the bacteria is made according to data from Bergey's Manual of Determinative Bacteriology (Baltimore: Williams & Wilkins, 1957).

In order to maintain a basic insight into the reaction readiness and the experimental conditions to be used, we performed appropriate preliminary experiments with a series of different strains:

a. TTC was added to the culture media in a concentration of 5 mg., 10 mg., 25 mg. and 50 mg. per 100, then inoculation was undertaken and growth was controlled;

b. several drops of a 0.025% TTC solution in a buffer solution at pH 7.0 were put into the cultures already growing on a solid cultre medium and the reaction result was observed at room temperature after 30, 60 and 180 minutes;

c. a culture sample, about 5 mg., taken with a wire loop from a culture on a solid nutrient medium, or from a liquid culture medium after centrifuging, was mixed with the TTC solution on a hollow slide and the reaction continued as in b.

As the result of these preliminary experiments, we were able to establish that the addition of TTC directly to the culture medium causes a very different inhibition of growth from strain to strain, especially with concentrations of over 10 mg. per 100. Otherwise the reaction was mostly positive with strains that were growing well. On the other hand, the addition of TTC solution to already growing cultures, whether they were taking place on solid nutrient medium or on a hollow slide, yielded clear results due to the formation of formazan that was occurring intracellularly and that occurred to a different degree according to the strain.

Experiments pertaining to the concentration of this TTC solution added to the culture on the culture medium or "in vitro" showed that an increase in concentration up to 0.05% of TTC produced a certain intensification of the reaction result, without our detecting disturbing incidental phenomena.

p

After we were able already to detect a strong dependency of the TTC reaction on the pH in Mycobacteria, we also worked with the present strains in a broad pH range. Here also it was basic that the reaction became positive or that an increase in the color intensity was found in a strong alkaline medium.

Our observations with respect to the duration of the reaction and therefore to the corresponding read-off time showed that in the strains with a positive TTC reaction a red coloration appears, setting-in mostly after 10-15 minutes and increasing at room temperature up to 60 minutes, that then, however, in the subsequent time between 1-24 hours a slight deepening of the coloration was barely detectable without a change in the reaction result. Fundamental changes no longer appeared, moreover, after one hour in strains with a weak positive or a negative TTC reaction.

Therefore, the following experiment technique was created for performing the definitive experiments: TTC Reagent: 0.05 g. of 2, 3, 5-triphenyltetrasoliumchloride were dissolved in 100 ml. of M/30 buffer solution (pH 2.8, 5.0, 7.0, 9.1, 12.5). The reagent is stable for about one week when it is preserved in a cool, dark place. — Performance: Three to four drops of TTC reagent were added directly to the growing culture on a solid culture medium or were well mixed on a hollow slide with about 5 mg. of bacteria substance. Readings were taken of the reaction at room temperature after 60 minutes, and are indicated as follows: - = no coloration of the cell mass; ± = pink tinting; + = red coloring; ++ = purple to violet coloring.

The findings obtained in this way from the 95 different strains used are entered separately in the tables [appended at the end], by means of which a clear difference can be discovered in the result of the TTC reaction between the individual genera, while within them, with the exception of the genera Pseudomonas, Klebsiella, Mycobacterium and Candida, no marked difference can be detected in the behavior of the strains. The result of the TTC reaction shows up, depending on the pH value. Therefore, TTC is not reduced by any strain at pH 2.8 and is reduced by all of them at pH 12.5. The correct range of study lies, therefore, between pH 5 and pH 9, according to our experience.

Discussion of the Results and Conclusions

From the technical point of view, there is always the fundamental question of the addition of a substrate in performing these or similar enzyme experiments. The majority of the authors listed in the bibliography at the end used in their work one that we rejected, however, in this series of studies, in order to obtain a concise survey of the metabolic capacity with reference to TTC, without increasing the danger of giving preference to particular strains. Our studies prove, on the one hand, that TTC reduction is also possible without the addition of a substrate, which could be demonstrated particularly in Mycobacteria (Hirsch-

Cattaneo (2)), and that no difference can be ascertained in the reaction result in the presence of the culture medium in comparison with the experiments "in vitro". The question, "substrate or no substrate", cannot however be disposed of definitively, therefore, because it is entirely conceivable that precisely in the genera that let no possibility of differentiation be established on the existing conditions the addition of a specific substrate, that causes the activation of specific enzymes, permits the discovery of strain differences. Therefore, it is clear that this may only be expected by using truly specific substrates, since the metabolic decomposition of the normal substances, which can be used by most of the strains, may merely lead to a further assimilation of the results. Closely connected with this question is the question of the dependency of the TTC reaction on the pH, which usually only affords the possibility of a differentiation. It is explained, on the one hand, as the consequence of the close connection of pH to all enzymatic processes, whether in relation to the activation of potentially present enzymes, or for the fermentation process itself, on the other hand, perhaps also on the basis of differences in permeability.

If the results of our studies are compared with the data of other writers, it is seen that there are experiments on the TTC reduction ability of only individual strains from the families of the Pseudomonadaceae (Antopol-Glaubach-Goldman, Diamanti), of the Enterobacteriaceae (Antopol-Glauback-Goldman, Diamanti, Gargani-Marracini, Gheorghiu-Alexa Petrovanu, Huddleson-Baltzer, Kopper, Lederberg, Marahara-Quittner-Goldman-Antopol, Nordmann-Jude-Nordmann-Servant-Gauchery, Stolp, Wundt), of the Brucellaceae (Huddleson-Baltzer, Thiago de Mello-Silva), of the Micrococcaceae (Diamanti, Gargani-Marracini, Gheorghiu-Alexa Petrovanu, Huddleson-Baltzer, Sevag-Forbes, Wundt), of the Lactobacillaceae (Antopol-Glaubach-Goldman, Barnes, Huddleson-Baltzer, Wundt), of the Corynebacteriaceae (Thibaut-Welsch, Wundt), of the Bacillaceae (Antopol-Glaubach-Goldman, Gargani-Marracini, Gheorghiu-Alexa Petrovanu, Huddleson-Baltzer, Soru-Sternberg), of the Mycobacteriaceae (Arima-Yamamoto-Kakimoto-Takahashi (1, 2), Castanbida Odier-Smith, Hirsch-Cattaneo (2, 3), Kanai, Kanai-Yanagisawa, Kochweser-Barclay-Ebert (1, 2), Kochweser-Ebert, MacVandiviere-Gentry-Willis, Segal-Bloch, Winterscheid-Glick-Mudd), of the Spirillaceae (Gargani-Marracini, Soru-Sternberg), of the Torulopsidaceae (Nagai, Pagano-Levin-Trejo) and of the Saccharomycetaceae (Nagai). There are no data on Neisseriaceae, Actinomycetaceae and Streptomycetaceae, whose representatives are also included in our material. It would be far beyond the limits of this article for us to want to go into detail on all these studies. Rather it seems proper to make a comparison not of the individual results but rather of the attempts at interpretation or of the conclusions.

Depending on the starting point of their experiments, many writers see the TTC reaction only under the point of view of a simple vital coloration or in comparison with counting the growing colonies, increase of the nephelometer values, etc., as an expression of viability and

permeability. Authors who are more biochemically oriented emphasize the possibility of drawing on the enzymatically controlled production of formazan for studying metabolic processes that characterize the behavior of the various microorganisms. Various others, primarily pure bacteriologists, studied the toxic action of TTC and NTC on bacterial cultures and they discuss the more or less strong inhibitory effects that show up. In addition to these publications that barely go beyond the framework of general observations, other writers took a decisive step ahead by resorting to the differences found in the enzyme family or in growth disturbances for the purpose of differentiation. Thus, on the basis of their different metabolic capabilities on culture media with the addition of TTC and enriched with a great amount of carbohydrate, individual strains of Streptococci (Barnes), Salmonella and Escherichia (Huddleson-Baltzer, Lederberg), Brucella (Huddleson-Baltzer, Thiago de Mello-Silva), Shigella (Lederberg) and Candida (Pagano-Levin-Trejo) were successfully differentiated. It was also possible to accomplish a species and strain differentiation in vitro or on culture media with the addition of TTC, based on the different degree of formazan production (Hirsch-Cattaneo (2, 3)). It was also determined in these experiments that the differences found in the TTC reduction ability, either depending on the pH value of on the substrate used, make it possible to pass judgment on the question, parasite or saprophyte, or pathogenic or not, as could clearly be demonstrated in Mycobacteria (Hanke, Hirsch-Cattaneo (2, 3)), in Escherichia, Salmonella and Shigella (Lederberg), as well as in various Candidae (Pagano-Levin-Trejo). The studies that were also conducted for differentiation purposes on the growth inhibiting effect of tetrazolium salts, among which NTC stands out particularly due to its toxic action, permitted a separation of a typical and avian strains from other Mycobacteria strains (Castaneda Odier-Smith), the differentiation of Corynebacteria of the gravis type from the mitis type (Thibaut-Welsch) and the general determination that gram-positive bacteria are more sensitive to TTC than gram-negative bacteria (Gargani-Marracini).

In order to touch only briefly on the reaction fundamental of TTC reduction (cf. Hirsch-Cattaneo (2)), it is a question here of the action of dehydrogenase systems, which are tied in with the most primitive life processes of cells (respiration, etc), in which there is, without doubt, a certain dependency on coenzyme I and II (Mattson-Jensen-Durcher, Smith) as well as on the flavoprotein portion of cytochrome C (Kun). Amino acid dehydrogenase (Kun), phosphoglyceraldehyde dehydrogenase (Brodie-Cohn), succinate dehydrogenase and pyridine nucleotide dehydrogenase (Mattson-Jensen-Dutcher). Therefore, it may be assumed that all cells have the corresponding enzymes, at least in the potential stage, that their activation, however, takes place under very different conditions.

If the results of our experiments are considered to be definitive, then it may be established, basically, that definite differences in the TTC reduction capability of the various strains, determined to be dependent on the pH value, were found in these experiments, that were performed

under precisely the same conditions without using a substrate. Every strain was able to produce formazan in a more strongly alkaline environment; however in neutral or acid environments only certain genera or individual strains accomplished this. The last fact, precisely, appears to us, therefore, to be most deserving of mention, since it indicates the existence of a means of differentiation. It can, therefore, be assumed that in TTC reduction it indeed is a question of a reaction basically possible in all strains but strongly dependent on the experiment conditions, which affords a conclusion on the metabolic processes of the individual strains or genera. From the point of view of the classification of microorganisms based on Gram's staining, on the one the gram-positive strains generally also show a positive TTC reaction at low pH values, while with gram-negative strains this was only found in certain saprophytes; on the other hand, the tendency was also clearly established in the former that saprophytic strains reduce TTC more strongly, which show up most clearly in Mycobacteria and Candidae. Under the given conditions, that were to provide us with the basis for comparison in a preliminary study, a clear differentiation in parasitic and saprophytic strains shows up with certainty only in these two genera; it seems likely, however, that further experiments performed with different, specific substrates will also permit a corresponding differentiation in strains of the other families. This differentiation is based on the different metabolic processes in indirect dependency on the redox potential of the systems and it gives an insight into the behavior of microorganisms with regard to their environment, that is, practically, into the question of parasite or saprophyte and the consideration of pathogenetic capabilities closely connected with it.

Summary

After a preliminary discussion of the problem of the behavior of a microorganism with respect to its environment with reference to its dissimilatory-metabolic processes, the authors take up the biological reduction processes and their measurement by transferring tetrazolium salts into formazan and then point out the reaction potentialities by using 2, 3, 5-triphenyltetrazoliumchloride (TTC) on 95 different strains that belong to 12 families of microorganisms. The TTC test, performed without the addition of a substrate, yielded clear differences in the reduction ability of the various strains, which were found to depend on the pH value. It could be determined that TTC reduction, appearing to be connected with the dehydrogenation processes and, therefore, with the fundamental life processes of the cell, is, indeed, a reaction that is possible in all strains but one that is very dependent on the conditions, and by means of which, under the given experiment conditions, a sure differentiation into parasitic and saprophytic strains occurs only with Mycobacteria and Candidae. Starting from these research results, it seems likely, however, that a differentiation of the strains of the other families will also be possible by means of appropriate experiments using specific substrates.

It depends on the different metabolic processes and gives an insight into the behavior of the microorganism with respect to its environment and into the problem of parasite or saprophyte and, therefore, into the pathogenetic potentialities.

Bibliography

ANTOPOL, W., GLAUBACH, S. and GOLDMAN, L., "Effects of a New Tetrazolium Derivative on Tissue, Bacteria and Onion Root Tips," Publ. Hlth. Rep. (Wash.), 63, 1948, p 1231.

ARIMA, J., YAMAMOTO, K., KAKIMOTO, S. and TAKAHASHI, Y., (1), "The Tetrazolium-Reducing Action of BCG. I. Fundamental Experiments on the Measurement of the Reaction," Jap. J. Tubercu., 2, 1954, p 222.

——, ——, ——, ——, (2), "The Tetrazolium-Reducing Action of BCG. II. Relation between Tetrazolium-Reducing Action and Viability of the Bacilli," Jap. J. Tubercu., 2, 1954, p 278.

BALDWIN, E., Dynamic Aspects of Biochemistry. Second edition. Cambridge: Cambridge University Press, 1952.

BARNES, E., "Tetrazolium Reduction as a Means of Differentiating Streptococcus faecalis from Streptococcus faecium," J. Gen. Microbiol., 14, 1956, p 57.

BROOKE, A. and COTS, J., "Effects of an Isolated Dehydrogenase Enzyme and Flavoprotein on the Reduction of Triphenyltetrazolium Chloride," Science, 1951, p 40.

BURTNER, H., BARR, R. and LONGLEY, J., "The Reduction and Quantitation of Neotetrazolium," J. Histochem. Cytochem., 5, 1957, 127.

DIANZANI, M., "Colorazione vitale di alcuni microorganismi con ditetrazolio cloruro" ("Vital Staining of Some Microorganisms with Ditetrazoliumchloride"), Nuovi Ann. Ig., 4, 1953, p 392.

GABRIELI, G. and MARRACCINI, G., "Attivita batteriostatica del 2, 3, 5-trifeniltetrazolio-cloruro sui batteri gram-positivi," ("Bacteriostatic Activity of 2, 3, 5-triphenyltetrazolium-chloride on Gram-Positive Bacteria"), Nuovi Ann. Ig., 5, 1954, p 809.

GASTAMBIDE-ODIER, M. and SMITH, D., "A Rapid Means of Differentiating Atypical Acid-Fast Bacilli and Avian Tubercle Bacilli from Other Mycobacteria. The Neotetrazolium Inhibition Test," Amer. Rev. Tubercu., 77, 1958, p 662.

GHEORGHIU, C. and ALEXA-PETROVANU, M., "Reduction of the Tetrazolium Salts," Acad. Rep. Pop. Romane, Stud. cercet. stiint 4, 1953, p 187.

HANKS, J., "Measurement of the Hydrogen Transfer Capacity of Mycobacteria," J. Bact., 62, 1951, p 521.

HIRSCH, A. and CATTANEO, C., (1), "I micobatteri: costituzione chimica e virulenza. II. Il potenziale di ossido-riduzione dei micobatteri" ("Mycobacteria: Chemical Composition and Virulence. II. The Oxidation-Reduction Potential of Mycobacteria"), G. Microbiol., 4, 1957, p 175.

----, ----, (2), "Die Mycobakterien: Chemischer Aufbau und Virulenz. III. Die Reduzierung von Triphenyltetrazolium-chlorid durch Mycobakteriumstamme verschiedener Species" ("Mycobacteria: Chemical Composition and Virulence. III. Reduction of Triphenyltetrazoliumchloride by means of Mycobacteria Strains of Various Species"), Z. Hyg. Infekt.-Kr., 145, 1958, p 71.

----, ----, (3), "Sulla differenziazione dei ceppi micobatterici per mezzo di un terreno contenente 2, 3, 5-trifeniltetrazolio-cloruro" ("On the Differentiation of Mycobacteria Strains by means of a Medium Containing 2, 3, 5-Triphenyltetrazoliumchloride"), Nuovi Ann. Ig. (in press).

HUDDLESON, I. and BALTZER, B., "Differentiation of Bacterial Species and Variation within Species by means of 2, 3, 5-triphenyl-tetrazolium chloride in Culture Medium," Science, 1959, p 651.

JAHOR, R., "Mechanism of the Reduction of Tetrazolium Salts," Nature, 176, 1955, 603.

KANAI, K., "The Tetrazolium Reduction as a Measure to Examine the Total Viability of Suspensions of Tubercle Bacilli," Jap. J. Bact., 9, 1954, 95.

KANAI, K. and YANAGISAWA, K., "Tetrazolium Reduction Test as a Measure to Examine the Total Viability of Suspensions of Tubercle Bacilli. I. Technique," Jap. J. Med. Sci. Biol., 7, 1954, p 155.

KOCHMESER, D., BARCLAY, W. and HEBERT, R., (1), "Reduction of Tetrazolium by Growing Tubercle Bacilli," J. Clin. Invest., 31, 1952, p 644.

----, ----, ----, (2), "The Influence of Isoniazide and Streptomycin on Acid-Fastness, Tetrazolium Reduction, Growth and Survival of Tubercle Bacilli," Amer. Rev. Tuberc., 71, 1955, p 556.

KOCHMESER, D. and HEBERT, R., "Tetrazolium Reduction by Tubercle Bacilli," J. Lab. Clin. Med., 46, 1955, 608.

KOPPER, P., "Studies on Bacterial Reducing Activity in Relation to Age of Culture," J. Bact., 63, 1952, p 639.

KUN, E., "Mechanism of Enzymatic Reduction of Triphenyl Tetrazolium Chloride," Proc. Soc. Exp. Biol. (N.Y.), 78, 1951, p 195.

KUHN, R. and JERCHEL, D., "Über Invertseifen. VIII. Mitteilung: Reduktion von Tetrazolium salzen durch Bakterien, garende Hefe und keimende Samen" ("On Invert Soaps. VIIIth Report: Reduction of Tetrazolium Salts by Bacteria, Fermenting Yeasts and Germinating Seeds"), Ber. Dtsch. Chem. Ges., 74b, 1914, p 949.

LEDERBERG, J., "Detection of Fermentative Variants with Tetrazolium," J. Bact., 56, 1948, p 695.

MAC VANDIVIERE, H., GENTRY, W. and WILLIS, H., "A Rapid Chemical Test of Total Viability for Suspensions of Tubercle Bacilli," Amer. Rev. Tuberc., 66, 1952, 95.

MATTSON, A., JENSEN, C. and DUTCHER, R., "Triphenyltetrazoliumchloride as a Dye for Vital Tissues," Science, 1917, 294.

NAGAI, S., "Sur la reduction de chlorure de triphenyl-tetrazolium par les levures," ("On the Reduction of Triphenyl-tetrazoliumchlorids by Yeasts"), C. R. Soc. Biol. (Paris), 149, 1955, p 2047.

NARAHARA, H., QUITTNER, E., GOLDMAN, L. and ANTOPOL, W., "The Use of Neotetrazolium in the Study of E. coli Metabolism," Trans. N. Y. Acad. Sci., 11-12, 1950, p 160.

NEILANDS, J. and STUMPF, P., Outlines of Enzyme Chemistry. New York: John Wiley and Sons, Inc., 1955.

NORDMANN, J., JUDA, A., NORDMANN, R., SERVANT, P. and GAUCHERY, C., "Reduction of 2, 3, 5-triphenyl-tetrazolium-chloride (TPT) by Escherichia coli," Rev. Immunol. (Paris), 16, 1952, p 157.

PAGANO, J., LEVIN, J. and TREJO, W., "Diagnostic Medium for Differentiation of Species of Candida," Antibiot. Ann., 1957-58, p 137.

SIEGAL, M. and BLOCH, H., "Biochemical Differentiation of Mycobacterium tuberculosis Grown in vivo and in vitro," J. Bact., 72, 1956, p 132.

SILVAG, M. and FORBES, M., "Oxydative Reactions between Glucose and Nitrogenous Substances," Arch. Biochem., 29 1950, p 229.

SMITH, F., "Tetrazolium Salt," Science, 1951, p 751.

SORU, E. and STEINBERG, N., "Oxidative Metabolism of Anaerobic Microorganisms by Triphenyltetrazolium Chloride," Acad. Rep. Pop. Romine, Stud. cercet. Chim., 4, 1956, p 263.

STOLP, H., "Über das scheinbare Versagen des Tetrazolium-testes bei Bacterium coli" ("On the Apparent Failure of the Tetrazolium Test with Bacterium coli"), Arch. Mikrobiol., 17, 1952, p 209.

THIAGO DE MELLO, N. and SILVA, N., "Use of Triphenyltetrazolium Chloride in the Study of Dehydrogenase Activity of Brucella," Mem. Inst. Osw. Cruz, 53, 1955, p 54.

THIBAUT, J. and WELSCH, M., "Sensibilite des Corynebacteries ou chlorure de 2, 3, 5-triphenyl-tetrazolium" ("Sensitivity of Corynebacteria or 2, 3, 5-triphenyltetrazoliumchloride"), C. R. Soc. Biol. (Paris), 145, 1951, p 159.

WINTERSCHEID, L., GLICK, H. and ST. MUDD, "The Use of Neotetrazolium Chloride in Cultures of Tubercle Bacilli," Amer. Rev. Tuberc., 68, 1953, p 625.

WURDT, W., "Untersuchungen uber die Reduktionswirkung von Bakterien auf Triphenyl-tetrazoliumchlorid" ("Studies on the Reduction Action of Bacteria on Triphenyltetrazolium-Chloride"), Dtsch. Med. Wschr., 1950, p 1471.

[Tables follow]

TABLE 1a. Schizomycetes

	a) TTC-Reaktion				
b)	[illegible]				
Pseudomonadales					
Pseudomonadaceae					
Pseudomonadeae					
Pseudomonas					
Pseudomonas aeruginosa Rec	--	+	+	++	-
Pseudomonas aeruginosa Pantem	-	-	-	+	-
Pseudomonas aeruginosa 2.1	-	+	++	++	+
Pseudomonas aeruginosa Rita	-	-	+	+	+
Pseudomonas aeruginosa Le Jacono	-	-	+	+	-
Eubacteriales					
Enterobacteriaceae					
Escherichieae					
Escherichia					
Escherichia coli [illegible]	-	-	-	-	+
Escherichia coli [illegible]	-	-	-	+	+
Escherichia coli 111	-	-	-	-	+
Escherichia coli 1	-	-	-	-	+
Escherichia coli 4	-	-	-	+	
Escherichia coli 3	-	-	-	-	
Escherichia coli Dandermann	-	-	-	+	-
Escherichia coli X 12	-	-	-	-	+
Escherichia coli Lazarpi	-	-	-	-	+
Escherichia coli Nivenni	-	-	-	+	+
Escherichia coli 0110	-	-	-	-	+
Escherichia coli [illegible]	-	-	-	+	+
Escherichia coli 0:10 B37/R8	-	-	-	-	+
Klebsiella					
Klebsiella pneumoniae T) p N 8033	-	-	+	+	+
Klebsiella rhinoscleromatis 147	-	-	-	-	+
Proteeae					
Serratia					
Serratia marcescens 2	-	+	+	++	++
Serratia marcescens 3	-	+	++	++	++
Proteae					
Proteus					
Proteus X 12 0 Amsterdam	-	-	-	+	+
Salmonelleae					
Salmonella					
Salmonella paratyphi A	-	-	-	-	+
Salmonella paratyphi A 1018	-	-	-	-	+
Salmonella paratyphi N M 1834	-	-	-	-	+
Salmonella typhi H 901	-	-	-	-	+
Salmonella typhi 0 901	-	-	-	-	+

[Legend:] a) TTC Reaction
b) pH 2.8; pH 5.0; pH 7.0; pH 9.1; pH 12.5

TABLE 1a. (continued)

b)	a) TTC-Reaktion				
	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]					
[illegible]					
Shigella dysenteriae 1953	-	-	-	±	±
Shigella similigna Schmitz II	-	-	-	-	±
[illegible]	-	-	-	-	±
Shigella Ceylonensis A	-	-	-	-	±
Shigella Ceylonensis B	-	-	-	-	±
[illegible]					
[illegible]					
[illegible]					
Proteus mirabilis Hauser	-	-	-	±	+
Proteus mirabilis NCTC	-	-	-	-	±
[illegible]					
[illegible]					
[illegible]					
Micrococcus myxoides Castellani 3	-	±	±	+	+
[illegible]					
[illegible]					
Staphylococcus albus TDR	-	+	-	+	++
Staphylococcus albus INV 31/102	-	±	+	++	-+
Staphylococcus albus Oxford 131	-	±	±	+	+
Staphylococcus T 3.1	-	+	++	++	++
Staphylococcus albus	-	±	±	+	++
Staphylococcus albus INV 48/104	-	+	+	+	++
Staphylococcus AS	-	±	-	-	++
Staphylococcus Gerlach	-	+	+	++	++
Staphylococcus Proell	-	±	±	+	++
Staphylococcus Rosin	-	±	++	++	++
[illegible]					
[illegible]					
Sarcina lutea PCI 1001	-	-	++	++	++
Sarcina mobilis [illegible]	-	±	++	++	++
[illegible]					
[illegible]					
[illegible]					
Neisseria gonorrhoeae 2933	-	-	±	±	+
Neisseria meningitidis 8239	-	-	-	±	+
[illegible]					
[illegible]					
[illegible]					
[illegible]					
Lactobacterium pneumoniae 6227	-	-	-	±	+
Lactobacterium homoiochii Typ II	-	-	-	±	+
[illegible]					
[illegible]					
Streptococcus pyogenes 61	-	-	-	±	+
Streptococcus hemolyticus Rowe	-	-	-	±	+
Streptococcus hemolyticus Petrangeli	-	-	-	±	+
Streptococcus hemolyticus 24	-	-	±	±	+
Streptococcus [illegible] 24	-	-	-	±	+
Streptococcus faecalis [illegible]	-	-	-	-	±

[Legend:] a) TTC Reaction
b) pH 2.8; pH 5.0; pH 7.0; pH 9.1; pH 12.5

TABLE 1a. (continued)

	a) TTC-Reaktion				
b)	2.8 pH	5.0 pH	7.0 pH	9.1 pH	12.5 pH
Corynebacteriaceae					
Corynebacterium					
Corynebacterium diphtheriae S	–	–	±	±	±
Corynebacterium diphtheriae 62	–	–	±	+	+
Corynebacterium diphtheriae 63	–	–	±	±	±
Corynebacterium xerose 9010	–	±	±	+	+ –
Corynebacterium V gamma	–	±	+	+	+ +
Bacillaceae					
Bacillus					
Bacillus subtilis ATCC	–	±	+	+ +	+ +
Bacillus subtilis Miedrell	–	+	+ +	+ +	+ +
Bacillus subtilis Mirel	–	±	±	+	+
Bacillus subtilis Pal	–	±	+	+ +	+ +
Bacillus subtilis Roma	–	±	+ +	+ +	+ +
Bacillus megatherium	–	±	+	+ +	+ –
Bacillus megatherium GP	–	±	+	+ +	+ +
Bacillus megatherium Paris	–	+	+ +	+ +	+ –
Bacillus megatherium RI	–	±	+	+	+ –
Bacillus cereus B 10	–	±	+	+ +	+ +
Bacillus cereus 569	–	+	+ +	+ +	+ +
Bacillus cereus 10702	–	±	+	+ +	+ +
Bacillus mycoides	–	+	+ +	+ +	+ +
Bacillus clavatus	–	±	+	+ +	+ +
Bacillus mesenterious vulgatus	–	±	+	+	+
Bacillus mesenterious vulgatus 207	–	±	+	+	±
Clostridium					
Clostridium novyi 0070	–	–	–	–	+
Clostridium paraputrificum G 100	–	–	–	+	+ +
Actinomycetales					
Mycobacteriaceae					
Mycobacterium					
Mycobacterium tuberculosis H 37	–	–	±	±	±
Mycobacterium bovis Vallée	–	–	±	±	±
Mycobacterium avium Colon. anal.	–	–	±	+	±
Mycobacterium fortuitum Cox 18	–	±	+	+	+ +
Mycobacterium marianum Chaudré	–	–	±	±	+
Mycobacterium phlei Moellmann	–	+	+ +	+ +	+ +
Mycobacterium smegmatis Smegma 10	–	+	+ +	+ +	+ –
Mycobacterium phlei Fleel	–	+ +	+ +	+ +	+ +
Actinomycetaceae					
Nocardia					
Nocardia asteroides 113	–	–	–	–	+
Nocardia erythropolis 80	–	–	–	–	+

[Legend:] a) TTC Reaction
b) pH 2.8; pH 5.0; pH 7.0; pH 9.1; pH 12.5

TABLE 1a. (continued)

	a) TTC-Reaktion				
b)	pH 2.8	pH 5.0	pH 7.0	pH 9.1	pH 12.5
Streptomycetaceae					
Streptomyces					
Streptomyces albus 115	-	+	+	++	++
Streptomyces griseus 111	-	+	+	+	++

TABLE 1b. Deuteromycetes

	a) TTC-Reaktion				
b)	pH 2.8	pH 5.0	pH 7.0	pH 9.1	pH 12.5
Thallosporales					
Tuberculariaceae					
Sporotrichum					
Candida					
Candida albicans	-	-	-	-	+
Candida Guilliermondi	-	+	+	+	++

[Legend:] a) TTC Reaction
b) pH 2.8; pH 5.0; pH 7.0; pH 9.1; pH 12.5

- END -